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BIOANTIBIO: BIOSOURCED ANTIBACTERIAL AND ANTIFUNGAL MOLECULES

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HEI INGÉNIEURS POUR LE MONDE | yncréa — Université Catholique de Lille — LIRIC — Faculté de Pharmacie de Lille

La science n'a pas de patrie, parce que

le savoir est le patrimoine de l'humanité,

le flambeau qui éclaire le monde

Louis Pasteur

Acknowledgments

Within these rows, I would like to express my thanks to several people and institutions.

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Va multumesc pentru ca ati acceptat sa fiu departe, va multumesc pentru tot.

To my friends

As long as I'm writing names down, I cannot neglect my best, constant friends since primary school or highschool: **Larisa**, **Ramona**, **Roxana**, **Andrei** who were there, always providing me a lot of support and encouragement over the years and with who I never missed an opportunity to get in perment record during my nice holidays back home or their visities in France. Growing together without growing apart.

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Preface

The results obtained within this project of thesis were obtained in collaboration with the following laboratories:

- Laboratory of Sustainable Chemistry and Health (INSERM LIRIC, UMR 995), Ecole des Hautes Etudes d'Ingénieur, Yncréa Hauts-de-France, Lille, France.

PhD director: Dr. Alina GHINET

PhD supervisor: Dr. Muriel BILLAMBOZ

- Laboratory of Analytical Chemistry, Faculty of Pharmacy, University of Lille.

PhD co-director: Dr. Emmanuelle LIPKA

The activities were carried out as part of the research project *BIOANTIBIO: Bio-sourced antibacterials and antifungals*. Partners: HEI, Faculty of Pharmacy, IFMAS.

Financement: HEI, Yncréa Hauts-de- France and Catholic Fondation of Lille.

The biological tests and results within this project were obtained in collaboration with the following institutions:

- University of Queensland, Australia, within the CO-ADD project (The Community for Antimicrobial Drug Discovery), funded by the Wellcome Trust (UK) and The University of Queensland (Australia).

- Pasteur Institute of Lille, Center of Infections and Immunity of Lille (CIIL) – Team Chemical Biology of Antibiotics, lead by Dr. Ruben HARTKOORN.

- University of Technology of Compiègne, Microbial Activities and Bioprocesses group - Integrated Renewable Material Transformations team - EA4297, research activities followed by Dr. Antoine FAYEULLE.

The solid state characterization of the potential additives for paint and varnishes, was carried out within the laboratories of IFMAS (Institut Français Des Matériaux AgroSourcés), an institute which until 2017 has developed new technologies to create innovative materials from plant resources.

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Abbreviations

AMB:	Amphotericin B
ATP:	Adenosine triphosphate
BINAP:	2,2′-Bis(diphenylphosphino)-1,1′-binaphthyl
BHT:	Butylated hydroxytoluene
DCC:	*N*, *N*'-Dicyclohexylcarbodiimide
DCU:	Dicyclohexylurea
DCM:	Dichloromethane
DPPH:	2,2-Diphenyl-1-picrylhydrazyl
DMSO:	Dimethyl sulfoxide
DNA:	Deoxyribonucleic acid
DSC:	Differential scanning calorimetry
DVS:	Dynamic vapor sorption
EFISH:	Electric field-induced second harmonic
ESBLs:	Extended spectrum β-lactamases
Et_2O:	Diethyl ether
EtOH:	Ethanol
FDA:	The Food and Drug Administration
FSSC:	*Fusarium solani* complex species
FTase:	Farnesyltransferase
FTI:	Farnesyltransferase inhibitor
HGT:	Horizontal gene transfer
HIT:	High-throughput screening
HMF:	Hydroxymethylfurfural
HPLC:	High-performance liquid chromatography
HRP:	Horseradish peroxidase
IC:	Inhibitory Concentration
IR:	Infrared radiation
KPC:	*Klebsiella pneumoniae* carbapenemase
LC:	Liquid chromatography
LPS:	Lipopolysaccharides
MeOH:	Methanol
MDR:	Multidrug-resistant
MIC:	Minimal Inhibitory Concentration

mRNA: Messenger ribonucleic acid

NAH: *N*-acylhydrazone

NAG: *N*-acetylglucosamine

NAM: *N*-acetylmuramic acid

NLO: Nonlinear optical

NMR: Nuclear magnetic resonance

PGA: Pyroglutamic acid

PTSA: *p*-Toluenesulfonic acid

RNA: Ribonucleic acid

ROS: Reactive oxygen species

SAR: Structure–activity relationship

SFC: Supercritical fluid chromatography

TAS-fluoride: (Tris(dimethylamino)sulfonium difluorotrimethylsilicate fluoride

TBAF: Tetra-*n*-butylammonium fluoride

TBHP: *tert*-Butyl Hydroperoxide

TEA: Triethylamine

TGA: Thermogravimetric analysis

THF Tetrahydrofuran

TLC: Thin-layer chromatography

tRNA: Transfer ribonucleic acid

UPLC: Ultra-performance liquid chromatography

UV-VIS: Ultraviolet–visible spectrophotometry

WHO: World Health Organization

BIOANTIBIO : Antibactériens/Antifongiques biosourcés

Résumé

En février 2017, l'Organisation Mondiale de la Santé (OMS) publie la toute première liste « d'agents pathogènes prioritaires », microorganismes résistants aux antibiotiques, présentant un risque élevé pour la santé humaine. Cette liste, divisée en trois catégories en fonction de l'urgence du besoin de nouveaux antibiotiques - priorité critique, haute et moyenne- comprend 12 microorganismes résistants à un grand nombre d'antibiotiques, parmi lesquels les carbapénèmes et céphalosporines de troisième génération, pourtant reconnus pour le traitement des bactéries multirésistantes. L'un des objectifs définis par l'OMS vise à garantir que la recherche et le développement répondent à ce besoin urgent de nouveaux antibiotiques. Le projet BIOANTIBIO s'inscrit dans cette optique de découverte et de développement de nouveaux agents thérapeutiques visant les bactéries résistantes.

Ce projet a été initié par le Laboratoire de Chimie Durable et Santé – INSERM U995-LIRIC (YNCREA-Hauts de France), représenté par le Dr. Alina Ghinet (HDR) en partenariat avec le Laboratoire de Chimie Analytique, Faculté des Sciences Biologiques et Pharmaceutiques de Lille, représenté par le Dr Emmanuelle Lipka (HDR), et l'Institut Français des Matériaux AgroSourcés (IFMAS), sous l'encadrement du Dr Muriel Billamboz (Laboratoire de Chimie Durable et Santé). En corrélation avec l'apparition de bactéries multirésistantes et en raison du manque d'alternatives thérapeutiques, ce projet a été proposé, afin de découvrir de nouveaux composés thérapteutiques. Lorsque le projet a été initié, deux applications principales avaient été envisagées :

• La première entrait dans le cadre de la formulation de matériaux innovants (peintures et revêtements, par exemple) afin de garantir leur durabilité en stockage (travail en collaboration avec IFMAS). Il était envisagé de concevoir des composés antifongiques et antibactériens capables d'être intégrés en tant qu'additifs aux peintures et vernis afin de les protéger de toute dégradation lors de leur phase de stockage (plusieurs années). Bien que les travaux aient débuté dans cette optique et que certains résultats préliminaires soient présentés en fin de manuscrit, la cessation d'activité de l'IFMAS en 2017 nous a incités à concentrer nos efforts sur la seconde application.

• La seconde application concerne la conception et le développement d'agents antimicrobiens et antifongiques avec une application en santé humaine. Au fur et à mesure de nos avancées, nous avons particulièrement développé des agents antibactériens visant la bactérie multirésistante aux médicaments, *Acinetobacter baumannii*.

Afin d'évaluer les activités biologiques des composés synthétisés, nous avons bénéficié de l'expertise des équipes de l'Institut des biosciences moléculaires (IMB), de l'Université du Queensland et de l'Institut Pasteur de Lille, ainsi que de l'équipe « Activités Microbiennes et Bioprocédés » de l'Université de Technologie de Compiègne.

Présentation générale

Les travaux de recherches décrits dans cette thèse ont donc été effectués au sein de plusieurs laboratoires : synthèse organique des composés au Laboratoire de Chimie Durable et Santé d'Yncréa Hauts-de-France, analyse chromatographique au Laboratoire de Chimie Analytique de la Faculté de Pharmacie de Lille, tests antifongiques *in vitro* au laboratoire MAB de l'UTC, Compiègne et analyses en phase solide à l'IFMAS, Lille.

Dans le **chapitre I**, les différentes stratégies de synthèse et les efforts visant à modifier et moduler l'acide pyroglutamique et ses dérivés ont été présentés et discutés. Cinq voies chimiques majeures ont été décrites, ainsi que des solutions alternatives connues dans la littérature.

Le **chapitre II** compile l'ensemble des résultats biologiques obtenus lors de ce travail. Les deux cibles d'intérêt - bactéries et champignons – sont présentées de façon séquentielle comprenant une introduction présentant les antibiotiques et antifongiques connus et leur mode d'action puis les résultats nouveaux obtenus lors de ces traveaux. Les discussions et les relations structure-activité sont réalisées et commentées par séries chimiques de molécules. À la fin de ce deuxième chapitre sur les cibles et résultats biologiques, une autre cible est présentée, la farnésyltransférase, dans le contexte où des résultats très intéressants ont été obtenus au sein de deux séries de composés.

Le **chapitre III** est consacré à l'analyse de certaines molécules sélectionnées. Comme beaucoup des molécules synthétisées lors de ce projet présentent un centre de chiralité, le premier sous-chapitre porte sur les techniques chromatographiques, et l'optimisation de la séparation énantiomérique de molécules sélectionnées. Le deuxième sous-chapitre porte sur l'analyse thermique de molécules sélectionnées, compte tenu en particulier de leur stabilité thermique.

Le **chapitre IV** regroupe les conclusions générales et les perspectives proposées. Les résultats sur le panel de 200 molécules synthétisées sont résumés et les résultats biologiques les plus importants sont récapitulés.

Le dernier **chapitre V** présente toute la partie expérimentale : procédures de tests biologiques, méthodes chromatographiques, protocoles d'analyse à l'état solide et caractéristiques physico-chimiques des nouveaux composé

INTRODUCTION

BIOANTIBIO:

BIOSOURCED ANTIBACTERIAL AND ANTIFUNGAL MOLECULES

GENERAL INTRODUCTION

Bacteria, fungi and molds are living microorganisms whose uncontrolled growing-up can cause several issues such as diseases (for human health) or degradation of the properties (for material science). In this general introduction, antibacterial and antifungal agents will be sequentially presented, from their discovery, to their development and the limitations which recently appeared for their use.

Emerging from these restrictions, the objectives of the BIOANTIBIO project, the design of targeted molecules and the contribution to the work of the different partners will be detailed.

BACTERIA AND ANTIBACTERIAL AGENTS

Antibiotics – greatest discovery of the 19th century

Bacteria, first identified in the 1670s by van Leeuwenhoek, are simple organisms, constituted from one cell. During the 19th century, strong correlations between bacteria and diseases attracted the interest of researchers, not only to answer some mysterious questions about infectious diseases, but also to find substances that could kill, inhibit, or at least slow down the growth of such disease-causing bacteria.

In 1941, Selman Waksman introduced the term "*antibiotic"* in order to describe a small molecule made by a microbe that inhibits the growth of other microbes. Even if antibiotics have been used for over one millennia by earliest civilizations such as Egyptians, it wasn't until the late 19th century that scientists began to observe antibacterial chemicals in action. Paul Ehrlich, a German physician, noted that certain chemical dyes colored some bacterial cells but not others. He concluded that, according to this principle, it must be possible to create substances that can kill certain bacteria selectively without harming other cells. In 1909, he discovered that a chemical called arsphenamine was an effective treatment for syphilis. This became the first modern antibiotic, although Ehrlich himself referred to his discovery as 'chemotherapy', as the use of a chemical to treat a disease.

Sir Alexander Fleming, a bit disorderly in his work, accidentally discovered penicillin in 1928, when a piece of mold fortuitously contaminated a petri dish. After isolation, he found that *P. notatum* proved extremely effective even at very low concentrations, preventing *Staphylococcus* growth, being less toxic than the disinfectants used at the time. In less than 12

years of research, Fleming and his collaborators had turned this finding into the "miracle" drug of its time, which cured patients with bacterial infections.

By the end of World War II, penicillin was nicknamed "the wonder drug" and had saved many lives. Further antibiotics were discovered and went on to revolutionize healthcare, becoming the bedrock of many of the greatest medical advances of the 20th century. In the post-World War II period following Alexander Fleming's breakthrough discovery along with the partnership between industry and government to produce this lifesaving drug on an industrial scale, new antibiotics were discovered and developed at a breathtaking pace. Such efforts led to great advances in human health, as antibiotics were used to treat a wide range of infections, allowing the evolution of the complex medical care that is now taken for granted, such as intensive care medicine dialysis, cancer treatment and hip replacement (Figure 1).

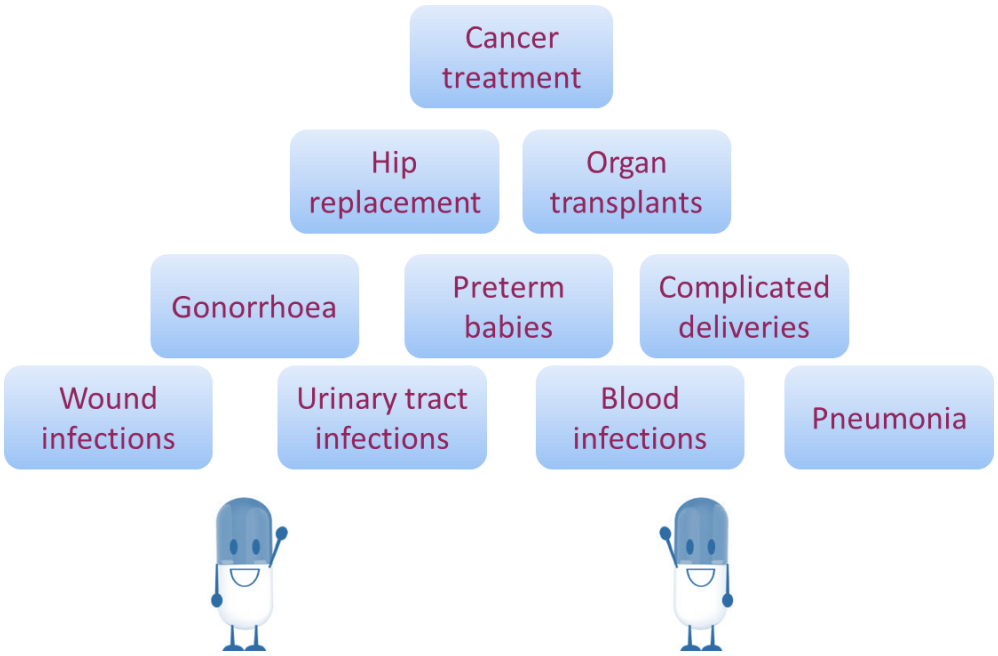


Figure 1 *Pyramid of antibiotic uses*

The scientists in Oxford were instrumental in developing the mass production process, and Howard Florey and Ernst Chain shared the 1945 Nobel Prize in Medicine with Alexander Fleming for their role in creating the first mass-produced antibiotic. The "golden era" of antibiotics ranged from the 1930s to 1960s and gave rise to many antibiotics.[1] Unfortunately, that era ended because researchers were unable to maintain the pace of antibiotic discovery in the face of emerging resistant pathogens.

[1] Nathan C., Cars O. Antibiotic resistance-problems, progress, and prospects. *N. Engl. J. Med*. 2014, 371, 19, p. 1761–1763.

Classification of antibacterial drugs based on their mode of action

Among the major functions, which are responsible for bacterial growth, are cell wall synthesis, cell membrane function, protein synthesis, nucleic acid synthesis or essential metabolites synthesis. Things considered, antibacterials, which disturb these processes, can be subdivided into five groups: i) agents acting on cell wall formation; ii) plasma membrane disruptors; iii) protein synthesis inhibitors; iv) DNA replication disruptors and v) essential metabolites synthesis inhibitors (**Figure 2**).

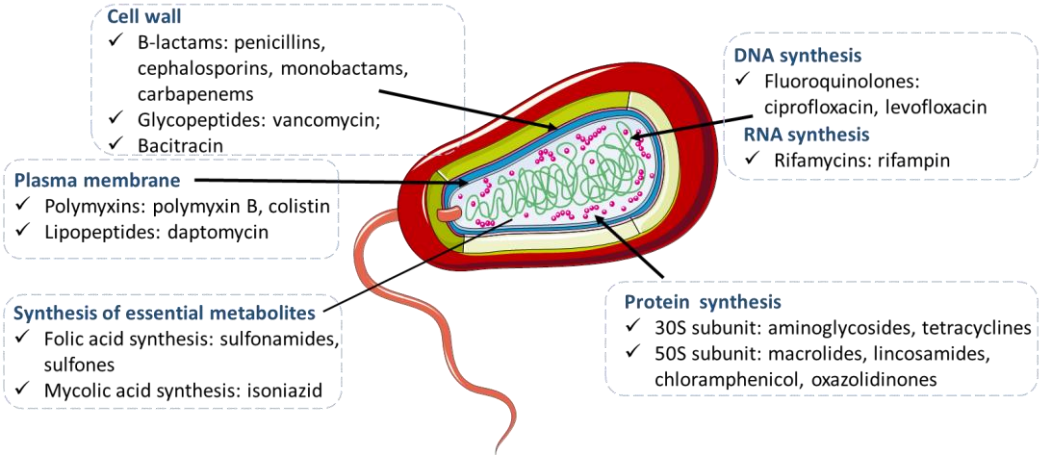


Figure 2 *Antibiotic classes based on their bacterial target*

i) Cell wall formation (peptidoglycan synthesis)

The bacterial cell wall offers structural completion to the cell, therefore, the most important process for avoiding bacterial growth is to stop cell wall synthesis by inhibiting the peptidoglycan layer of bacterial cell walls. In Gram-positive and Gram-negative bacteria the cell wall is formed from a cross-linked chain of alternating units of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM), known as peptidoglycan or mucopeptide (**Figure 3**).

In Gram-positive bacteria the cell wall structure is thick (about 30 nm), tightly cross-linked and embedded with polysugarphosphates (teichoic acids), some of which have a lipophilic tail buried in the cell membrane (lipoteichoic acids). Gram-negative bacteria, on the other hand, have a relatively thin (2-3 nm), loosely cross-linked peptidoglycan layer without teichoic acid, but have other components like lipid A. External to the Gram-negative peptidoglycan is a membrane-like structure, composed mainly of lipopolysaccharide and

lipoprotein, which prevents large molecules such as glycopeptides from entering the cell. Small hydrophilic molecules enter Gram-negative bacilli through aqueous channels called porins.

β-Lactam drugs, including penicillin derivatives, cephalosporins, monobactams, and carbapenems, are the major antibiotics that inhibit bacterial cell wall synthesis, some known exemples can be seen in **Figure 3**.

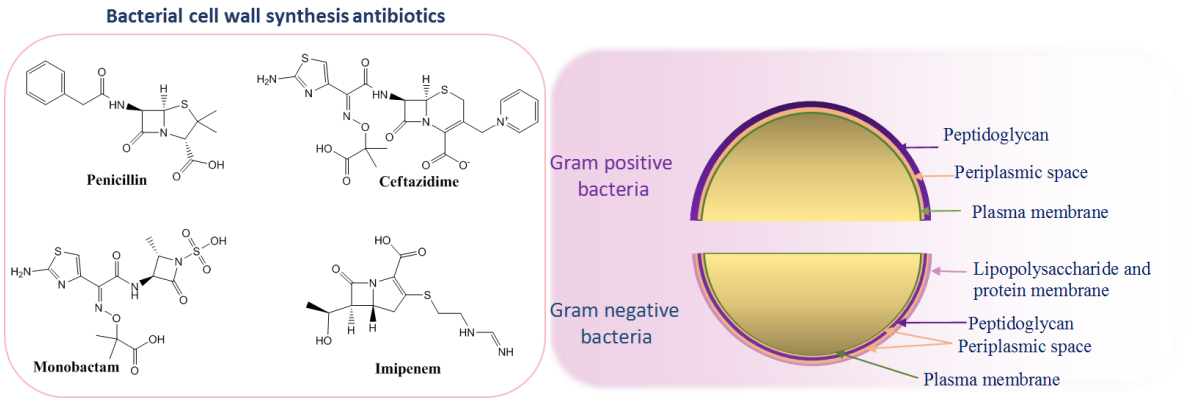


Figure 3 *Differences between Gram-positive and Gram-negative cell walls*

ii) Plasma membrane

The cytoplasmic membrane, which covers the cytoplasm, serves as a selective barrier and controls the internal composition of the cell. Whenever these functional roles of the cytoplasmic membrane get disturbed, macromolecules and ions will outflow, which will result in cell destruction or death.

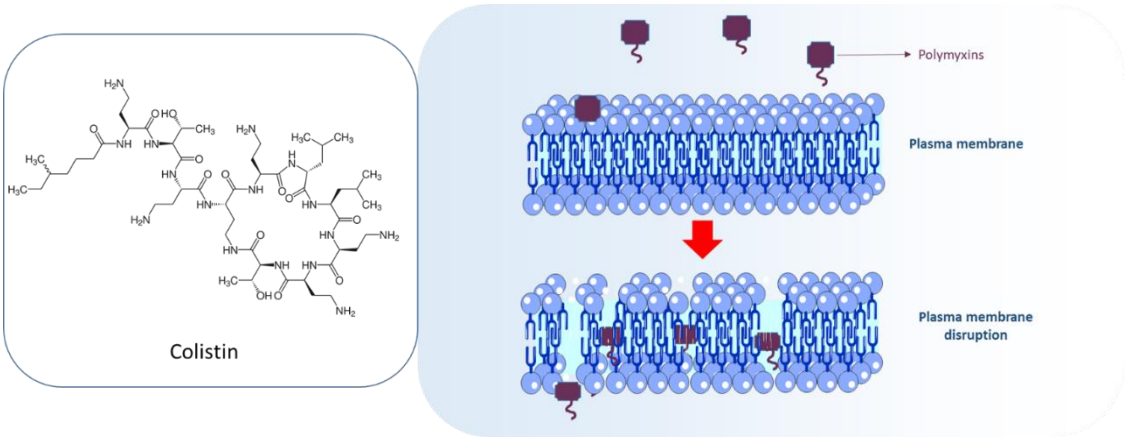


Figure 4 *Polymyxins binding to LPS, leading to the plasma membrane disruption*

Polymyxins are antibacterial agents, which are cyclic peptides, with a long hydrophobic tail. Polymyxins show their specificity for polysaccharide molecules, which are present in the outer membrane of many Gram-negative bacteria, this way polymyxins are considered to be selectively toxic for Gram-negative bacteria. Mechanistically, after association with the lipopolysaccharide substrate (LPS) in the outer membrane of Gram-negative bacteria, polymyxins change the membrane structure so that its permeability increases, which results in disruption of the osmotic balance (**Figure 4**). As Gram-positive bacteria have a too thick cell wall, which denies the access of these molecules to the Gram-positive bacterial cell membrane, polymyxins have less or even no effect on Gram-positives.

iii) Protein synthesis inhibitors (mnemonics)

The ribosome is one of the most sophisticated macromolecular machines of the cell. It is composed of two unequal subunits, a small 30S and large 50S in bacteria, which join together to form a 70S ribosome. There is a diverse range of clinically important antibiotics that interfere with protein synthesis by binding at various functional centers of the ribosome and either freezing a particular conformation of the ribosome or hindering the binding of its ligands.

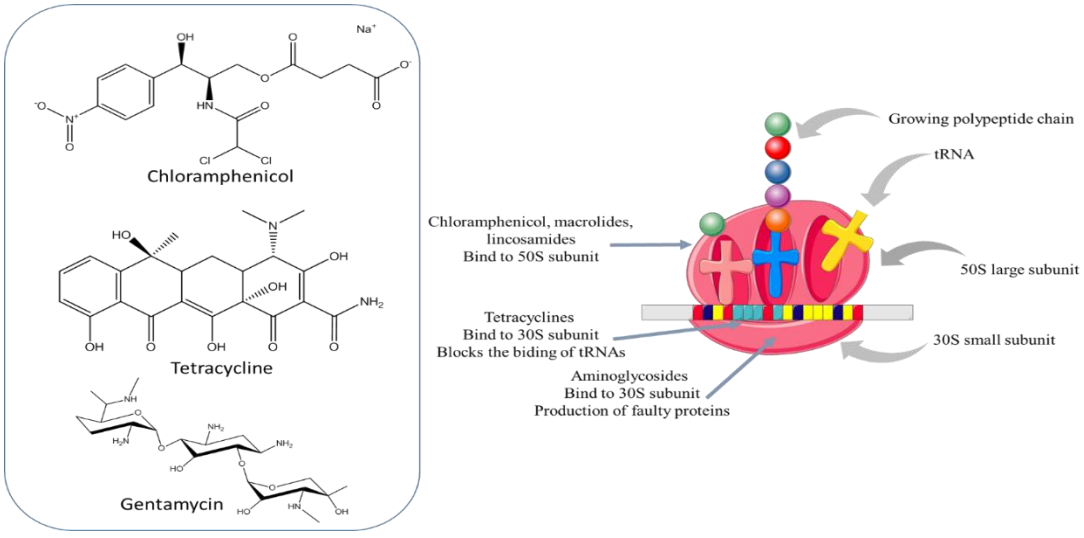


Figure 5 *The major classes of protein synthesis inhibitors*

Antibiotics predominantly interact with the messenger RNA (mRNA)-transfer RNA (tRNA) decoding region on the 30S subunit, or the peptidyltransferase center on the 50S subunit (**Figure 5**). Protein synthesis can be divided into four main steps: initiation, elongation,

termination, and ribosome recycling, each of which is targeted by a range of different antibiotics.[2]

Inhibitors That Bind the 30S Subunit

In this category enter aminoglycosides, highly polar antibacterial drugs that impairs the proofreading ability of the ribosomal complex, causing mismatches between codons and anticodons, resulting in the production of proteins with incorrect amino acids and shortened proteins that insert into the cytoplasmic membrane.

Another class of antibacterial compounds that bind to the 30S subunit is tetracyclines, bacteriostatic drugs that block the association of tRNAs with the ribosome during translation.

Inhibitors That Bind the 50S Subunit

There are several classes of antibacterial drugs that work through binding to the 50S subunit of bacterial ribosomes. One of them is the macrolides, broad-spectrum, bacteriostatic drugs that block elongation of proteins by inhibiting peptide bond formation between specific combinations of amino acids.

Another such family of antibiotics, having the same mechanism of action as macrolides are the lincosamides, which include the naturally produced lincomycin and semisynthetic clinda-mycin.

Another structural distinct class, but with the same mechanism of action of peptide bond formation inhibition is the chloramphenicol. Chloramphenicol, a natural antibiotic, was the first broad-spectrum antibiotic that was approved by the FDA.

The oxazolidinones, are the newest broad-spectrum class of synthetic protein synthesis inhibitors that bind to the 50S ribosomal subunit of both gram-positive and gram-negative bacteria.

iv) DNA and RNA replication

[2] Wilson D.N. Ribosome-targeting antibiotics and bacterial resistance mechanisms. *Nat Rev Microbiol*, 2014, 12, p. 35–48.

Another mechanistic pathway of antibiotics involves the inhibition of nucleic acid synthesis. These classes of drugs are working either by interfering with DNA replication in the target cells, the case of nitroimidazole family, or by blocking RNA polymerase activity in bacteria, the last one being the case of rifamycin family. The RNA polymerase enzymes in bacteria are structurally different from those in eukaryotes, making the rifamycins selective against bacterial cells.

In the same family of DNA synthesis inibitiors, enters the quinolone family which are selectively inhibiting the activity of bacterial DNA gyrase, blocking DNA replication.

v) Synthesis of essential metabolites

The sulfonamides, the last important class of antibiotics are synthetic, functioning as antimetabolites, competitive inhibitors for bacterial metabolic enzymes. Their mechanism of action consists in the inhibition of the enzyme which is involved in the production of the dihydrofolic acid, blocking the biosynthesis of folic acid, subconsequently blocking the synthesis of pyrimidines and purines required for nucleic acid synthesis.

Isoniazid is an antimetabolite, specific for mycobacteria, being used in the treatment of tuberculosis in combination with rifampin or streptomycin.

Antibiotic resistance

"The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug, make them resistant" – 1945, Alexander Fleming, during his Nobel Prize acceptance speech. True to his prediction, penicillin resistance began to emerge after 10 years of a long fight against bacterial infections, the main reason being the widescale introduction of it.

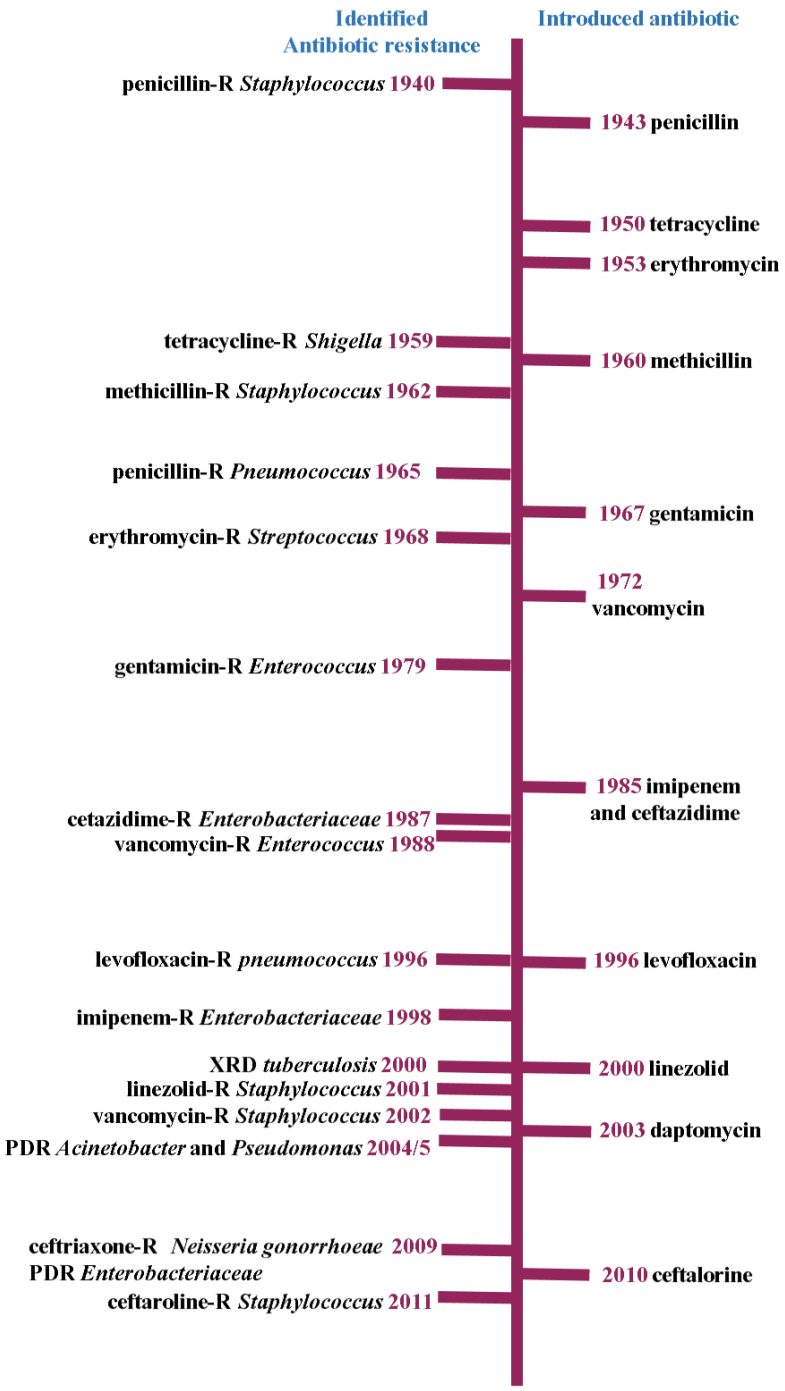


Figure 6 *Roadmap of antibiotics and resistance*

Due to the mishandling and misprescription of antibiotics, the bacterial population was transformed such that many drugs have lost their efficacy, and now, after 74 years since Fleming warning, important resistance is upon us (**Figure 6**). The "golden era" of antibiotics lasted from the 1930s to 1970s and gave rise to many antibiotics. This era ended because researchers were unable to maintain the pace of antibiotic discovery in the face of emerging resistant pathogens, a "golden" pipeline which rapidly dried out. In recent decades, the discovery and development of new antibiotics have decreased dramatically as scientific barriers

to drug discovery, regulatory challenges, and poor investment have led major drug companies to scale back or abandon their antibiotic research. As a consequence, antibiotic discovery, which peaked in the 1950s, has dramatically dropped. A greater concern is the fact that approximatively all antibiotics which saw the market in the past 30 years have been modifications on existing drugs ("me too" drugs), with no innovative skeletons.[3] As a result, currently available antibiotics are derivatives of a class discovered between 1920s and 1984.

Antimicrobial resistance (AMR) poses a serious global threat to the public health, threating the effective treatment of a wide range of infections caused by bacteria, parasites, and fungi no longer susceptible to the available drugs used to treat them. The most important resistance is now in bacteria. Since the penicillin was discovered, in different manners, bacteria have developed resistance to each new marketed antibiotic. Bacteria which are resistant to many different antibiotics, are now called multidrug-resistant (MDR). Infections with MDR bacteria are hard to treat since the treatment lacks or there are very few options left. The current multidrug-resistance facilitates the spread of antibiotic resistance, posing one more risk.

In the present, antimicrobial resistance-related deaths have reached alarming rates throughout the world. Statistics suggest that more than 700,000 people are annually dying from drug-resistance infections; this number could rise to 10 million by 2050, a number that is winning over cancer, the second major cause of death worldwide after cardiovascular diseases.[4] Bacteria use two main genetic strategies to acquire antibiotic resistance:

i) Acquisition of foreign DNA coding for resistance through horizontal gene transfer (HGT). There are three different ways in which this can occur: a) transformation, b) transduction, c) conjugation. The simplest type of HGT is the transformation of genetic material of antibiotic-resistant bacteria to other bacterial cells, making them resistant to antibiotics as well. The bacterial DNA can be found in chromosomes and plasmids.[5] The genetic material known to provide protection against antibiotics is commonly found in plasmids. A single plasmid can have several resistance genes as R1, R2 and R3 (**Figure 7**). As a consequence, the use of only one antibiotic, can maintain all the antibiotic resistance mechanisms as they are all linked on the plasmid. This being the so called phenomenon, of co-selection of resistance genes. Co-selection phenomenon makes almost impossible to reverse resistance once it has been settled

[3] Lynn L. Challenges of Antibacterial Discovery. *Clin. Microbiol. Rev.* 2011, 24, p. 71–109.
[4] Ragheb M.N., Thomason M.K., Hsu C. Nugent P., Gage J., Samad pour A.N., Kariisa A., Merrikh C.N., Miller S.I., Sherman D.R. Inhibiting the Evolution of Antibiotic Resistance. *Mol. Cell,* 2018, 73, p. 157-165.
[5] Hughes V.M, Datta N. Conjugative plasmids in bacteria of the "pre-antibiotic" era. *Nature*, 1983, 302, 5910, p. 725–726.

in a bacterial population. If multidrug-resistance plasmids are transferred to other bacteria, these last become also resistant to antibiotics. In healthcare institutions and large production animal farms for example, where bacteria are continuously exposed to antibiotics, multidrug-resistance is favored and in consequence selected and spread further.

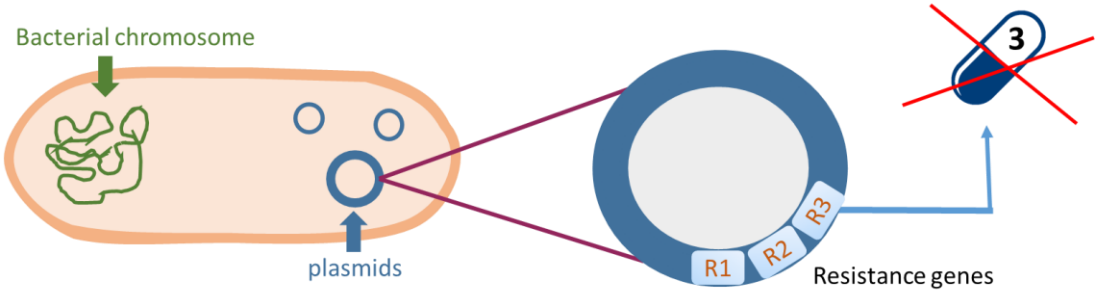


***Figure 7** Plasmids and resistance genes*

Another strategy of defense against antibiotics, is the mutational resistance.[6] This could be happening *via* mechanisms like: a) modifications of the antibiotic[7] (enzymes chemically inactivate the antibiotic or destroy the molecule itself, becoming unable to recognize the target), b) drug excretion by activation of efflux pumps (mechanisms which prevents the antibiotic of reaching the intracellular target, notably through porins alteration or the overexpression of pump efflux[8]), c) important changes in the target sites (protection of the binding site, alteration or sometimes complete replacement of it,[9] resulting in decreased or no affinity of the drug) (**Figure 8**). Resistance due to acquired mutational changes varies in complexity and resistance to one antibiotic class can usually be achieved through more than one biochemical pathway, one bacteria being capable of using multiple mechanisms in order to survive an antibiotic "attack".

[6] Munita J., Arias C.A. Mechanisms of Antibiotic Resistance. *Microbiol. Spectr.* 2016, 4, 2.
[7] Wilson D.N. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nat. Rev. Microbiol*. 2014, 12, 1, p. 35–48.
[8] Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev*. 2003, 67, 4, p. 593–656.
[9] Miller W.R., Munita J.M., Arias C.A. Mechanisms of antibiotic resistance in enterococci. *Expert Rev. Anti Infect. Ther*. 2014, 12, 10, p. 1221–1236.

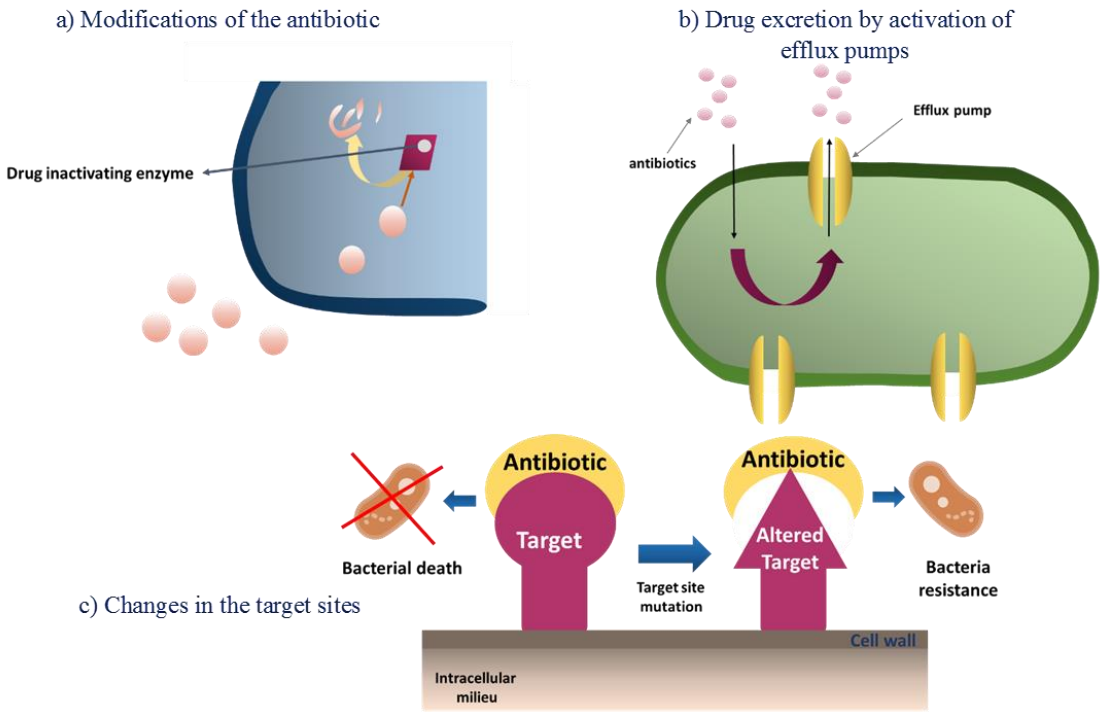


***Figure 8** Mutational resistance mechanisms*

The discovery of the "wonder drugs" is very young compared to the emergence of organisms such as bacteria, on this planet. As a consequence, resistance is a normal, adaptive phenomenon and moreover a clear example of Darwinian principles of evolution. In order to survive the antibiotic attack, bacteria have developed creative strategies, resistance which has evolved fast in the last few decades, becoming one of the most important threats of 21st century. As a consequence, researchers have to adapt their strategies to design adapted therapeutic agents.

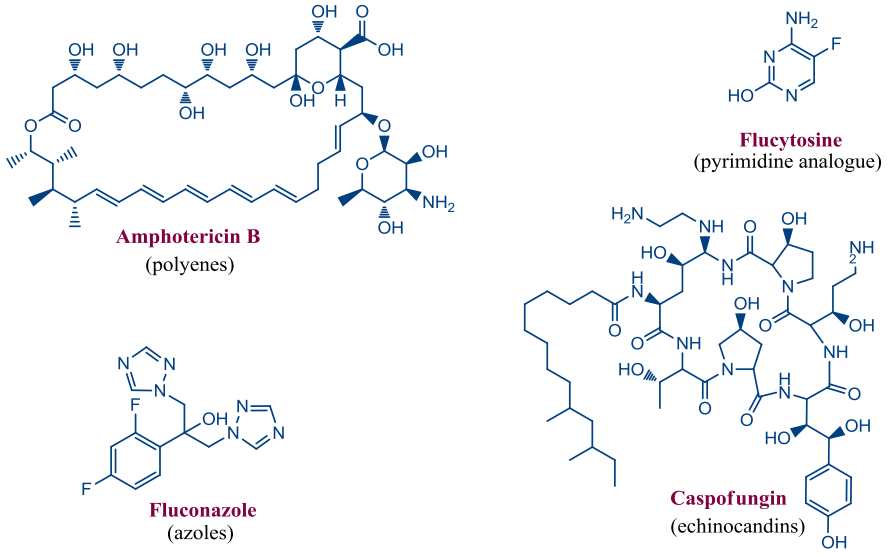
FUNGI AND ANTIFUNGAL AGENTS

The first antifungals date back in 1900 when potassium iodide was used for the treatment of sporotrichosis. Although its mechanism of action is unknown, it is still used as a therapeutic alternative as a 1g.mL^{-1} aqueous solution. Between 1940 and 1950, topical treatments with mainly exfoliating and keratolytic action and a weak antifungal power emerged. These include Whitfield's ointment consisting of 6% benzoic acid and 3% salicylic acid and the Castellani tincture consisting of a 0.3% fuchsine solution, gentian violet at 0.5%, undecylenic acid at 5% and selenium sulfide at 2%.[10]

[10] Diehl K.B. Topical antifungal agents: an update. *Am. Fam. Physician*, 1996, 54, 5, p. 1687-1692.

Antifungal therapies evolved really slowly during the early years of the 20th century, the development of antifungal agents lagging behind that of antibacterial agents. This is a predictable consequence of the difference between the two organisms, bacteria and fungi. As a prokaryotic organism, the bacteria offers more structural and metabolic targets which differ from the human host. In contrast, fungi are eukaryotes, in consequence, the antifungal agents which are toxic to fungi are also toxic to human due to inhibition of closely related human targets. Besides this fact, fungi generally grow slowly, in multicellular forms, being generally more difficult to quantify than bacteria. Consequently, this complicates the *in vitro* or *in vivo* experiments designed to evaluate the potential antifungal agents.

Introduced in the 1950s, amphotericin B (AMB) has long been considered as a gold standard for invasive fungal infections. Then in the 1970s, fluocytosine was introduced, but its emergence was rapidly limited by its toxicity, narrow spectrum of activity and the development of frequent resistance when it was used as monotherapy. However, it made the entrance to the first generation of azole antifungals in the 1990s. The second generation antifungal azoles appeared in the 2000s.[11] During this same period, echinocandin-type antifungals, which have little drug interaction and a good safety profile, found their place in the therapeutic arsenal. Starting with the early 1990s, systemic triazole antifungals became the first treatment choice in the management and prophylaxis of invasive fungal infections. The current therapeutic arsenal for systemic treatment of antifungal infections includes polyenes, azoles, echinocandins and pyrimidines (**Figure 9**).



[11] Nett J.E., Andes D.R. Antifungal Agents: Spectrum of Activity, Pharmacology, and Clinical Indications. *Infect. Dis. Clin. N. Am.* 2015, 30, 1, p. 51-83.

Figure 9 *Structures of systematically used antifungals*

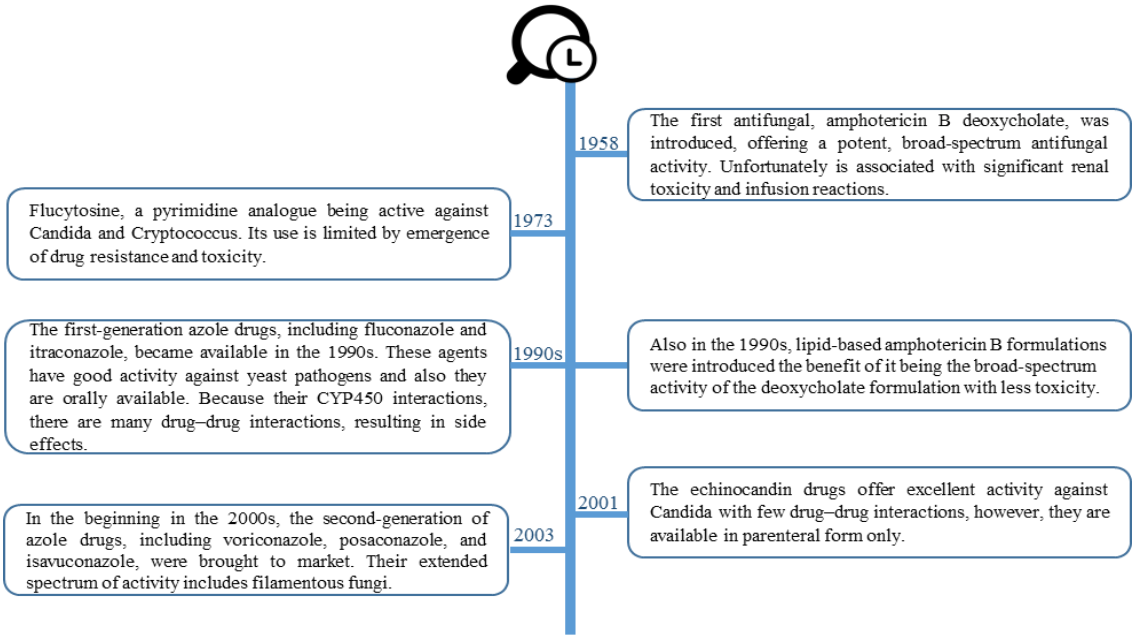


Figure 10 *History of antifungal therapies*[12]

Antifungal antibiotics are now facing the same threat that antibacterial compounds: the development of antifungal resistance in medical care facilities, environments concomitant with their use. Fungal infections have emerged as an important clinical threat, with high associated morbidity and mortality rate. During the last decade, clinical needs for novel antifungal agents have altered steadily with the change in spectrum of fatal disseminated fungal infections. Considering allergies in particular, in comparison with pollen, fungal spores and mycelial cells can cause type I allergy as well as a large number of other illnesses, as allergic sinusitis, bronchopulmonary mycoses, hypersensitivity, pneumonitis and also atopic dermatitis. Besides this fact, in up to 80% of asthmatic patients, fungal allergies are frequently linked with allergic asthma, while pollen allergies are not.

Identifying new drugs is challenging because of the fungal diversity, as well because the drug should remain well tolerated in human subject. Up to date, there are only three different classes from a mechanistic point of view which are currently used to treat serious infections due to both yeast and molds.

[12] Roemer T., Krysan D.J. Antifungal Drug Development: Challenges, Unmet Clinical Needs, and New Approaches. *Cold Spring Harb Perspect Med.*, 2014, 4, 5.

Known antifungal agents and their mechanism of action

Fungal cells are complex organisms sharing many common biochemical targets with other eukaryotic cells. As a consequence finding a target which won't result in drug-interactions or toxicity is difficult. The fungal cell wall is a unique organelle and a target which is fulfilling the criteria for selective toxicity and even if each organism has a different biochemical composition, their gross cell wall structure is similar. The major components of the fungal cell wall are chitin, glucans and glycoproteins. Although variations can exist from species to species, the cell wall components are thought to be arranged as shown in the above figure. The β-(1,3)-glucan extends throughout the cell wall. The proteins, glucans and chitin components are integrated into the wall by crosslinking the chitin, glucans and protein-associated oligosaccharides.

As the fungal cell wall is very different from the bacterial cell wall, it is not affected by antibacterial cell wall inhibitors like β-lactams or vancomycin. There are three general mechanisms of action for the antifungal agents: cell membrane disruption, inhibition of cell division and inhibition of cell wall formation.

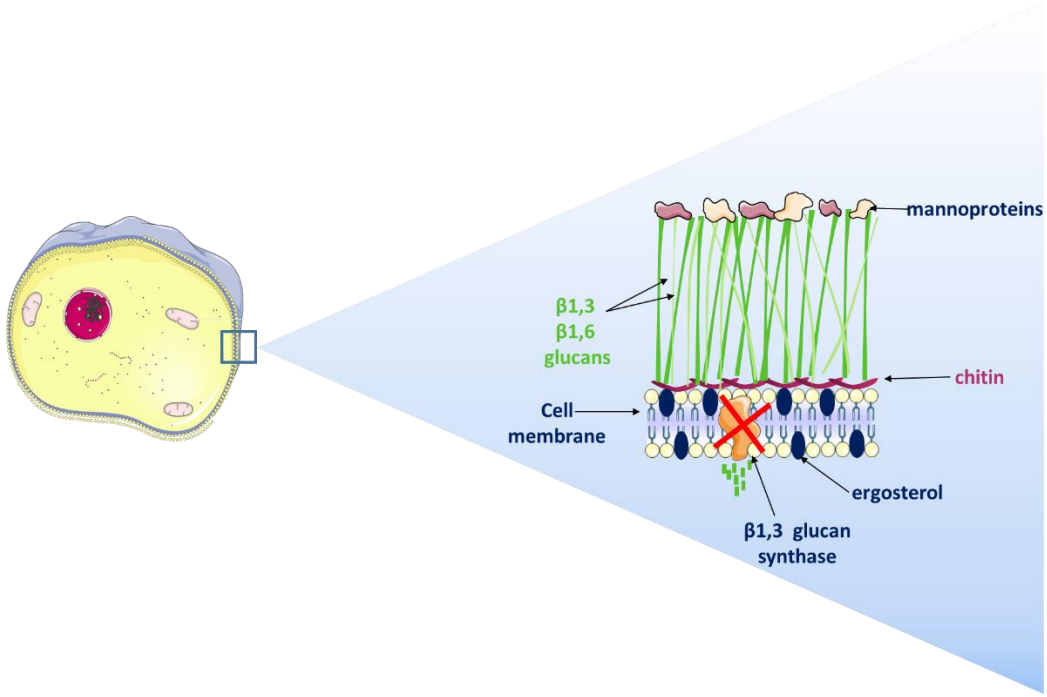


Figure 11 *Representation of the fungal cell wall*

Cell membrane disruption

There are two possible mechanisms for the cell membrane disruption, both targeting ergosterol, which is the most abundant sterol in fungal cell membranes, regulating permeability

and fluidity. Because of its crucial functions, unique structural properties, and particular biosynthetic steps, the ergosterol is one important target of many antifungals which are either binding to the sterol, forming pores which has as a result a leaky membrane (the class which is binding the sterol is polyene class), or are inhibiting the ergosterol biosynthesis (the case of azole antifungal agents). The only problem in targeting ergosterol is its similarity to mammalian cholesterol, and as a consequence agents binding ergosterol may be cytotoxic in the host tissue. Compared to cholesterol for example, the mammalian sterol, the ergosterol has two additional conjugated double bonds.

Polyenes

The most known antifungal of the family of macrocyclic polyenes is AMB (**Figure 9**), which is extracted from *Streptomyces nodosus*. Polyenes are amphoteric organic molecules that have a high affinity for sterols. They attach to sterols of the fungal cell membrane, making it permeable and thus causing its lysis by potassium depletion and sodium entry.[13] Although its spectrum of action is broad, its administration is accompanied by adverse effects and sometimes significant toxicity.

Another member of this family is nystatin (**Figure 12**), the first topical antifungal to be used in clinical practice. It has activity on yeast and is indicated in mucosal candidiasis and in the prophylaxis of oropharyngeal candidiasis in immunocompromised patients. Recently, the liposomal form of nystatin has been developed for the intravenous treatment of invasive fungal infections.

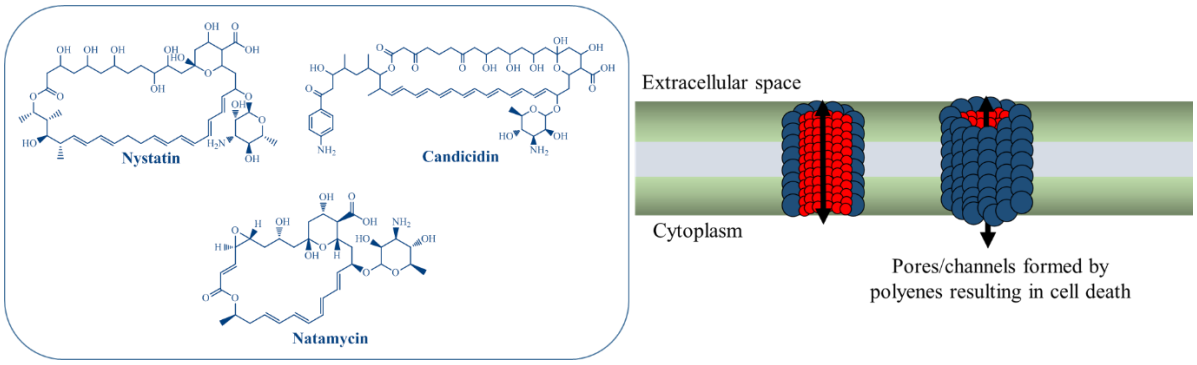


Figure 12 *Pore formation by ergosterol binding*

[13] Valiante V., Macheleidt J., Föge M, Brakhage A.A. The *Aspergillus fumigatus* cell wall integrity signaling pathway: drug target, compensatory pathways, and virulence. *Front. Microbiol.* 2015, 6, p. 325.

Azoles

Since the 1990s, azole antifungals are by far the most clinically used. Depending on the number of nitrogens in the ring, they are divided into imidazoles: miconazole and ketoconazole and triazoles: fluconazole, itraconazole, voriconazole (**Figure 13**). These antifungals target ergosterol synthesized from lanosterol *via* 14-α demethylase (ERG11) enzyme. This enzyme is encoded by the CYP51A gene. The accumulation of lanosterol and the lack of ergosterol in the cell results in high toxicity and leads to lysis of the cell. Imidazole antifungals are little used therapeutically for the treatment of invasive infections because of their wide spectrum of activity but also because of their toxicity and significant subsequent drug interactions.

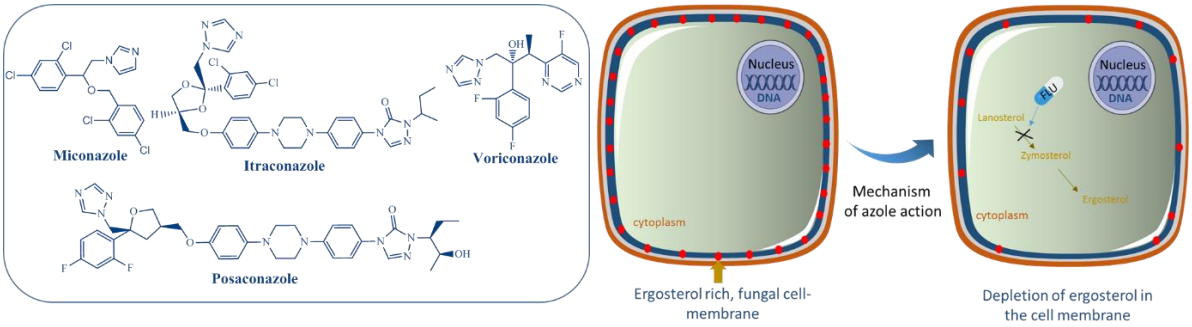


Figure 13 *Ergosterol biosynthesis inhibition*

Inhibition of Cell Division

Nucleoside antifungal agents affect cell division by targeting the microtubule effects in forming the mitotic spindle. The first such antifungal was flucytosine. The drug enters the fungal cell through active transport on ATPases which normally transports pyrimidines. After that, the fungal cytosine deaminase converts the drug into 5-fluorouracil which is further incorporated into RNA causing faulty RNA synthesis or inhibit the thymidylate synthase which causes the inhibition of DNA synthesis. Mammalian cells do not contain cytosine deaminase; in this way such antifungals can act selectively towards fungi (**Figure 14**).

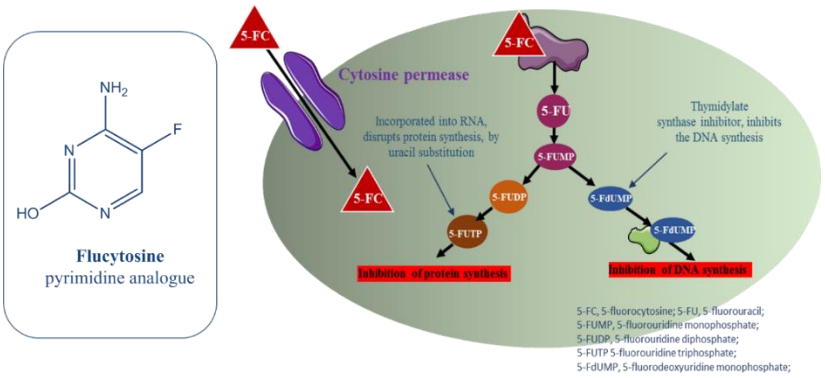


Figure 14 *Ergosterol biosynthesis inhibition*

Inhibition of Cell Wall Formation

Many chemicals have been discovered that interfere with various steps in fungal cell wall synthesis with excellent antifungal activity *in vitro*. Unfortunately, development of these agents into useful drugs proved to be very difficult. Many of these agents were developed to target β-glucan synthesis.

The fungal wall is constituted by different substances highlighting glucans, chitin, and mannoproteins. Glucans are carbohydrate polymers formed by glucose units linked by β-1,3 (linear) and β-1,6 (branched) bonds. β-(1,3)-glucan synthase is an essential enzyme for the integrity of the cell wall of the fungus. Any mutation that prevents the synthesis of β-(1,3)-glucan is lethal. Another very important factor is that there is no β-(1,3)-glucan in the cells of mammals. Some families of compounds inhibiting the synthesis of glucans which has been studied are papulacandins and the echinocandins (**Figure 15**). These antifungals are both natural products derived from fungi, papulacandins being glycolipids while echinocandins are lipopeptides.

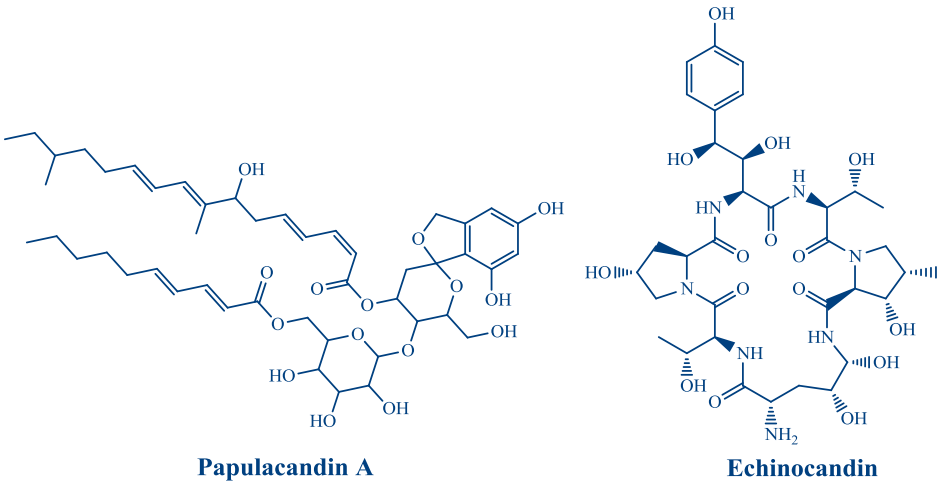


Figure 15 Structures of Papulacandin A, respectively Echinocandin E

The BIOANTIBIO project

In February 2017, the World Health Organization (WHO) publishes the first-ever list of "priority pathogens" resistant to antibiotics, posing a high risk to human health. The lack of therapeutic innovations or new chemical families in the pipe for the discovery of therapeutic alternatives reached a critical level. This issue, correlated with the appearance of multidrug-resistant bacteria underlined the need for new approaches towards the discovery of innovative antibiotics. As a consequence, the BIOANTIBIO project was proposed.

The two main initially envisioned applications for the most active compounds were:

• Additives for the formulation of innovative materials (*eg* paints and coatings) in order to guarantee their durability in storage (working in collaboration with IFMAS).

• Drugs: antibacterial and antifungal agents, particularly for the development of antimicrobial agents against drug-resistant bacteria for human health protection.

Employing natural molecules to develop novel antifungal or antimicrobial classes can be challenging, especially as novel scaffolds and families are required as a measure to decrease fungi and bacteria resistance to drugs. Nevertheless, nature is known to inspire chemists in the total or semisynthesis of analogues with improved biological properties and there are still new resources to be exploited. In the context of sustainable development and green chemistry, many efforts have focused on the recovery of natural resources or industrial waste.

Pyroglutamic acid (PGA), also known as 5-oxoproline or pidolic acid is a derivative of the amino acid glutamine and glutamic acid which was discovered in 1882 by Haitinger,[14] who observed that when it was heated, glutamic acid lost a molecule of water to give a new product, namely PGA. The exact structure of the molecule was only determined in 1892 by Menozzi and Appiani.[15] However, the actual development of the acid did not begin until after the Second World War. In the 60s, Drs Monge, Bocher, Harnist and Ciaceri, pioneers in this field, conducted studies on PGA and its salts. Since then, numerous patents and articles have been dedicated to this remarkable molecule all over the world, and there are now more than 10,000 publications on PGA and its derivatives.

[14] Haitinger L. Vorlauge Mittheilung iiber Glutaminsaure und Pyrrol. *Monatsh. Chem*. 1882, 3, p. 228-229.
[15] Menozzi, A., Appiani G. Sopra alcuni derivati dell' acido glutammico, Acidi piroglutammici e piroglutammidi. *Gazz. Cim. Ital.* 1892, 22, 14.

PGA is a constituent of many plant and animal tissues, which conquered the pharmaceutical and cosmetic fields as well, being one of the reasons why our team continued to study and modify this chemical synthon. Gertrud Pascher was the first to note in 1956 the high concentration of PGA in the human stratum corneum (97% PGA present in the skin is localized in the stratum corneum).[16] Since then, numerous studies have been done on the presence of this unusual metabolite in the human body, revealing the presence of PGA in free state, acid or salt, in most tissues and biological fluids.

PGA is obtained by thermal cyclization of glutamic acid (dehydration reaction). It is exclusively obtained in the L form, thanks to a synthesis process allowing to perfectly control the stereochemistry of the molecule. This is a most natural process possible since during the various stages, no solvent or chemical additive intervenes being obtained from sugar beet molasses. In the past 4 decades, the HEI laboratory has developed a great deal of expertise around pyroglutamic acid derivatives, which is now continuing through BIOANTIBIO, being the first project having as research and development subject, antibacterial and antifungal molecules.

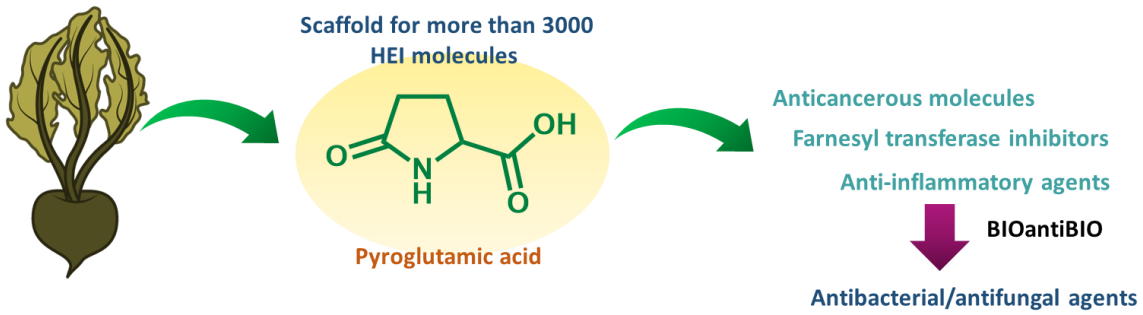


Figure 16 *Main motif of the project and HEI expertise – Pyroglutamic acid*

During the course of the project, some decisions had to be taken. Indeed, in 2017, IFMAS activity ended and, in parallel, exciting results on human health were obtained. So, from the initially envisioned applications, the formulation part was not fully evaluated and it was decided to put our efforts on further developing human drugs.

Structural arrangement of the manuscript

[16] Pascher G. Die wasserlöslischen Bestandteile der peropheren Hornschicht (Hautoberfläche) Quantitative Analysen III, a-pyrrolidoncarbonsaüre., *Archiv. Klein. Dermatol.* 1956, 203, p. 234-238.

In **Chapter I**, different synthetic strategies and efforts to modify and modulate pyroglutamic acid and its derivatives are presented and discussed.

Chapter II presents the two targets of interest bacteria and fungi, with known antibiotics and antifungals and their mode of action. Each part, antibiotics or antifungals, is followed by the obtained results concerning the designed compounds. At the end of this second chapter on biological targets and results, another target is presented, farnesyltransferase.

Chapter III is devoted to the analysis of certain selected molecules. The first subchapter deals with chromatographic techniques, and the optimization of the enantiomeric separation of selected molecules. The second sub-chapter deals with the thermal analysis of selected molecules, especially in view of their thermal stability.

Chapter IV brings together the general highlights and the proposed perspectives.

And the last **chapter V** presents the overall experimental part: biological test procedures, chromatographic methods, solid state analysis protocols and physicochemical characterization of the new compounds.

CHAPTER I

SYNTHETIC EFFORTS TOWARDS DEVELOPING NEW ANTIBACTERIAL AND ANTIFUNGAL DRUGS

General chemical strategies

As stated in the introduction of the manuscript, the goal of the project was the research and development of new antifungal and antibacterial agents, which could further be used either in paints and stabilizing agents, either in the human health. Within this first chapter, the chosen chemical pathways which were used to achieve such molecules will be discussed. In **Figure 17**, all the chemical modifications around our "pyro" synthon, are depicted.

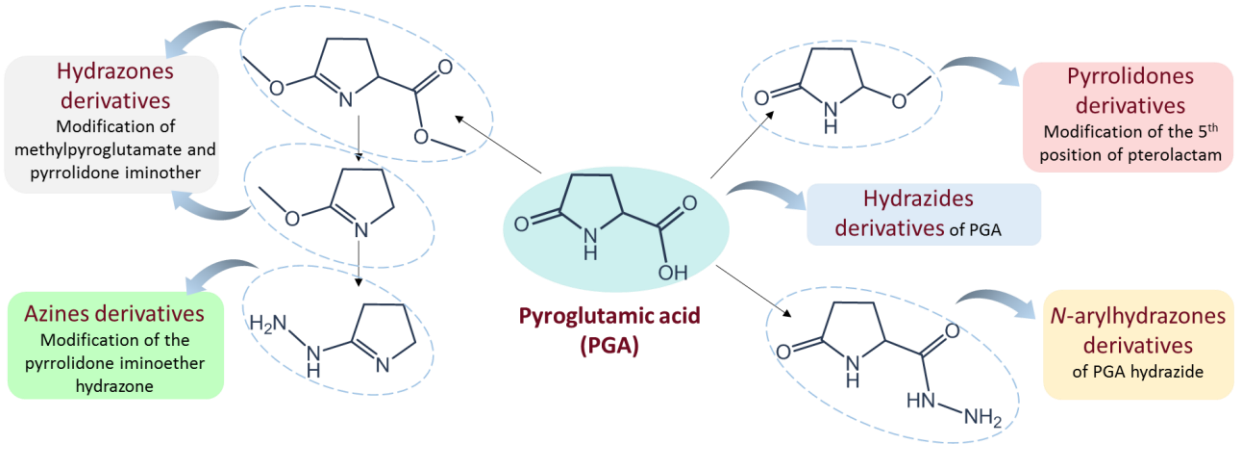


***Figure 17** Chemical families around the pyroglutamic acid and derivatives*

Considering the chemical pathways, we firstly started with the modification of the 5th position of pterolactam, by synthesizing novel γ-lactam molecules – pyrrolidones derivatives, which is going to be further discussed.

I.1 The γ-lactam core: modifications in the 5th position of 5-methoxypyrrolidin-2-one

The γ-lactam core is naturally occurring in plants alkaloids,[17,18] fungi,[19, 20] and bacteria[21] (**Figure 18**). This lactam is also a well-known privileged structure in medicinal chemistry as a versatile heterocycle, present in the scaffold of compounds with a wide spectrum of biological

[17] Zhang Y., Zhao X.C., Xie Y.G., Fan C., Huang Y.Y., Yan S.K., Zhang Y., Jin H.Z., Zhang W.D. Eight new γ-lactam alkaloids from the roots of the Hemerocallis minor Mill. *Fitoterapia*, 2017, 118, p. 80–86.
[18] Natio T., Honda M., Miyata O., Ninomiya I. Total syntheses of (±) anatine and (±) isoanatine via thiyl radical addition cyclization reaction, *Chem. Pharm. Bull.* 1993, 41, p. 217–219.
[19] Huang P., Li C., Sarotti A.M., Turksonc J., Cao S. Sphaerialactonam, a γ-lactam–isochromanone from the Hawaiian endophytic fungus Paraphaeosphaeria sp. FT462. *Tetrahedron Lett.* 2017, 58, p. 1330–1333.
[20] Zhang W., Wenhao Z., Dehai X., Wang B. Stereoselective synthesis of spirofuranone-γ-lactam core of cephalimysin A. *Tetrahedron Lett.* 2018, 59, p. 3507–3510.
[21] Ogawa H., Iwasaki A., Sumimoto S. Iwatsuki M., Ishiyam A., Hokari R., Otoguro K., Ōmura S., Suenaga K. Isolation and total synthesis of hosinolactam, an antitrypanosomal lactam from marine cyanobacteium. *Org. Lett.* 2017, 19, p. 890−893.

activities including anti-tuberculosis[22], anti-inflammatory[23], asthma and agents in respiratory diseases treatment. [24, 25]

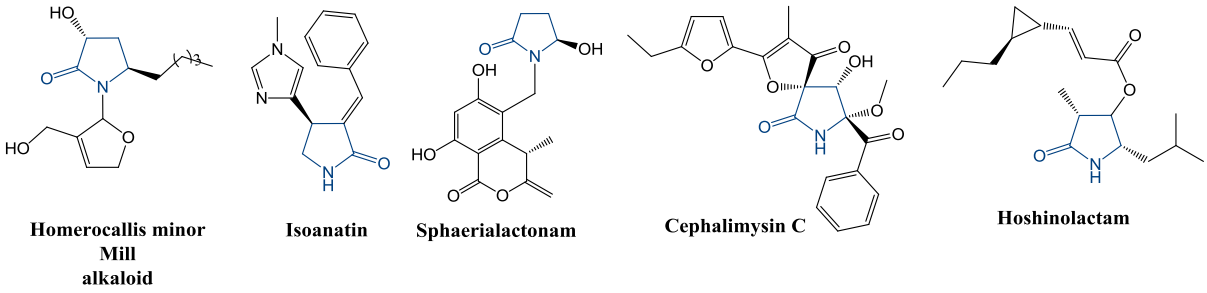


Figure 18 *Naturally occurring γ-lactams*

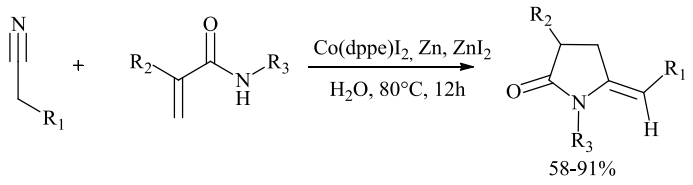
In view of sustainable chemistry, the derivatization of 2-pyrrolidones in the 5th position through the functionalization of C-O bond of pterolactam, under mild conditions seemed an attractive tool in organic synthesis. The main objective of this functionalization is opening up new pathways for the synthesis of novel biological active molecules, previously difficult to achieve through traditional methods, the C-N, C-O, C-S, and C-C bond formation deserves making the process simpler by avoiding the environmentally benign approaches, offering an economical synthesis.

Despite the great synthetic possibilities of such chemistry, catalytic versions have been developed only quite recently. In this context of obtaining different lactam derivatives, several efficient protocols were reported, particularly for 1-, 2- and 5-derivatives of γ -lactams. Among various strategies adopted for the construction of C5-substituted γ-lactams, the most successful strategies of obtaining such derivatives are as following:

I.1.1 Building of the γ-lactam core and structural modifications

Cobalt-catalyzed reductive coupling of nitriles with acrylamides[26]

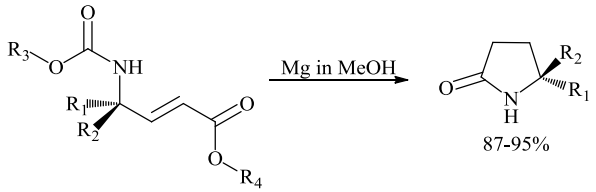
[22] Singh S., Phukan S., Sinha B. Assesing ethnobotanical values and threat status of wild asparagus (stemona tuberosa lous): a case study in eastern Hymalaya, India. *Int. J. Conserv. Sci.* 2012, 3, p. 319-324.
[23] Tonew E., Tonew M., Grafe U., Zöpel P. On the antiviral activity of diffusomycin (oxazolomycin). *Acta Virol.* 1992, 36, p. 166-169.
[24] Pilli R.A., De Oliveira M.D. Recent progress in the chemistry of the Stemona alkaloids. *Nat. Prod. Rep.* 2000, 17, p. 117-127.
[25] Pilli R.A., Rosso G.B., De Oliveira M.D. The chemistry of Stemona alkaloids: An update. *Nat. Prod. Rep*. 2010, 27, p. 1908-1937.
[26] Wong, Y.C., Parthasarathy, K. Cheng, C.H. Cobalt-catalyzed regioselective synthesis of pyrrolidinone derivatives by reductive coupling of nitriles and acrylamides. *J. Am.Chem. Soc.* 2009, 131, 18252-18253.



Scheme 1 *Cobalt-catalyzed reductive coupling of nitriles and acrylamides*

Cobalt complexes have also been employed as catalysts in catalyzed coupling reactions. In 2009, Wong and Chen have reported a cobalt-catalyzed regioselective synthesis of pyrrolidinones from intermolecular reductive coupling of nitriles and acrylamides in one pot. Though this method, new 2-pyrrolidone derivatives were synthesized and the 1st, 3rd, and 5th positions of the lactam ring have been successfully modified.

Reduction of *N*-protected γ-amino-α,β-unsaturated esters with magnesium[27]



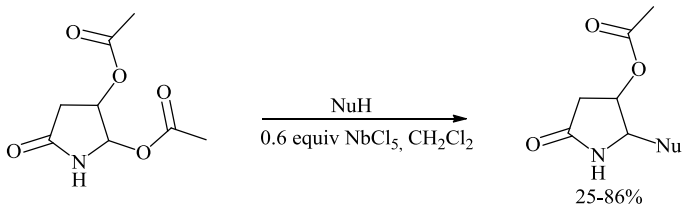
Scheme 2 *Reduction of N-protected γ-amino-α,β-unsaturated esters with magnesium*

Another synthesis pathway for C5-substituted 2-pyrrolidones, is described by Wei and Knaus, starting from the corresponding *N*-protected-γ-amino-α,β-unsaturated esters with magnesium in methanol. They report an efficient method to obtain novel C-C bonds in the 5th position of the lactam, but this procedure is limitated by the nature of the γ-amino-α,β-unsaturated ester.

Niobium pentachloride-nucleophilic additions[28]

In 2005, Andrade and Godoy, reported the use of niobium pentachloride as a novel and efficient Lewis acid in nucleophilic additions to cyclic *N*-acyliminium salts. $NbCl_5$ has proved to be an efficient Lewis acid in the formation of enantiopure *N*-acyliminium ions, but the only nucleophiles which were investigated were allyltrimethylsilane, silyl enol ether, and indoles.

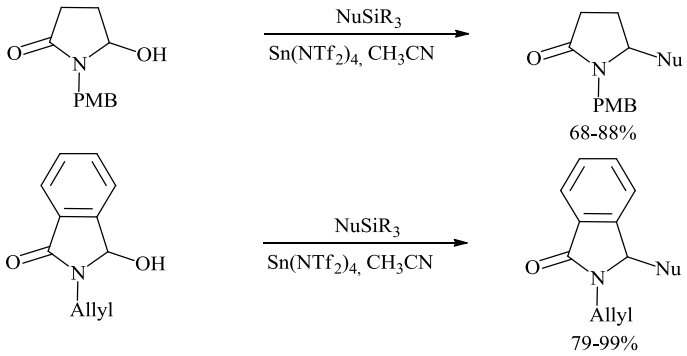
[27] Wei, Z.Y., Knaus, E. E. Synthesis of chiral 5-substituted 2-pyrrolidinones: An unusual one-step transformation. *Tetrahedron Lett*. 1993, 34, p. 4439-4442.
[28] Andrade C.K., Rocha R.O., Russowsky R., Godoy M.N. Studies on the Niobium Pentachloride-Mediated Nucleophilic Additions to an Enantiopure Cyclic *N*-acyliminium Ion Derived from (S)-malic acid. *J. Braz. Chem. Soc.* 2005, 16, p. 535-539.



Scheme 3 *Niobium pentachloride-nucleophilic additions*

Within this work, no other classes of nucleophiles were reported, presumably because the methodology might not be applicable, except from C-nucleophiles.

$Sn(NTf_2)_4$ catalyzed cross-coupling[29]



Scheme 4 *$Sn(NTf_2)_4$-catalyzed amidoalkylation of N,O-acetals with various silicon-based nucleophiles*

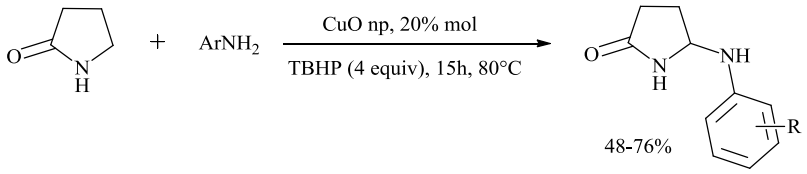
Another pathway for the synthesis of 2-pyrrolidone derivatives, employing C-nucleophiles, has been described by Dalla and coworkers. They have reported a general tin(IV) triflimidate catalyzed amidoalkylation reaction of hydroxy aminals, with various carbon-centered nucleophiles. Though an interesting choice of catalyst for the cross-coupling of cyclic *N,O*-acetals with C-nucleophiles, in this report, the methodology was not extended to other nucleophile types.

CuO nanoparticles catalyzed cross-coupling[30]

[29] Antoniotti S., Dalla V., Dunach E. Metalltriflimidate sind bessere Katalysatoren für die organische Synthese als Metalltriflate – der Effekt eines stark delokalisierten Gegenions. *Angew. Chem. Int. Ed. Engl.* 2010, 49, p. 7860-7888. b) Othman R.B., Affani R., Tranchant M.J., Antoniotti S., Dalla V., Dunach E. N-Acyliminium Ion Chemistry: Highly Efficient and Versatile Carbon–Carbon Bond Formation by Nucleophilic Substitution of Hydroxy Groups Catalyzed by Sn(NTf2)4. *Angew. Chem. Int. Ed.* 2010, 49, p. 776-780.

[30] Priyadarshini S., Amal Joseph P.J., Lakshmi Kantam M. Copper catalyzed oxidative cross-coupling of aromatic amines with 2-pyrrolidinone: a facile synthesis of *N*-aryl-γ-amino-γ-lactams. *Tetrahedron*, 2014, 70, p. 6068-6074.

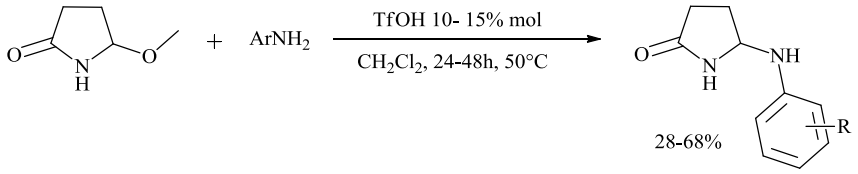
In 2014 Kantam and coworkers described a new synthesis of *N*-aryl-γ-amino-γ-lactams by the oxidative cross-coupling of amines with 2-pyrrolidinone under catalytic conditions employing CuO nanoparticles. The procedure utilizes a dual C-H and N-H activation strategy under oxidative conditions promoted by TBHP (Tert-Butyl Hydroperoxide) and catalytic amount of CuO nanoparticles. Although the method afforded the synthesis of various 5-amino-pyrrolidin-2-ones, nor oxygen or sulphur nucleophilic substrates were employed.



Scheme 5 *CuO np catalyzed cross-coupling*

Triflic acid catalyzed intermolecular α-amination

Recently, a new methodology, relying on the use of a triflic acid catalyzed intermolecular α-amination of pterolactams *via N*-acyliminium species was reported to produce 5-arylamino pyrrolidinones.[31] However, the scope of this reaction to other nitrogen or oxygenated nucleophiles is highly limited. The described protocol gave good yields with anilines but failed when using benzyl amine, hydrazine or benzyl alcohol derivatives.



Scheme 6 *Triflic acid catalyzed intermolecular α-amination*

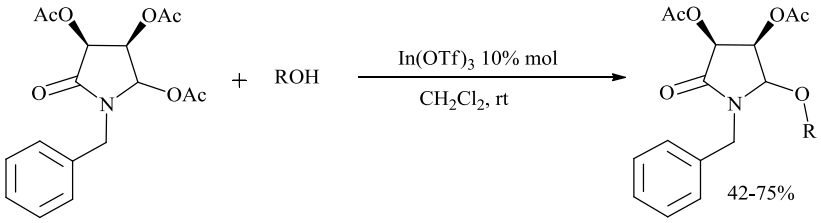
In(OTf)$_3$-catalyzed addition reaction of alcohols[32]

Involving the same acyliminium ion, Stefani *et al.* reported a pathway to obtain 5-ether-2-pyrrolidinones derivatives, *via* the addition reaction of primary, secondary, aromatic, and alkylic alcohols to *N*-acyliminum anion catalyzed by In(OTf)$_3$, in moderate to good isolated

[31] Dumitriu G.M., Bîcu E., Eryuruk U., Belei D., Rigo B., Daïch A., Ghinet A. Triflic Acid Catalyzed Intermolecular α-Amination of Pterolactams To Give 5-Arylaminopyrrolidinones via N-Acyliminium Species. *Synlett.*, 2016, 27, 06, p. 934-940.

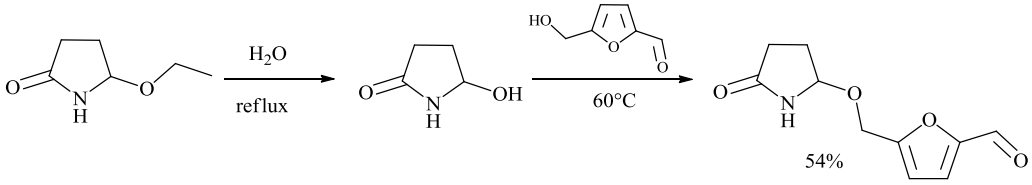
[32] Stefani H.A., Ali B., Ferreira F.P. Ultrasound-assisted addition of alcohols to *N*-acyliminium ions mediated by In(OTf)$_3$ and synthesis of 1,2,3-triazoles. *Tetrahedron Lett.*, 2014, 55, p. 3400-3405.

yields. Though they proved *N*-acyliminum anion can react with weaker electrophiles such as alcohols derivatives, the team hasn't investigated others classes.



Scheme 7 *In(OTf)$_3$-catalyzed addition reaction of alcohols*

The synthesis of sessiline[33]



Scheme 8 *The synthesis of sessiline*

In 2014, Ilkei *et al.* synthesized for the first time the sessiline, an alkaloid which was recently isolated from the fruits of *Acanthopanax sessiliflorus*.[34] The sessiline was obtained in the second step, from the reaction of 5-hydroxypyrrolidin-2-one and 5-hydroxymethylfurfural, in neat conditions at 60°C, without catalyst and a yield of 54%. However, the authors were interested only in the synthesis of this particular molecule and no other nucleophiles were tested.

I.1.2 Catalysis

The history of the catalysts use, in the life of the human being dates back in 5000 b.c. when for the wine obtaining, through fermentation, the zymase enzyme was involved, converting the sugars to alcohols. However, the concept of catalysis wasn´t introduced until 180 years ago by the Swedish chemist Jons Jacob Berzelius who defined a catalyst as any substance which is added in amounts much lower than stoichiometric, that increases the rate of a chemical reaction without getting consumed.

[33] Ilkei V., Faragó K., Sánta Z., Dékány M., Hazai1 L., Szántay C., Szántay C., Kalaus G. The First Synthesis of Sessiline. *IJOC*, 2014, 4, p. 309-313.
[34] Lee S., Ji J., Shin K.H, Kim B.K. Sessiline, A New Nitrogenous Compound from the Fruits of *Acanthopanax sessiliflorus*. *Planta Med*., 2002, 68, p. 939-941.

I.1.2.1 Cesium - a great metal discovery, CsF – a remarkable catalyst

Discovered in 1860 by the german scientists Gustav Kirchhoff and Robert Bunsen, CsF was firstly identified spectroscopically in samples of mineral water from Durkheim. In the past decades, cesium salts as catalysts became an excellent choice for short reaction, small amount of reagent being recognized to deliver high yields under milder reaction conditions.

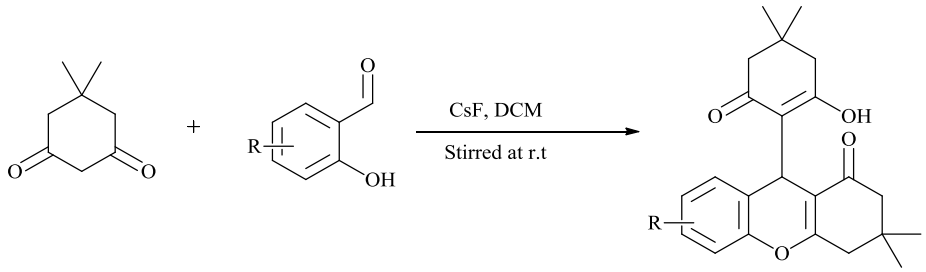
Cesium salts are utilized in nucleophilic substitution reactions, replacing the standard bases. Between the most known cesium salts we have mainly cesium carbonate, nitrate, fluoride, chloride, bromide, iodide, hydroxide and acetate. The most used salt in organic chemistry being cesium carbonate Cs_2CO_3, cesium fluoride CsF and cesium acetate CsOAc. In organic chemistry, CsF is known to be used as a source of fluoride anion.[35] From all the non-radioactive elements, the cesium has the lowest electronegativity, while fluoride has the highest electronegativity. As can be easily to dissociate compared to other bases, is a more reactive source of fluoride. Because of that and also because of its less hygroscopic nature, CsF usually comes as an alternative instead of TBAF (tetra-*n*-butylammonium fluoride) or TAS-fluoride (tris(dimethylamino)sulfonium difluorotrimethylsilicate fluoride).

Considering the fact that CsF may generate HF, the salt is considered moderately basic compared to other fluorides. However, fluoride's nucleophilicity makes it a good choice in several organic chemistry reactions. Between the most representative reactions where CsF works as a base there are few important to be mentioned.

Knoevenagel-Michael reaction

Khan *et al.* decribed an efficient pathway through a CsF-catalyzed tandem Knoevenagel–Michael,[36] an eco-benign method for the synthesis of highly functionalized 4,4' -(arylmethylene)bis(1*H*-pyrazol-5-ols), starting from the dimedone and various salicylaldehydes in presence of a catalytic amount of CsF.

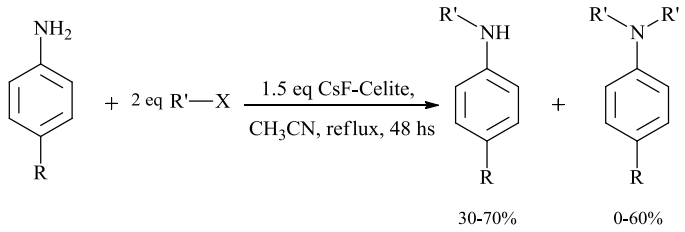
[35] Greenwood N.N, Earnshaw A. Chemistry of elements 2nd, Pergmon Press: Oxford UK, 1984.
[36] Khan K.M., Taj M., Khan I., Perveen S. Voelter W. Rapid cesium fluoride-catalyzed Knoevenagel condensation for the synthesis of highly functionalized 4,4′-(arylmethylene)bis(1H-pyrazol-5-ol) derivatives. *Monatshefte für Chemie*, 2015, 146, 9, p. 1587-1590.



Scheme 9 *Rapid cesium fluoride-catalyzed Knoevenagel condensation*

N-alkylation

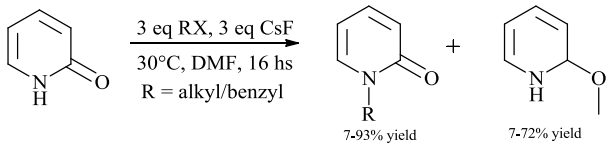
Hayat *et al.* reported an efficient method for *N*-alkylation of anilines, and aromatic compounds involving nitrogen heterocycles using cesium fluoride-celite as solid base in acetonitrile.[37] They proved that the chemistry of the CsF-Celite provides a clean and convenient method for such reaction, being superior in terms of handling.



Scheme 10 *N-alkylation of p-substituted anilines*

N or O-alkylation

The alkylation of 2-pyridone, under mild conditions was described by Sato *et al.*[38] They have reported that CsF affects the alkylation and the N/O selectivity can be tuned by using the appropriate alkyl halides.



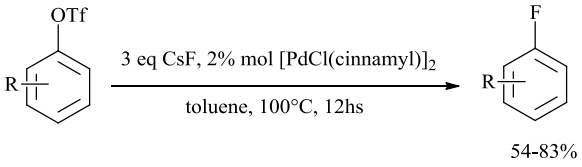
Scheme 11 *Non selective N/O-alkylation*

Fluorination reactions

[37] Hayat S., Rahman A., Choudhary M., Mohammed Khan K., Schumann A., Bayer E. *N*-Alkylation of anilines, carboxamides and several nitrogen heterocycles using CsF–Celite/alkyl halides/CH_3CN combination. Tetrahedron, 2001, 57, 50, p. 9951-9957.

[38] Sato T., Yoshimatsu K., Otera J. CsF in Organic Synthesis. Tuning of N- or O-Alkylation of 2-Pyridone. *Synlett*. 1995, 8, p. 845-846.

For the first time, in 2009, Buchwald *et al.* developed a palladium-catalyzed method for the preparation of aryl fluorides from aryl triflates imploying CsF that proceeds with high functional group tolerance under mild reaction conditions.[39]



Scheme 12 *Fluorination of aromatic triflates*

I.1.2.2 CsF a powerful catalyst for the development of new γ-lactam new derivatives

Within this subchapter, we will focus on extended chemical modification which was done in the 5th position of the pterolactam molecule (**Figure 19**), one of our starting goal being the design of new γ-lactams as antifungal/antibacterial molecules.

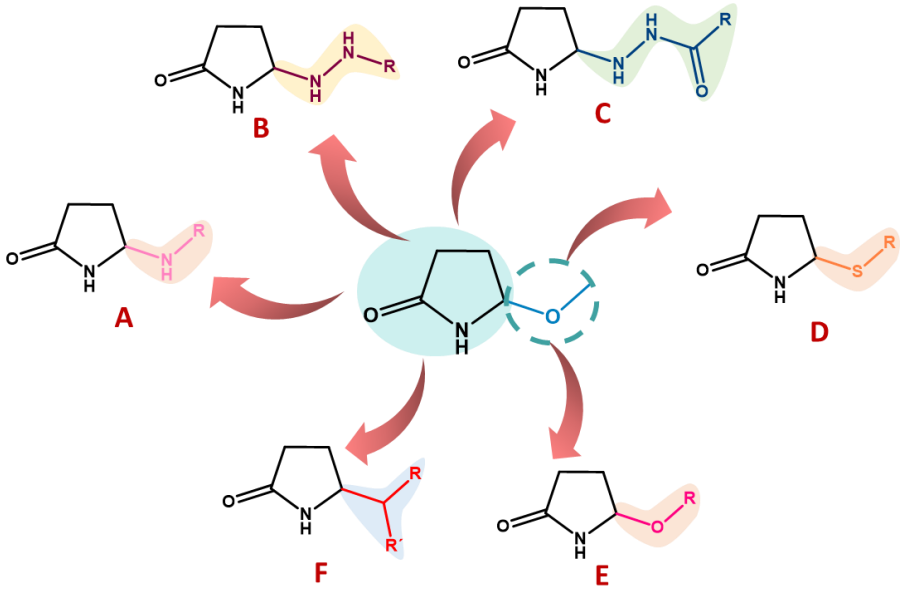


Figure 19 *Targeted modifications in the 5th position of the pterolactam*

As depicted in subchapter I.1.1, numerous synthetic strategies were developed for the modification or the building of γ-lactam derivatives. However, there is obviously a lack of general pathways to obtain such derivatives without harsh conditions, protection or activation of the substrate. The closest pathway which could had provided us the wanted scaffold molecules, is the triflic acid catalyzed intermolecular α-amination, which was also developed

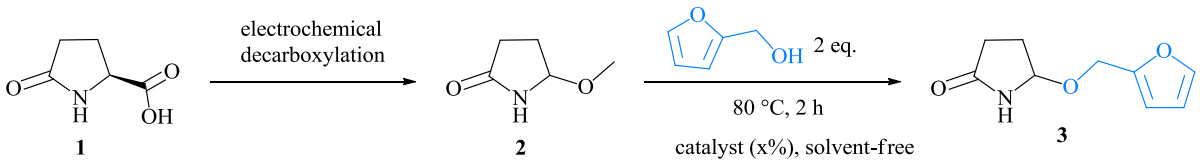
[39] Watson D.A.., Su M., Teverovskiy G., Zhang Y., García-Fortanet J., Kinzel T., Buchwald S.L. Formation of ArF from LPdAr(F): catalytic conversion of aryl triflates to aryl fluorides. *Science*, 2009, 325, 5948, p.1661-1664.

within our team as well. Unfortunately, that pathway would not be delivering us the wanted molecules type B, E, or probably neither C, D nor F, as the needed nucleophiles to obtain those links were not tested using triflic acid methodology.

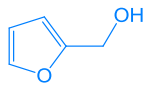
In the context of clean chemistry, we developed a chemical pathway which helped us tomodify the γ-lactam, originally obtained from the pyroglutamic acid through an electrochemical pathway. The original methodology came from our need to improve the reactivity of pterolactam, which allowed us employing a large range of nucleophiles under mild non-acidic conditions.

I.1.2.2.1 Efficiency comparison between CsF and other known catalysts

For beginning, in order to identify the advantages of using CsF as a catalyst in our nucleophilic substitution reaction, different catalysts such as methanesulfonic acid, Amberlyst H15, $ZrCl_4$ or $AlCl_3$ were tested in the reaction between furfuryl alcohol and pterolactam. The racemic pterolactam substrate, particularly of interest for us in order to develop new antifungal and antibacterial compounds, was prepared in a large scale, by electrochemical decarboxylation of *L*-pyroglutamic acid.



Scheme 13 *The reaction of furfuryl alcohol with pterolactam*

Entry	Nucleophile	Catalyst	Load (mol%)	Conversion (%)	Isolated yield (%)
1		-	-	22	11
2		CH_3SO_3H	10	100	0
3		Amberlyst H15®	10	100	0
4		$ZrCl_4$	10	100	0
5		$AlCl_3$	10	100	0
6		CsF	5	31	22
7		CsF	10	52	52
8		CsF	20	68	60
9		**CsF[a]**	**10**	68	65

[a] *The time of reaction was extended to 4 hours.*

Table1 *Various catalysts used for the promotion of furfuryl alcohol as nucleophile*

For the comparison, the reactions were carried out at 80°C during 2 hours under stirring. The conversion % refers to the consumed pterolactam substrate which was followed by NMR.

When Brönsted acids were used (**Table 1**, entries 2-3), though a complete conversion was seen, it was actually corresponding to a total degradation of the pterolactam, and in fact the expected product was not isolated. The same scenario while employing Lewis acid such as zirconium and aluminium chloride, total degradation of the substrate was observed, with no isolation of the wanted product (**Table 1**, entries 4-5). On the other side, while using CsF, even 5%, we could see a conversion, without any degradation and an actual isolated yield of 22% was obtained. Further in the study, the impacts of the load of catalyst was examined. The outcome of the test was that indeed, the quantity of the CsF has an influence on the reaction, and by doubling the quantity in a first step we double as well our conversions and isolated yields. However, going up to 20% of CsF we lose the proportional results, but we still see a slight improvement of 16% in conversion and 8% in isolated yield, compared to the previous screening at 10% CsF. As 10% of CsF seemed the best option, the reaction was extended up to 4 hours, when 68% of conversion and 65% isolated yield were obtained, proving the fact that for such type of nucleophile, we need to extend the time of reaction, especially when the conversion is not complete.

I.1.2.2.2 Solvent influence

Further, as initially the screening was starting without any solvents, to see if there was actually a need or an improvement in the promotion of the reaction, different solvents, referred as "green", such as water, *tert*-butanol, methyltetrahydrofuran and methyltetrahydropyran were tested (**Table 2**). In a first instance, water as a solvent led to the partial degradation of pterolactam to 5-hydroxypyrrolidin-2-one, and a modest conversion of 16% with 10% product recovery (**Table 2**, entry 1). Considering the other tested solvents, conversions and yields were similar to those in water. Giving these results, as there was not any actual need of using solvents and the importance to develop a solvent-less methodology from an environmental point of view, the study was continued in 10% CsF in neat conditions.

Entry	Solvent	Conversion (%)	Isolated yield (%)
1	H_2O	16	10
2	t-BuOH	16	16
3	Me-THF	18	18

4	MTHP	19	19

Table 2 *The screening results of different solvents, when using 10% CsF, at 80°C for 2 hours*

I.1.2.2.3 Cesium salts comparison based on the furfuryl alcohol reaction

Further in this study, we wanted to find out whether if the fluoride is the key for the promotion of the reaction or the Cs cation plays also a role (**Table 3**). Considering this, KF among other Cs salts such as CsCl, CsBr, CsI, Cs_2CO_3, were tested. As observed, KF was less efficient, leading to degradation with only 28% of yield, meaning twice less than CsF. For the other Cs salts, considering the isolated yield, all are quite the same, at around 30% except Cs_2CO_3, which was particular, causing degradation of the substrate with a low final isolated yield of 17% (**Table 3**, entry 6). Interesting fact is that when the reaction under vacuum was performed, a slight conversion/yield improvement was observed, of 6%, which probably was caused by a faster methanol elimination consequently a faster substitution rate, being a kinetic consequence (**Table 3**, entry 1).

Entry	Catalyst	Conversion (%)	Isolated yield (%)
1	CsF[a]	58	58
2	KF	58	28
3	CsCl	36	30
4	CsBr	35	28
5	CsI	36	27
6	Cs_2CO_3	95	17

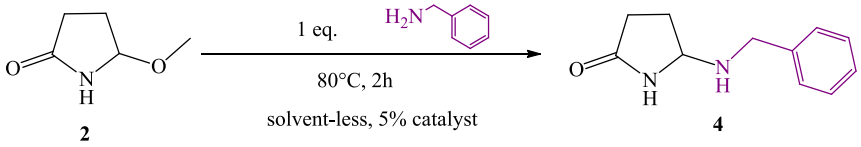
[a]The reaction was realized under vacuum (20 mmHg)

Table 3 *Other catalysts screening results using 10% CsF at 80°C, after 2 hours*

I.1.2.2.4 Cesium salts comparison based on the benzylamine reaction

In order to get a preliminary idea on the reactivity of different nucleophiles, especially a nitrogen containing one, benzylamine was tested in the same conditions of using 5% CsF in solvent-less medium, at 80°C (**Scheme 15**). The overall outcome is similar to that of furfuryl alcohol, in the presence of CsF the wanted product was obtained in 57% yield, being the best

halogenated cesium salt in the current study. If vacuum was used, a slight improvement was observed, as reported before in presence of furfuryl alcohol (**Table 4**, entry 6). Using benzylamine as nucleophile, the yields were lower when CsCl, CsBr and CsI were used instead of CsF as catalysts. On the other hand, Cs_2CO_3 exhibited a superior yield of 92% (**Table 4**, entry 5). Replacing Cs by K resulted in decreasing the yield with 11%. Concerning the other tested fluorinated catalysts, KF did not show much efficiency, the conversion respectively isolated yield being 31% (**Table 4**, entry 7). TBAF as fluoride provider proved to be a good alternative to CsF as similar yield and conversion were obtained.



Scheme 14 *The reaction of benzyl amine and pterolactam*

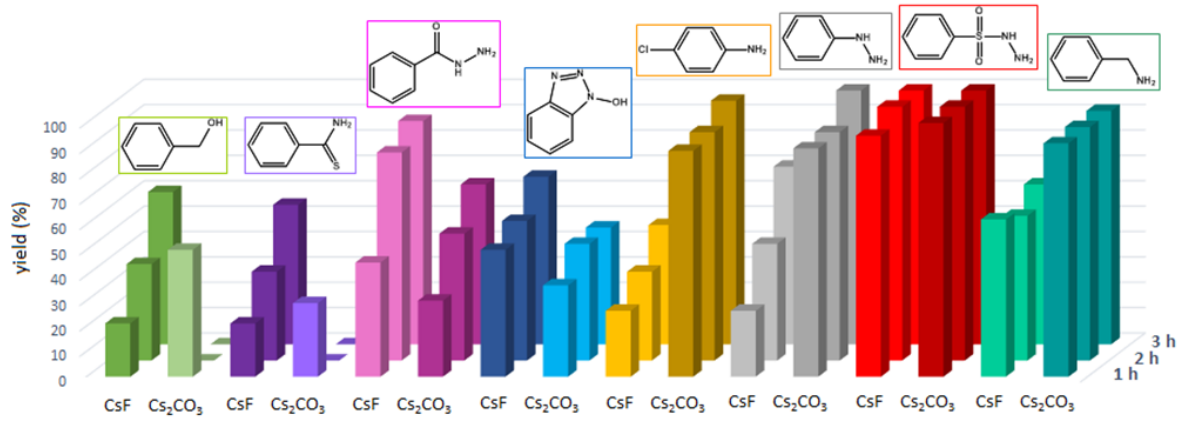
Entry	Catalyst	Conversion (%)	Isolated yield (%)
1	CsF	57	57
2	CsCl	16	16
3	CsBr	15	15
4	CsI	15	15
5	Cs_2CO_3	92	92
6	CsF[b]	65	65
7	KF	31	31
8	K_2CO_3	81	81
9	TBAF	58	58

b) Under vacuum conditions

Table 4 *Screening results of different catalysts (5%) for the reaction of benzyl amine*

Considering the preliminary results, CsF and Cs_2CO_3 emerged as leading catalysts. In order to explore their full capacity and extend the methodology, a large range of nucleophiles were screened to evaluate the potential of these two pre-selected catalysts, some general selected ones are presented in Graph 1. The choice of nucleophiles was based on our antifungal drug design research as well, which was also one of the initial goals of this methodology, finding a more accessible way to design γ-lactams.

I.1.2.2.5 General screening of a larger number of nucleophiles



Graph 1 *Selected nucleophiles for the evaluation of the two catalysts*

All the reactions were carried out in the same conditions, using 5% mol of catalyst, at 80°C without any solvent, as we previously decided as the best alternative, and the samples were analyzed after 1, 2 and 3 hours respectively. From our observations, concerning certain reactions, big differences between the efficiency of the two salts were noted, which is the case of benzohydrazide, 4-chloroaniline and phenylhydrazine. In some cases, as the reaction is slower, the reaction time has to be extended, the yields increasing in time, being the case of benzyl alcohol, thiobenzamide, benzohydrazide and phenylhydrazine, the last one being particularly for CsF use. Overall, we could clearly notice that the yield is linked to the nucleophilicity of the reactant.

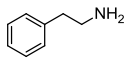
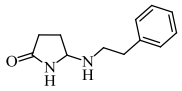
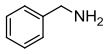
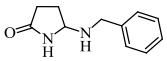
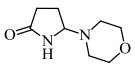
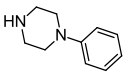
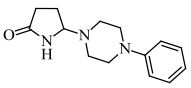
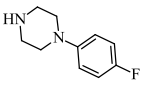
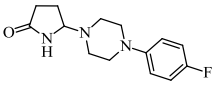
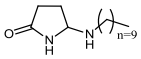
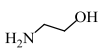
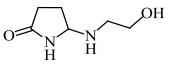
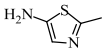
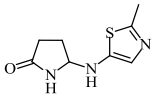
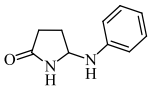
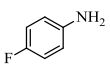
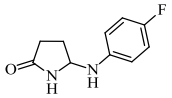
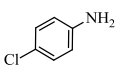
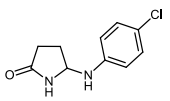
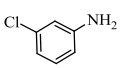
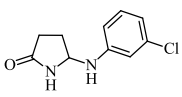
Though the Cs_2CO_3 seemed a very good alternative for some of the selected nucleophiles, such as 4-chloroaniline, hydrazine, benzylamine and benzenesulfonohydrazide, in other reactions such as those involving benzylalcohol and benzothioamide, Cs_2CO_3 proved to be a poor choice. In these reactions, which apparently were slower for CsF, Cs_2CO_3 seemed to produce degradation of the substrates. Taking into account the unpredictable behavior of Cs_2CO_3 mediated reaction, we continued our synthesis of pterolactam derivatives by imploying CsF as main catalyst, keeping the carbonate salt as a backup.

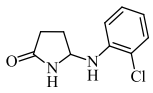
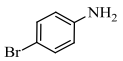
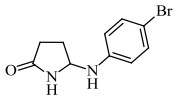
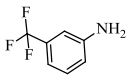
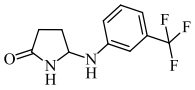
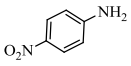
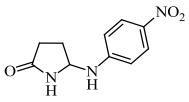
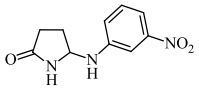
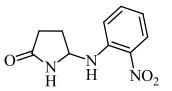
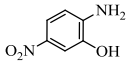
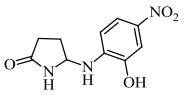
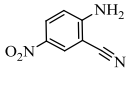
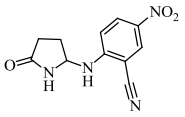
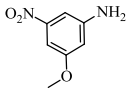
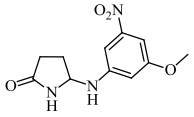
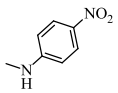
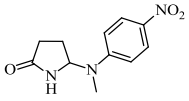
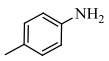
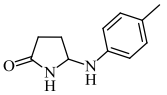
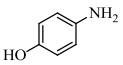
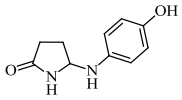
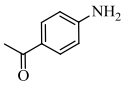
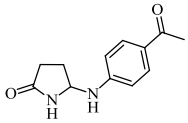
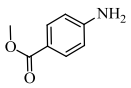
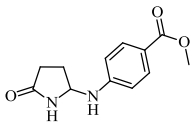
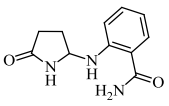
I.1.2.2.6 Amino-nucleophiles reactivity

As previously mentioned, due to our need to design new antibacterial and antifungal compounds, a large range of nucleophiles which allowed us to modulate position 5 of the lactam ring, were used in the synthesis. **Table 5** is summarizing the data concerning the amino-

derivatives tested in the optimized conditions. The reactions were conducted in solvent-less conditions at 80°C under moderate vacuum (20 mmHg) in the presence of 5 mol% of CsF.

Primary aliphatic and aromatic amines had overall great results, without important degradation, the reaction happening smoothly. Aliphatic amines such as dodecylamine reacted well, and 5-(decylamino)pyrrolidin-2-one was isolated in 78% yield (**Table 5**, entry 6). Further, the 2-aminoethanol reaction with pterolactam was selective towards the amino- group, the isolated yield being 70% (**Table 5**, entry 7). On the other hand, secondary aliphatic cyclic amines such as morpholine and phenylpiperazine derivatives (**Table 5**, entries 3, 4, 5) led also to very good yields for the formation of compounds **6**, **7**, and **8** respectively proving the applicability of this methodology for secondary amines as well.

Entry	Nucleophile	Product	Product N°	Time of reaction (h)	Conversion (%)	Isolated yield (%)
1			5	14	86	68
2			4	3	88	79
3			6	6	76	70
4			7	24	100	95
5			8	5	80	78
6			9	36	100	78
7			10	5.5	90	32
8			11	2	60	54
9			12	5	99	88
10			13	2	85	82
11			14	2	85	82
12			15	3	87	77

13			16	15	95	85
14			17	3	85	81
15			18	4	60	33
16			19	4	75	60
17			20	24	80	50
18			21	48	60	30
19			22	17	80	55
20			23	48	50	35
21			24	12	68	55
22			25	24	76%	4
23			26	5	90	88
24			27	8	83	50
25			28	3	75	70
26			29	2	90	86
27			30	15	100	85

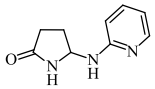
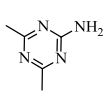
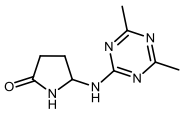
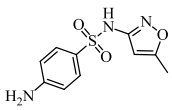
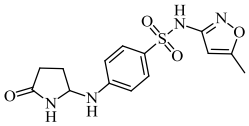
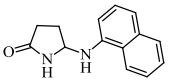
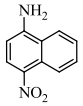
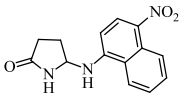
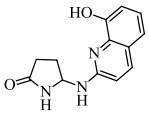
28			31	6	80	60
29			32	12	73	51
30			33	24	75	55
31			34	2.5	100	42
32			35	20	58	53
33			36	5	90	62

Table 5 *Amino-pterolactam derivatives*

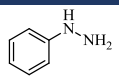
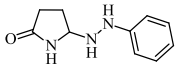
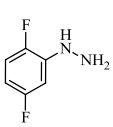
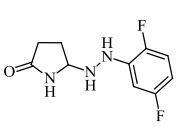
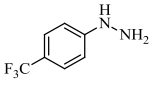
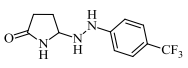
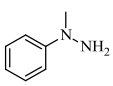
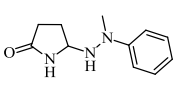
Concerning aromatic amines, aniline as example, a week nucleophile which was expected to react with a moderate conversion, happened to have an excellent conversion and very good yield of 88% affording compound **12** (**Table 5**, entry 9). While employing anilines with moderate deactivating substituents such as F, Cl, and Br in *para* position, a slightly lower 85% conversion is observed, being the same for the synthesis of **13**, **14**, **17** (**Table 5**, entries 10, 11, 14). As we were particularly interested in chlorinated derivates, being more "drugable" and also in order to assess the reactivity differences between *ortho*-, *meta*- and *para*-chloroaniline, **15** and **16** were designed as well. The difference of yield was not notable, the *ortho*-derivative **16** being obtained in improved 95% yield.

Regarding weaker nucleophiles such as 3-trifluoromethylaniline, the strong deactivation effect of fluorine was reflected in lower results, of 60% conversion and 30% isolated yield for the compound **18** (**Table 5**, entry 15). Going further with the reactions of deactivating substituents in the *para* position, 4-nitroaniline compared to halogenated amines, turned to be a little less efficient, having a conversion of 75%, compound **19** being obtained in 60% yield (**Table 5**, entry 16). Among 2-nitro, 3-nitro and 4-nitro anilines, the less efficient was 2-nitroaniline, the reaction happening slower and the purification being more difficult, resulting in a yield of 30% for compound **21** (**Table 5**, entry 18). Moreover, secondary amines such as *N*-methyl-4-nitroaniline proved to be very little reactive, being degradated during the reaction, in the end leading to 4% of isolated yield for compound **25** (**Table 5**, entry 22). Additional

activating substituents such as hydroxyl- and methoxy- on nitroaniline, had from little to no influence compared to the simple nitroanilines reactivity with pterolactam, the compounds **22**, respectively **24** being obtained with a yield of 55% (**Table 5**, entries 19, 21). However, as mentioned before in the case of ethanolamine, the reaction with 4-aminophenol, was also selective for the amino group. The same behavior could be seen in the case of 2-aminobenzamide and sulfamethoxazole which reacted with the amino moiety to give exclusively compounds **30**, respectively **33**, in quantitative yields. Pyridine-2-amine and 4,6-dimethyl-1,3,5-triazin-2-amine reacted also well, under such conditions, producing the desired γ-lactams **31** and **32** in satisfactory yields (**Table** 5, entries 28,29). Bulky amines such as 1-naphtylamine, 4-nitronaphthalen-1-amine and even 2-aminoquinolin-8-ol, proved to have a very good reactivity with the pterolactam in presence of CsF, giving average yields between 50 and 60% (**Table 5**, entries 31-33).

I.1.2.2.7 More complex amino-groups reactivity

Further, the goal was of course to enrich our panel of nucleophiles which are able to react with pterolactam, but also we wanted to see which type of link would render more biological properties. As seen in the case of anilines, aromatic hydrazines proved to be also good nucleophiles, most of them having complete conversions and good yields, allowing us to obtain compounds **37**-**41** (**Table 6**, entries 1-5). The only exception from this series, as in the aniline series, is the 4-trifluoromethylhydrazine which was degradated and made the purification more difficult, resulting in a modest final yield of 20%. Whithin the hydrazines series, the reaction occurred selectively on the primary amine.

Entry	Nucleophile	Product	Product N°	Time of reaction (h)	Conversion (%)	Isolated yield (%)
1			37	12	90	85
2			38	4	90	80
3			39	6	66	20
4			40	19	98	60

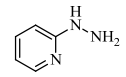
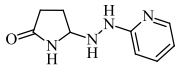
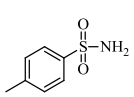
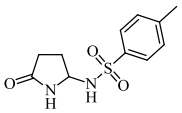
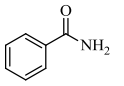
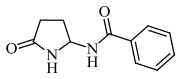
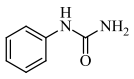
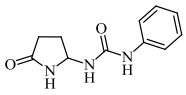
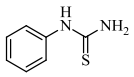
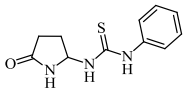
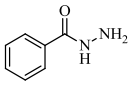
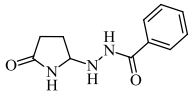
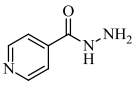
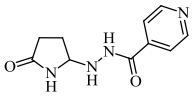
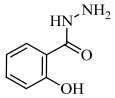
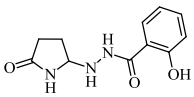
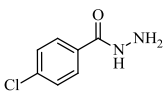
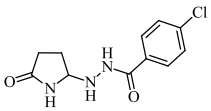
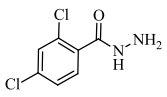
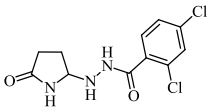
5			41	6	100	75
6			42	24	80	60
7			43	24	35	0
8			44	24	0	0
9			45	20	90	60

Table 6 *Hydrazino and amido- pterolactam derivatives*

Among the other nucleophiles which were tested there are: 4-methyl benzenesulfonamide, benzamide, phenylurea and thiophenylurea. While a good conversion and yield were obtained for the reaction of 4-methylbenzenesulfonamide, when benzamide was used as a substrate, a very poor conversion was observed, in the end being impossible of isolating the compound (**Table 6**, entry 7). This could be attributed to the low nucleophilicity of benzamide. For similar reasons, neither the phenylurea did not react with **3**, no conversion being observed (**Table 6**, entry 8). On the other hand, phenylthiourea reacted quantitatively *via* the primary amine and 1-(5-oxopyrrolidin-2-yl)-3-phenylthiourea **45** was isolated in 60% yield (**Table 6**, entry 9).

Entry	Nucleophile	Product	Product N°	Time of reaction (h)	Conversion (%)	Isolated yield (%)
1			46	3	100	95
2			47	9	62	35
3			48	24	85	80
4			49	5	62	50
5			50	8	78	40

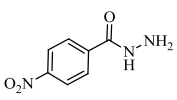
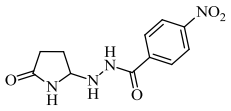
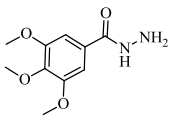
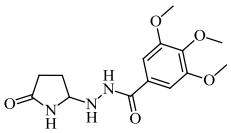
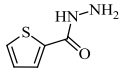
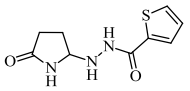
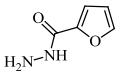
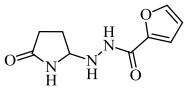
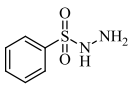
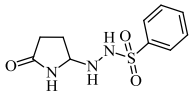
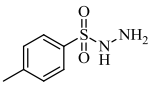
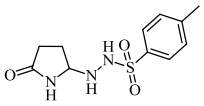
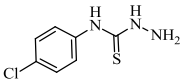
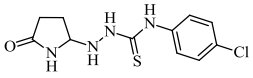
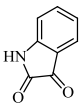
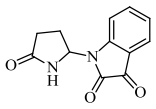
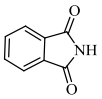
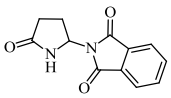
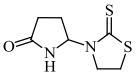
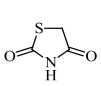
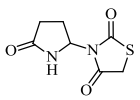
6			51	1	60	54
7			52	3	75	60
8			53	16	75	55
9			54	24	60	38
10			55	3	100	80
11			56	6	90	65
12			57	4	40	30
13			58	20	90	65
14			59	9	65	30
15			60	4	95	75
16			61	30	77	67

Table 7 *Hydrazido- and other pterolactam derivatives*

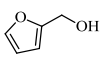
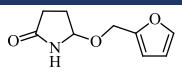
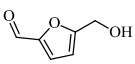
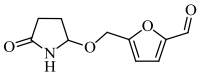
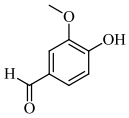
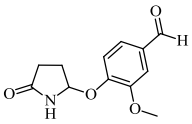
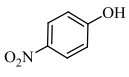
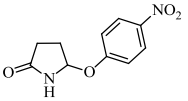
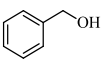
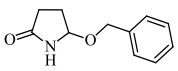
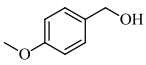
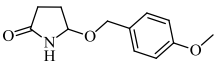
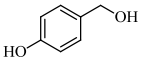
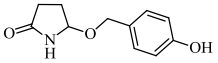
Another type of nucleophile that interested us, were the hydrazides, particularly because of the new formed link which we learnt that has an important impact on antibacterial activity. Things being said, 9 preliminary aromatic hydrazides were employed, which to our delight had very good yields, reacting quite fast with our substrate. Benzohydrazide and 2-hydroxybenzohydrazide turned to have the most efficient reaction with pterolactam, allowing the synthesis of **46** and **48** with isolated yield of 95%, respectively 80% (**Table 7**, entries 1 and 3). Concerning the rest, it is worth mentioning that withdrawing substituents are causing an important yield decrease, 4-chloro-*N*'-(5-oxopyrrolidin-2-yl)benzohydrazide **49** for example, was isolated with only 50% yield while the 2,4-dichloro derivative **50** was isolated in a modest 40% yield. Heterocyclic hydrazides such as thiophene-2-carbohydrazide and furan-2-

carbohydrazide, compared to the benzohydrazide, reacted slower, with poor yields, however allowing the formation of **53** and **54** (**Table 7**, entries 8-9). Benzenesulfonylhydrazide, was also submitted to our optimized conditions, having a total conversion and an isolated yield a little lower than the benzohydrazide derivative (**Table 7**, entry 10). Besides hydrazides, very acidic lactams like indoline-2,3-dione, isoindoline-1,3-dione, thiazolidine-2-thione or thiazolidine-2,4-dione, allowed us as well to obtain the wanted lactam derivatives, in quantitative yields, led to the desired products in good yields (**Table 7**, entries 13-16).

Observing the results, we can confirm that the optimized solvent-less conditions are widely applicable for the reaction of pterolactam **3** with a large range of nitrogen nucleophiles. With reference to the reactivity, the general behavior and results are proportional with the nucleophilicity of the substrate. In order to demonstrate the extended applicability of CsF, we chose to add more nucleophiles in the study.

I.1.2.2.8 Oxygenated nucleophiles reactivity

Consequently, next in line were the alcohols, which were used as substrates for the reaction with pterolactam **3**. As alcohols are weaker nucleophiles compared to the amines tested so far, moderate yield or no reaction were expected. Furfuryl alcohol reacted with an excellent conversion and yield of 80% (**Table 8**, entry 1).

Entry	Nucleophile	Product	Product N°	Time of reaction (h)	Conversion (%)	Isolated yield (%)
1			3	6	90	80
2			62	4	95	75
3			63	10	25	15
4			64	48	30	10
5			65	12	35	30
6			66	8	55	46
7			67	12	58	33

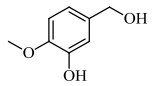
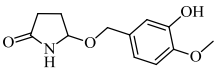
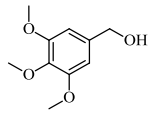
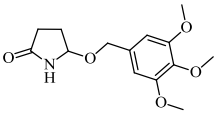
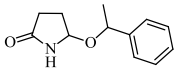
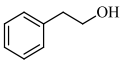
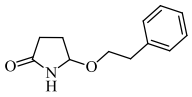
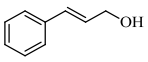
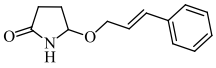
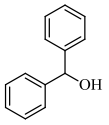
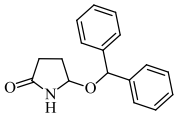
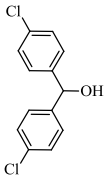
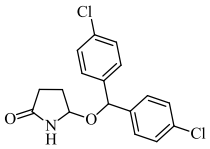
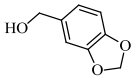
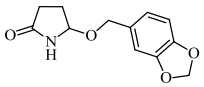
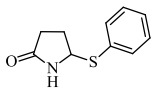
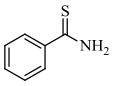
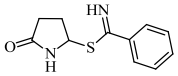
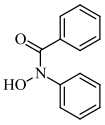
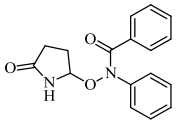
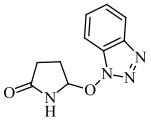
8			68	8	40	30
9			69	24	60	40
10			70	24	45	10
11			71	12	47	12
12			72	15	60	53
13			73	8	45	24
14			74	5	20	8
15			75	16	100	10
16			76	12	50	30
17			77	15	75	55
18			78	8	30	20
19			79	4	90	81

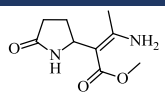
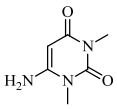
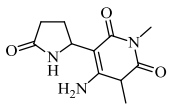
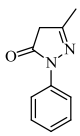
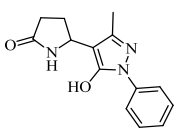
Table 8 *Oxy- pterolactam derivatives*

Besides this, we wanted to obtain as well using our methodology the sessiline alkaloid, which to our surprise, reacted fast, with a better isolated yield of 75%, compared to the previously reported method.[33] Going further to aromatic substrates, simple phenol for reagent, was a challenge, and no conversion could be seen. Vanillin and 4-nitrophenol on the other part, unexpectedly reacted better than the phenol, and though the conversions were not so high, we were happy with a small isolated yield (**Table 8**, entries 3-4). On the contrary, benzyl alcohol

derivatives proved to be a lot more efficient compared to phenols, and we obtained isolated yields up to 30% for the 5-(benzyloxy)pyrrolidin-2-one **65** (**Table 8**, entries 5). Activating substituents such as methoxy- seem to have positive influence, the yield being actually increasing up to 46% (**Table 8**, entry 5). Other reactions which confirmed us the phenol lack of reactivity are the reactions of 4-(hydroxymethyl)phenol or 5-(hydroxymethyl)-2-methoxyphenol with pterolactam. As expected, the reaction took place on the benzylic alcohol and not on the phenol, affording compounds **67** and **68** (**Table 8**, entries 7-8). Other primary alcohols such as 2-phenylethanol and 2-phenylethenol reacted in similar yields to benzyl alcohol (**Table 8**, entries 11-12). The hindrance of the alcohol did not impact on the yield, as diphenylmethanol, a secondary alcohol, gave similar results as benzyl alcohol (**Table 8**, entry 11). However, the *para*- chlorinated derivative of diphenylmethanol gave only 8% yield, being considerable less nucleophile. Overall, oxygen containing nucleophiles reacted moderately under such conditions, allowing a large range of new lactam derivative with satisfactory yields.

Compared to phenol, thiophenol reacted better, under the same conditions, leading to 5-(phenylthio)pyrrolidin-2-one **76** (**Table 8**, entry 16). When benzothioamide is used as nucleophile, the conversion was moderate and a single product was isolated, being exclusively linked by the sulfur atom. *N*-Hydroxy-derivatives were also obtained employing our methodology, affording compounds **78** and **79**, *N*-phenylbenzamide leading to the desired product in moderate yield (**Table 8**, entry 18), while *1H*-benzotriazol-1-ol reacted smoothly, conducting to compound **79** in excellent conversion and yield after only three hours (**Table 8**, entry 19).

I.1.2.2.9 Carbon nucleophiles reactivity

Entry	Nucleophile	Product	Product N°	Time of reaction (h)	Conversion (%)	Isolated yield (%)
1			80	24	55	50
2			81	24	100	98
3[a]			82	5	85	65

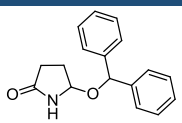
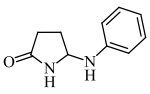
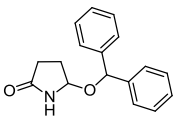
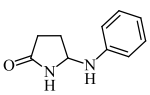
[a]Cs_2CO_3 was used as a catalyst

Table 9 *Pterolactam derivatives obtained from the reaction of methylene active reactants*

Our CsF-mediated protocol was further applied for the reaction of pterolactam **3** with various polyfunctionalized methylene active compounds, and we were able to successfully isolate compounds **80**-**82**, in good to quantitative yields (**Table 9**, entries 1-3). Interesting fact is that (*Z*)-methyl 3-aminobut-2-enoate and 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione reacted exclusively *via* the methylene group and not the amino one, to give compound **80** and **81** as the only products, in good yields, underlining the high reactivity of such substrates under solvent-less cesium mediated reaction. Considering these three methylene active substrates selected to study the selectivity of the reaction, it is notable than C5-subtituted-γ-lactams are preferentially formed compared with *N*,*N* or *N*,*O* acetals under such cesium-catalyzed optimized conditions.

I.1.2.2.10 Extension of the protocol to other substrates

In the end, the same protocol using CsF was applied to differents substrates. For starters, we wanted to see if the departing group can be a problem whether is a little more bulky, so subtrates such as 5-(benzhydryloxy)pyrrolidin-2-one, *N*-((5-oxopyrrolidin-2-yl)oxy)-*N*-phenylbenzamide and 5-(phenylthio)pyrrolidin-2-one and others were tested (**Table 10**, entries 1-4). The outcome was that yields were moderate compared when using pterolactam because of the competition between aniline and new-formed alcohol as nucleophiles. However the reactions were a success proving that bulky groups can also be displaced by aniline. The best leaving group according to the yields is the *N*-hydroxy moiety (**Table 10**, entry 3), followed by thiophenol and then alcohol derivatives. On the contrary, we also tested benzylamine efficiency in displacing aniline, which indeed was possible, but with moderate yields (**Table 10**, entry 5).

Entry	Substrate	Nucleophile	Product	Product N°	Time of reaction (h)	Conversion (%) (*Isolated yield* (%))
1				12	6	45
2				12	6	44

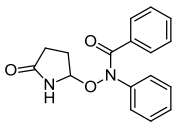
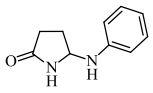
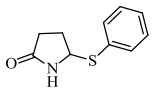
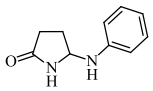
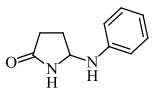
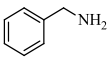
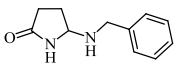
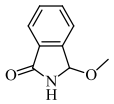
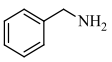
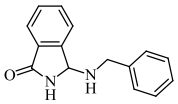
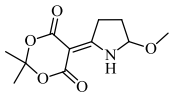
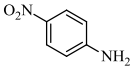
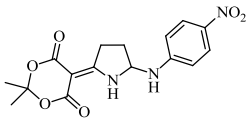
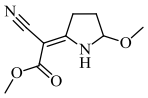
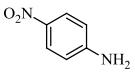
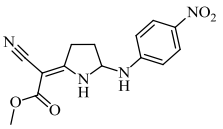
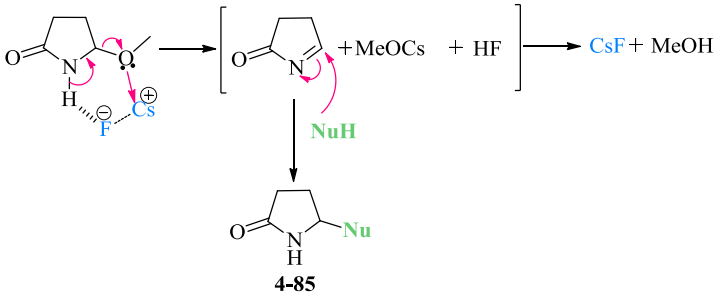
3				12	6	65
4				12	6	56
5				5	6	43
6[b]				83	48	100 *(61)*
7				84	24	100 (*65*)
8				85	10	100 (*51*)

Table 10 *Extended substrates*

Besides the different leaving groups, we wanted to see if the cyclic lactam can influence the nucleophilic substitution. Things being said, substrates such as 3-methoxyisoindolin-1-one, 5-(5-methoxypyrrolidin-2-ylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione and (*Z*)-methyl 2-cyano-2-(5-methoxypyrrolidin-2-ylidene)acetate were submitted to the screening (**Table 10** , entries 6-8). Compound **83** was formed with a moderate yield compared to the results obtained with lactam **3** while compounds **84** and **85** were formed approximately with the same yield as pterolactam based related nitroaniline derivatives, proving that the carbonyl modification had little or no influence over the leaving ability of the methoxy group. To conclude, various groups and substrates can be used under the optimized CsF-mediated methodology proving the large range of applicability of the CsF.

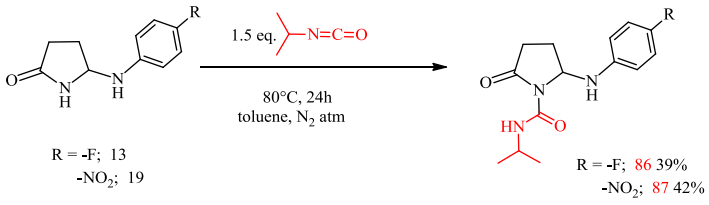
I.1.2.2.11 Proposed mechanism of reaction for the CsF catalyst



Scheme 15 Proposed mechanism of the reaction via 3,4-dihydro-2H-pyrrol-2-one.

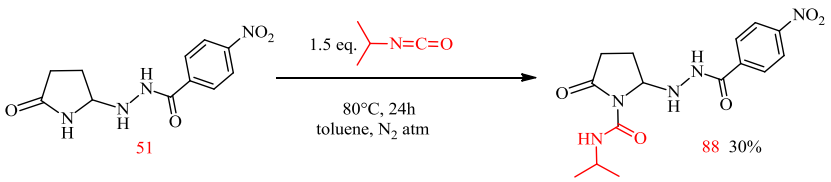
As previously mentioned, one of the use of CsF in organic synthesis, is as a source of the fluoride anion. However, when compared to the other soluble fluorides, CsF is moderately basic, giving the weak acid HF. Thing being considered, the low nucleophilicity of the fluoride ion makes it as a useful base in several organic transformations. Based on this, a possible mechanism can be proposed for the transformation of 5-methoxypyrrolidin-2-one **3**. The catalytic role of CsF could occur *via* a 6-members ring pathway involving cation-σ interactions between CsF and oxygen's pair of electrons, allowing the fluoride anion to free and act as a base, affording the formation of 3,4-dihydro-*2H*-pyrrol-2-one as intermediate (**Scheme 16**). This imine intermediate then suffers a nucleophilic attack by the nucleophile, leading to the pyrrolidine-2-one derivatives **4-85**. CsF is reformed and methanol is released as side product, which can be continuously eliminated to increase both conversion and yield by displacing the equilibrium to the right side.

I.1.3 Isocyanation reactions of selected pyrrolidin-2-ones



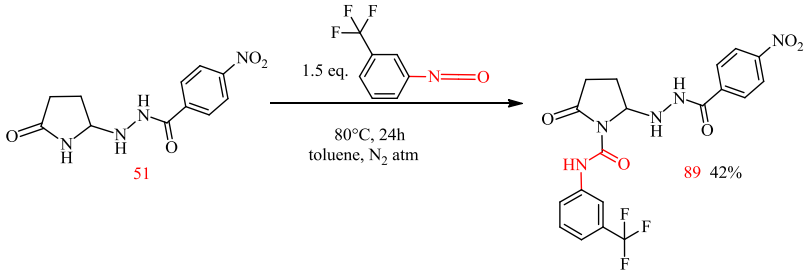
Scheme 16 N-substitution of some selected pyrrolidin-2-ones

Compounds **13** and **19** were further submitted to the isopropyl isocyanate reaction, and as expected, the *N*-isopropylacetamide moiety has been linked to the *NH*- group of the lactam ring, affording derivatives **86** and **87** in 39% respectively 42% of yield.



Scheme 17 *N-substitution of a hydrazide derivative with 2-isocyanatopropane*

4-Nitro-*N*'-(5-oxopyrrolidin-2-yl)benzohydrazide **51** was also subjected to the same reaction as **13** and **19**, resulting in the same type of substitution, however the reaction being less efficient, compound **88** being obtained in only 30% yield. Keeping the substrate **51**, another isocyanate, bulkier, was employed, allowing us the formation of compound **89**, which was slightly more efficient, being formed with 42% of isolated yield.



Scheme 18 *N-substitution of a hydrazide derivative with 1-isocyanato-3-(trifluoromethyl)benzene*

I.1.4 Conclusions

To conclude this subchapter, a simple, clean, widely applicable, CsF-mediated protocol for the formation of *N,N'*-aminals, *N,O* or *N,S*-acetals and C_5-substituted γ-lactams has been optimized under solvent-less conditions, being in the frame of sustainable chemistry. We finished by proving the efficiency of CsF even with very weak nucleophiles and different lactam substrates. Besides this, one of the most important aspect is that CsF allowed us to explore biosourced pterolactam and show that there are easy pathways of obtaining γ-lactams derivatives. The overall modifications which were done around the scaffold are illustrated in **Figure 20**.

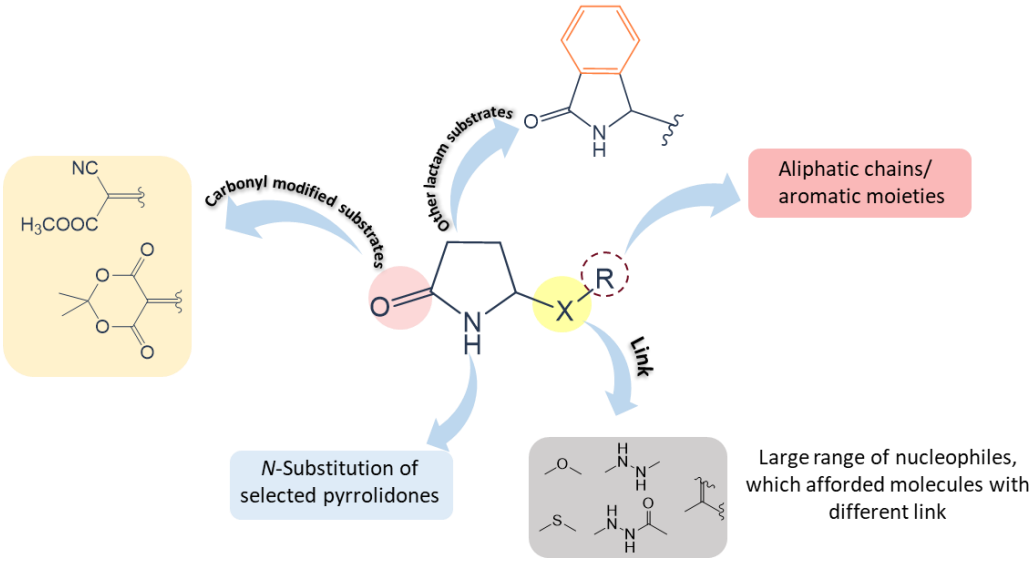


Figure 20 *Pyrrolidones series which were obtained and described in this subchapter*

Nevertheless, this large range of new derivatives made a great difference in the continuous interest of finding new antibacterial and antifungal agents, being largely sufficient for having a good idea on a possible structure-activity relationship study.

I.2 Modifications of the carboxylic group of pyroglutamic acid. The synthesis of new 5-oxopyrrolidine-2-carbohydrazide

I.2.1 Hydrazides – rich sources of biological activities

Hydrazides are the acylated derivatives of hydrazines, belonging to the heterocyclic family of compounds containing carbon and nitrogen as active centers of their biological properties. More precisely, hydrazide chemicals are sharing a common functional group characterized by a nitrogen to nitrogen covalent bond with four substituents, with at least one of them being an acyl group, whereas the related hydrazines do not carry an acyl group. From the crystallographic point of view it is expected that the hydrazides link is driven by H-bonding, as is in the case of amides. However, the presence of the additional amino group, which introduces one more H-bonding donors and one acceptor, should result in a more rich set of possible H-bonding patterns[40] and possible more biological interactions with biological targets.

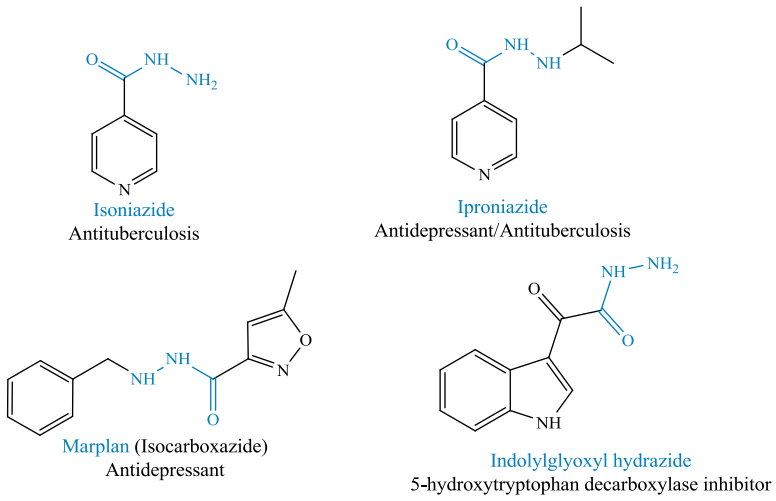


Figure 21 *Hydrazides derivatives and their pharmaceutical applications*

One of the most known acid hydrazide, which is commercially available, is isonicotinic acid hydrazide (INH, isoniazide), (**Figure 21**), known as the most effective agents against tuberculosis since 1952,[41] which however, loss its value today. Iproniazid, though it was designed for the tuberculosis treatment, now is used as an irreversible MAO inhibitor as well. Isocarboxazide, also known as Marplan (**Figure 21**), is used as antidepressant, being a powerful

[40] Narang R., Narasimhan B., Sharma S. Review on Biological Activities and Chemical Synthesis of Hydrazide Derivatives. *Curr. Med. Chem*. 2012, 19, 4, p. 569-612.
[41] Norman A. Effect of Isonicotinic Acid Hydrazide on Some Plant Systems. *Science*, 1955, 121, 3154, p. 834.

monoamine oxidase (MAO) inhibitor.[42] The last drug, indolylglyoxylyl hydrazide has been reported by Heinzelman and Szmuszkovicz as a potent 5-hydroxytryptophan decarboxylase inhibitor.[43]

I.2.2 Synthesis pathways for carbohydrazides

Synthesis starting from esters, halides and anhydrides

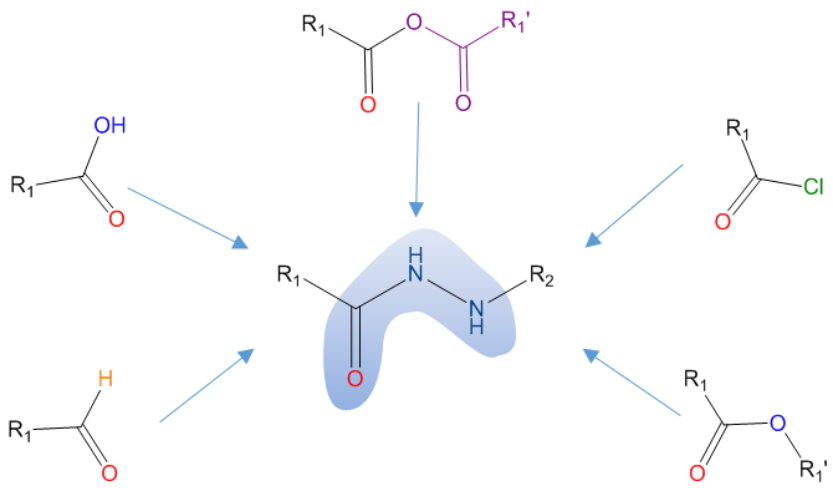


Figure 22 *Synthesis pathways to obtain carbohydrazides*

According to literature, a variety of procedures have been developed to prepare carbohydrazides. A very used method involves the preparation of acyl anhydrides and acid chlorides which subsequently react rapidly with hydrazine to give the corresponding carbohydrazides. The one inconvenient for using acid chlorides or anhydrides as substrates is their reactivity, being difficult to stop the reaction at the end of the monoacylation step.

An attractive method to prepare carbohydrazides is the hydrazinolysis of the corresponding esters which has a requirement of preparing esters from corresponding acids. Conversion of esters to hydrazides is a generally simple process, although the conditions of hydrazinolysis vary. Acid hydrazides are mostly tractable materials, often insoluble in organic solvents from which they separate as they form. There are cases when the conditions of hydrazinolysis are too drastic. As an example, in peptides which have valine or isoleucine at

[42] Vardanyan, R., Hruby V. Synthesis of Essential Drugs, 1st ed. *Elsevier Science: Maryland Heights*, 2006, p 111.
[43] Heinzelman, R.V., Szmuszkovicz J. Recent studies in the field of indole compounds. *J. Prog. Drug Res*. 1963, 6, p. 75-150.

their C-terminal residue, the reactivity of the ester group is greatly reduced by the combination of electron release by the branched alkyl side chain and steric hindrance. Such peptide esters are resistant to hydrazinolysis and complete conversion to hydrazides requiring many hours of heating to the boiling point of the mixture. Less hindered esters will afford the desired hydrazide within hours at room temperature. Difficulties usually can arise from the insolubility of protected amino acid or peptide esters in solvents such as alcohol or dioxane.[44]

A less attractive, however efficient, pathway to form hydrazide bond is the use of "coupling reagents". The most successful coupling reagent, dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), which was introduced by Sheehan and Hess.[45] In spite of numerous attempts to replace this powerful reagent with more efficient or less drastic materials, DCC, together with its water soluble derivatives,[46] are leading the field along with diisopropylcarbodiimide,[47] later introduced which is more expensive.

Synthesis starting from the corresponding *N*-acylhydrazone

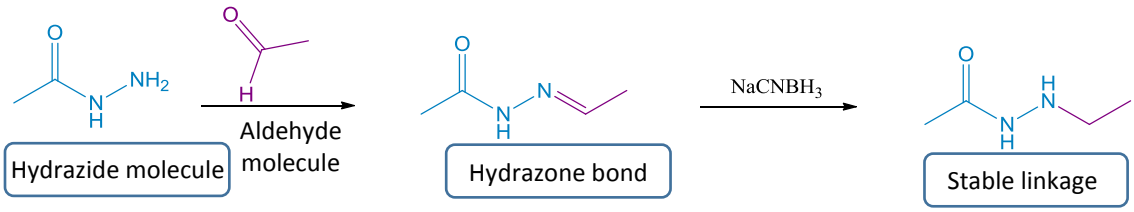


Figure 23 *Hydrazides derivatives synthesis through corresponding hydrazone reduction*

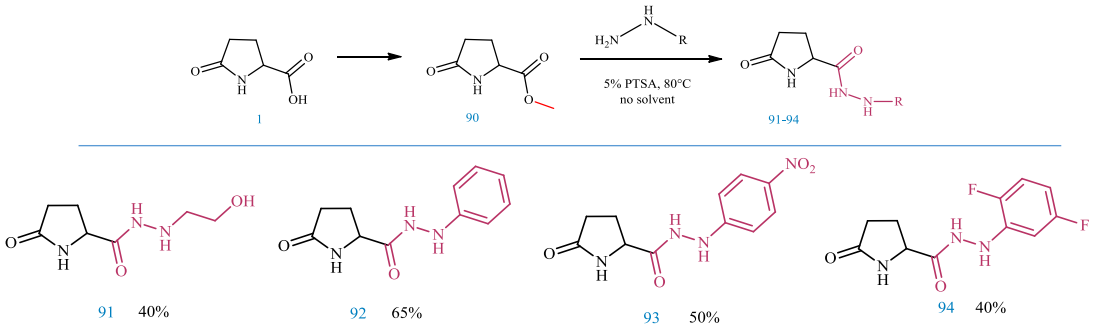
Another interesting pathway of obtaining hydrazides is by reducing the corresponding hydrazones because of its simplicity and the great diversity allowed by the large number of available aldehydes and ketones. Such reduction can be realized by using BH_3THF,[48] catalytic hydrogenation[49] and Et_3SiH, however these require long reaction times and harsh conditions.

[44] Bodanski M. Principles of peptide synthesis. Springer-Verlag Berlin Heidelberg, 1984, 16.
[45] Sheehan J. C., Hess G. P. A New Method of Forming Peptide Bonds. *J. Am. Chem. Soc.* 1955, 77, 4, p. 1067-1068.
[46] Sheehan, J. C., Hlavka J. J. The Use of Water-Soluble and Basic Carbodiimides in Peptide Synthesis. *J. Org. Chem*. 1956, 21, 4, p. 439-441.
[47] Sheehan, J. C. Activated cyclic derivatives of amino acids in peptide synthesis. *Ann. N. Y. Acad. Sci.* 1960, 88, p. 665.
[48] Ghali M.I., Venton D.L. High-yielding synthesis of monoalkylhydrazines. *J. Org. Chem.* 1981, 46, 26, p. 5413-5414.
[49] Burk M.J., Feaster J.E. Enantioselective hydrogenation of the C:N group: a catalytic asymmetric reductive amination procedure. *J. Am. Chem. Soc.* 1992, 114, 15, p. 6266-6267.

Similar agents as sodium borohydride,[50] or sodium cyanoborohydride[51] are also known, however with poor yields and long reaction times.

I.2.3 The synthesis of pyroglutamic acid carbohydrazides *via* hydrazinolysis

We began our study with the commercially available pyroglutamic acid **1**. We obtained the new hydrazides **91-94** in moderate yields (40-65%) by synthesizing firstly methyl pyroglutamate **90** according to a reported methodology developed in the laboratory.[52] As a first strategy, methyl pyroglutamate **90** is firstly employed in a hydrazinolysis reaction with phenylhydrazine derivatives furnishing the wanted hydrazides (**Scheme 20**).



Scheme 19 *Hydrazinolysis reaction of methylpyroglutamate*

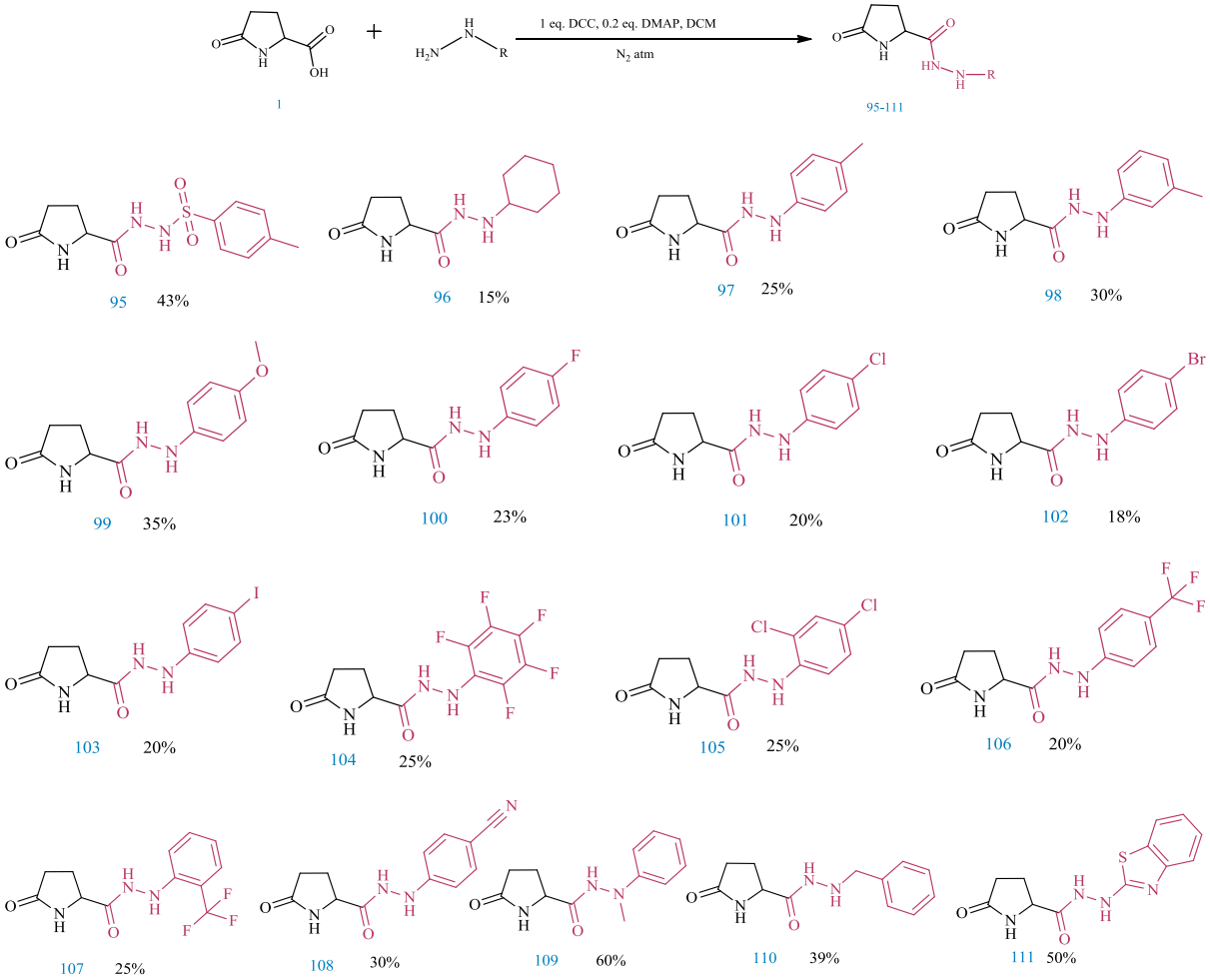
The conditions were selected accordingly to a previous report from our laboratory, which however corresponded to an aminolysis reaction.[53] Within this series, we can notice that the nucleophilicity has an impact on the hydrazinolysis, and deactivating substituents on the hydrazine substrate, such as nitro- and fluoro- are resulting in a decrease of the yield. Indeed, *N*'-(4-nitrophenyl)-5-oxopyrrolidine-2-carbohydrazide **93** was obtained with a yield of 50% and *N'*-(2,5-difluorophenyl)-5-oxopyrrolidine-2-carbohydrazide **94** with 40% yield. However efficient for the isolation of the products, this methodology has an inconvenient, being applicable only for free hydrazines, hydrochloride forms not reacting under these conditions. Though prior releasing of the hydrazine with sodium methoxide is efficient, this would ultimately lead to protocol failure as the release works only *in situ* without further purifications.

[50] Condon F. E. Selective acetylation of methylhydrazine. 1-Acetyl-1-methyl- and 1-acetyl-2-methylhydrazine. *J. Org. Chem.* 1972, 37, 23, p. 3608-3615.
[51] Calabretta R., Gallina C., Giordano C. Sodium Cyanoborohydride Reduction of (Benzyloxycarbonyl)- and (tert-Butoxycarbonyl)hydrazones. *Synthesis*, 1991, 7, p. 536-539.
[52] Cauliez P., Rigo B., Fasseur D., Couturier D. Studies on pyrrolidones. Convenient syntheses of methyl, methyl N-methyl- and methyl *N*-methoxymethylpyroglutamate. *J. Heterocycl. Chem.* 1991, 28, p. 1143-1146.
[53] Baudelet D., Daïch A., Rigo. B. Lipka E., Gautret P., Homerin G., Claverie C., Rousseau J., Abuhaie C.-M., Ghinet A. Impact of Functional Groups on the Copper-Initiated N-Arylation of 5-Functionalized Pyrrolidin-2-ones and Their Vinylogues. *Synthesis*, 2016, 48, p. 2226-2244.

Considering this matter, a different protocol had to be adapted for the synthesis of such carbohydrazides, this series containing one important hit that had to be further developed.

I.2.4 The synthesis of pyroglutamic carbohydrazides *via* a peptidic coupling protocol

The next pathway adopted for the design of carbohydrazides was the peptidic coupling using *N*,*N*'-dicyclohexylcarbodiimide (DCC) and 4-DMAP as a base. The reason of choosing this peptidic coupling agent is because of its low cost and because is one of the most known, a classical one, which can be applied to a large range of acids if needed being a versatile organic reagent. The main drawback of its use is the formation of *N*,*N*-dicyclohexylurea (DCU), which partially remains in solution with the product. The low solubility of DCU in most organic solvents complicates purifications, especially in the peptide synthesis.[54]



Scheme 20 *Carbohydrazides obtained from PGA through peptidic coupling*

[54] Kvasnica M. Dicyclohexylcarbodiimide (DCC). *Synlett*. 2007, 14, p. 2306–2307.

The protocol consists in the DCC (1 eq) addition to the dichloromethane solution of the carboxylic acid and the hydrazine. Before adding the acid, the hydrazine is released by addition of a stoichiometric amount of a methanol solution of sodium methanoate 30% to the substrate, releasing *in situ* the wanted hydrazine. As it is known that the reaction rate is increased by the use of a catalyst such as 4-DMAP, 0.2 eq were added as well. All reactions were conducted under nitrogen atmopshere. Compared to the hydrazinolysis reaction, the peptidic coupling method though more extendable to other hydrazines, was less efficient, involving tedious purifications. However, starting from the pyroglutamic acid, we were able to isolate derivatives **95**-**111**.

Cyclohexylhydrazine reacted slowly with **1** to afford compound **96** in a poor 15% of yield, being the least efficient hydrazine for the formation of new carbohydrazides. Aromatic hydrazines on the other part, reacted a little better than cyclohexylhydrazine, depending of the ring substituents. *para*-Methylphenylhydrazine reacted with the pyroglutamic acid and afforded derivative **97** in 25% yield, which was lower than the *meta*-methylphenylhydrazine reaction yield with **1**, compound **98** being obtained in 30% yield. Concerning other activating substituents such as methoxy-, the yield went up a little bit more, compound **99** being obtained with 35% of yield.

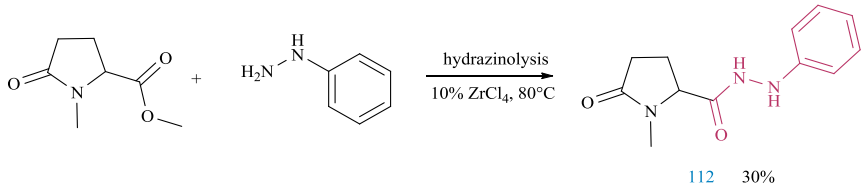
Considering the deactivating substituents such as halogenated ones, we were able to isolate a series of eight such derivatives, **100**-**107**. Between the fluoro-, chloro- and bromo-derivatives we can notice a slight yielding difference, compound **100** which is obtained from the 4-fluorohydrazine being formed with 23% yield while **101** and **102** are formed with 20% and respectively 18% yields. The iodophenylhydrazine however, being a free hydrazine was firstly submitted to a hydrazinolysis reaction, which was unsuccessful and so, the peptidic coupling pathway was applied, compound **103** being isolated in 20% yield.

Surprisingly, the pentafluorophenyl hydrazine reacted approximatively the same as 4-fluoro- substrate, affording compound **104** in 25% yield. The other fluorinated derivatives which we were interested in, were trifluoromethyl derivatives, things being said, 4-trifluoromethyl- and 2-trifluoromethylphenylhydrazine were submitted to the DCC peptidic coupling method with the pyroglutamic acid. Between the two hydrazines, 5% of difference can be noted among the yields, compound **107** being formed with 20% yield while **108** being formed with 25% yield. The 2,4-dichlorophenylhydrazine was also submmitted to our protocol, compound **105** being obtained in 25% yield. 4-Cyanophenylhydrazine seemed to react a little better with pyroglutamic acid, allowing the formation of compound **108** in 30% yield. 1-Methyl-1-phenylhydrazine derivative **109** turned to be a surprise in terms of reactivity and

purification facility, being isolated with 60% yield through the peptidic coupling method. The last substrates submitted to the protocol were benzylhydrazine and 2-hydrazinylbenzo[*d*]thiazole that reacted moderately well compared to other aromatic hydrazines, furnishing compounds **110** and **111** in 37 and 50% of yield.

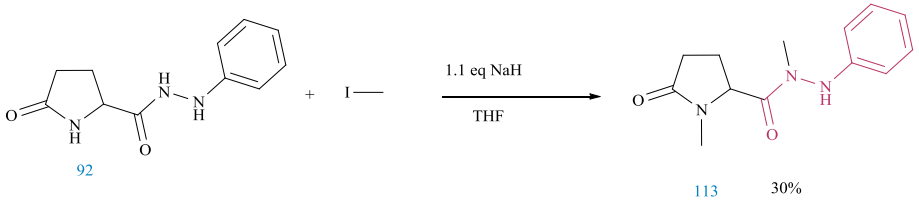
I.2.5 Modifications around the pyroglutamic acid hydrazine **92** - *NH*- group modifications

As we had preliminary informations regarding the antibacterial activity of derivatives such as **92**, we decided to modulate different positions of the molecule in order to have a more comprehensive structure-activity relationship study. Things being said, in a first instance, through a hydrazinolysis reaction of 1-methyl-5-oxo-*N*'-phenylpyrrolidine-2-carbohydrazide and phenylhydrazine, in presence of a catalytic amount of $ZrCl_4$[55], we were able to obtain derivative **112**, however with a low yield of 30%.



Scheme 21 *Hydrazinolysis reaction of the N-methylated PGM*

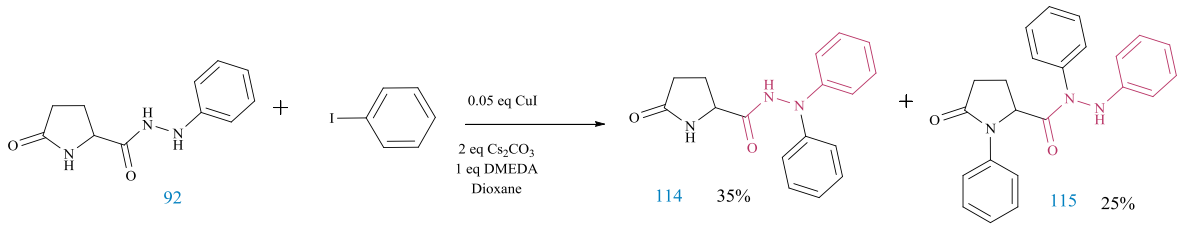
As a different path to obtain our alkylated derivative, methyl iodide in presence of sodium hydride (60% dispersion in mineral oil), were used for derivative **92**, having THF as a solvent. The method, as expected, was not selective for the formation of a mono-alkylated derivative, but conducted to the formation of **113** as a di-methylated derivative of **92**, obtained in 30% yield (**Scheme 23**).



Scheme 22 *N-methylation reaction of derivative **92***

[55] Homerin G., Baudelet D., Dufrénoy P., Rigo B., Lipka E., Dezitter X., Furman C., Millet R., Ghinet A. $ZrCl_4$ as a new catalyst for ester amidation: an efficient synthesis of h-P2X7R antagonists. *Tetrahedron Lett.* 2016, 57, 10, p. 1165-1170.

Besides small groups such as methyl, we also wanted to include some more bulky moieties such as phenyl groups. Considering this type of modification, we chose to perform a copper-catalyzed *N-arylation*[56] of the *NH*-group, directly on **92** substrate molecule.



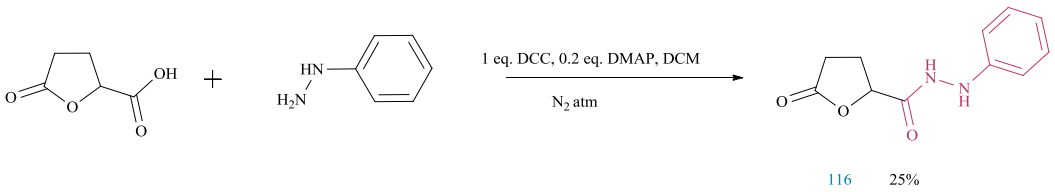
92 114 35% 115 25%

Scheme 23 *N-Arylation reaction of derivative **92***

The outcome was, as expected, a non selective *N*-arylation, which furnished us a mixture of two molecules, one mono-arylated and one di-arylated with 35 and 25% of yield for the new molecules **114** and **115** (**Scheme 24**).

I.2.6 Synthesis of other hydrazide derivatives

Another way which could prove the importance of having a lactam ring, was replacing the nitrogen atom by an oxygen one. Things being said, the L-enantiomer of 5-oxotetrahydrofuran-2-carboxylic acid was employed, following the same protocol as for the pyroglutamic acid, which furnished carbazide **116** with a modest yield of 25% (**Scheme 25**).

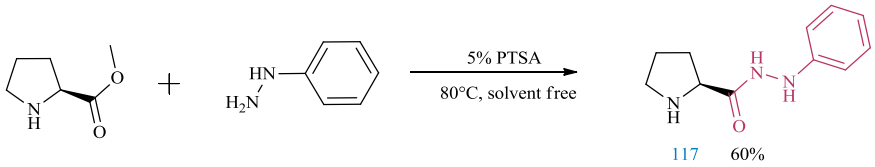


116 25%

Scheme 24 *Peptidic coupling of 5-oxotetrahydrofuran-2-carboxylic acid with phenylhydrazine*

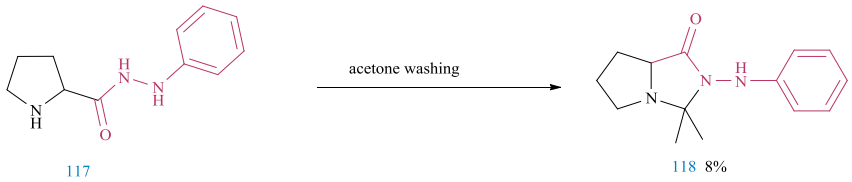
Moreover, to study the importance of the lactam carbonyl moiety, we decided to start from the L-proline methyl ester and follow the same hydrazinolysis protocol as employed for methylpyroglutamate. Fortunately the protocol helped us obtain derivative **117**, in a moderate yield of 60% (**Scheme 26**).

[56] Baudelet D., Daïch A., Rigo B., Lipka E., Gautret P., Homerin G., Claverie C., Rousseau J., Abuhaie C.M., Ghinet A. Impact of Functional Groups on the Copper-Initiated N-Arylation of 5-Functionalized Pyrrolidin-2-ones and Their Vinylogues. *Synthesis* 2016, 48, 14, p. 2226-2244.



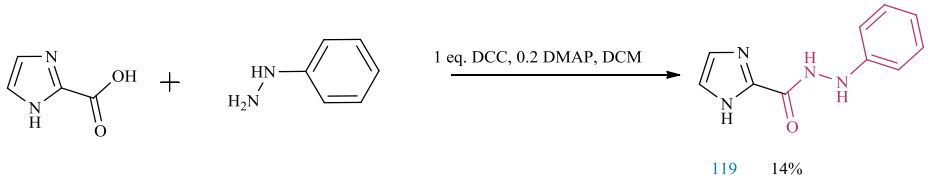
Scheme 25 *Hydrazinolysis reaction of L-proline methyl ester*

Further, by a simple acetone washing, derivative **117** was rigified, by performing a 5 members ring closure, and **118** was obtained.



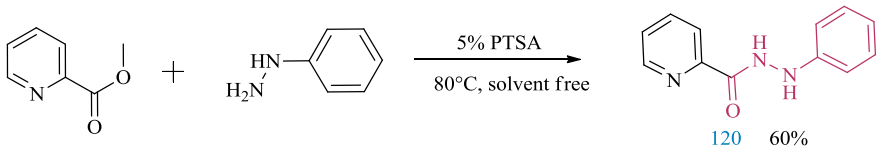
Scheme 26 *Rigidification of derivative 117*

Among the other cycle replacements which were designed, is also the imidazole. In order to synthesize such a carbazide, 1*H*-imidazole-2-carboxylic acid was used as a substrate, to which the phenylhydrazine was linked though the peptidic coupling method employing DCC, as seen before. For this reaction, the purification procedures were particularly difficult and **119** was furnished with a poor 14% yield.



Scheme 27 *Peptidic coupling of 1H-imidazole-2-carboxylic acid with phenylhydazine*

The last ring modification was the replacement of the pyroglutamic acid moiety by the picolinic acid. In this context, the methyl picolinate was engaged in the hydrazinolysis reaction with phenylhydrazine, in presence of PTSA, pathway which allowed us to obtain product **120** in 60% of yield.



Scheme 28 *Hydrazinolysis reaction of methyl picolinate with phenylhydrazine*

I.2.7 Conclusions

To conclude, within this series, 21 hydrazides derivating from the pyroglutamic acid were obtained, and we saw that the classic protocols such as peptidic coupling with DCC, can be applied to the biosourced PGA.

Moreover, nine ring and link modifications were done around the reference molecule **92**, for a structure-activity relationship study, considering the antibacterial activity. The summarized hydrazides series is depicted in **Figure 24**.

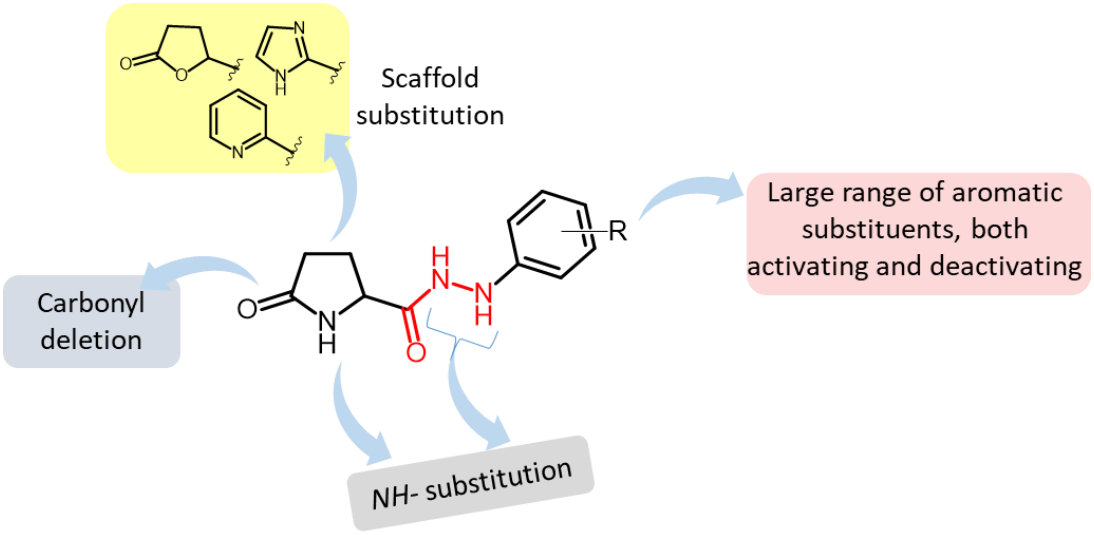


Figure 24 *Summarized hydrazide series*

I.3 Modifications of pyroglutamic acid hydrazide. The synthesis of new *N*-acyl hydrazones

I.3.1 *N*-acylhydrazones generalities

N-acylhydrazones constitute a class of organic compounds, extremely attractive because of their azomethine group (–NH–N=CH–) that is connected to a carbonyl group, making it responsible for their different and wide pharmaceutical applications. In the past two decades, the biological active core of *N*-acylhydrazone (NAH) has been extremely investigated as a functional group in medicinal chemistry and many hit and lead compounds were identified with different pharmacological properties.

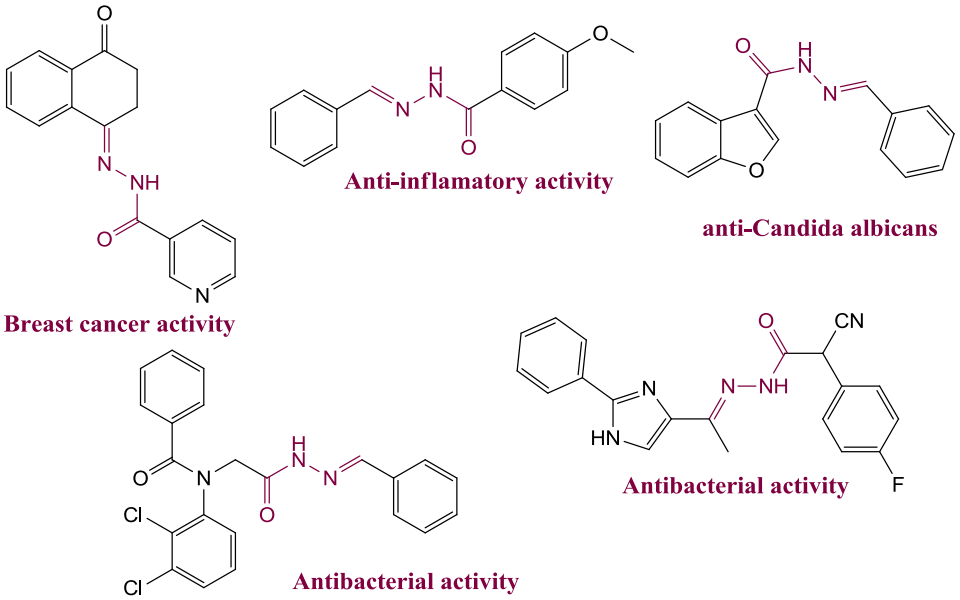


Figure 25 *Biological activities of some N-acylhydrazones*

A variety of hydrazone derivatives has been synthesized with the potential pharmacological activities like anticancer,[57] anti-inflammatory,[58] antibacterial,[59,60] analgesic,[61]

[57] Dandawate P., Khan E., Padhye S., Gaba H., Sinha S., Deshpande J., Venkateswara Swamy K., Khetmalas M., Ahmad A., Sarkar F.H. Synthesis, characterization, molecular docking and cytotoxic activity of novel plumbagin hydrazones against breast cancer cells. *Bioorg. Med. Chem. Lett.* 2012, 22, p. 3104-3108.

[58] Kümmerle A.E., Vieira M.M., Schmitt M. Miranda A.L., Fraga C.A., Bourguignon J.J., Barreiro E.J. Design, synthesis and analgesic properties of novel conformationally-restricted N-acylhydrazones (NAH). *Bioorg. Med. Chem. Lett.* 2009, 19, p. 4963-4966.

[59] Sharma R.N., Sharma K.P., Dikshit S.N. Synthesis, characterization and biological activities of some new hypophosphorous adducts of acid hydrazones derived from 2-[(*N*-benzoyl) 2, 3 dichloroanilido] acetohydrazide. *Asian J. Chem.* 2012, 24, 3, p. 1271-1275.

[60] Abdel-Wahab B.F., Awad G.E., Badria F.A. Synthesis, antimicrobial, antioxidant, anti-hemolytic and cytotoxic evaluation of new imidazole-based heterocycles. *Eur. J. Med. Chem.* 2011, 46, p. 1505-1511.

[61] Gökçe M., Utku S., Küpeli E. Synthesis and analgesic and anti-inflammatory activities 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(*p*-substituted/nonsubstituted benzal)hydrazone derivatives. *Eur. J. Med. Chem.* 2009, 44, p. 3760-3764.

antifungal,[62] antihypertensive,[63] antiplatelet,[64] antimalarial,[65] antidepressant,[66] anticonvulsant[67] and antiviral[68]. Among the multiple biological properties of this class of compounds, antimicrobial activity[69] is the most frequently encountered in scientific literature, this being one of the reason why we chose to design this type of derivatives.

Even though this small scaffold NAH-based drug discovery has progressed dramatically in the past decade, only few have reached the market. The two first compounds belong to a chemical class related to NAHs, the semicarbazones of the 2-nitrofuran derivatives (nitrofurazone and nitrofurantoin).[70]

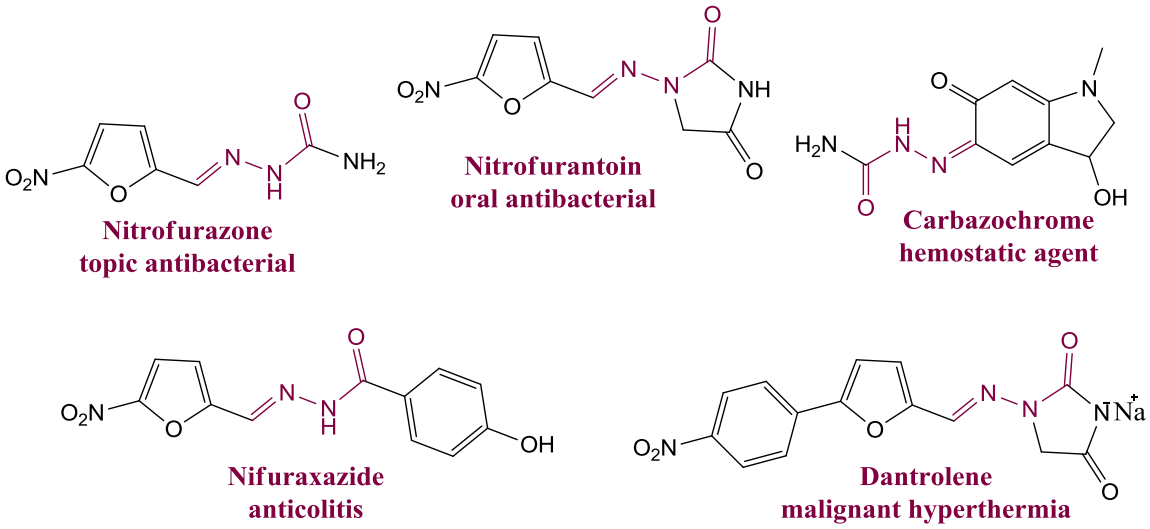


Figure 26 *Structures of approved NAH motif-bearing scaffolds*

[62] Telvekar V.N., Belubbi A., Bairwa V.K., Satardekar K. Novel N'-benzylidene benzofuran-3-carbohydrazide derivatives as antitubercular and antifungal agents. *Bioorg. Med. Chem. Lett.* 2012, 22, p. 2343-2346.
[63] Leal C.M., Pereira S.L., Kümmerle A.E., Leal D.M., Tesch R., de Sant'Anna C.M., Fraga C.A., Barreiro E.J., Sudo R.T., Zapata-Sudo G. Antihypertensive profile of 2-thienyl-3,4-methylenedioxy benzoylhydrazone is mediated by activation of the A2A adenosine receptor. *Eur. J. Med. Chem.* 2012, 55, p. 49-57.
[64] Mashayekhi V., Haj Mohammad Ebrahim Tehrani K., Amidi S., Kobarfard F. Synthesis of novel indole hydrazone derivatives and evaluation of their antiplatelet aggregation activity. *Chem. Pharm. Bull. (Tokyo)*, 2013, 61, p. 144-150.
[65] Fattorusso C., Campiani G., Kukreja G. Persico M., Butini S., Romano M.P., Altarelli M., Ros S., Brindisi M., Savini L., Novellino E., Nacci V., Fattorusso E., Parapini S., Basilico N., Taramelli D., Yardley V., Croft S., Borriello M., Gemma S. Design, synthesis, and structure-activity relationship studies of 4-quinolinyl-and 9-acrydinylhydrazones as potent antimalarial agents. *J. Med. Chem*. 2008, 51, p. 1333-1343.
[66] Cutshall N.S., Onrust R., Rohde A., Gragerov S., Hamilton L., Harbol K., Shen H.R., McKee S., Zuta C., Gragerova G., Florio V., Wheeler T.N., Gage J.L. Novel 2-methoxyacylhydrazones as potent, selective PDE10A inhibitors with activity in animal models of schizophrenia. *Bioorg. Med. Chem. Lett.* 2012, 22, 17, p. 5595-5599.
[67] Kulandasamy R., Adhikari A.V., Stables J.P. A new class of anticonvulsants possessing 6 Hz activity: 3,4-dialkyloxy thiophene bishydrazones. *Eur. J. Med. Chem.* 2009, 44, p. 4376-4384.
[68] el-Sabbagh O.I., Rady H.M. Synthesis of new acridines and hydrazones derived from cyclic beta-diketone for cytotoxic and antiviral evaluation. *Eur J Med Chem.* 2009, 44, p. 3680-3686.
[69] L. Popiołek. Hydrazide–hydrazones as potential antimicrobial agents: overview of the literature since 2010. *Med. Chem. Res.* 2017, 26, 287–301.
[70] Guay D.R. An update on the role of nitrofurans in the management of urinary tract infections. *Drugs*, 2001, 61, p. 353–364.

The main use of these two derivatives is to fight bacterial infections. Nitrofurazone being used as a topical antibacterial agent, while nitrofurantoin is used as an oral antibacterial agent to treat infections in the genitourinary tract. Beside these two candidates, there is also carbazochrome, which has been used as a hemostatic agent and is specifically indicated for capillary and parenchymal hemorrhage.[71] Another antibiotic which has a NAH is nifuroxazide, which was approved for the treatment of colitis and diarrhea in adults which unfourtunatly has been withheld from routine practice in recent years.[72] Dantrolene sodium salt is another NAH-based drug which has been successfully approved for clinical use, in the treatment of malignant hyperthermia.[73]

I.3.2 *N*-acylhydrazones synthesis

The versatility of *N*-acylhydrazones in medicinal chemistry is based on their ease of synthesis, the usual main route to synthesize such derivatives is by heating of appropriate hydrazides of carboxylic acids with different aldehydes or ketones in various organic solvents like ethanol, methanol or *tert*-butanol (**Scheme 28**).[74,75,76]

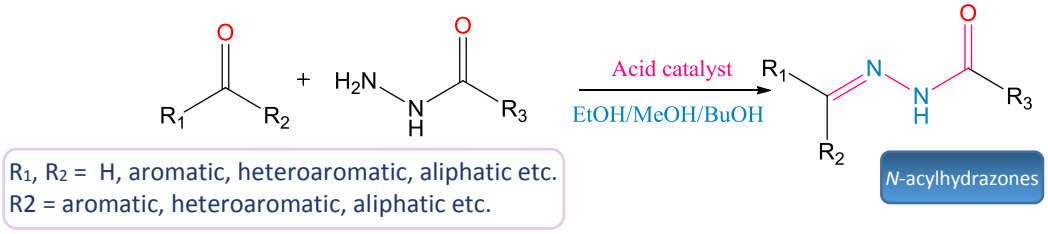


Figure 27 *General synthetic route for the preparation of N-acylhydrazones*

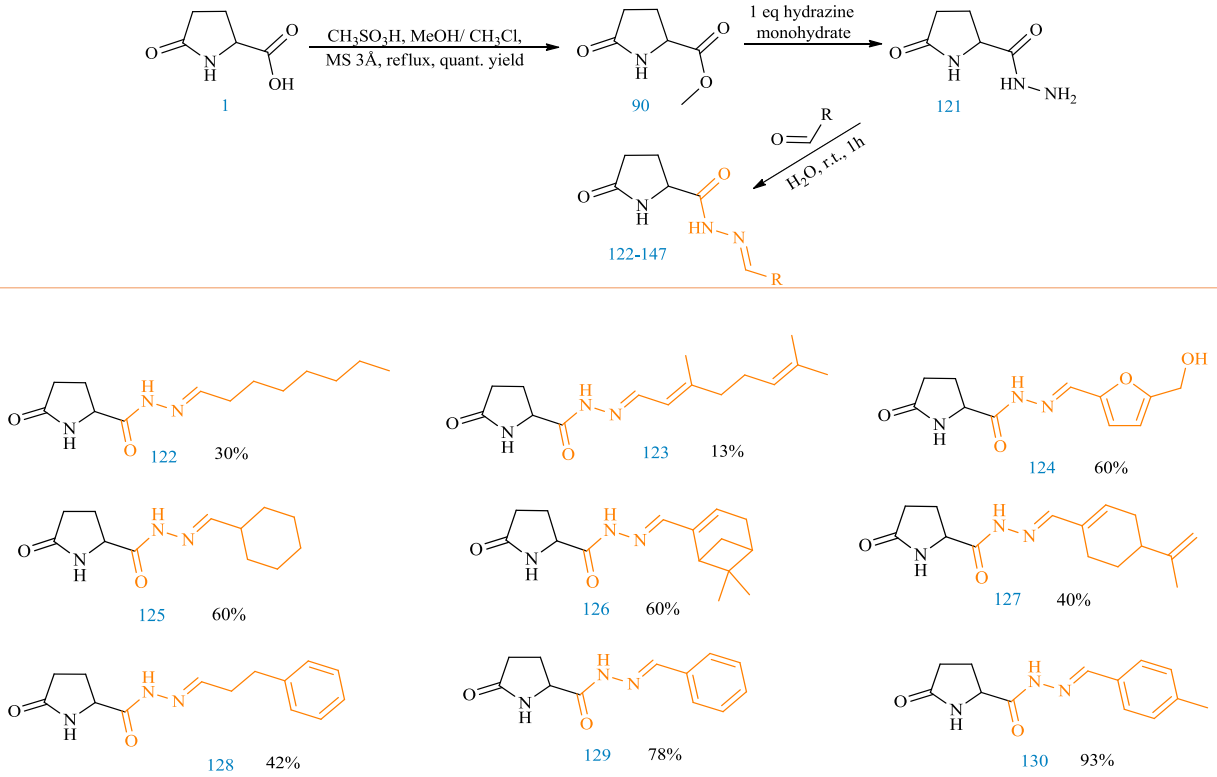
Considering their reactivity and also their importance, hydrazide-hydrazones compounds proved to be very effective organic intermediates, their active hydrogen component

[71] Imlitemsu M.M. Drug treatment of haemorrhoids. *Drugs*, 2005, 65, p. 1481–1491.
[72] Begovic B., Ahmedtagic S., Calkic L., Vehabović M., Kovacevic S.B., Catic T., Mehic M. Open clinical trial on using nifuroxazide compared to probiotics in treating acute diarrhoeas in adults. *Mater. Sociomed*. 2016, 28, p. 454–458.
[73] Krause T., Gerbershagen M.U., Fiege M., Weisshorn R., Wappler F. Dantrolene–a review of its pharmacology, therapeutic use and new developments. *Anaesthesia*, 2004, 59, p. 364–373.
[74] Bala S., Uppal G., Kajal A., Kamboj S., Sharma V. Hydrazones as promising lead with diversity in bioactivity-therapeutic potential in present scenario. *Int. J. Pharm. Sci. Rev. Res.* 2013, 18, p. 65–74.
[75] Popiołek Ł., Biernasiuk A., Malm A. Synthesis and antimicrobial activity of new 1,3-thiazolidin-4-one derivatives obtained from carboxylic acid hydrazides. *Phosphorus Sulfur*, 2015, 190, p. 251–260.
[76] Popiołek Ł., Stefańska J., Kiełczykowska M., Musik I., Biernasiuk A., Malm A., Wujec M. Synthesis, dissociation constants, and antimicrobial activity of novel 2,3-disubstituted-1,3-Thiazolidin- 4-one derivatives. *J. Heterocycl. Chem*. 2016, 5, p. 393–402.

of –CONHN=CH- azometine group can be reduced with $NaBH_4$ affording this way *N*-alkyl hydrazides.[77]

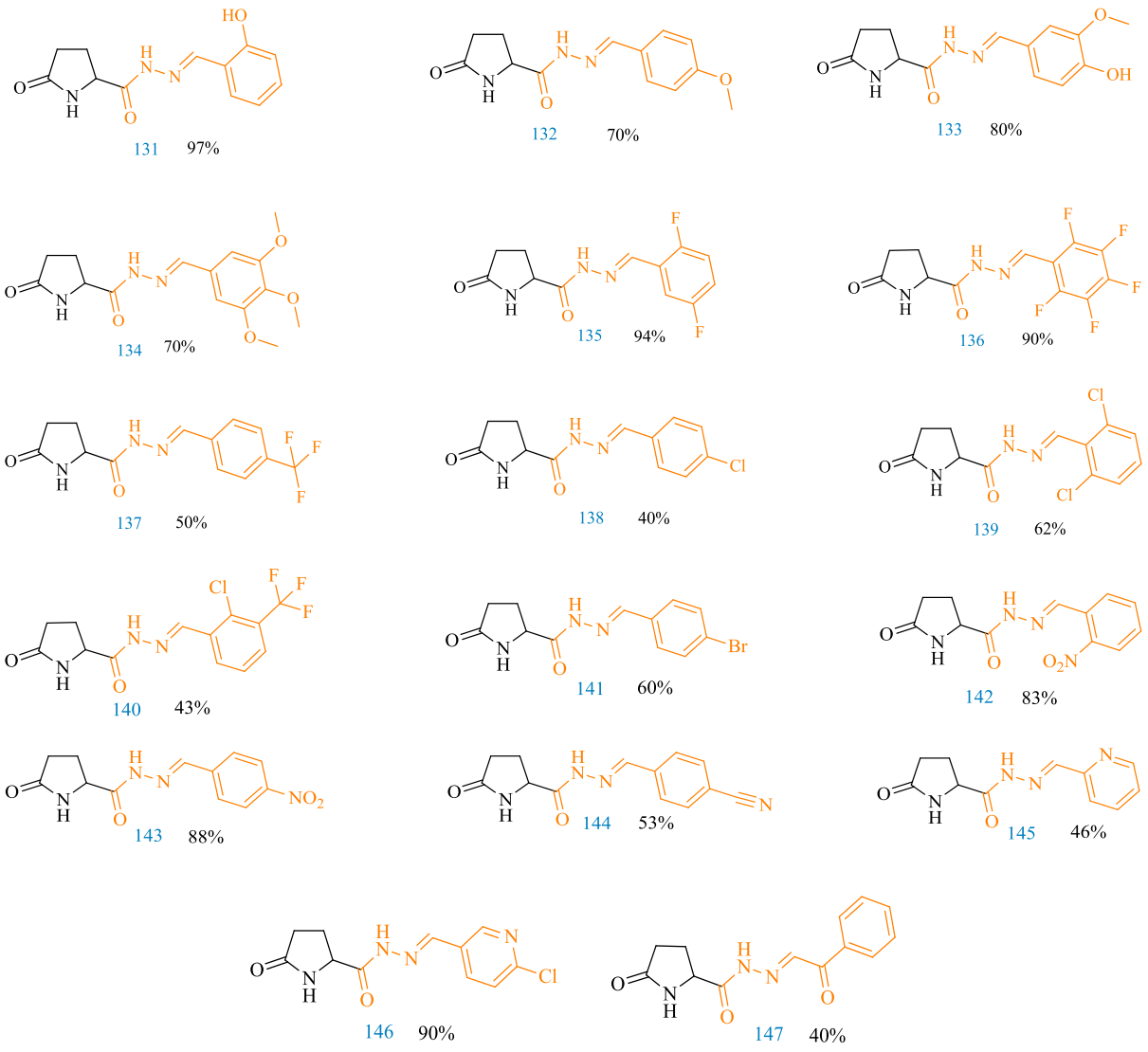
I.3.3 Synthesis of pyroglutamic acid *N*-acylhydrazones

The routes utilized for the synthesis of these new potential antifungal compounds are depicted in the following scheme. We obtained the new isomeric hydrazones **122-147** in moderate to very good yields (23-97%) by synthesizing firstly methyl pyroglutamate **90**,[54] which was then converted to the known pyroglutamyl hydrazide **121**[78] before reaction with the corresponding aldehydes in water or ethanol at room temperature, depending on aldehydes solubility. Those which were not soluble in water, were submitted to the ethanol as solvent protocol. The easiness of obtaining this type of derivatives comes from the facility of the protocol, short reaction time and simple purification for most of the compounds.



[77] Doğan H.N., Duran A., Rollas S. Synthesis and structure elucidation of some new hydrazones and oxadiazolines : anticonlsant activitites of 2-(3-acetyloxy-2-naphtyl)-4-acetyl-5-substituted-1,3,4-oxadiazolines. *Med. Sci. Res.* 1998, 26, p. 755–758.

[78] Cauliez P., Rigo B., Fasseur D., Couturier D. Studies on pyrrolidinones. Catalyst induced selectivity during heterocyclizations of organosilicon compounds into 1,3,4-oxadiazoles or 1,2,4-triazines. *J. Heterocycl. Chem*. 1996, 33, 4, p. 1073-1077.



Scheme 29 *Synthesis of pyroglutamic acid N-acylhydrazones*

Analyzing the results we can notice that aliphatic aldehydes did not react so well, octanal aldehyde for example reacted with **121** affording **122** only in a 30% of yield. Moreover, citral, a natural aldehyde which can be found in citrics and has some proven pharmacological effects itself, such as antibacterial, antifungal, sedative, expectorant, spasmodic and diuretic activities,[79,80] was also submitted to the protocol. However, compound **123** was yielded only in 13%, one reason being also the difficulty of purification besides citral reactivity. Concerning heteroaromatic aldehydes, hydroxymethylfurfural was employed and reacted moderately, NAH **124** being obtained with 60% of yield. Similar yield was obtained for the reaction between

[79] Silva-Angulo A.B, Zanini S.F., Rosenthal A., Rodrigo D., Klein G., Martínez A. Comparative study of the effects of citral on the growth and injury of Listeria innocua and Listeria monocytogenes cells. *PLoS One*, 2015, 10, e0114026.

[80] Carvalho P.M.M., Macêdo C.A.F., Ribeiro T.F., Silva A.A., Da Silva R.E.R., de Morais L.P1, Kerntopf M.R., Menezes I.R.A., Barbosa R. Effect of the Lippia alba (Mill.) N.E. Brown essential oil and its main constituents, citral and limonene, on the tracheal smooth muscle of rats. *Biotechnol. Rep.* 2018, 17, p. 31-34.

cyclohexanecarboaldehyde and our substrate, derivative **125** being obtained in 60% of yield. Moreover, from the same category of cyclic aldehydes we used myrtenal, which is an active principle of essential oils found in plants, such as spearmint, cardamom, orange, lemon, pepper and ginger, widely used as flavoring agent in food industry.[81] The latter one reacted satisfyingly with **121**, furnishing **126** with 60% of yield. Perillaldehyde, another natural cyclic aldehyde, was also used as substrate and afforded the formation of **127** in 40% yield. Perillaldehyde is a known monoterpenoid abundant in the herb perilla such as *Perilla fructescens*,[82] which is frequently used as Chinese traditional treatment of major depression.[83]

Further, a wide range of aromatic aldehydes were used to obtain the aromatic *N*-acylhydrazones. Firstly, 3-phenylpropanal was submitted to the protocol, which led to the formation of **128** in 42% of yield, as a more lipophilic reprezentative of the aromatic ring containing NAHs. Then, benzaldehyde, which is generally extracted from fruit kernels such as apricots, was used in the condensation reaction with pyroglutamic acid hydrazide, which allowed the formation of **129** in good yield of 78%. Introducing activating groups on the aromatic rings, leads us to a general reaction time decrease and also to a yield increase. Electron donating groups such as methyl- in the *para*-position led to very good yields, compound **130** being obtained with 93%. Salicylaldehyde was another natural occurant aldehyde which we submitted to the hydrazide condensation, and which successfully led to compound **131** with 97% yield. Though having a stronger activating group, *para*-methoxybenzaldehyde was less efficient in the reaction with **121**, **132** being obtained with 70% of yield. Furthermore, vanillin was used as an aldehydic substrate, a natural aldehyde known to accumulate in the pods of the orchid *Vanilla planifolia* (vanilla) as the glycoside, being considered an antimicrobial and antioxidant agent.[84] The outcome was satisfying, the reaction underwent fast, furnishing compound **133** in 80% of yield. The last aromatic aldehyde presenting activating groups to which we applied the protocol is trimethoxybenzaldehyde which reacted well with **121** affording **134**.

[81] Martins B.X., Arruda R.F., Aparecida Costa G. Jerdy H., de Souza S.B., Santos J.M., de Freitas W.R.., Kanashiro M.M., de Carvalho E.C.Q., Sant'Anna N.F., Antunes F., Martinez-Zaguilan R., Souad S., Okorokova-Façanha A.L., Façanha A.R. Myrtenal-induced V-ATPase inhibition - A toxicity mechanism behind tumor cell death and suppressed migration and invasion in melanoma. *BBA*, 2019, 1863, 1, p. 1-12.

[82] Masahiro T., Risa M., Harutaka Y., Kazuhiro C. Novel Antioxidants Isolated from Perilla frutescens Britton var. crispa (Thunb.). *Biosci. Biotechnol. Biochem.* 1996, 60, 7, p. 93-95.

[83] Li J.M., Kong L.D., Wang Y.M., Cheng C.H., Zhang W.Y., Tan W.Z. Behavioral and biochemical studies on chronic mild stress models in rats treated with a Chinese traditional prescription Banxia-houpu decoction. *Life Sci*. 2003, 74, 1, p. 55-73.

[84] Saadi A.H., Abdullah A.A., Talay P.P., Yardım Y., Şentürk Z. Simultaneous voltammetric determination of vanillin and caffeine in food products using an anodically pretreated boron-doped diamond electrode: Its comparison with HPLCDAD. *Talanta*, 2017, 170, p. 384-391.

Considering the benzaldehyde derivatives bearing deactivating groups, some reactions worked astonishingly well, being well tolerated. Two exemples are the fluorinated benzaldehydes, 2,5-difluorobenzaldehyde and pentafluorobenzaldehyde which reacted very well with hydrazide **121**, furnishing NAHs **135** and **136** in 95, respectively 90% yields. However, the *para*-trifluoromethyl NAH derivative **137** was obtained with a lower yield of 50%, the reason being the strongly deactivating effect of the trifluoromethyl group. Among the halogenated benzaldehyde derivatives, the chlorinated ones had the lowest reactivity rate, *para*-chlorobenzaldehyde leading to **138** and while 2,6-dichlorobenzaldehyde led to **139** with 40, respectively 62% of yield, the last one being formed more efficiently despite the hindrance effect of the *ortho*-substituents. A similar reactivity can be observed for the formation of compound **140**. Considering bromo-derivatives, *para*-bromobenzaldehyde was the only one subjected to the protocol, **141** being formed with a moderate yield of 60%.

Going further with π-acceptors groups which are strongly deactivating, *ortho*- and *para*-nitrobenzaldehydes reacted surprisingly well with the substrate, furnishing **142** and **143** with 83 and 88% of yield, while the 4-cyano-NAH **144** was formed less efficiently, as expected. Besides benzaldehydes, the picolinaldehyde and 6-chloronicotinaldehyde were included in the series, in the frame of a structure-activity relationship study, the first one leading to the formation of **145** with 46% of yield while the chlorinated nicotinic NAH **146** was successfully obtained with 90%. Further, also for the SAR study, phenylglyoxal was used as an aldehydic substrate, and permitted the formation of **147**, though with a poor yield of 40%.

To summarize, we have developed a benign, simple and practical synthetic approach for the synthesis of pyroglutamic acid *N*-acylhydrazones by the condensation reaction of the pyroglutamic acid hydrazide and aldehydes, in green solvents such as water or ethanol. The desired products were obtained in moderate to very good yields, and mostly were very simple to isolate. The one-pot strategy is tolerant to a broad range of aldehyde substrates and performs well in a mild environment. This work provides an efficient way to synthesis new NAHs, designed as potential antifungals.

I.4 Hydrazones – an exploitable source of antimicrobial agents

Hydrazones are a class of organic compounds which possess the C=N-N- link, being very easy to access compounds with a straightforward synthesis, very useful in organic synthesis. Within the link, the imine C-atom in hydrazone has both electrophilic and

nucleophilic character, while the nucleophilic imine and amino-type nitrogens are nucleophilic (**Figure 28**). These structural motifs give the hydrazone group its physical and chemical properties, in addition of playing a crucial part in determining the range of applications it can be involved in.

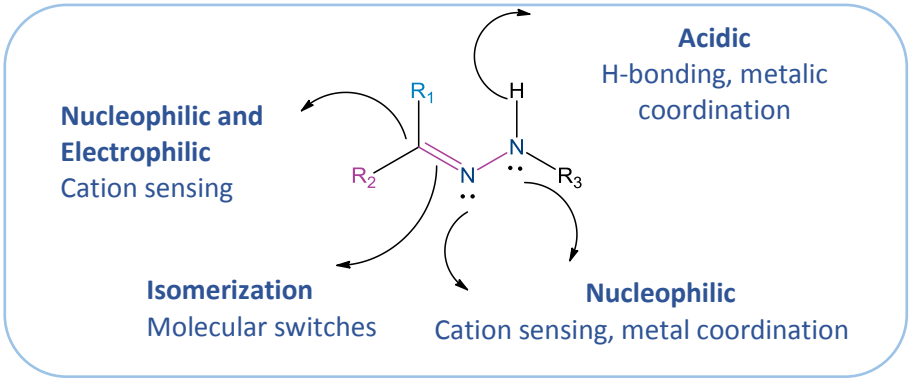


Figure 28 *The structural hydrazone group*

They allow and can participate in a large number of reactions, including radical, nucleophilic or electrophilic addition reactions. The triatomic structure C=N-N enables its use in various fields, acting as ligands for metal complexes, organocatalysis and synthesis of organic compounds. They have an important role in synthesis of heterocyclic precursors of many nitrogenous heterocycles (Fischer indole synthesis). They also act as intermediate in Wolff-Kishner,[85] as reactants in various important reactions such as hydrazone iodination, Shapiro reaction[86] and Bamford Stevans[87] reaction to form vinylic compounds. Nonetheless, hydrazones are also precursors of many functional groups highly sought after by the pharmaceutical or agrochemicals, particularly for the conversion of aldehydes to hydrazines, amines, nitriles and nitrogenous heterocyclics such as pyrazoles.[88, 89]

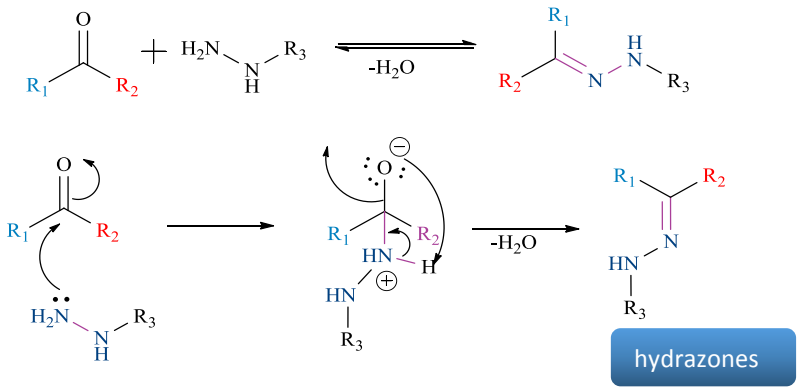
I.4.1 Chemical synthesis pathways of hydrazones

The synthesis of a hydrazone by the condensation of a hydrazine with a carbonyl derivative

[85] Szmant H.H., Harmuth C.M. The Wolff-Kishner Reaction of Hydrazones. *J. Am. Chem. Soc.* 1964, 86, 14, p. 2909-2914.
[86] Maruoka K., Oishi M., Yamamoto H. The Catalytic Shapiro Reaction. *J. Am. Chem. Soc.* 1996, 118, 9, p. 2289-2290.
[87] Farnum D. G. Preparation of Aryldiazoalkanes by the Bamford-Stevens Reaction. *J. Org. Chem.* 1963, 28, 3, p. 870-872.
[88] Brehme R., Enders D., Fernandez R., Lassaletta J. M. Aldehyde *N*,*N*-Dialkylhydrazones as Neutral Acyl Anion Equivalents: Umpolung of the Imine Reactivity. *Eur. J. Org. Chem*. 2007, p. 5629-5660.
[89] Lazny R., Nodzewska A. *N*,*N*-Dialkylhydrazones in Organic Synthesis. From Simple *N*,*N*-Dimethylhydrazones to Supported Chiral Auxiliaries. *Chem. Rev.* 2010, 110, p. 1386-1434.

One of the most frequently used method for the synthesis of hydrazones is through the condensation of corresponding hydrazine with a carbonyl derivative such as aldehyde or ketone. Easy to implement, the reaction is usually carried out at room temperature for the hydrazones of aldehydes. Nevertheless, high temperatures and the use of Brönsted acids are often required for synthesis hydrazones of ketones. This method allowed to obtain mono- or *N*, *N*-disubstituted hydrazones.

This type of condensation is usually described at neutral pH, but sometimes was reported to react slowly, this being one particular issue that limits the practical utility of these imine-forming and their relatively slow rate.[90] However, in a general manner, the decrease of pH influences the reaction rate which is increased through the activation of the carbonyl at the addition step and by accelerating the dehydration by protonation.[91]



Scheme 30 *The synthesis of a hydrazone by the condensation of a hydrazine with a carbonyl derivative*

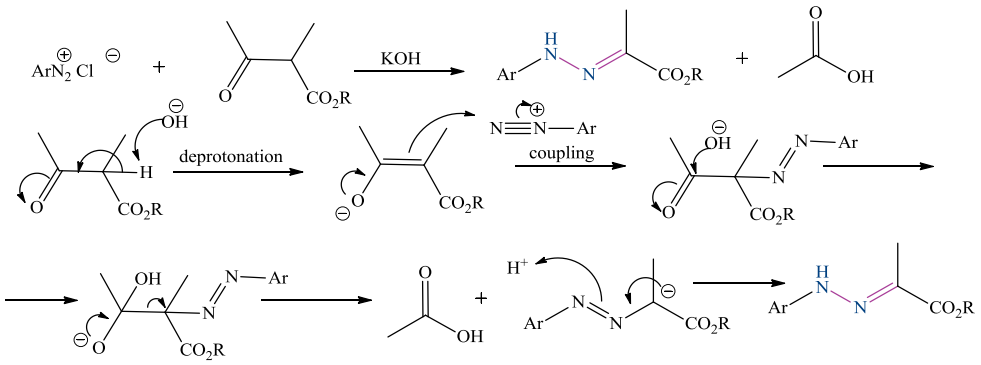
A rather large screen of reactants for the formation of hydrazones was done by Kool and coworkers. A kinetic study employing a range of hydrazines where they have measured the relative rate of formation at the physiological pH in water was reported.[92] They discovered that substrates with acid/base-active moieties always accelerated the reaction, but also some more general trends. They discovered that by weakening the nucleophilicity of the amino- reactant, either with electron-poor aromatics or electron-withdrawing substituents, the reaction slightly slowed down.

[90] Kool E.T., Park D., Crisalli P. Fast Hydrazone Reactants: Electronic and Acid/Base Effects Strongly Influence Rate at Biological pH. *J. Am. Chem. Soc.* 2013, 135, p. 17663-17666.
[91] Levrand B., Fieber W., Lehn J.M., Herrmann A. Controlled Release of Volatile Aldehydes and Ketones from Dynamic Mixtures Generated by Reversible Hydrazone Formation. *Helv. Chim. Acta*, 2007, 90, p. 2281-2314.
[92] Kool E.T., Crisalli P., Chan K.M. Fast Alpha Nucleophiles: Structures that Undergo Rapid Hydrazone/Oxime Formation at Neutral pH. *Org. Lett.* 2014, 16, p. 1454-1457.

Considering the carbonyl reactant, ketones are generally slower reactants than aldehydes. Among aldehydes, the aliphatic types are known to be the fastest. However, within the aryl-aldehydes the presence of electron-withdrawing moieties can enhance the rate of formation, but the effects are modest. The lower reactivity of aryl-aldehydes is due to the conjugation of the carbonyl with the aromatic ring, which is interrupted upon the formation of the tetrahedral intermediate.

Japp-Klingemann reaction

Hydrazones can also be synthesized by the Japp-Klingemann reaction, which is usually preferable for the preparation of ketohydrazones difficult to access by condensation, due to the dicarbonyl substrates.



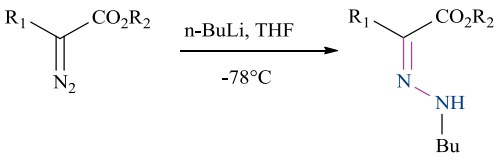
Scheme 31 *Japp–Klingemann hydrazone synthesis - from β-ketoesters and diazonium salts*

The reaction involves the use of aryldiazonium salts and compounds with methylene protons activated by electron-withdrawing groups in which at least one of such activating groups is an acyl-, carboxyl-, or ester group to form an unstable azo intermediate that rearranges into a stable hydrazone derivative by expelling of the above acyl-, carboxyl- or ester group. The reaction usually occurs in mild and almost neutral conditions. As it can be seen from the equation (**Scheme 32**), the Japp–Klingemann reaction is a special case of the coupling of diazonium salts, distinguished by the fact that the coupling product undergoes solvolysis as rapidly, or almost as it is formed.[93]

[93] Japp F.R., Klingemann F. Ueber Benzolazo- und Benzolhydrazofettsäuren. *Ber. Dtsch. Chem. Ges.*,1887, 20, p. 2942–2944. (b) F Japp F.R., Klingemann F. Zur Kenntniss der Benzolazo- und Benzolhydrazopropionsäuren. *Ber. Dtsch. Chem. Ges.* 1887, 20, p. 3284–3286.

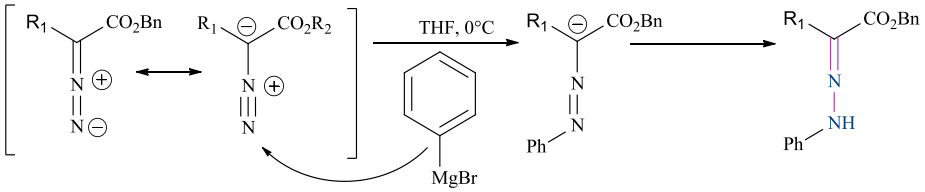
Nucleophilic addition to a diazonium salt

In 1973, Takamura's group described the reaction of an α-diazo ester and a base which allows the formation of an alkylhydrazone. An example of their work is the reaction of butyl lithium which acts as a nucleophile and adds to the diazo derivative (**Scheme 33**).[94]



Scheme 32 *Hydrazone synthesis through the addition of n-butyllithium on α-diazo ester*

In 2009, the same group reported that this type of reaction also gives good yields by employing organomagnesium derivatives, while organozincs proved to be inefficient (**Scheme 34**).[95]

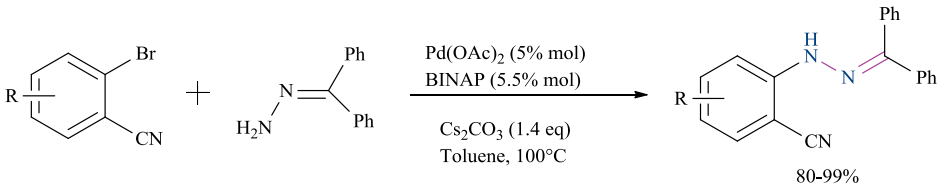


Scheme 33 *Hydrazone synthesis through the addition of a Grignard reagent on an α-diazo ester*

Aryl halide substitution

This methodology is allowing the access to diverse aryl- or heteroarylhydrazone derivatives by avoiding the use of conventional methods such as reduction of diazonium salts or SNAr reactions using hydrazine derivatives. This third pathway to obtain hydrazones involves the use of a palladium catalyst and a base. In 2010 Fabis and coworkers reported the synthesis of substituted 3-aminoindazoles from 2-bromobenzonitriles having as a first step the synthesis of arylhydrazones from benzophenone hydrazone and 2-bromobenzonitrile.[96] Within this work, they proved that the use of $Pd(OAc)_2$ (5 mol %) and BINAP (5.5 mol %) in toluene at 100°C and by using cesium carbonate as base gave 99% yields (**Scheme 35**).

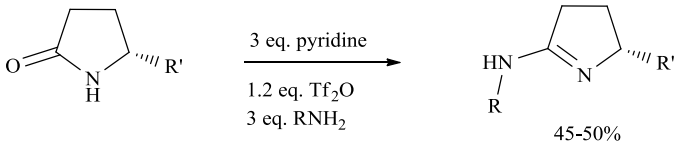
[94] Takamura N., Mizoguchi T., Yamada S. Stereoselective syntheses of trans- and cis-cinnamic acid esters from the phenylalanine derivatives. *Tetrahedron Lett.* 1973, 14, p. 4267-4270.
[95] Yasui E., Wada M., Takamura N. Novel method for synthesis of aryl hydrazones from α-diazo esters: scope and limitations of nucleophiles. *Tetrahedron*, 2009, 65, p. 461-468.
[96] Lefebvre V., Cailly T., Fabis F., Rault S. Two-Step Synthesis of Substituted 3-Aminoindazoles from 2-Bromobenzonitriles. *J. Org. Chem.* 2010, 75, p. 2730–2732.



Scheme 34 *Aryl halide substitution example*

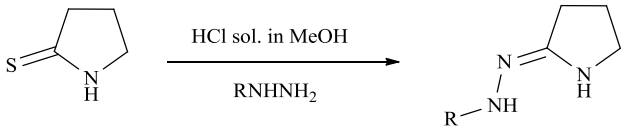
Lactam activation

In 2006, Lutz H. Gade *et al.* [97] reported the synthesis of some 2-aminopyrrolines through a highly reactive intermediate, which was obtained in a first step with trifluoromethanesulfonic (triflic) anhydride in the presence of pyridine and then treated *in situ* with the corresponding arylamine (**Scheme 36**). Though this procedure they were able to isolate four amidines in around 25% overall yield as pale yellow oils.



Scheme 35 *Trifluoromethanesulfonic (triflic) anhydride activation*

In 1982, Prochazka Michael[98] patented some amidine and hydrazidine derivatives of 1-aza-1-cyclopentene, having fungitoxic activity and give synergic effect with 7 conventional pesticides in application into soil, on plants, or seeds. The pathway they have developed was starting from the 1-azacyclopentane-2-thione in an HCl methanolic solution, which in reaction with the corresponding hydrazines, allowed them to obtain the wanted amidines (**Scheme 37**).

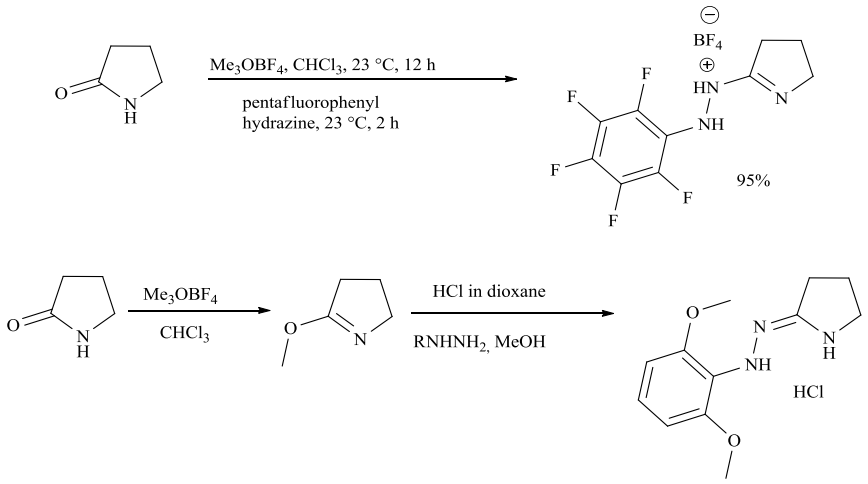


Scheme 36 *1-Azacyclopentane-2-thione reaction with corresponding hydrazines*

Another worth mentioning agent used in order to activate the amide would be Meerwein's reagent (trimethyloxonium tetrafluoroborate) (**Scheme 38**). Tomislav Rovis *et al.* reported the synthesis of hydrazinium tetrafluoroborate which they obtained from the *in situ* reaction of hydrazine and the amidate derived from Meerwein's reagent and 2-pyrrolidinone in

[97] Ward B.D., Risler H., Weitershaus K., Laponnaz S.B., Wadepohl H., Gade L.H. 2-Aminopyrrolines: New Chiral Amidinate Ligands with a Rigid Well-Defined Molecular Structure and Their Coordination to TiIV. *Inorg. Chem*. 2006, 45, 19, p. 7777–7787.
[98] Prochazka J., Prochazka M. Amidine and hydrazidine derivatives of 1-aza-1-cyclohexene. *CS 197976*, 1980.

dichloromethane. Frank Glorius on the other part, reported the use of a catalytic amount of HCl in dioxane, for the addition of hydrazine.

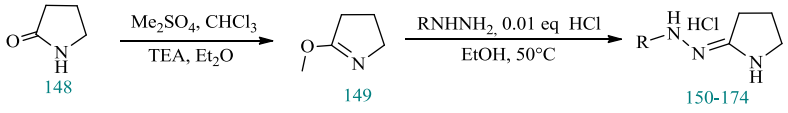


Scheme 37 *Synthesis of lactim ethers using Meerwein's reagent* [99,100]

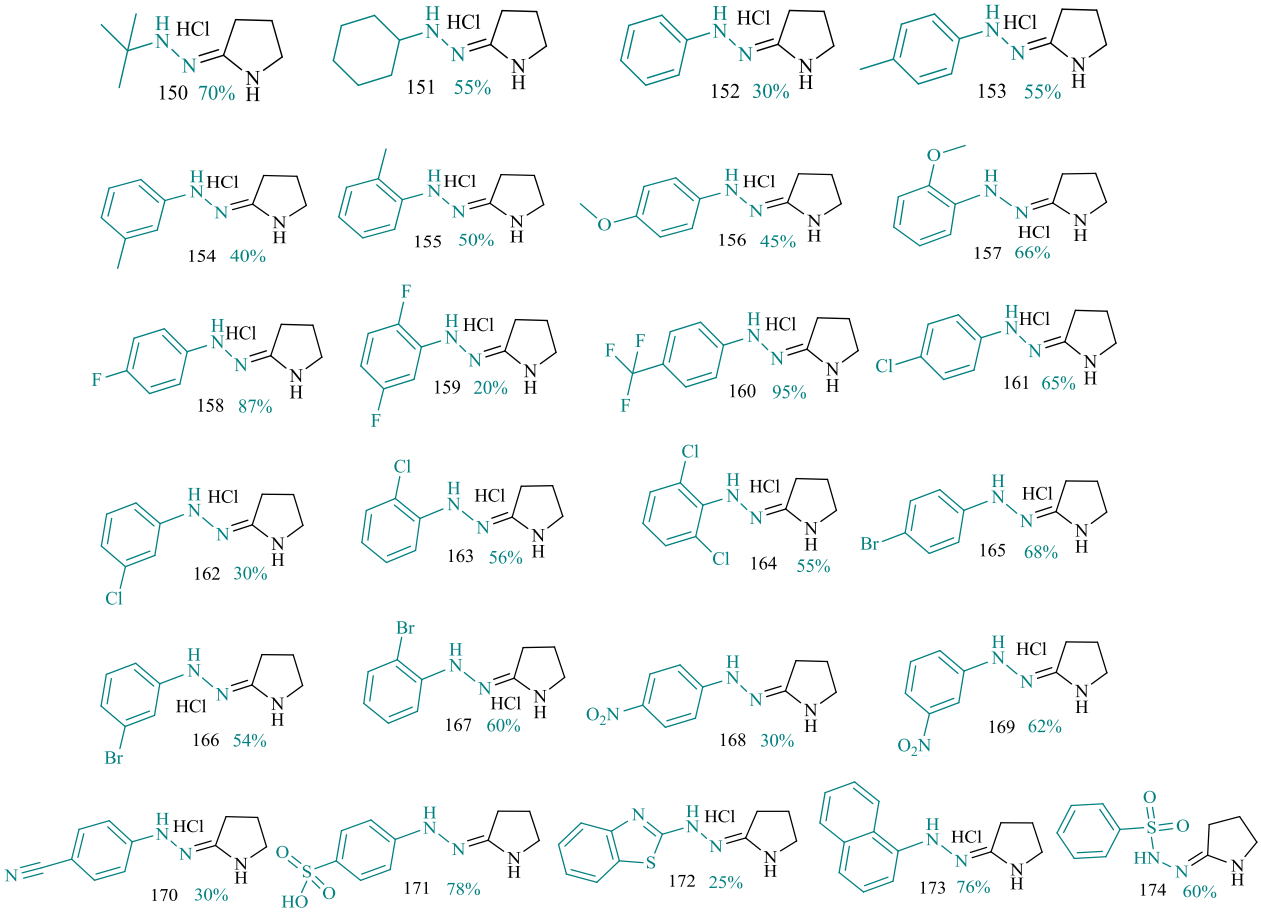
Another way to obtain the wanted amidate is by refluxing the amide in acetonitrile with dimethyl sulfate, method which provides the desired amidate in 90% yield, was reported by Frank Glorius in the same paper.[103] The latter one can be either released by employing one equivalent of trimethylamine or treated directly with the wanted amino nucleophile *in situ*.

I.4.2 Pyrrolidine hydrazones synthesis starting from proline iminoethers

Among the previously seen methods to obtain the lactim ether intermediate, the fastest way which we applied was the **148** refluxing with dimethyl sulfate. The former salt being released with one equivalent of triethylamine affording the free iminoether **149** quantitatively. Further, the iminoether was employed in a large number of hydrazines reactions, in presence of a catalytic amount of HCl (0.01 eq) leading us to the formation of 25 hydrazonopyrrolidine derivatives.



[99] Mark S.K., de Alaniz J.R., Rovis T. An Efficient Synthesis of Achiral and Chiral 1,2,4-Triazolium Salts Bench Stable Precursors for *N*-Heterocyclic Carbenes. *J. Org. Chem.* 2005, 70, 14, p. 5725–5728.
[100] Schedler M., Fröhlich R., Daniliuc C., Glorius F. 2,6-Dimethoxyphenyl-Substituted *N*-Heterocyclic Carbenes (NHCs): A Family of Highly Electron-Rich Organocatalysts. *Eur. J. Org. Chem.* 2012, 22, p. 4164-4171.



Scheme 38 *The synthesis of (2-phenylhydrazono)pyrrolidine derivatives*

The reactions were fast, and new products could be seen forming just after a few minutes, but nevertheless, the reaction were let for 3 hours.

Regarding the yielding, there are big differences depending on the hydrazine type, this synthetic route can be problematic with certain aryl hydrazines. Among the hydrazonopyrrolidine series, you can notice that there was not a big interest in designing aliphatic derivatives, our focus in the early stage of this research pivoted around aromatic members, particularly due to our knowledge regarding their biological activity. Nevertheless, compounds **150** and **151** were successfully formed with moderate to good yields, *tert*-butylhydrazonopyrrolidine **150** being isolated with 70% of yield while the cyclohexyl derivative was formed with 55% of yield.

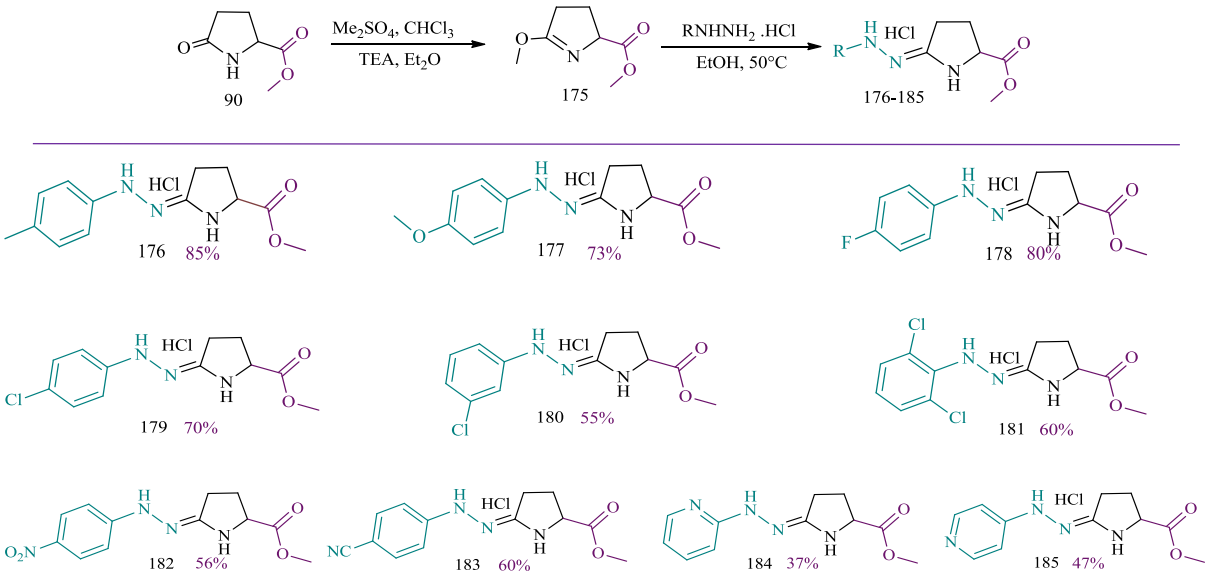
Going to aromatic derivatives, the simple phenylhydrazine derivative presented difficulties in purification, and this is how the low yield is explained, compound **152** being recovered with 30% yield. It has been observed that the aromatic ring of hydrazines bearing electron-withdrawing groups generally favoured the formation of hydrazones, in contrast to

those with electron-donating groups. Tolylhydrazines reacted moderately with the lactim ethers, furnishing compounds **153**, **154** and **155**. Interestingly, we can observe that *ortho*-methylsubstituted hydrazine gave almost the same yield as *para* or *meta* indicating that the steric hindrance has little influence on the reaction, **155** being formed with 50% of yield while (*p*-tolyl)hydrazono)pyrrolidine is formed with 55% and the *m*-tolylhydrazine furnishes **154** with 40% yield. Further, we can notice that methoxyphenyl hydrazines, performed equally moderate, providing the corresponding hydrazonopyrrolidine compounds. *Ortho*-methoxy hydrazine seems to work better than *ortho*-tolylhydrazine, furnishing **157** with 66% of yield, while *para*-methoxy has a lower yield compared to it, of 45%.

Para-fluorinated phenylhydrazine reacted smoothly with **149** giving the corresponding hydrazone **158** in very good yield, same for the 4-trifluorophenylhydrazine, which afforded **160** in 97% yield. 2,5-Difluorophenylhydrazine gave a low yield of **159,** indicating that the electronic nature of hydrazine has a certain influence on this protocol, which should be improved for this particular type of substrate. 4-Chlorophenylhydrazine on the other part, proved to be less efficient in the synthesis of **161**, compared to the previous *para*-halogenated derivatives, (4-chlorophenyl)hydrazonopyrrolidine being formed with 65% of yield. *Meta* and *ortho*-chlorophenylhydrazones were also synthesized, the less efficient reaction being that of *meta*-chloro substrate, **162** being furnished with 30% of yield while **163** yield is similar to **161**. 2,6-Dichlorophenylhydrazine also reacted quantitatively, giving **164**. Bromo-derivatives **165**, **166** and **167** were obtained as well, with yields similar to those of chlorinated products, proving the extended applicability and also facility of the protocol with different nucleophilicity hydrazines. Interestingly, 4-nitrophenylhydrazine gave the desired product **168** in a lower yield of 30%, compared to the *meta*-substrate which afforded **169** in 62% yield. Further, similar to 4-nitro- substrate, 4-cyanophenylhydrazine led to only 30% of yield for compound **170**. (2-(Pyrrolidin-2-ylidene)hydrazinyl)benzenesulfonic acid **171** was also synthesized with a surprising yield of 78%, being better than expected compared to previously seen withdrawing groups hydrazine reactions. Bulky aromatic hydrazines such as 2-hydrazinobenzothiazole or 1-naphthylhydrazine reacted in a very different manner, the first one being efficient in affording **172** while **173** was formed with a good yield of 78%. For the curiosity of the structure activity relationship, we also employed benzenesulfonylhydrazine, which furnished **174** in 60% of yield.

I.4.3 Pyrrolidine hydrazones synthesis starting from methyl pyroglutamate iminoether

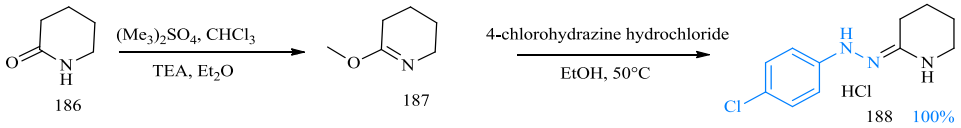
In order to have a better idea about the structure activity relationship, and also because the pyroglutamic acid ester is also a very stable and drugable synthon, we decided to design the same type of hydrazones, but starting from the methyl pyroglutamate **90**. As we observed that HCl did not influenced so much the promotion of the reaction, we decided to stop using it, and to carry the hydrazine reaction in a simple manner, just by mixing it with the iminoether in ethanol, at 50°C.



Scheme 39 *The synthesis of methyl (5-hydrazono)pyrrolidine-2-carboxylate derivatives*

In reaction with electron-donating groups hydrazines as 4-methyl- or methoxy-, **175** gave the desired product under milder reaction conditions in higher yields compared to **149**. Compounds **176** and **177** being formed with 85% respectively 73% yield. 4-Fluoro- and 4-chloro- hydrazines proved to be good nucleophiles in the reaction with the iminoether, affording **178** and **179** in 80% and 70% yields. Having a chlorine atom in the *meta* position of the phenylhydrazine, seems to deactivate more the substrate, compound **180** being formed with a lower yield, of 55%. *Ortho*-substituted (dichlorophenyl)hydrazine furnished **181** in 60% of yield, while surprisingly, the 4-nitro- and 4-cyano-hydrazine reacted better with **175**, providing **182** and **183** with doubled yields compared with simple lactim ether reaction. 2-hydrazinylpyridine and 4-hydrazinylpyridine reacted moderately with **175**, but allowed the isolation of **184** and **185**, the first one though being more difficult to recover, being non-hydrochlorated.

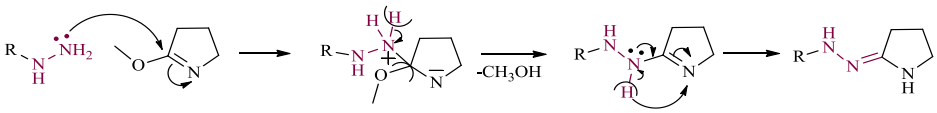
I.4.4 The synthesis of methyl (5-hydrazono)pyrrolidine-2-carboxylate derivatives



Scheme 40 *The synthesis of (2-(4-chlorophenyl)hydrazono)piperidine*

Going further with other scaffolds, as we could observe, **188** was formed with full conversion of 100%, compared to the five membered ring **161** which was formed with only 65% of yield. Presumably, the construction of systems involving six membered rings is energetically more favorable than the construction of systems involving five membered rings and occurs more rapidly, consequently resulting in the predominant formation of (2-(4-chlorophenyl)hydrazono)piperidine.

The mechanism pathway (**Scheme 42**) for the formation of the hydrazones involves an addition proceeded by an elimination of the methanol. The latter one is followed by a proton interchange allowing the formation of the hydrazone. In the case of the pyroglutamate iminoether, having and electron withdrawing group in the right side, can promote the reaction, however little difference was seen.



Scheme 41 *The proposed mechanism for the formation of the hydrazone*

To conclude, we successfully designed 36 derivatives though a simple protocol which did not involved tedious purifications, the final hydrazones being easy to purify. Moreover, we noticed that the nucleophilicity of the hydrazines did not seem to have a strong impact on the reaction promotion. Also it should be noted that the reactions of lactim ethers with hydrazines substantially depends on the lactam ring size.

I.5 Azines linker derivatives synthesis

I.5.1 Azines generalities

Azines are a class of interesting compounds undergoing a wide variety of chemical processes. Due to their two imine bonds that form the azine moiety, azine represent an important class of nitrogen donor ligands in organometallic complexes with pharmacological and

biological activity.[101] Their binding and modulating capacity make them suitable candidates for drug development.

Azines were studied for a large range of biological activities, among which there are antitumoral,[102,103] antibacterial,[104,105] or antihypertensive[106] properties. Besides this, mixed azines were synthesized from opioid antagonists showing various biological effects, including ultralong opioid antagonist activity.[107]

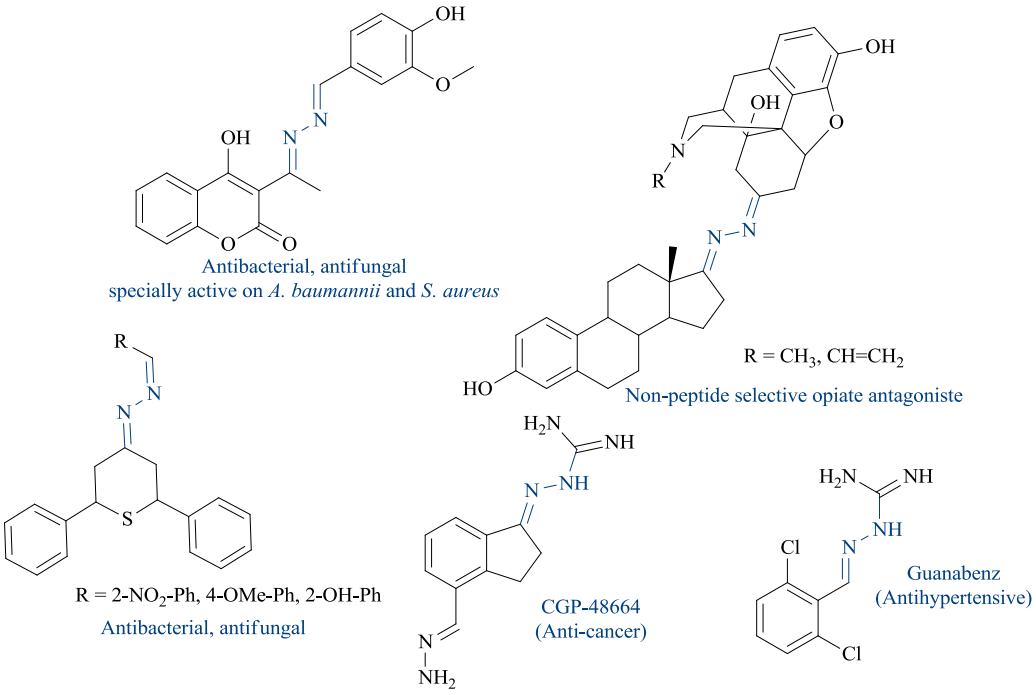


Figure 29 *Biologically active azines*

Moreover, mixed azines from estrone and naloxone, were discovered to be non-peptide selective opiate antagonists.[108] Furthermore, diazines (*N*–*N*-linked diimines) have recently

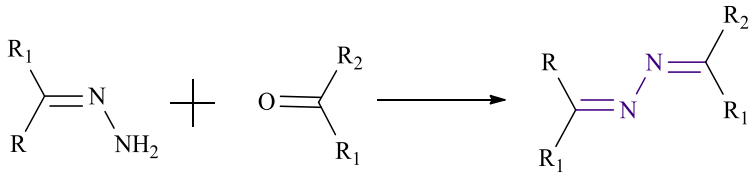
[101] Khodair A.I., Bertrand P. A new approach to the synthesis of substituted 4-imidazolidinones as potential antiviral and antitumor agents. *Tetrahedron*, 1998, 54, 19, p. 4859–4872.
[102] Murdock K.C, Child R.G., Lin Y.I., Warren J.D., Fabio P.F., Lee V.J., Izzo P.T., Lang S.A., Angier R.B., Citarella R.V., Wallace R.E., Durr F.E. Antitumor agents. II. Bis(guanylhydrazones) of anthracene-9,10-dicarboxaldehydes. *J. Med. Chem.* 1982, 25, 5, p. 505–518.
[103] Regenass U., Mett H., Stanek J., Mueller M., Kramer D., Porter C.W. CGP 48664, a new S-adenosylmethionine decarboxylase inhibitor with broad spectrum antiproliferative and antitumor activity. *Cancer Res.* 1994, 54, p. 3210–3217.
[104] Jayabharathi J., Thanikachalam V., Thangamani A. Synthesis and microbial evaluation of novel *N*(1)-Arilidene-*N*(2)-t(3)-methyl-r(2),c(6)-diaryl-piperidin-4-one azine derivatives. *Med. Chem. Res.* 2007, 16, 6, p. 266–279.
[105] Ristic M.N., Radulovic N.S., Dekic B.R., Dekić V.S., Ristić N.R., Stojanović-Radić Z. Synthesis and Spectral Characterization of Asymmetric Azines Containing a Coumarin Moiety: The Discovery of New Antimicrobial and Antioxidant Agents. *Chem. Biodiv.* 2019, 16, 1, e1800486.
[106] Diamant S., Agranat I., Goldblum A., Cohen S., Atlas D. β-adrenergic activity and conformation of the antihypertensive specific α2-agonist drug, guanabenz. *Biochem. Pharmacol.* 1985, 34, p. 491-498.
[107] Godara M., Maheshwari R., Varshney S., Varshney A.K. Synthesis and characterizaton of some new coordination compounds of boron with mixed azines. *J. Serb. Chem. Soc.* 2007, 72, 4, p. 367–374.
[108] Kolb V.M., Hua D.H., Duax W. L. Stereochemistry of long-lasting opiates. Selective opiate antagonists and their agonist analogs. *J. Org. Chem.* 1987, 52, 14, p. 3003–3010.

attracted attention because of their pharmacological properties as antidepressant, anticonvulsant, anti-inflammatory or antiviral.[109]

I.5.2 Synthesis pathways to obtain azinic coumpounds

There are different approaches for the synthesis of symmetrical and unsymmetrical azines which are described in the literature. Symmetrical azines are readily synthesized, directly or indirectly, by the reaction of hydrazine with excess of aldehyde or ketone, while the preparation of their unsymmetrical counterparts is more challenging. Some methods for the synthesis of mixed azines are here presented:

Reaction of hydrazines with various carbonyl coumpounds



Scheme 42 *General formation of aldazines of ketazines*

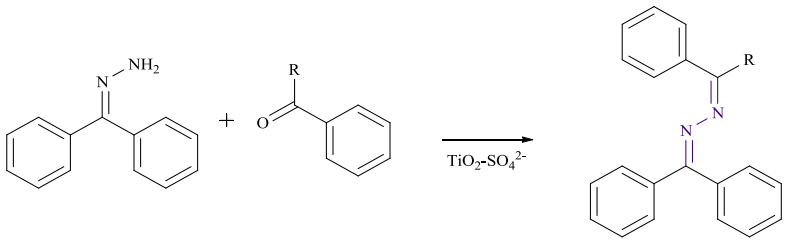
One of the first and simplest azides, the formaldehyde azine, was prepared in 1959 by Neureiter.[110] It is known that the rate of reaction of hydrazine with various carbonyl compounds decrease in the following order: aldehyde > dialkyl ketone > alkaryl ketone > diaryl ketone. Things considered, the aldazines form more quickly than do ketazines, and sometimes ketazines require the presence of excess ketone together with acetic or formic acid as catalyst (**Scheme 43**).[111]

Synthesis from benzophenone hydrazone and ketones or aldehydes[112]

A good catalyst described in 2011 by Swaminathan and coworkers, for the reaction of benzophenone hydrazine and aldehydes or ketones, is the solid acid catalyst, sulfated anatase

[109] Easmon J., Purstinger G., Heinisch G., Roth T., Fiebig H.H., Holzer W., Jäger W., Jenny M., Hofmann J. Synthesis, Cytotoxicity, and Antitumor Activity of Copper(II) and Iron(II) Complexes of 4*N*-Azabicyclo[3.2.2]nonane Thiosemicarbazones Derived from Acyl Diazines. *J. Med. Chem.* 2001, 44, 13, p. 2164–2171.
[110] Neureiter T. P. Monomeric formaldazine-synthesis of 1,3,4-thiadiazolidine-a new heterocycle. *J. Am. Chem. Soc.* 1959, 81, 11, p. 2910.
[111] Barluenga J., Fustero S., Gomez N., Gotor V. A New Method for the Synthesis of Unsymmetric Azines: Alkylidene Group Exchange between Azines and lmines. *Synthesis,* 1982, 11, p. 966–967.
[112] Krishnakumar B., Swaminathan M. An expeditious and solvent free synthesis of azine derivatives using sulfated anatase–titania as a novel solid acid catalyst. *Catal. Commun.* 2011, 16, 1, p. 50–55.

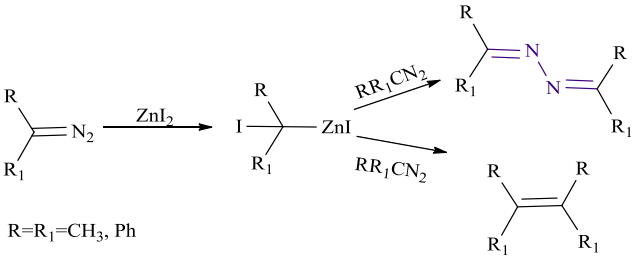
titania (TiO_2–SO_4^{2-}). Azine derivatives were synthesized at room temperature by simple physical grinding in the presence of sulfated titania, at room temperature.



Scheme 43 *Azine formation, in presence of TiO_2–SO_4^{2-}*

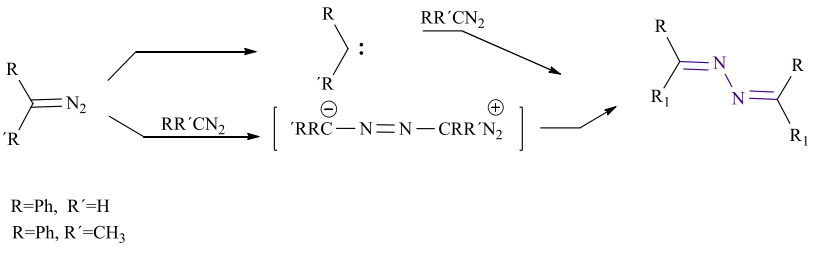
Synthesis from iodoalkylzinc iodide[113]

Further, another method to obtain symmetrical azines was developed in 1962 by Applequist and Babad, who used iodoalkylzinc iodide. The zinc iodide intermediate was obtained from the reaction of diphenyldiazomethane, 2-diazopropane with ZnI_2. The former iodoalkylzinc iodide, reacts fastly with the diazo derivative, affording the wanted azine, but also the alkene as a side product, making this method less appealing.



Scheme 44 *Azine synthesis from iodoalkylzinc iodide*

Reaction of phenyldiazomethane with 1-diazo-1-phenylethane[114]

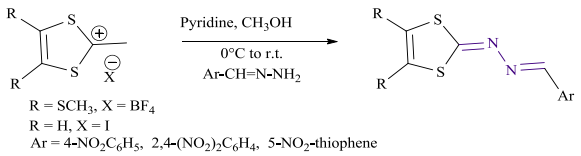


Scheme 45 *Azines synthesis by reaction of phenyldiazomethane*

[113] Applequist D.E., Babad H. Reactions of Diphenyldiazomethane and 2-Diazopropane with Zinc Iodide. *J. Org. Chem.* 1962, 27, 1, p. 288–290.
[114] Abelt C.J., Pleier J.M. Stereoselective azine formation in the decomposition of phenyldiazomethanes. *J. Am. Chem. Soc.* 1989, 111, 55, p. 1795–1799.

During their bimolecular dimerization study of phenyl diazomethane and 1-diazo-1-phenylethane, in 1989, Abelt and Pleier used them to synthesize azines. Azines often come as major products of thermal decomposition of diazo compounds, resulting from the reaction of a carbene with a diazo compound and from the bimolecular reaction of two diazo compounds. The mechanism of azine formation involves nucleophilic attack of the carbon atom of the first diazo compound on the terminal nitrogen of the second compound. The former intermediate is then losing N_2, forming the final azine.

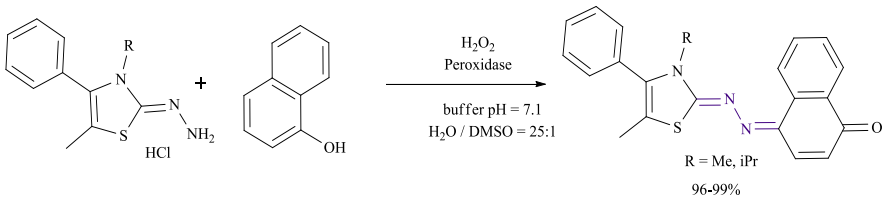
Synthesis by reaction of aromatic aldehydes with dithiolium salts[115]



Scheme 46 *Azines synthesis from dithiolium salts*

Another pathway described in 2001 by Moreno-Manas *et al.* is the synthesis and characterization of the first push–pull 1,3-dithiol-2-ylidene derivatives which had an azine linker. Such azines were formed from the reaction of nitro-substituted aromatic aldehydes with 2-methylthio-1,3-dithiolium salts. The authors investigated their electrochemical and second order nonlinear optical (NLO) properties by electric field-induced second harmonic (EFISH) measurements.

Synthesis by oxidative coupling catalyzed by horseradish peroxidase[116]



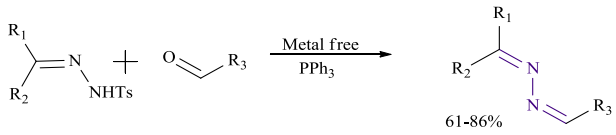
Scheme 47 *Oxidative coupling catalyzed by horseradish peroxidase*

In 2005, Pfeiffer and coworkers reported the synthesis of azine pigments by HRP-catalyzed oxidative coupling of a hydrazono-dihydrothiazole derivative and α-naphthol in the

[115] Moreno-Manas M., Pleixats R., Andreu R., Garín J., Orduna J., Villacampa B., Levillaind E., Salléd M. The first 1,3-dithiol-2-ylidene donor–π–acceptor chromophores containing an azine spacer: synthesis, electrochemical and nonlinear optical properties. *J. Mater. Chem.* 2001, 11, p. 374–380.
[116] Bodtke A., Pfeiffer W.D., Ahrens N., Langer P. Horseradish peroxidase (HRP) catalyzed oxidative coupling reactions using aqueous hydrogen peroxide: an environmentally benign procedure for the synthesis of azine pigments. *Tetrahedron*, 2005, 61, 46, p. 10926–10929.

presence of hydrogen peroxide. These mild, benign conditions of transformations allowed the formation of *p*-naphthoquinone-thiazol-2-one-azines.

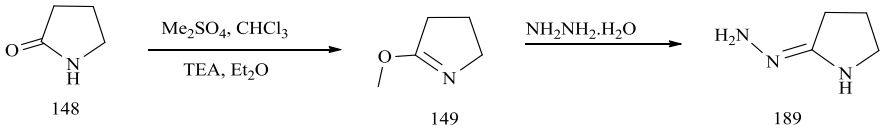
Azines synthesis starting from *N*-tosylhydrazones



Scheme 48 *Azines synthesis from N-tosylhydrazones*

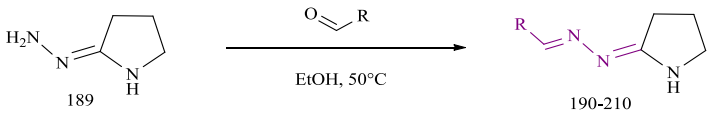
In 2013, Wei's team developed a one pot method to synthesize azines, under free catalyst conditions. Within this pathway the triphenylphosphine captures the diazo compounds which are generated *in situ*, affording the corresponding azines in good yields.

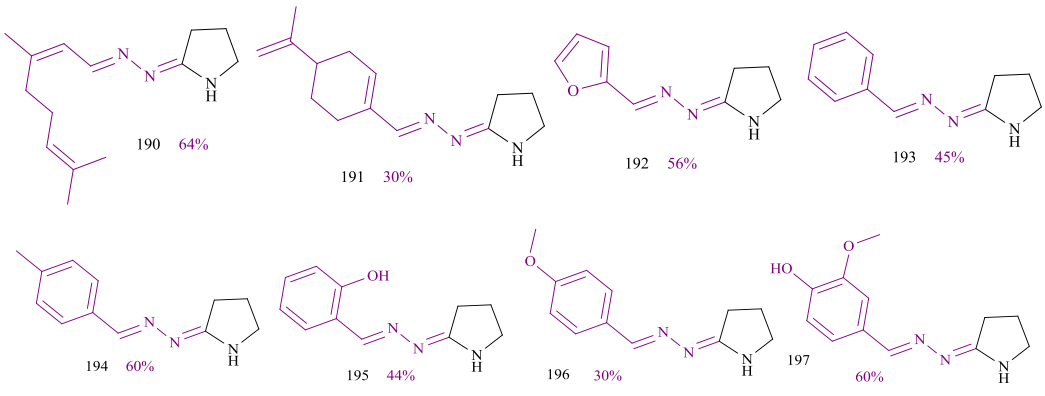
I.5.3 Synthesis of pyrrolidino-azines starting from the 2-hydrazonopyrrolidine

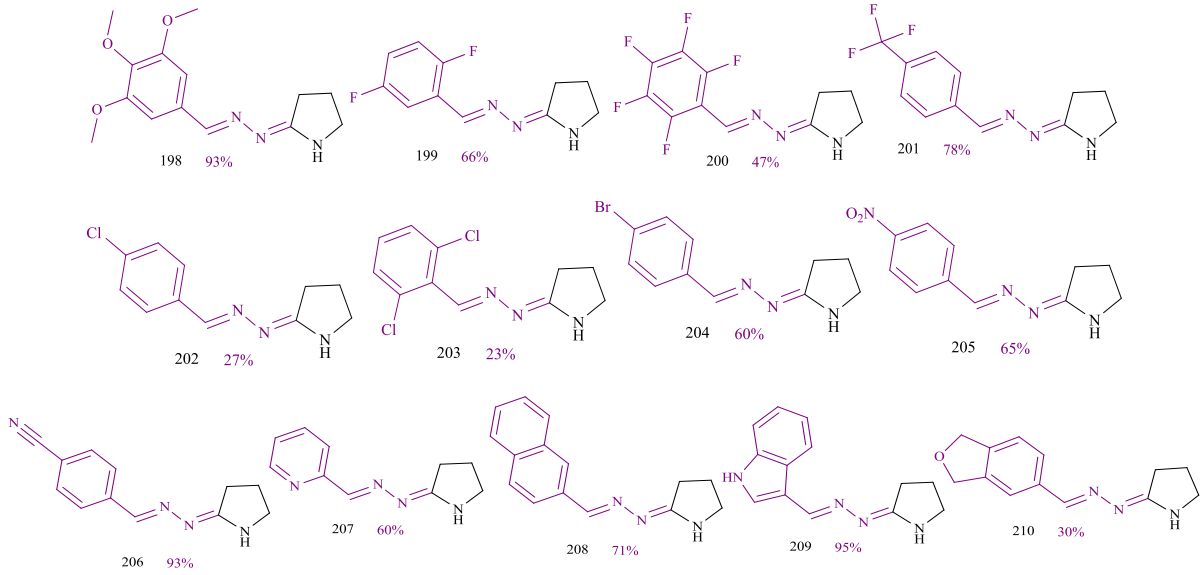


Scheme 49 *The synthesis of 2-hydrazonopyrrolidine*

The same iminoether which was previously used to form hydrazonopyrrolidine derivatives, was employed in the reaction with hydrazine hydrate, to afford derivative **189** in quantitative yield. Without any purification, **189** was used directly in reactions with different aldehydes, giving access to a wide variety of azine linker derivatives.







Scheme 50 *The synthesis of pyrrolidino-azines* ***190-210***

The reaction occurs at 50°C in air without any catalyst, in ethanol, being a benign pathway. This approach tolerates a wide range of aldehydes, and we were able to isolate azines **190-210** in yields between 23 and 95%. Alkenes derivatives such as citral, a natural aldehyde found in citric juice has reacted well with **189**, providing **190** in 60% yield. Another natural aldehyde which was submitted to the simple protocol is perillaldehyde, which delivered **191**, unfortunately in lower yields. Heteroaromatic aldehydes such as furaldehyde reacted as well with **189**, furnishing **192** in a moderate yield of 56%.

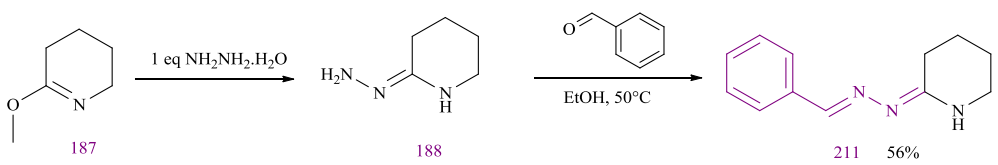
Further, under these conditions, moderate to good yields were obtained with a good substituent group tolerance for aromatic aldehydes. Benzaldehyde has reacted moderately with the hydrazine, affording **193** in 45% yield. Electron-rich groups generally accelerated the reaction rate but the yields have not particularly increased. 4-Methylbenzaldehyde reacted a little better than simple benzaldehyde, but no important benefit of this substituent could be seen, **194** being formed with 60% of yield. The reaction with salicylaldehyde was sensitive to steric hindrance of the hydroxyl- group on the aryl aldehyde, compound **195** was obtained with 44% yield. Furthermore, compared to the methyl group from the *para*- position, the methoxy- group on the aromatic aldehyde in the same position, seems to lower the yield for **196**. However, having two electron-donating groups on the ring, as for vanillin, another natural aldehyde, the reaction went better, allowing the formation of **197** with 60% of yield. The last electron-rich aldehyde which was employed for this series is the 3,4,5-trimethoxybenzaldehyde that reacted smoothly with **189**, furnishing **198** with 93% of yield.

The effect of electron-withdrawing substituents was also surveyed and various halogeno-aldehydes were employed. Fluorinated benzaldehydes have reacted moderately with **189**. 2,5-Difluorobenzaldehyde as an example, reacted generally better compared with certain electron-donating benzaldehydes which were supposed to have a better reactivity, compound **199** being obtained with 66% of yield. Further, three additional fluorine atoms on the aromatic group of the benzaldehyde substrate, led to slightly lowering of the yield, **200** being obtained in 47% yield. Surprisingly, 4-(trifluoromethyl)benzaldehyde reacted well with **189**, compared to other fluorinated substrates, affording **201** in 78% of yield. Chlorinated aldehydes, on the other part, showed significantly reduced activity under the same reaction conditions, and probably a catalyst should be added, 4-chlorobenzaldehyde and 2,6-dichlorobenzaldehyde reacting slow with **189**, furnishing derivatives **202** respectively **203** with 27 and 23% of yield. The only brominated benzaldehyde which was submitted to these conditions was the 4-bromobenzaldehyde which reacted similar to fluorinated benzaldehydes, affording derivative **204** in 60% yield.

Going further, some other electron-deficient benzaldehydes were used. 4-Nitrobenzaldehyde for instance, reacted well with **189**, however the purification was difficult, azine derivative **205** being obtained with 65% of yield. 4-Cyanobenzaldehyde was also successfully submitted to the protocol, reacting smoothly with **189**, furnishing **206** in 93% yield.

Other aldehydes reactants such as 2-pyridinecarboxaldehyde, reacted better compared to benzaldehyde, and azinic compound **207** was obtained with 60% of yield. Changing the aromaticity of the aldehyde, we can observe that the yield is increasing up to 71% for naphthyl derivative **208**. In the perspective of a structure activity relationship study, indole-3-carboxaldehyde and piperonal were also submitted to the reaction with **189**, the first one leading smoothly to the formation of **209**, which was isolated with 95% of yield, while the second aldehyde led to the formation of **210** which was isolated with a modest yield of 30%.

I.5.4 Synthesis of benzylidenehydrazono piperidine starting from the 2-hydrazono piperidine



Scheme 51 *Condensation reaction of 2-hydrazonopiperidine with benzaldehyde*

In the frame of a structure activity relationship study, we also employed the six-member ring hydrazone **188**, which allowed us to obtain derivative **211**, in a moderate yield, which however was slightly superior compared to the five-member ring hydrazone. As seen before, the construction of systems involving six membered rings might be energetically more favorable than the construction of systems involving five membered rings and that may explain the difference.

In summary, we have synthesized twenty-one representative examples of potentially biologically interesting pyrrolidino-azines and one piperidine representative, containing pyrrole respectively a piperidine moiety, and an azine linker. The novel skeleton was constructed by a condensation between the hydrazone and different aldehydes, being the first time to introduce the azine link next to a pyrrole or a piperidine core.

I.6 Conclusions

To summarize, within this chapter, all the chemical pathways which allowed us to obtain a large range of biological molecules were presented and discussed. In the frame of the project we were able to synthesize mostly novel molecules, such as γ-lactam derivatives, pyroglutamic acid carbazides, pryoglutamic acid hydrazones, imino-hydrazones and novel azines as well. According to each substrate reactivity, reaction type as well as the easiness of purifying the final product, the yields ranged from low to excellent.

As depicted in **Figure 30**, through 5 chemical pathways, 5 different families were obtained, with a total of around 200 molecules which have been tested for different biological

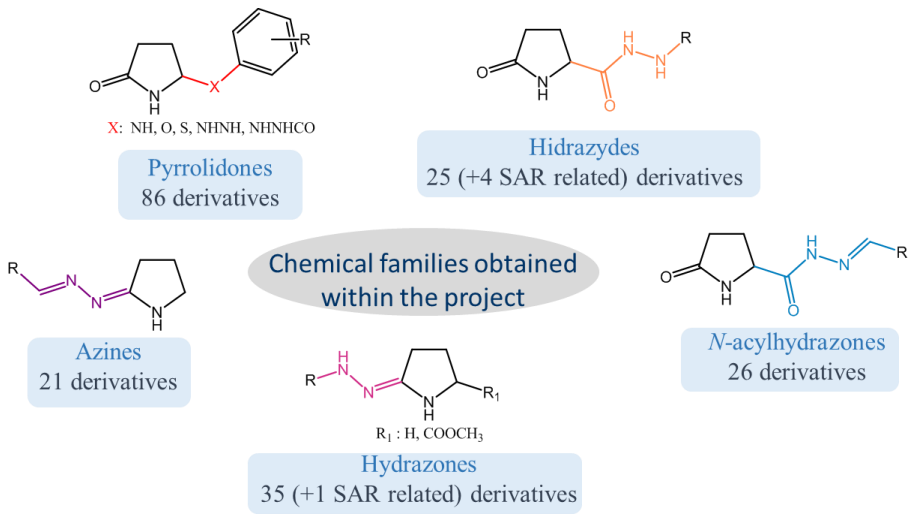


Figure 30 *Chemical families obtained within the project*

activites which will be discussed in the next chapter.

In what perspectives are concerning, in the context of a possible scale-up, the efficiency of certain chosen chemical pathways would have to be improved and other agents should be tested, if the corresponding pathways are not efficient enough. All the experimental data and chemico-physical description of the synthesized molecules are included in the experimental section.

CHAPTER II

BIOLOGICAL EVALUATION

OF THE SYNTHESIZED MOLECULES

WITHIN THE PROJECT

II.1. ESKAPE and WHO alert

In 2008, the ESKAPE pathogens were highlighted for the first time, as critical bacteria capable of "escaping" the action of the antibiotics, representing a new paradigm in pathogenesis, transmission and resistance (**Figure 31**).[117]

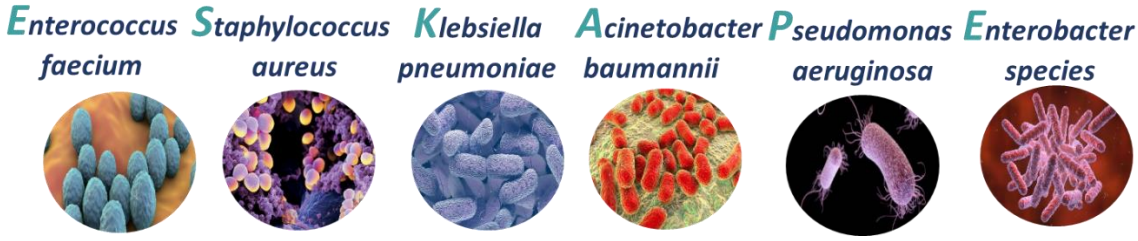


***Figure 31** ESKAPE pathogens*

In February 2017, the World Health Organization (WHO) published the first ever list of antibiotic-resistant "priority pathogens" which are posing a great risk for human health.[118] The list which is divided into three categories according to the urgency of need for new antibiotics: critical, high and medium priority, comprises 12 microorganisms which are resistant to a large number of antibiotics, including carbapenems and 3rd generation cephalosporins, known to be the best antibiotics for treating multi-drug resistant bacteria.

Priority 1: CRITICAL

- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing

Priority 2: HIGH

- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant

[117] Rice L.B. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J. Infect. Dis.* 2008, 197, 8, p. 1079–1081.
[118] WHO publishes list of bacteria for which new antibiotics are urgently needed. 2017, www.who.int

- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant

Among the many purposes of this list, one of them was to find a new tool that could ensure R&D in order to respond to this urgent public need of new antibiotics and to direct attention towards the most tenacious and resistant microorganisms and novel antimicrobial agents. The following chapter is going to focus on the top of the list bacteria, *Acinetobacter baumannii*, which was classified by WHO as the most critical microorganism for which we need new antibiotics.

Acinetobacter species

Back from the middle ages, some philosophers suspected "invisible organisms" to be causing the spread of certain diseases such as plague or tuberculosis. It was not until 1675, that Leeuwenhoek observed for the first time microorganisms, then called "animalcules". While some exhibited very distinct characteristics, others seem to have a similar appearance. However, the first classification of species microbials, mainly based on morphological criteria, will not be implemented until 1774 by Müller.[119] He has divided the microorganisms he observed in two groups: *Monas* and *Vibrio*. This was the first time that the notion of "gender" was introduced in the microbial language. Later, other criteria have been used to establish an order in the bacterial kingdom. We learned to describe a microorganism according to its appearance, its smell and its behavior towards certain natural compounds, in order to give it a panel of features that will define it. The best known criterium which remains today is the distinction between gram positive (one membrane) and negative (2 membranes) bacteria that was established by Hans C. J. Gram in 1884. Even if this method remains imperfect, it marked the arrival of a new type of test for microbial classification.

[119] Müller O.F. Vermium terrestrium et fluviatilium seu animalim infusoriorum, helminthicorum, et testaceorum, non marinorum, succincta historia, 1774.

This is the context in which the history of *Acinetobacter baumannii* began. In 1911 Beijerinck, a german microbiologist described for the first time a micro-organism extracted from the sol, having an oval form and which can grow in a calcium and acetate medium. He gave it the name of *Micrococcus calcoaceticus* (Beijerinck, 1911).[120] It was not until 1954 that this little bacterium received the name of *Acinetobacter* (Brisou and Prevot, 1954).[121] At that time, the *Acinetobacter* genus (from Greek "akinetos"- non-mobile) regrouped heterogenous species which presented a double phospholipidic membrane, possessing or not one oxidase having the particularity of not moving and being non-pigmentated (Bergogne-Bérézin and Towner, 1996).[122]

The species classification was mostly done by morphological and metabolical criteria. At that time, the *Acinetobacter* bacteria had multiple characteristics in common with *Nesseria*, *Branhamella* and *Moraxella*. In this context, the bacteria nomenclature committee, proposed the temporarily integration of this genus of *Acinetobacter*, within the family of *Nesseriaceae* (Catlin, 1970).[123]

It was not until 1986, when new DNA based technologies came to resolve the taxonomy problems. In that moment, the "false *Nesseria"* joined *Moraxellaceae* family which regroups the bacteria genus as *Moraxella* and *Psychrobacter* (Rossau et al., 1991) which are a branching family of proteobacteria.[124] Things considered, it took almost a century to establish the taxonomy of *Acinetobacter*.

Taxons	Nomenclature
Domain	Bacteria
Kingdom	Eubacteria
Phylum	Proteobacteria
Class	Gamma-proteobacteria
Order	Pseudomonadales
Family	Moraxellaceae

[120] Beijerinck A. Pigmenten als oxydatieproducten gevormd door bacterien. 1911, Amsterdam.
[121] Brisou, J., Prevot A.R. Studies on bacterial taxonomy. X. The revision of species under Acromobacter group. *Ann. Inst. Pasteur*, 1954, *86*, p. 722–728.
[122] Bergogne-Bérézin E., Towner K.J. Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin. Microbiol. Rev.* 1996, 9, p. 148–165.
[123] Catlin B.W. Report (1966-1970) of the Subcommittee on the Taxonomy of the Neisseriaceae to the International Committee on Nomenclature of Bacteria. 1970, Mexico.
[124] Rossau R., Van Landschoot A., Gillis M., de Ley J. Taxonomy of Moraxellaceae fam. nov., a New Bacterial Family To Accommodate the Genera Moraxella, Acinetobacter, and Psychrobacter and Related Organisms. *Int. J. Syst. Bacteriol.* 1991, 41, p. 310–319.

Genus	Acinetobacter

Table 11 *Taxonomic classification of Acinetobacter*

Acinetobacter spp. habitats are multiple and because of that, this bacterial genus is said to be "ubiquitous". *Acinetobacter* colonizes the environment (water, soil, plants) as well as food, animals or humans.[125] Today, the hypothesis that humans could be a natural reservoir of *Acinetobacter*-like bacteria remains possible. Some species such as *A. bereziniae, A. calcoaceticus, A. haemolyticus, A. nosocomialis* or *A. baumannii* specifically colonize animals and humans when they are not part of their natural flora.[126] In contexts such as these, the species represent a human pathogenic flora and in this sense, they can be responsible for infections and diseases. Among them, the most frequently isolated species in hospitals is *A. baumannii*.

II.1.1 History of a species causing a global epidemic

A. baumannii is recognized as an opportunistic pathogen responsible for infections related to care since the 1970s. Years later, Europe suffers from the first outbreaks of nosocomial infections associated with *A. baumannii*.[127] At this time, *A. baumannii* accounted for 2-10% of Gram-negative bacterial infections.[128] The multiplication of epidemic peaks and the prolongation of endemic phases led to the genotypic analysis of the strains involved in these infectious periods. In 2009, *A. baumannii* infections have reached 20% of the counted cases in intensive care units. Mortality rates that are associated with these infections are constantly increasing,[129] in part because the proposed treatments are less and less effective and fewer and fewer. The multiplicity of resistance systems developed by *A. baumannii* places this bacterium ranking among the most problematic pathogens in the world. As such, it joined the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas*

[125] Al Atrouni A., Joly-Guillou M.L., Hamze M., Kempf M. Reservoirs of Non-baumannii *Acinetobacter* Species. *Front. Microbiol.* 2016, 49.
[126] McConnell M.J., Pérez-Romero P., Lepe J.A., Pérez-Ordóñez A., Valencia R., Vázquez-Barba I., Pachón J. Positive predictive value of Leeds acinetobacter medium for environmental surveillance of Acinetobacter baumannii. *J. Clin. Microbiol.* 2011, 49, p. 4416.
[127] Peleg A.Y., Seifert H., Paterson D.L. Acinetobacter baumannii: emergence of a successful pathogen. *Clin. Microbiol. Rev.* 2008, 21, p. 538–582.
[128] Hanberger H., Garcia-Rodriguez J.A., Gobernado M., Goossens H., Nilsson L.E., Struelens M.J. Antibiotic Susceptibility Among Aerobic Gram-negative Bacilli in Intensive Care Units in 5 European Countries. *JAMA*, 1999, 281, p. 67–71.
[129] McConnell M.J., Actis L., Pachón J. Acinetobacter baumannii: human infections, factors contributing to pathogenesis and animal models. *FEMS Microbiol. Rev.* 2013, *37*, p. 130–155.

aeruginosa and Enterobacter sp.) proposed by Rice in 2008,[130] which defines the 6 agents responsible for a large majority of cases of nosocomial infections in the US, able to "escape" antibacterial treatments.[131]

II.1.2 Infections and pathogenesis

According to the World Health Organization (WHO) recent report, *Acinetobacter baumannii* occupies the first place on the list of the most critical pathogens, for which we are in great need of new antibiotics. *A. baumannii* is known to cause nosocomial infections and mostly experienced by the patients in intensive care units. There are different infections caused by it such as: meningitis, pneumonia, urinary tract infections, endocarditis, bacteremia, and skin infections. The mortality rate among *A. baumannii* infected patients is about 75%, because of restricted treatment options due to high resistance of this pathogen towards various classes of antibiotics.[132]

Today, the main rising incidence of multidrug-resistant *A. baumannii* infections is in health-care settings, due to its extraordinary capacity to colonize and spread.[133] *Acinetobacter* spp are known to survive on dry inanimate surfaces for a long time, studies reporting their survival from 1 month to 5 months.[134] Moreover, *A. baumannii* can easily colonize patients or equipment used in medical care. Some strains can develop biofilms in contact with plastic or glass surfaces,[135] attach to human epithelial cells through lipopolysaccharide side chains or fimbriae and also some specific strains can bind to salivary mucins.[136]

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[130] Rice L.B. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J. Infect. Dis.* 2008, *197*, p. 1079–1081.

[131] Boucher H.W., Talbot G.H., Bradley J.S., Edwards J.E., Gilbert D., Rice L.B., Scheld M., Spellberg B., Bartlett J. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* 2009, 48, p. 1–12.

[132] Dijkshoorn L., Nemec A., Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat. Rev. Microbiol. 2007, 5, p. 939-951.

[133] Van den Broek P.J., Arends J., Bernards A.T., De Brauwer E., Mascini E.M., van der Reijden T.J., Spanjaard L., Thewessen E.A., van der Zee A., van Zeijl J.H., Dijkshoorn L. Epidemiology of multiple acinetobacter outbreaks in the Netherlands during the period 1999–2001. *Clin. Microbiol. Infect.* 2006, 12, p. 837–843.

[134] Kramer A., Schwebke I., Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect. Dis.* 2006, 6, 130.

[135] Lee H.W., Koh Y.M., Kim J., Lee J.C., Lee Y.C., Seol S.Y., Cho D.T., Kim J. Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. *Clin. Microbiol. Infect.* 2008, 1, p. 49-54.

[136] Koeleman J.G., Van der Bijl M.W., Stoof J., Vandenbroucke-Grauls C.M., Savelkoul P.H. Antibiotic resistance is a major risk factor for epidemic behavior of Acinetobacter baumannii. *Infect. Control Hosp. Epidemiol*. 2001, 22, p. 284–288.

The colonization itself can be happening through the simple mechanical ventilators [137] or simply by linen articles as pillows, bed linen, curtains and everything surrounding a patient.[138] Moreover, once an object is touched by hands might also become colonized with *A. baumannii*, for example keyboards, door handles, sinks and cleaning equipments. Besides this, various medical equipment were found to be contaminated with multidrugresistant *A. baumannii* during outbreaks. Wound treatment and surgical interventions being also related to infections of *A. baumannii*, presumably because of the use of already infected materials or equipments.

II.1.3 *Acinetobacter baumannii* treatment

The list of potential treatment remains limited, because the resistance rates of *A. baumannii* to many antibacterial agents can be very high. The current trend of *A. baumannii* infections treatment are polymyxins, as colistin (polymyxin E) and polymyxin B. However, there is some hesitancy to their use. In fact, in the '80s, polymyxins have been abandoned because of their toxicity. As a consequence many clinicians are preferring antibacterial treatment based on other agents including sulbactam and tigecycline, in monotherapy or synergism with other antibiotics for carbapenem resistant isolates.

Some antibacterial classes which are potentially active against *A. baumannii* are:

- **Sulbactam**
- **Polymyxins**
- **Anti-pseudomonal carbapenems**
- **Cephalosporins**
- **Tetracyclines**
- **Aminoglycosides**
- **Rifamycins**
- **Fluoroquinolones**

[137] Dealler S. Nosocomial outbreak of multi-resistant Acinetobacter sp on an intensive care unit: possible association with ventilation equipment. *J. Hosp. Infect.* 1998, 38, p. 147–148.
[138] Wilks M., Wilson A., Warwick S., Price E., Kennedy D., Ely A., Millar M.R. Control of an outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus colonization and infection in an intensive care unit (ICU) without closing the ICU or placing patients in isolation. *Infect. Control Hosp. Epidemiol.* 2006, 7, p. 654-658.

1. Sulbactam

Sulbactam is among the most active of the β-lactamase inhibitors and remains a solution to treat *A. baumannii* infection. In synergism with ampicillin, cefoperazone, or antipseudomonal penicillins it has enhanced antibacterial activity against *A. baumannii*. The mode of action of sulbactam is mediated *via* binding to penicillin-binding protein PBP2 of *A. baumannii*.[139] Studies reported a substantial decline of the antimicrobial activity of sulbactam against *A. baumannii* isolates.[140]

2. Carbapenems

The carbapenems imipenem and meropenem have been regarded as the treatment of choice for severe *A. baumannii* infections. However, several recent microbiological studies have found susceptibility rates of *A. baumannii* isolates to carbapenems of nearly 90%.[141] Many mechanisms are involved in carbapenem resistance as the production of carbapenem-hydrolyzing β-lactamase ("carbapenemase"), penicillin-binding protein alterations, reduced permeability of the outer membrane (porins), and efflux pump mechanisms.

3. Cephalosporins

Most clinical isolates of *A. baumannii* are resistant to cephalosporins. There are basically two categories of enzymes which are responsible for conferring resistance to cephalosporins, they include the Extended Spectrum Beta-Lactamases (ESBLs) and AmpC Beta-lactamases.

4. Cyclines (tetracyclines and glycylcyclines)

Cyclines bacteriostatic action concerns in reversibly binding to the 30S ribosomal subunit and inhibiting protein translation.[142] *A. baumannii* resistance to this class is well known and documented. The mechanism is involving the efflux pumps proteins, through two tetracycline

[139] Krizova L., Poirel L., Nordmann P., Nemec A. TEM-1 β-lactamase as a source of resistance to sulbactam in clinical strains of Acinetobacter baumannii. *J. Antimicrob. Chemother.* 2013, *68, 12, p. 2786-2791.*
[140] Insa R., Cercenado E., Goyanes M.J., Morente A., Bouza E. *In vitro* activity of tigecycline against clinical isolates of Acinetobacter baumannii and Stenotrophomonas maltophilia. *J. Antimicrob. Chemother.* 2007, 59; p. 583–585.
[141] Van Looveren M., Goossens H. Antimicrobial resistance of *Acinetobacte*r spp in Europe. *Clin. Microbiol Infect*, 2004, 10, p. 684–704.
[142] Speer B. S., Shoemaker N. B., Salyers A. A. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. *Clin. Microbiol. Rev.* 5, p. 387–399.

resistance determinants TetA and TetB, the tetracyclines being removed from the cell and not reaching the active site.

5. Aminoglycosides

The activity of aminoglycosides is low for multidrug-resistant isolates. However, on carbapenem resistant isolates, aminoglycosides can show some activity.[143] Aminoglycosides activity mechanism is to bind to the 16S ribosomal RNA of the 30S ribosomal subunit and inhibit protein synthesis. In the case of *A. baumannii*, the mechanism of action is to produce different aminoglycoside modifying enzymes. However, the use of aminoglycosides in combination with other classes of antimicrobial agents has been reported, referring to meningitis.[144]

6. Polymyxins

Because their toxicity concerns, polymyxins were used only for the treatment of patients with cystic fibrosis. However, a newer version of colistin (polymyxin B), colistimethate sodium, a non-active prodrug is now used because of its lower toxicity. This antibacterial class are cationic polypeptides which interact with the lipopolysaccharide layer of Gram-negative bacteria, disrupting the negatively charged outer-membrane.[145] There are two mechanisms of resistance; the first one being the simple proteolytic cleavage of the antibiotic and exclusion of peptides by efflux-pump. The second mechanism is by the reduction of the net negative charge of the outer-membrane protein through the modification of lipid A, one essential component of the bacterial lipopolysaccharide.

7. Quinolones and fluoroquinolones

Fluoroquinolones have moderate antimicrobial activity against *A. baumannii*, with high susceptibility rates. Considering this class, their antibacterial activity comes from the inhibition of bacterial gyrase (encoded by gyrA and gyrB genes) and topoisomerase IV (encoded by parA and

[143] Gales A.C., Jones R.N., Sader H.S. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of Gram-negative bacilli: report from the SENTRY antimicrobial surveillance programme (2001–2004). *Clin. Microbiol. Infect.* 2006, 12, p. 315–321.
[144] Rodriguez Guardado A., Blanco A., Asensi V., Pérez F., Rial J.C., Pintado V., Bustillo E., Lantero M., Tenza E., Alvarez M., Maradona J.A., Cartón J.A. Multidrugresistant acinetobacter meningitis in neurosurgical patients with intraventricular catheters: assessment of different treatments. *J. Antimicrob. Chemother.* 2008, 61, p. 908–913.
[145] Landman D., Georgescu C., Martin D. A., Quale J. Polymyxins revisited. *Clin. Microbiol. Rev.* 2008, 21, p. 449–465.

parC genes) enzymes.[146] Resistance to this class was acquired due to mutations in the target sites of quinolones and fluoroquinolones, particulary mutations resulting in a Ser-86-Leu substitution in GyrA, and a Ser-80-Leu substitution in ParC.[147]

8. Rifampicins

The mechanism of action of this class consists in binding to conserved amino acids in the active site of the bacterial RNA polymerase in consequence, blocking the transcription initiation. However, important resistance has been assessed for this family. The *A. baumannii* resistance to rifampicin comes from chromosomal mutations leading to amino acid changes in the active site. Another identified resistance mechanism concerns a transferable gene which encodes a rifampicin ADP-ribosylating transferase, called arr-2 gene. This gene is the cause of rifampicin inactivation, by ribosylation of it. [148]

Besides all the antibiotic classes mentioned above, resistance to heavy metals, dyes, and hospital disinfectants has also been reported. If not applied for an adequate period of time, and not done meticulously in order to achieve sterilization, the agents can only induce more alteration to *A. baumannii* organisms to a more pathogenic resistant phenotype.[149] The main mechanism is mediated by intrinsic efflux pumps. There are several operons with genes encoding efflux pumps that confer resistance to different heavy metals and disinfectants. An example of one, is theoperon encoding resistance to mercury which is encoded by transposon Tn21.[150]

9. New drugs against multiresistant *Acinetobacter baumannii* in trial phases

Eravacycline (TP-434) is a compound of the tetracyclines in phase III of clinical trials with modified C-7 and C-9 positions, which inhibits the synthesis of bacterial proteins through binding to the 30S ribosomal subunit. It has been shown to be an effective treatment in infections caused

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[146] Nordmann P., Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J. Antimicrob. Chemother*. 2005, 56, p. 463–469.
[147] Vila J., Marti S., Sanchez-Cespedes J. Porins, efflux pumps and multidrug resistance in Acinetobacter baumannii. *J. Antimicrob. Chemother.* 2007, 59, p. 1210–1215.
[148] Towner K.J. Antibiotic resistance in *Acinetobacter* spp. In *Acinetobacter* Molecular Biology, 2008, p. 331–343, Caister Academic Press, Norfolk, UK
[149] Edwards J., Patel G., Wareham D.W. Low concentrations of commercial alcohol hand rubs facilitate growth of and secretion of extracellular proteins by multidrug-resistant strains of *Acinetobacter baumannii*. *J. Med. Microbiol.* 2007, 56, p. 1595–99.
[150] Fournier P.E., Vallenet D., Barbe V., Audic S., Ogata H., Poirel L., Richet H., Robert C., Mangenot S., Abergel C., Nordmann P., Weissenbach J., Raoult D., Claverie J.M. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. *PLoS Genet.* 2006, 2, p.7.

by Gram-negative, Gram-positive and anaerobic multidrug-resistant organisms.[151] The activity of the drug is not hindered by efflux pumps, nor by the ribosomal protection mechanism of drug resistance. There is evidence that its efficacy is superior to tigecycline against *Acinetobacter* spp. MDR and *Enterobacteriaceae* producing ESBLs.[152]

DS-8587 is a new fluoroquinolone that acts by inhibiting DNA topoisomerase. It has a potent activity against pathogens that cause nosocomial infections. Studies show that DS-8587 has an excellent antibacterial activity against strains of *A. baumannii* carrying mutations in the gyrA / parC genes, with a MIC of 0.015 to 0.06 µg/ml. These MICs were 4 to 8 times and 8 to 16 times lower than those of levofloxacin and ciprofloxacin, respectively.[153]

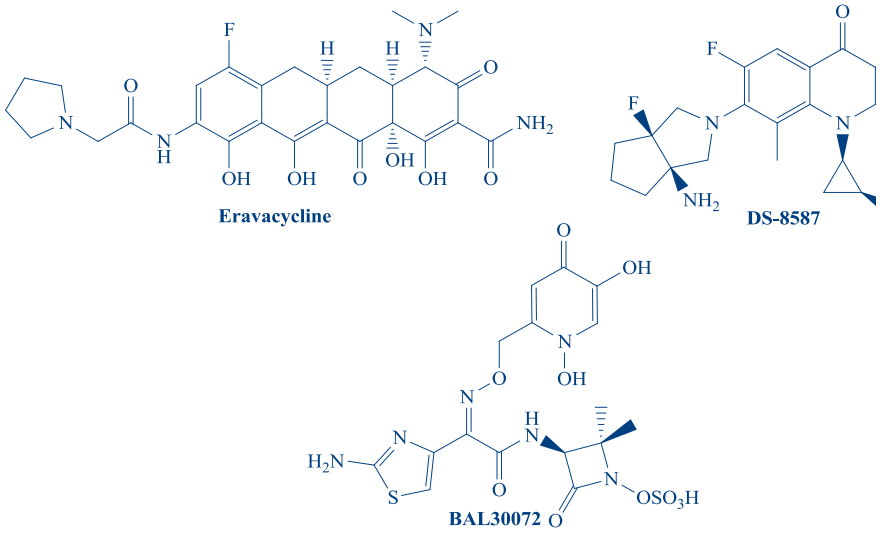


Figure 32 *Novel drugs for A. baumannii treatment*

BAL 30072 is a compound that is in phase I, belonging to the group of monosulfactams. It is active against many Gram-negative bacteria, including those that produce β-lactamases and *Klebsiella pneumoniae* carbapenemase (KPC), and has a synergistic effect with carbapenems. It penetrates into bacteria through iron and porin transport systems. The drug consists of a siderophore residue that confers activity against *A. baumannii*. Landman and colleagues showed a

[151] Taneja N., Kaur H. Insights into Newer Antimicrobial Agents Against Gram-negative Bacteria. *Microbiol Insights.*, 2016, 9, 1, p. 9-19.
[152] Zhanel G.G., Cheung D., Adam H., Zelenitsky S., Golden A., Schweizer F., Gorityala B., Lagacé-Wiens P.R., Walkty A., Gin A.S., Hoban D.J., Karlowsky J.A. Review of Eravacycline, a Novel Fluorocycline Antibacterial Agent. *Drugs*, 2016, 76, 5, p. 567-588.
[153] Higuchi S., Onodera Y., Chiba M., Hoshino K., Gotoh N. Potent in vitro antibacterial activity of DS-8587, a novel broad-spectrum quinolone, against *Acinetobacter baumannii*. *Antimicrob. Agents Chemother*. 2013, 57, 4, p. 1978-1981.

decrease of up to four times for the MIC, both for *A. baumannii* and *K. pneumoniae*, when combined with meropenem.[154]

II.2 *Acinetobacter baumannii* antibacterial results and discussion

II.2.1 Antibacterial hydrazides

High-throughput screening (HTS) is a huge contributory factor to the drug discovery of many new drugs within large pharmaceutical companies, in the past decade becoming an available method to smaller companies and even academic institutions. Though HTS is often thought of as the opposite of "rational" drug design, stifling creativity and innovation, it has a high success rate. In this context, through the laboratory´s latest collaborator, a large antimicrobial screening was performed within CO-ADD (The Community for Antimicrobial Drug Discovery), within the University of Queensland (Australia). One antimicrobial preliminary HIT, found as *A. baumannii* antimicrobial is compound **92** (HEI 2840, **Figure 33**) making it a promising hit compound for the development of novel antimicrobial agents, targeting particularly *A. baumannii*.

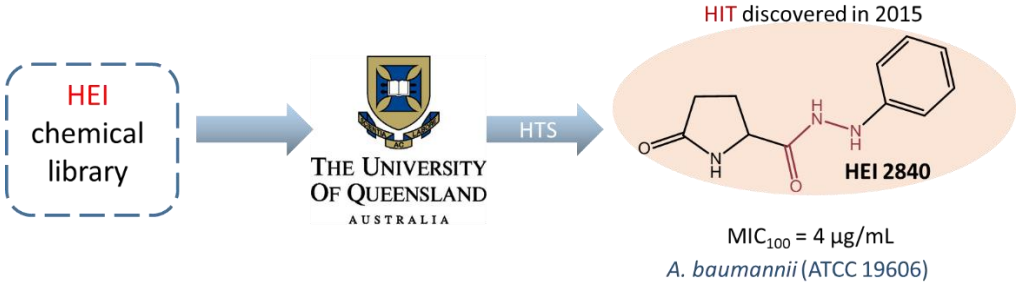


***Figure 33** HIT compound HEI 2840*

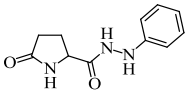
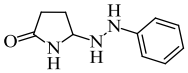
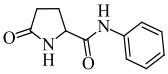
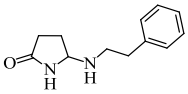
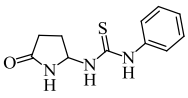
Considering the fact that testing the compounds in University of Queensland is more time consuming and that their goal is only screening, another partner was found within Pasteur Institute of Lille, able of screening the activity and identify the mode of action of the compounds. The following presented results being obtained within the institute, by the Chemical Biology of Antibiotics Group (CBA Group), led by Dr Ruben Hartkoorn.

[154] Landman D., Singh M., El-Imad B., Miller E., Win T., Quale J. *In vitro* activity of the siderophore monosulfactam BAL30072 against contemporary Gram-negative pathogens from New York City, including multidrug-resistant isolates. *Int. J. Antimicrob. Agents*. 2014, 43, p. 6, 527-532.

II.2.1.1. Link switch influence

HEI 2840 has been discovered as a very potent *A. baumannii* antibacterial agent. As a consequence, efforts have been put on its structural modifications in order to improve as best as possible its activity. The compounds synthesized in this series allowed us to conduct a well covering structure-activity relationship (SAR) analysis.

To better understand if the chemical space between the pyroglutamic moiety and phenyl group is important and to explore novel links for an improved antibacterial activity and pharmaceutical properties, we initially envisioned to exchange the hydrazide (**92**) with a different type of hydrazine, amide, amine, thiourea, sulfonylhydrazide, or the reversed hydrazide, which mostly resulted in a great decrease of activity, indicating the high influence of the spacer on the activity (**Table 12**).

Entry	N° compound	N° HEI	N° compound	Liquid MIC (µg/mL)	Cytotoxicity analysis HepG2 cells µg/mL	
					Impact on cell proliferation	Impact on cell proliferation
1	92	2840		0.625< MIC < 1.563	6.04 (Bottom=83.6%)	
2	37	2909		3.125		5.07 (Bottom=1.6%)
3	Chemical library	29		25	Safe (Variability < 10%)	
4	5	2971		> 100	Safe (Variability < 10%)	
5	45	2956		> 100	Safe (Variability < 10%)	

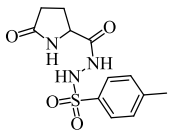
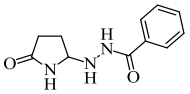
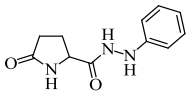
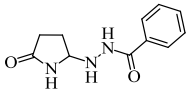
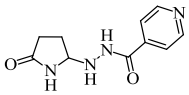
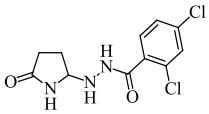
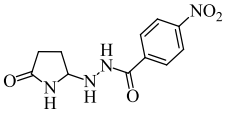
6	56	3038		6.25	Safe (Variability < 10%)
7	46	2970		1.56	55.46 (Bottom=34.7%)

Table 12 *A. baumannii (ATCC179778) screening results of different pyro-derivatives with different linkers*

From the first results, we can clearly observe the importance of the hydrazide moiety, but our next question was: it the order link really important? As it can be seen, the "reverse" hydrazide **46** (**Table 12**, entry 7) has a very close MIC_{100} of 1.56 µg/mL to the HIT compound, **92**. In order to explore also the lead, several hydrazides derivatives from pterolactam, were tested as well (**Table 13**). The substitution of the ring causes a dramatically loss of potency, except derivative **46**, the other hydrazides of this series having no antibacterial activity.

Entry	N° compound	N° HEI	N° compound	Liquid MIC (µg/mL)
1	92	2840		0.625< MIC < 1.563
2	46	2970		1.56
3	47	2947		> 100
4	50	3008		> 100
5	51	2940		50

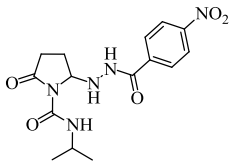
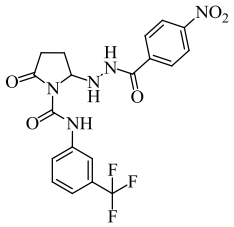
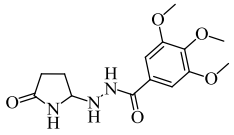
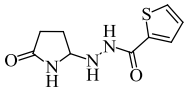
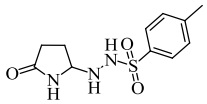
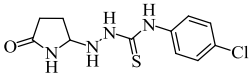
6	88	3013		> 100
7	89	3016		> 100
8	52	2941		> 100
10	53	2944		> 100
11	56	3035		> 100
12	57	2946		25

Table 13 *A. baumannii (ATCC179778) screening results of pterolactam reversed-hydrazides*

II.2.1.2 Ring substitution of the HIT compound HEI 2840

Figure 34 *Second step in the SAR study: R group tolerance*

A series of new carbohydrazides derivatives were designed and tested. Their *in vitro* anti-*A. baumannii* activities are summarized in **Table 14**, being expressed as the MIC to achieve 100% inhibition of the bacterium. Holding the lactam ring constant, we systematically explored SAR of

the R substituent (Fig. 27). Our initial lead optimization focused on exploring substituents such as alkyl chains. However, to our disappointment, derivatives such as (2-hydroxyethyl)-5-oxopyrrolidine-2-carbohydrazide **91** or carbohydrazide having a cyclohexyl ring as a R group, failed to deliver the same activity as the HIT compound, **91** having a more than 10 times higher MIC_{100} compared to **92**, while **96´**s MIC_{100} is bigger than 100 µg/mL (**Table 14**, entries 1, 2). These results demonstrate that the alkyl nature as an R group has a decreasing effect of *A. baumannii* antibacterial potency.

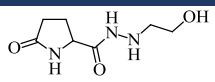
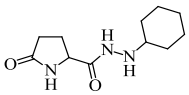
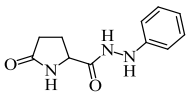
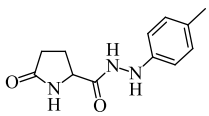
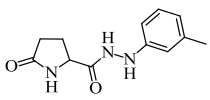
As the phenyl ring seemed to be delivering better results, the next step in the SAR study was to test different aromatic substituents pyroglutamic carbohydrazides. Activating groups such as methyl- and methoxy- were firstly tested (**Table 14**, entries 4, 6). It appeared that both derivatives such as **97** and **99** retained the potency against all *A. baumannii* being actually more potent than HIT compound **92** with MIC_{100} of 0.313 µg/mL. Moreover, the SAR data did indicate that substitutions in the *para*-position of the phenyl ring were most favorable as for example hydrazide **98** (*meta*-methyl) was moderately active (MIC_{100} = 1.56 µg/mL), having the same antibacterial activity as the HIT compound, while hydrazide **97** (*para*-methyl) demonstrated a more potent *A. baumannii* growth inhibition (MIC_{100} = 0.313 µg/mL) compared to the *meta*-substituted analogue (**Table14**, entries 4, 5).

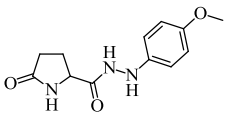
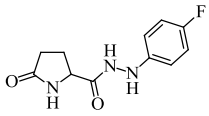
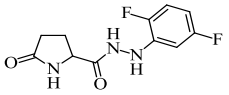
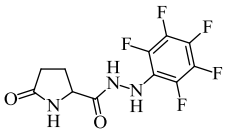
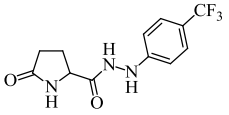
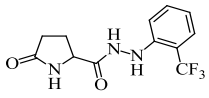
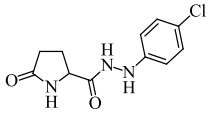
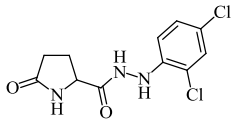
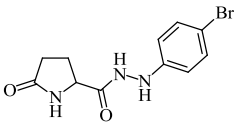
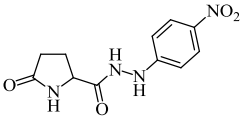
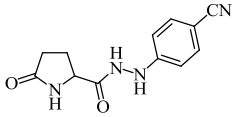
Among the tested substituents on the benzene ring, halogen substituents provided very good activities as well. While the *para*-fluorinated carbohydrazide **100** provided a very good activity of 0.313 µg/mL (**Table 14**, entry 7), multi-substitution of the ring seemed to decrease the antibacterial activity, derivatives such as 2,5-difluorinated or pentafluoro- analogues, having MIC_{100} of 2 respectively 10 times bigger than derivative **100** (**Table 14** entries 8, 9). Trifluoromethyl product seemed to be less efficient than the simple fluorinated analogue, having a MIC_{100} equal to the HIT compound (**Table 14**, entry 10). In the context of *para*-position being more favorable, another example is given by the CF_3 group, which also shows sensitivity to substitution pattern. While the *para*-position derivative **106** kept the antibacterial activity, the *ortho*-position derivative **107** MIC_{100} jumped to 25 µg/mL (**Table 14**, entries 10, 11).

Based on the improved effects acquired from 4-fluoro, we envisaged the introduction of 4-chloro- substituent in order to improve the activity. Therefore, compound **101** was designed and tested, which, to our delightfulness, resulted in the maximum effects, leading us to a 4 folded

MIC_{100} of 0.078 µg/mL compared to **100** (**Table 14**, entry 12) against *A. baumannii*, suggesting that the halogen from the 4 position of the benzene ring is required for a potent antibacterial activity. Moreover, the size of the halogen might have an impact on the activity, as the brominated derivative **102** also presented a low MIC_{100}, similar to that of **101**, the potency order could be (Br ≥ Cl > F >> H). However, it seemed that a larger group such as iodine caused a regression to the starting MIC value of the HIT compound **92**. The same behavior regarding the multi-substitution of the benzene ring as seen in the case of the fluorinated derivatives, can be seen for the chlorinated ones. Indeed, as well, 2,4-disubstitution being less well tolerated leading to 20-fold decrease in potency for derivative **105** compared to **101** (**Table 14**, entry 13).

We also explored potential substituents group such as nitro- and cyano- (derivatives **93**, **108** – **Table 14**, entries 15,16). These groups were suitable replacements with MIC_{100} values of 0.313 µg/mL, the main biological activity difference between them seems to be the impact on cell proliferation. While the cyanophenyl hydrazides presented a safe profile, with no important impact on cell proliferation, the nitro- derivative **93** exhibited a certain influence; as known, most of the times the nitro derivatives are not the best candidates and often cytotoxic.

Entry	N° compound	N° HEI	Structure	Liquid MIC (µg/mL)	Cytotoxicity analysis HepG2 cells µg/mL	
					Impact on cell proliferation	Cytotoxicity
1	91	3232		12.5		
2	96	3231		> 100		
3	92	2840		0,313 < MIC < 1,563	6.04 (Bottom=83.6%)	
4	97	3136		0.313	30.72 (Bottom=8.9%)	
5	98	3227		1.56	8.86 (Bottom=65.3%)	

6	99	3128		0.312		31.59 (Bottom=4.4%)
7	100	3213		0.312	Safe (Variability < 10%)	
8	94	3036		0.625	Safe (Variability < 10%)	
9	104	3122		3.125	Safe (Variability < 10%)	
10	106	3126		1.56		67.58 (Bottom=0.9%)
11	107	3212		25	6.28 (Bottom=79.8%)	
12	101	3216		0.078	0.474 (Bottom=82.3%)	
13	105	3133		1.56	99.35 (Bottom=87.5%)	
14	102	3230		0,078< MIC< 0,156	Safe (Variability < 10%)	
15	93	3037		0.312	52.44 (Bottom=86.9%)	
16	108	3219		0.312	Safe (Variability < 10%)	

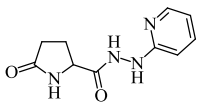
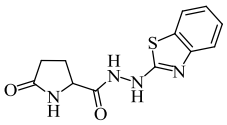
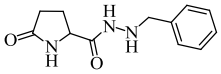
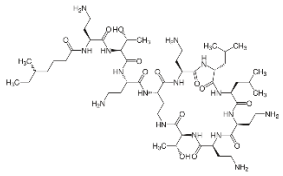
17	112	3129		0.39	ND
18	111	3270		6.25	ND
19	110	3269		25	ND
					0.625<MIC_{95}<1.25

Table 14 *A. baumannii (ATCC179778) screening results of different pyroglutamic acid carbohydrazides*

Extension to other R groups such as 2-pyridine, improved the activity of the HIT compound, increasing its potency with 4-5 folds (**Table 14**, entry 17). However, if aryl ring is replaced by a benzothiazole, the activity is decreased by approximately 4 folds (**Table 14**, entry 18). Going further with replacing the phenyl with a benzyl, causes a 20 folds decrease of potency, hydrazide derivative **110** having a MIC_{100} of 25 µg/mL.

II.2.1.3 *N*-substitution of the HIT compound

To further study the SAR around the HIT molecule, the three NH- groups depicted in **Figure 35**, were substituted with either light groups such as methyl-, acetyl- or bulky ones such as phenyl-, and tested for their antibacterial potency (**Table 15**).

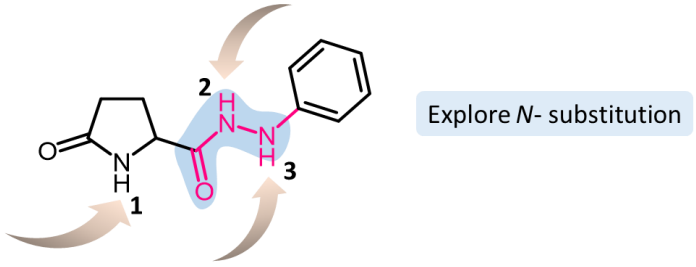


Figure 35 *Explored structural modifications regarding NH-* groups

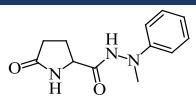
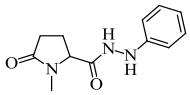
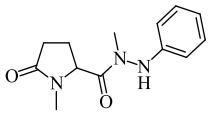
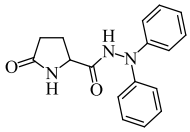
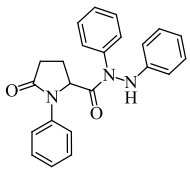
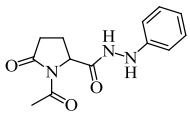
Entry	N° compound	N° HEI	Structure	Liquid MIC (µg/mL)	Cytotoxicity analysis HepG2 cells µg/mL	
					Impact on cell proliferation	Cytotoxicity
1	109	3132		25	Safe (Variability < 10%)	
2	113	3187		0.625	6.37 (Bottom=72.9%)	
3	114	3205		6.25	Safe (Variability < 10%)	
4	115	3173		12.5	Safe (Variability < 10%)	
5	116	3181		1.56	55.56 (Bottom=37.5%)	
6	Chemical library	3334		0.195 < MIC < 0.625	18.45 (Bottom = 83.6%)	

Table 15 *A. baumannii (ATCC179778) screening results of NH-substituted molecules, derivated from the HIT molecule* ***92***

The obtained results led us to a better understanding of the SAR. What seemed clear is that link substitution with groups such as methyl-, are not improving the activity of **92**. While the 3rd NH group was methylated (derivative **109**), a strong decrease in activity was noticed, the MIC_{100} jumping to 25 µg/mL (**Table 15**, entry 1), whereas for derivative **115**, for the same modified position, now with a phenyl substituent, the activity decreased, but not as much as for **109**, the MIC_{100} being 12.5 µg/mL. If the lactam ring is methylated or acetylated, one fold of difference is observed (**Table 15**, entries 2, 6). However, when both 1st and 2nd NH- groups (lactam and link) are methyl substituted, a 5 folds decrease of antibacterial potency is observed (**Table 15**, entry 3).

Interestingly, as seen before, if we substitute the same positions with bulky phenyl groups, the activity is less impacted, the activity of the HIT compound being kept (**Table 15**, entry 5).

II.2.1.4 Lactam motif substitution on the HIT compound

In our continuous efforts to improve the pharmacological properties of the HIT compounds, and also to understand if the lactam ring is a key motif for the *A. baumannii* antibacterial activity, several molecules with different scaffolds were designed and tested for their potency.

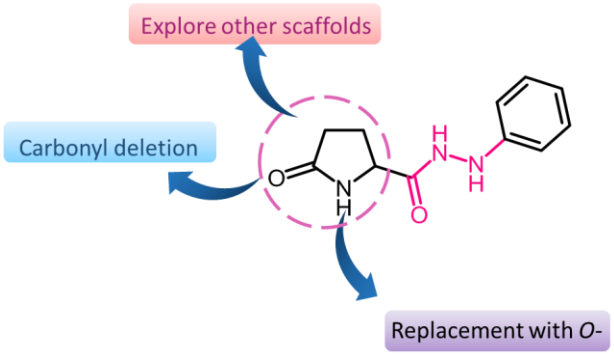


Figure 36 *Replacement of the lactam motif*

Interestingly, keeping the phenyl hydrazide moiety constant and changing the scaffold, the *A. baumannii* activity is not lost, and in some cases, the activity is actually slightly improved. Firstly, the NH- group was replaced with a simple oxygen atom, and as we have seen in the case of the methylated derivative, compound **117** is keeping the activity of the HIT derivative, improving it with one fold, proving the *NH* group might not be necessarily needed in the ring (**Table 16**, entry 1). However, the benefit/risk ratio changed, hydrazide **117** having a bigger impact on cell proliferation. The same thing could be said about **118**, which's efficiency is also well kept, while its safety has decreased. The result itself might though be a proof that the carbonyl is not really needed either to conserve the potency, or the mechanism of action might not be linked to the scaffold but mostly to the link. The imidazole and furan scaffold molecules had the same behavior as seen with the first two (**Table 16**, entries 3, 4), however the thiophene hydrazide is not in concordance with the previous results, completely losing potency, with a MIC_{100} of 100 µg/mL (**Table 16**, entry 5).

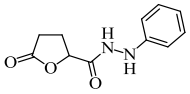
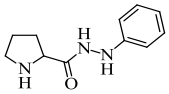
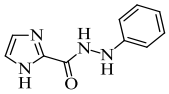
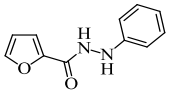
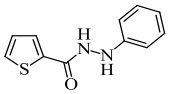
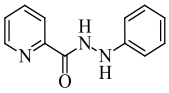
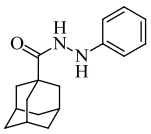
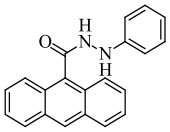
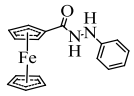
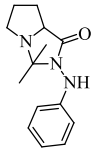
Entry	N° compound	Name	Structure	Liquid MIC (µg/mL)	Cytotoxicity analysis HepG2 cells µg/mL	
					Impact on cell proliferation	Cytotoxicity
1	117	3194		0.313< MIC <0.781	5.46 (Bottom=75.1%)	
2	118	3193		0.313< MIC <0.625		17.6 (Bottom=3.1%)
3	120	3211		0.313< MIC <0.781	82.03 (Bottom=53.1%)	
4	Chemical library	3305		0.195 < MIC < 0.625	ND	
5	Chemical library	3266		100	ND	
6	121	3177		0.313< MIC <0.625	58.54 (Bottom=56.9%)	
7	Chemical library	3293		1.563		47,47 (Bottom = 3%)
8	Chemical library	3288		6.25	61,11 (Bottom = 48,8%)	
9	Chemical library	3285		1.563		46.34 (Bottom = 16.1%)
10	119	3188		0.313	14.67 (Bottom=75.3%)	

Table 16 *A. baumannii (ATCC179778) screening results of different scaffolds hydrazides derivatives*

Enlarging the cycle, replacing it with a pyridine for example, did not shift the potency, just the cell proliferation was disturbed, whereas for bulkier groups such as an anthracene, activity was lost, proving that the dimensions of the groups might be too big (**Table 16**, entry 8). The ferrocene and the adamantane derivatives, on the other hand, seemed to both keep the activity, but not the ratio benefit/risk, presenting cytotoxicity. Closing the ring delivered approximately the same activity as the HIT compound, though the IC_{50} of the cell proliferation is lower (**Table 16**, entry 10). This test's conclusion is that replacing the lactam motif will not highly improve the activity, but sometimes decreased it depending on how bulky was the new substituent. However, the lactam replacement definitely had an impact on the benefit/risk ratio, the pyroglutamic acid which is known as a natural product, delivering better results in our perspective, being more "drug-like".

II.2.1.5 Enantiomeric influence on *A. baumannii* activity

All pyroglutamic molecules present an asymmetric carbon center on the fifth position on the lactam ring. Even if the synthesis has been developed from the L-pyroglutamic acid, we considered to investigate the stereochemistry biological impact of the HIT compound **92** (**Figure 37**).

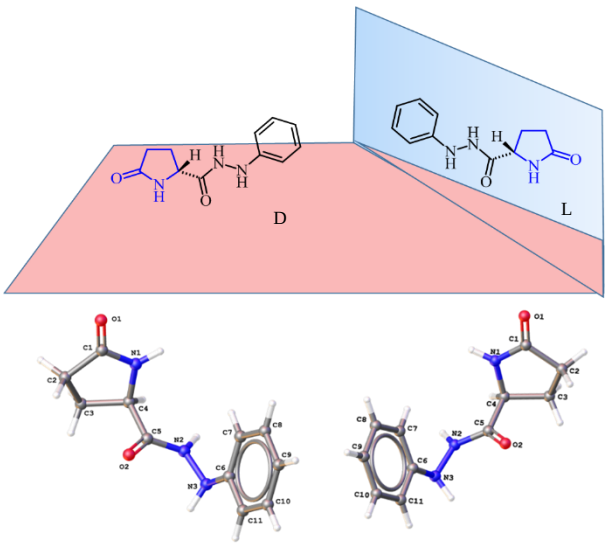


Figure 37 *D and L forms of the pyroglutamic acid*

However, within the series we presented so far, the center was blocked, the molecules being in the L departing form, as the natural pyroglutamic acid molecule which is found in the sugar beet. In order to test their both enantiomeric forms, the *D*, *L* and racemate as well were synthesized from the departing corresponding substrates and then tested for their *A. baumannii* potency, results being summarized in **Table 17**.

Comparing the data, in terms of activity there is only one fold of difference between the *D* and the *L* form, the *D* non-natural one being more potent compared to the natural *L*-form, while the racemic one it seems to be slightly improving the MIC, the difference between the two enantiomers could be linked to the mode of action of the molecules.

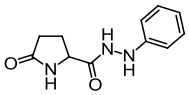
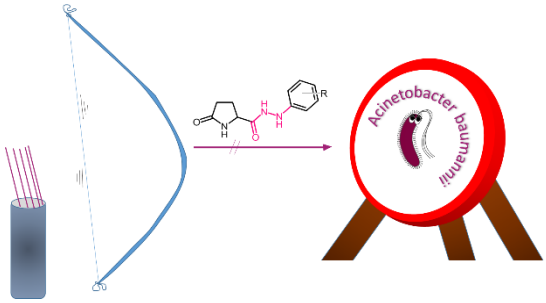
N° compound	Name	Structure	Form	Liquid MIC (µg/mL)
92	2840		*D*	1.25
			L	2.5
			D, L	0.625 < MIC < 1.563

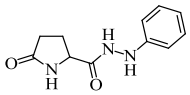
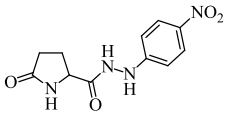
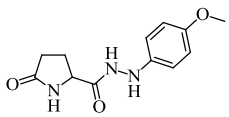
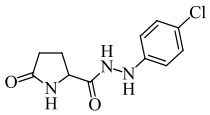
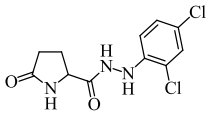
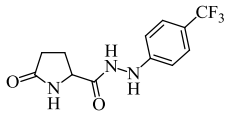
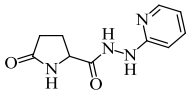
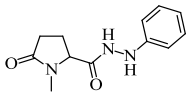
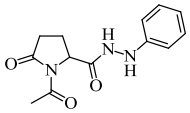
Table** 17 A. baumannii (ATCC179778) screening results of the different enantiomeric forms of the HIT compound **92

II.2.1.6 Bug specificity of the selected molecules



In order to probe the antibiotic spectrum of the most active molecules which presented anti-*A. baumannii* potency, MICs against 6 representative microorganisms - most of them being part of the ESKAPE panel - were performed: *Escherichia coli* with and without efflux pumps, methicillin resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (**Table 18**). To our delight, the HIT compound presented a MIC of ≥100

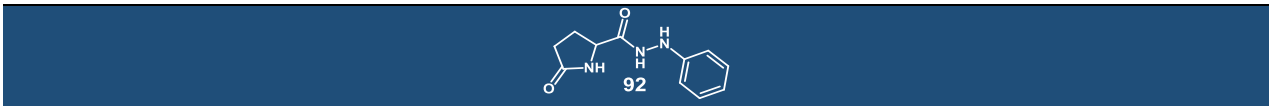
µg/mL for all bacterial species other than *A. baumannii*, establishing the fact that **92** is a narrow spectrum antibiotic. Considering the other tested derivatives we can notice a slight activity, but with more important MIC_{100}>12-25 µg/mL on *E. coli* with or without efflux pumps and *K. pneumonia*. This is the case of substituted derivatives: **93**, **99**, **101**, **105**, **106**, **113**. The most active compound **101** presents a high selectivity towards *A. baumannii* with ratios superior to 160.

N° compound	N° HEI	Structure	MIC_{100} (µg/mL)						
			E. coli BW25113	*S. aureus*	*S. pneumoniae*	*K. pneumonia*	*A. baumannii*	*P. aeruginosa*	*E. coli* ΔTolC
92	2840		>100	>100	>100	>100	**0.625**	>100	>100
93	3037		>100	>100	>100	12.5	**0.312**	>100	25
99	3128		25	>100	>100	25	**0.312**	>100	25
101	3216		12.5	>100	>100	12.5	**0.078**	>100	12.5
105	3133		25	>100	>100	25	**1.56**	>100	>100
106	3126		50	>100	>100	50	**1.56**	>100	>100
112	3129		>100	>100	>100	100	**0.39**	>100	100
113	3187		12.5	>100	>100	12.5	**0.313**	>100	12.5
Chemical library	3334		50	>100	>100	50	**0.313**	>100	50

*The determinated MIC was MIC_{95}

Table 18 *Bug specificity of selected molecules found to be A. baumannii antibacterials*

Moreover, the HIT compound was also tested for its antifungal activity on a large panel of strains. As expected, 5-oxo-*N*'-phenylpyrrolidine-2-carbohydrazide **92** did not exhibit any potent activity towards fungi and molds (**Table 19**).



C. albicans	*C. neoformans*	*G. candidum*	*A. alternata*	*A. oryzae*	*S. cerevisiae*	*P. variotii*	*C. cladosporiodes*
>32	>32	>50	>50	>50	>50	>50	>50

Table** 19 Antifungal results of HIT molecule **92

These informations with regard to the selectivity towards the bacteria microorganism and the specificity for *A. baumannii*, are considered important leads towards the elucidation regarding the mechanism of action.

II.2.1.7 *In vitro* metabolic stability

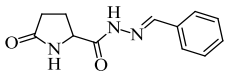
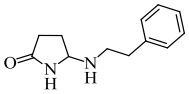
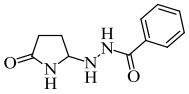
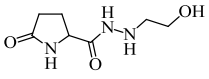
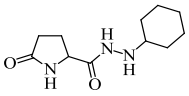
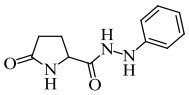
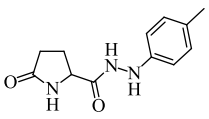
The *in vitro* metabolic stability using mouse liver microsomes was also conducted for hit compounds **92**, **99** and **101**. Due to its implications for both dose level and frequency, the clearance rate is an important pharmacokinetic parameter to be conserved when designing a drug candidate. In this context, we tested three possible candidates, one having a methoxy- activating group (compound **99**), one chlorine deactivating group (compound **101**) and also the HIT compound **92** for their liver clearance which is known to be one of the most important organs for such tests.

Preliminary, we assumed that this type of compounds are potential hydrolytic instable and a time-dependent stability study during 40 minutes was performed. Each compound was dosed in an adjusted buffer and incubated at 37 °C. The mixture was then analyzed by LC-MS/MS to measure its rate of degradation. It was determined that the compounds are very much stable, and no clearance could be determinated within the analysis time of 40 minutes and further studies should be done.

II.2.1.8 Mechanism of action leads

Reactive oxygen species (ROS) generation have been linked to provoke damage to multiple cellular organelles and processes causing direct injury to proteins, lipids or nucleic acids and

leading to cell death and stopping the normal physiology. The ROS production is implicated in the course of multiple pathologies such as cancer, pulmonary diseases, hypertension, asthma, or retinopathy. As a consequence, studying the ability of our antibacterial series for their antioxidant properties seemed useful. To evaluate this potency, the radical scavenging test using DPPH as a probe was applied. Vitamin C and BHT were used as references. To our delight, the molecules presenting an antibacterial profile were also found to be strong radical-scavenging molecules with IC_{50} below than the references. Moreover, a correlation between the antioxidant activities of the molecules to their antimicrobial potency has been established (**Table 20**).

Entry	N° compound	N° HEI	Structure	Liquid MIC (µg/mL) against *A. baumanii*	Antioxidant activity IC_{50} (µM)
1	130	261		> 100	> 1000
2	5	2971		> 100	> 1000
3	46	2970		1.56	38.6
4	91	3232		12.5	196.9
5	96	3231		> 100	207.9
6	92	2840		0.313 < MIC < 1.563	5.2
7	97	3136		0.312	7.5

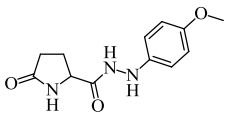
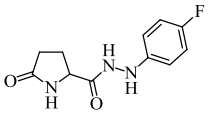
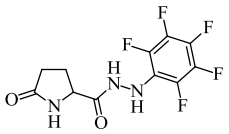
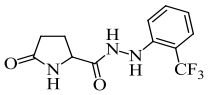
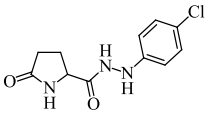
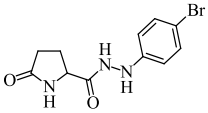
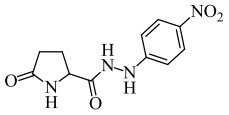
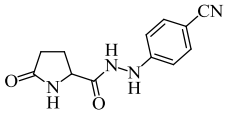
8	99	3128		0.312	23.0
9	100	3213		0.312	27.6
10	104	3122		3.125	31.0
11	107	3212		25	47.6
12	101	3216		0.078	4.2
13	102	3230		0.078< MIC< 0.156	4.3
14	93	3037		0.312	7.4
15	108	3219		0.312	3.6
		Vitamin C		> 100	15

Table 20 *Antioxidant activities of selected molecules*

As it can be observed, molecules having different links from the hydrazide were found not to be antibacterial and are not presenting any antioxidant activity neither (**Table 20**, entries 1-2). *N*'-(5-oxopyrrolidin-2-yl)benzohydrazide **46** or aliphatic carbohydrazides **91** and **96** exhibited moderate radical scavenging activities and moderate antibacterial potential (**Table 20**, entries 3-5). On the other hand, the molecules presenting good antibacterial profile, have also moderate to very good antioxidant activities. We hope to be able to elucidate if there is a link between the two and if there is any mechanistic path that involves ROS inhibition.

II.2.1.9 Conclusions

Within this subchapter, pyroglutamic hydrazides were described as a novel class of antimicrobial agents. Around the HIT molecule **92** we have generated an extensive SAR which is recapitulated as following:

(1) the hydrazide link is imperative for the *A. baumannii* activity;

(2) it has been observed that alkyl chains are not so well tolerated;

(3) considering the phenyl substitution, the SAR is quite broad, and many substituents are permissible without significant losses in potency;

(4) regarding the substituent, the chlorine derivative was found to be superior with a very good benefit/risk ratio;

(5) so far, we observed that groups are sensitive to position shift on the ring and multiple substitution is generally decreasing the potency of the molecule, however this lead should be more studied;

(6) lactam NH- substitution conserves the antibacterial potency, while the link can be sensitive to substitution;

(7) other motifs except lactam ring are tolerated, however the ration benefit/risk can be changed;

(8) D-enantiomer seems to be more potent, but other investigations should be done.

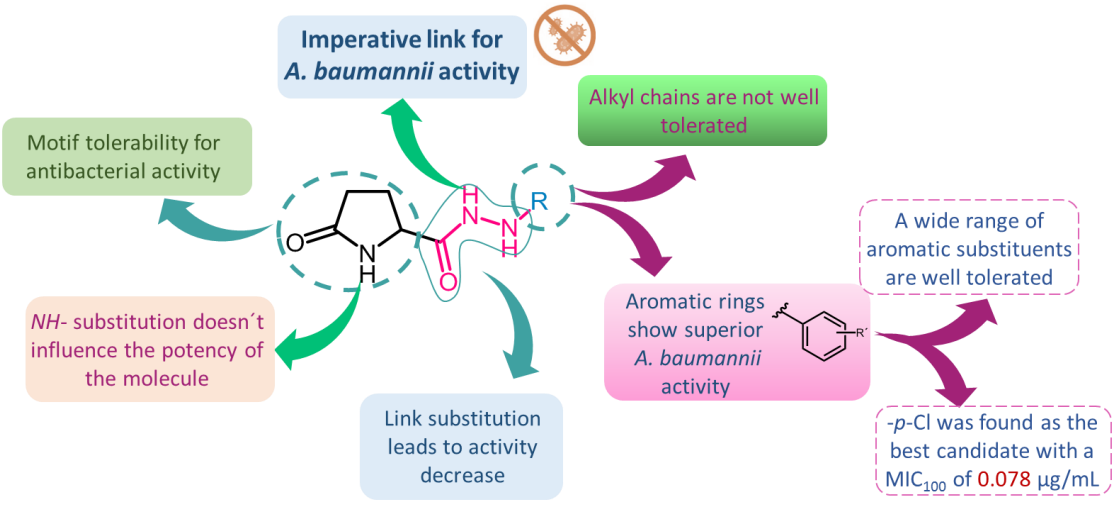


Figure 38** SAR regarding HIT molecule **92

The screen against ESKAPE and fungal pathogens revealed that these compounds were selective for *A. baumannii* as they were not active against the other microorganisms (MIC_{100}>12.5 μg/mL). Importantly, the majority of the compounds were not cytotoxic at the highest concentration tested against mammalian liver HepG2 cell lines.

Out of this effort, we identified **101** as a lead compound for further profiling. 4-(Chlorophenyl)-5-oxopyrrolidine-2-carbohydrazide **101** is 8 folds more potent compared to the HIT molecule **92**, on the drug resistant strain *A. baumannii*, presenting suitable selectivity over the drug resistant strains we tested, with a great stability as well and no cytotoxicity. Thus, compound **101** presents a promising lead for optimization as an antibiotic drug targeting specifically *A. baumannii*, with a low molecular weight, modest lipophilicity, excellent antibacterial potency, and we assume a long half-life oral bioavailability.

II.2.2 Hydrazones as antibacterials

Another HIT compound which was found by The University of Queensland through HTS, being active against *A. baumannii* is HEI 604 (**Figure 39**), becoming another promising hit for the development of a different family of antimicrobial agents, targeting *A. baumannii*.

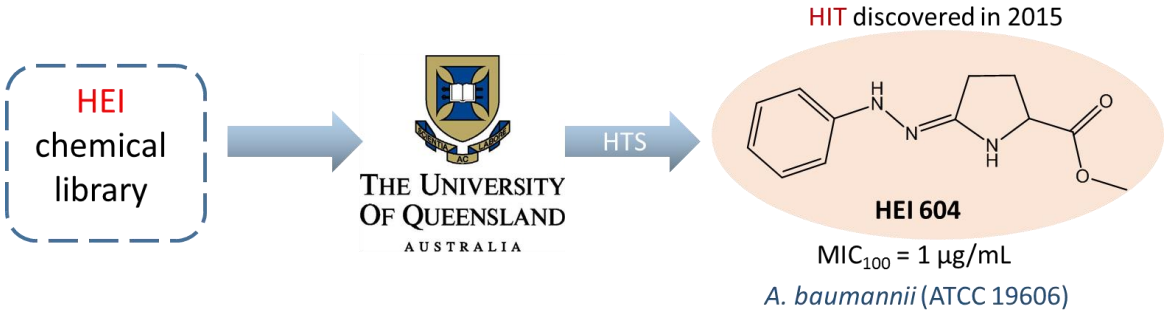


***Figure 39** HIT compound HEI 604*

After the identification of this type of compound, the new synthesized derivatives presented in the Chapter I, with a hydrazone link, were tested in the same laboratory from Pasteur Institute, and all the presented results were obtained there.

II.2.2.1 Link switch influence

Considering the second HIT compound, HEI 604, in order to improve its activity, and to understand better the SAR, as was described in the synthesis chapter, extensive modifications were done around the scaffold, link but mostly, on the aromatic ring.

As seen in the previous sub-chapter, the chemical space between the pyro moiety and the phenyl group is important. Considering this aspect, the hydrazone link was changed to an imine, azine, *N*-acylhydrazone, sulfonyl hydrazone as well as semicarbazide link. All derivatives, except *N'*-(pyrrolidin-2-ylidene)benzenesulfonohydrazide **175**, which kept a little activity, resulted in a loss of activity, indicating the fact that the hydrazine spacer is essential for keeping the antibacterial activity against *A. baumannii* (**Table 21**).

N° compound	N° HEI	Structure	Liquid MIC (µg/mL)

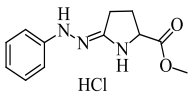
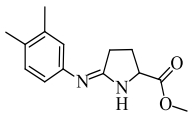
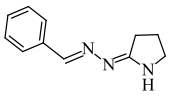
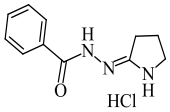
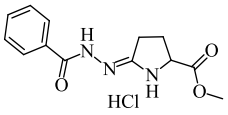
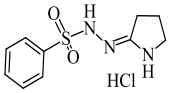
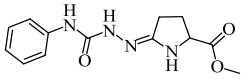
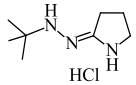
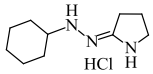
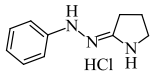
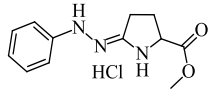
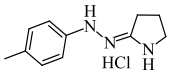
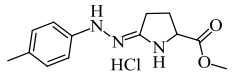
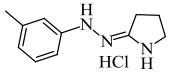
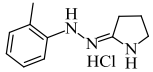
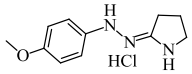
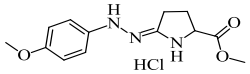
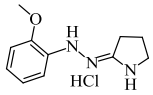
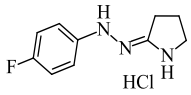
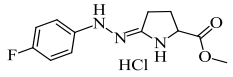
HEI library	604		0.625< MIC_{100} < 1.25
HEI library	596		>100
194	3427		>100
HEI library	369		>100
HEI library	378		>100
175	3348		3.125< MIC_{100} < 10
HEI library	815		>100

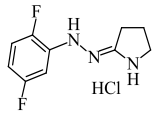
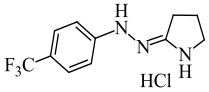
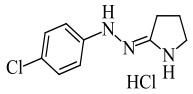
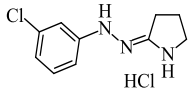
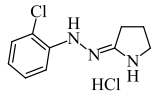
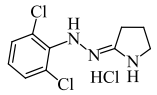
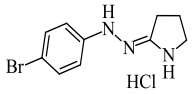
Table 21 *A. baumannii (ATCC179778) screening results of derivatives with different links*

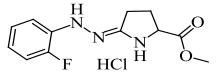
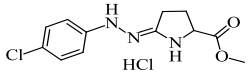
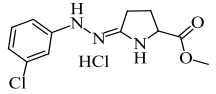
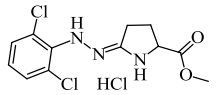
II.2.2.2 Substitution of the phenyl moiety of simple pyrrolidine or pyrrolidine-2-carboxylate hydrazones

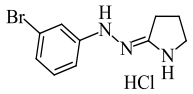
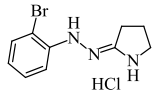
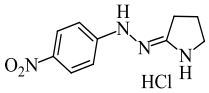
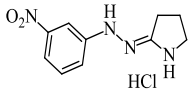
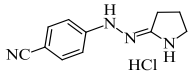
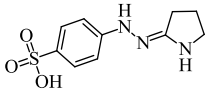
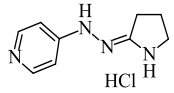
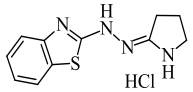
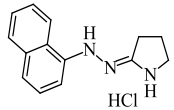
Two series of new pyrrolidine and pyrrolidine-2-carboxylate derivatives were designed and tested. Their *in vitro A. baumannii* activities are summarized in **Table 22**, being expressed as the MIC to achieve 100% inhibition of the bacterium. Holding either the pyrrolidine or the pyrrolidine-2-carboxylate ring constant, we systematically explored the SAR of the R substituent.

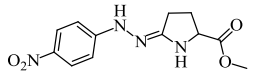
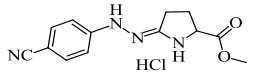
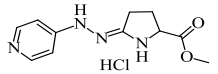
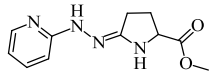
Things considered, as previously seen in our lead optimization, alkyl groups were introduced as R groups. Apparently, as observed for the carbazide family, alkyl moieties failed to provide the same *A. baumannii* activity, which is decreasing very much. As example, while the *iso*-butyl hydrazone **151** keeps a certain activity, the cyclohexyl derivative **152**, has a MIC_{100} of 25 µg/mL.

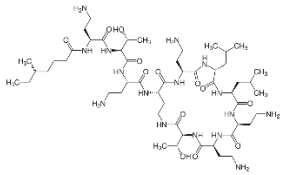
N° compound	N° HEI	Structure	Liquid MIC (µg/mL)	Cytotoxicity analysis HepG2		N° compound	N° HEI	Structure	Liquid MIC (µg/mL)
				Impact on cell proliferation	Impact on cell proliferation				
151	3347		3.125< MIC_{100}< 10	Safe (Variability < 10%)				/	
152	3349		25	84.6 (Bottom=20%)				/	
153	3337		0.391 < MIC_{100}< 1.25	47.7 (Bottom=10.5%)		HEI library	604		0.625 < MIC_{100}< 1.25
154	3343		0.391 < MIC_{100}< 1.25		1.93 (Bottom=8.9%)	177	3407		0.625
155	3344		0.195 < MIC_{100}< 0.625	3.43 (Bottom=36.4%)				/	
156	3345		0.391 < MIC_{100}< 1.25	6.29 (Bottom=50%)				/	
157	3341		0.391		10.44 (Bottom=8.1%)	178	3403		0.313
158	3421		5					/	
159	3340		0.195 < MIC_{100}< 0.625	1.68 (Bottom=65.8%)		179	3404		0.156 < MIC_{100}< 0.313

		/		
160	3346		0.156 < MIC_{100}< 0.313	82.4 (Bottom=34.4%)
161	3356		0.625	56.3 (Bottom=13.7%)
162	3338		0.156 < MIC_{100} < 0.313	
163	3413		0.156 < MIC_{100}< 0.313	
164	3415		0.313 < MIC_{100}< 0.625	15.2 (Bottom=62.5%)
165	3339		0.195	
166	3411		0.078 < MIC_{95} < 0.156	

HEI library	606		0.313 < MIC_{100}< 0.625
		/	
		/	
180	3402		0.078 < MIC_{95} < 0.156
181	3410		0.156 < MIC_{100}< 0.313
		/	
182	934		0.625 < MIC_{95} < 1.25
		/	

167	3412		0.156 < MIC_{100}< 0.313		
168	3422		0.625	16.07 (Bottom=44%)	
169	3342		0.078 < MIC_{100}< 0.156		
170	3414		0.156	100 (Bottom=89%)	
171	3352		0.078 < MIC_{100}< 0.313	Safe (Variability < 10%)	
172	3416		> 1.25		
HEI library	3472		0.313		
			/		
173	3353		25	37.46 (Bottom=0%)	
174	3420		10		

		/	
		/	
183	3406		0.156 < MIC_{100}< 0.313
		/	
184	3405		0.156
		/	
186	3408		0.313 < MIC_{100}< 0.625
185	3409		0.625 < MIC_{95} < 1.25

Colistin		$0,625 < MIC_{95} < 1,25$

>10	*1-10*	*0.156-1*	*0.15>*

Table 22 *A. baumannii (ATCC179778) screening results of pyrrolidine or pyrrolidine-2-carboxylate series*

These results revealed that once again, the aromatic molecules had a better antibacterial profile. Electron-donating groups, either methyl- or methoxy- were added to the phenyl group, both on the simple and pyrrolidine-2-carboxylate scaffolds. Starting with group such as methyl-, a first observation is that the *meta*-derivative is slightly more active, at possibly one fold compared to the *para*- or *ortho*- corresponding derivative. However, the carboxylate group did not seem to play much of a role so for, regarding the *A. baumannii* activity, molecules such as **154** and **177** presenting almost the same MIC values. Considering the second electron-donating tested group, methoxy-, while the *para*-derivative **157** entirely kept the previously seen MIC value of 0.391 µg/mL, the group seemed to be sensitive to position switch, as the MIC value of the *ortho*-methoxyphenyl hydrazone jumps to 5 µg/mL. Moreover, **157** was found to be cytotoxic. The result is quite unexplainable, as derivative HEI 606 which has a fluorine atom in the same position, presents an excellent activity between 0.313 and 0.625 µg/mL.

Considering other fluorinated molecules, we can observe one-fold improvement for the *para*- derivative **179** with a MIC between 0.156 and 0.313 µg/mL, while **159**, the simple pyrrolidine derivative goes back up to 0.313-0.625 µg/mL. Multi-substituted molecule **160**, seemed to improve the antibacterial profile of the simple pyrrolidine scaffold analogues, though not far better than the previous seen results. Other halogenated molecules were tested, retaining the potency against *A. baumannii*. Chlorinated analogues for example exhibited approximately the same efficiency as fluorinated ones. However, 2-(2-(4-chlorophenyl)hydrazono)pyrrolidine **162**, bearing a *para*-chlorine atom, was found to be one-fold more active than the fluorinated one.

Next, the brominated hydrazones were also tested for their antibacterial efficiency, and surprisingly, (4-bromophenyl)hydrazono)pyrrolidine **166** proved to be a very strong antibacterial candidate, with a MIC_{95} value between $0.078 < MIC_{100} < 0.156$. Considering bromo- group position tolerance, we can observe a certain decrease in activity for the *meta*-, followed by the *ortho*- position, at one-fold step.

Electron-withdrawing groups such as nitro- and cyano- were also added on the ring as substituents. Both *para*-nitro- and cyano-phenylhydrazones **169** and **171** presented very good biological activities lacking cytotoxicity, and more interesting, the methyl ester molecules **183** and **184** seemed to be one-fold less efficient then the simple pyrrolidines. Concerning the nitro-group, *para*-position derivative **169** is favored. The last phenyl substituted derivative is the

sulfonic acid analogue **172**, which failed to keep any activity of the HIT molecule, probably because of its solubility which was particularly low.

Further in this study, the phenyl ring was replaced with other moieties such as pyridine. From the obtained results we can assess that the biological activity of 4-pyridine derivative **186** is one-fold better than the 2-pyridine derivative **185.** However, while the pyridine ring successfully kept the *A. baumannii* activity, if bulkier moieties such as benzothiazolyl- or naphthyl- are integrated, a decrease of activity was observed, compounds **173** and **174** presenting MIC_{100} of 25 and 10 µg/mL, respectively.

II.2.2.3 Link and lactam motif modifications

In our efforts to improve the pharmacological properties of the HIT compound, and also to understand the key motifs for the *A. baumannii* antibacterial activity, some chemical modifications were done around the scaffold as depicted in **Figure 40**, the corresponding molecules being tested for their potency (**Table 23**).

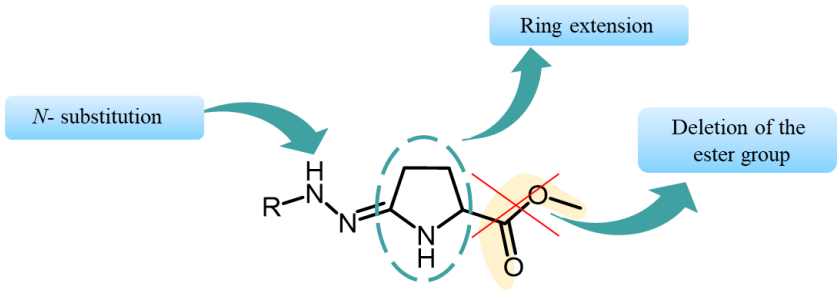


Figure 40 *Structural modifications around the HIT compound*

Interestingly, as seen in the case of the previous *A. baumannii* antibacterials family, the substitution of the second nitrogen from the link, led to the loss of the activity. However, considering the chemical differences between the two families, no link should be assumed, as probably their mechanisms of action might differ.

Another interesting fact is that the deletion of the ester groups did not clearly influence the activity, derivative **153** presenting a good antibacterial profile, with a MIC_{100} ranging between 0.391 and 1.25 mg/mL which is similar to the results of **HEI 604**. The low influence of the ester group has already been observed for different couples of derivatives (**Table 22**,

compounds **154/177**; **157/178**; **159/179**; **162/180**; **163/181**; **165/182**; **169/183**; **171/184**; **HEI3472/186**). Moreover, the modification of the ring apparently is not inducing an activity loss, 2-(2-(4-chlorophenyl)hydrazono)piperidine hydrochloride **189** being actually more active than the 5-membered ring analogue **162**, which will be included in **Table 23**.

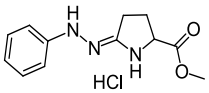
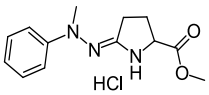
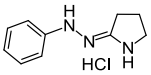
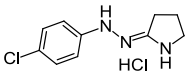
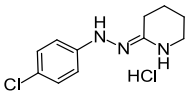
N° compound	N° HEI	Structure	Liquid MIC_{100} (µg/mL)
HEI library	604		0.625< MIC_{100} < 1.25
HEI library	619		25
153	3337		0.391 < MIC_{100} < 1.25
162	3338		0.156 < MIC_{100} < 0.313
189	3368		0.078

Table 22 *A. baumannii (ATCC179778) screening results of different hydrazones*

II.2.2.4 Bug specificity of the selected molecules

As depicted before for the carbazide family, in order to prove *A. baumannii* selectivity, a panel of fungal and bacterial strains were also tested (**Table 24**). We found that derivatives such as HEI 604 or 606, are lacking activity on the 6 screened strains, being completely selective against *A. baumannii*.

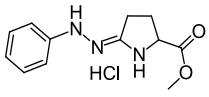
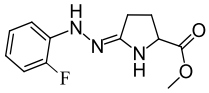
N° HEI	Structure	*MIC₁₀₀ (µg/mL)*						
		E. coli	*S. aureus*	*K. pneumoniae*	*A. baumannii*	*P. aeruginosa*	*C. albicans*	*C. neoformans*
604		>50	>50	>50	0.625 < MIC_{100}< 1.25	>50	>50	>50
606		>50	>50	>50	0.313 < MIC_{100}< 0.625	>50	>50	>50

Table 23 *Bug specificity of selected molecules*

II.2.2.5 Perspectives on optimized hits

Considering that in the previous sub-chapter, regarding antibacterial activity of hydrazides, we discovered a link between the antibacterial profile and their antioxidant activity, we considered it useful to test the active hydrazones for their efficiency as radical scavengers by the DPPH test.

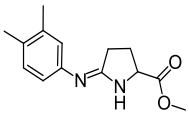
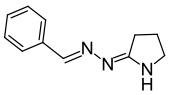
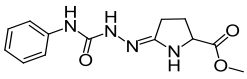
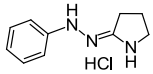
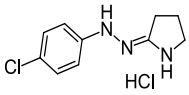
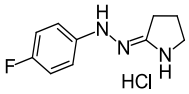
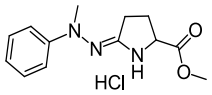
N° compound	N° HEI	Structure	Liquid MIC (µg/mL) against *A. baumanii*	Antioxidant activity IC_{50} (µM)
HEI library	596		>100	>100
194	3427		>100	>100
HEI library	815		>100	>100
153	3337		$0.391 < MIC_{100} < 1.25$	3.6
162	3338		$0.156 < MIC_{100} < 0.313$	4.6
159	3340		$0.195 < MIC_{100} < 0.625$	2.4
HEI library	619		25	25
	Vitamin C		> 100	15

Table 24 *Antioxidant activities of selected molecules*

In the same manner, considering the obtained results, we can assume that there is a direct link between the two activities: antibacterial (*A. baumannii*) and antioxidant. As we can observe, the activity loss induced by the imine, azine or *N*-acylhydrazone linkers is also

reflected in the radical scavenger capacity of these molecules. Whereas, considering **153**, **162** and **159** hydrazones, presenting anti-*A. baumannii* profile, we can notice a direct correlation of the two activities, however, not proportional. Moreover, as seen in the hydrazides family, if the second nitrogen from the link is substituted, we observe an antibacterial activity loss but also an antioxidant activity decrease. As well as for the previous family, the mechanistic pathway is still unelucidated, and these results are only a hint linking a possible ROS mechanism, which will have to be proved.

II.2.2.6 Conclusions

Within this subchapter, a series of hydrazonopyrrolidines was described as a novel class of antimicrobial agents. Around the HIT molecule, a well covering SAR was done, and the most important conclusions are depicted in **Figure 41**.

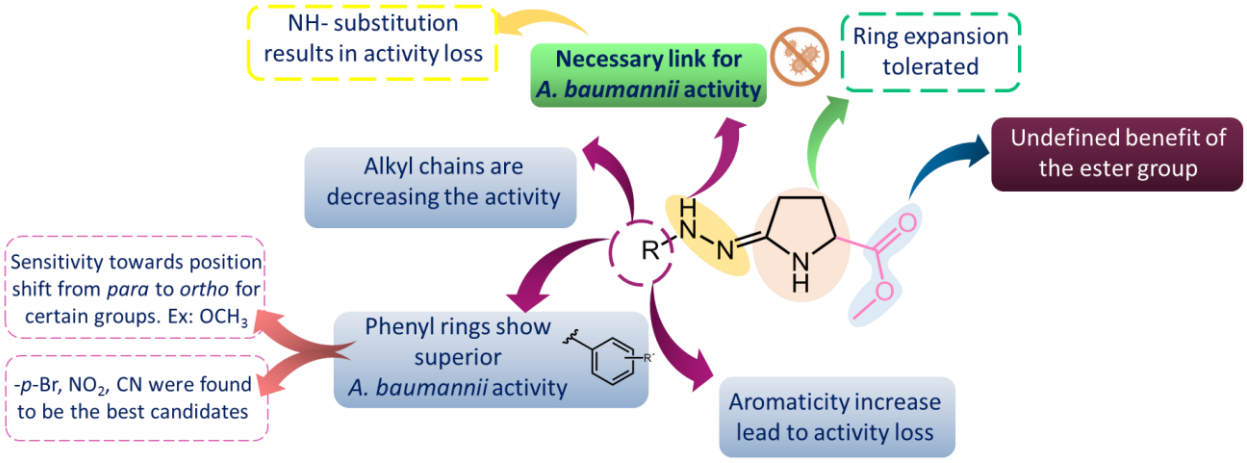


***Figure 41** SAR of hydrazones series regarding A. baumannii activity*

The structure-activity relationship is defined by the following:

(1) the hydrazone link is necessary for the *A. baumannii* activity;

(2) link NH-group substitution results in the loss of antibacterial potency;

(3) it has been observed that alkyl chains are leading to activity decrease;

(4) considering the phenyl substitution, the substitution is permissible, without significant losses in potency while the solubility is not strongly decreased, however the ratio benefit/risk is strongly impacted by electron-donating groups;

(5) the most tolerated position is *para*-, and certain groups such as methoxy- for exemple, are sensitive to position shift on the ring, decreasing the potency of the molecule, however this lead should be more studied;

(6) regarding the phenyl substituent, the bromine derivatives were found to be the most active, along with nitro- and cyano- hydrazones, all having very good benefit/risk ratios;

(7) considering the motifs, the ester group did not play an important role on activity, but it could be important for other pharmacological properties such as stability which will have to be studied.

(8) the ring expansion is very well tolerated.

The selectivity towards *A. baumannii* was also tested on a panel of 4 bacteria and fungi. The screening confirmed that this type of hydrazones are selective for *A. baumannii* strain, showing no activity under 50 μg/mL on other microorganisms. Another important feature of this series, is that except some electron-donating phenylhydrazones, most of the antimicrobials present no risk of cytotoxicity, having very good benefit/risk ratios.

As a result, from our modulation around the preliminary identified molecule, we found **166** as a lead compound for further profiling. 2-(2-(4-Bromophenyl)hydrazono)pyrrolidine is around 5 folds more potent compared to the HIT on the drug resistant strain *A. baumannii*, presenting suitable selectivity and no cytotoxicity. Therefore, compound **166** is the second promising lead for optimization as an antibiotic drug targeting specifically *A. baumannii*, with a low molecular weight, very good solubility being obtained as a salt and excellent antibacterial potency of 0.078-0.156 μg/mL.

II.2.3 *A. baumanii* leads obtained within the project

Acinetobacter baumannii is considered today one of the most drug resistant pathogen, associated with hospital-acquired infections, becoming a major threat to the global health, with a rapid resistance expansion to mostly all new antibiotics.[155] As respond to the call for "Research and Development of new antibiotics", within this project we have developed two series which are selectively targeting *A. baumannii in vitro*, while their potency *in vivo* is to be

[155] Antunes L, Visca P, Towner K. *Acinetobacter baumannii*: evolution of a global pathogen. *Pathogens and disease*. 2014, 71, 3, p. 292-301.

tested after the evaluation and confirmation that these molecules are active on a larger panel of drug resistant *A. baumannii* strains.

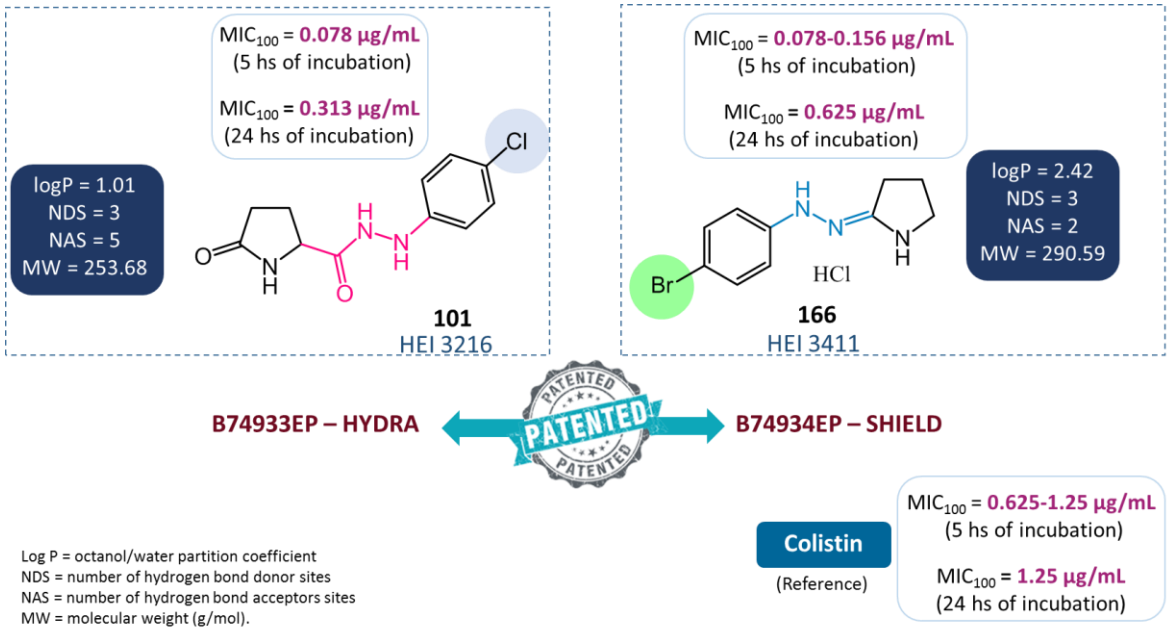


Figure 42 *Lead molecules as A.baumannii antibacterials*

As a result of the extended synthetic modifications of the HIT molecules which were found in University of Queensland, two lead molecules from each of the two series, were identified. *In vitro*, the two leads present a better antibiotic profile compared to the colistin which is considered as the last-resort therapy for the treatment of infection caused by Gram-negative bacilli, which is becoming less and less efficient due to the emergence of colistin-resistant strains. Besides this, the two lead compounds meet the Lipinski "Rule of five" criteria with LogP in −0.4 to +5.6 range, NDS < 5, NAS < 10 and MW < 500 g/mol, showing druggability. The herein results, afforded us to obtain two patents, one on each of the two discovered families, HYDRA being the first patented family of hydrazides while SHIELD is the second patented family, of hydrazones, around which one maturatation project was launched in January 2019.

II.3 Antifungal results and discussion

One of the initial goals of the project was the development of new antifungal agents which could be formulated into smart paints and coatings having antifungal properties. As a consequence, according to their industrial partners, IFMAS provided us a list of fungi and molds of interest to be screened. This list comprises 8 fungal strains: *A. oryzae*, *S. sclerotiorum*, *B. cynerea*, *F. solani*, *A. alternata*, *C. cladosporioides*, *P. variotii*, *P. ochrochloron*, to which 4 yeasts were added such as *G. candidum, C. krusei, C. tropicalis and C. pseudotropicalis*. In the context of paints and coatings, these species are of interest because they could provoke consumer allergies or diseases as well as modifications of the properties (odor, color, viscosity) of the paint with the development of fungal colonies.

II.3.1 Fungi of our interest

Aspergillus oryzae

Aspergillus is one of the oldest genus of fungi that received its name from Pier Antonio Micheli, a Catholic priest and a famous botanist, in 1729. The name, *Aspergillus*, comes from "Aspergill" which is a brush used in the Catholic Church, to sprinkle holy water. Nowadays, the fungus is found worldwide, colonizing walls whether water damage occurred, being also found in decomposing organic matter.[156] Airbone spores, which are small enough to get into alveoli, are responsible for pulmonary *Aspergillus* infection, especially if the host is predisposed to disease.

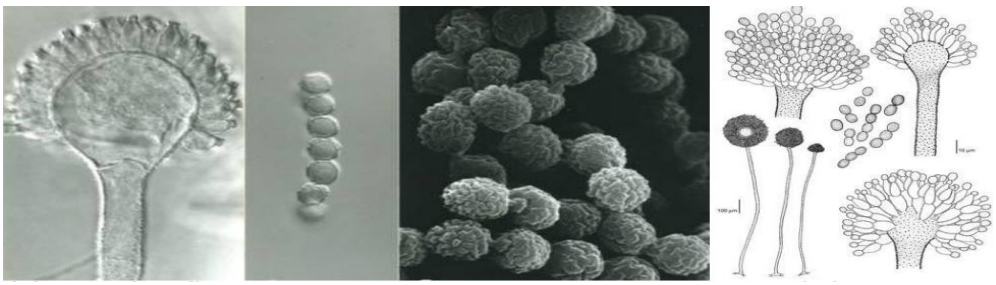


Figure 43 *Aspergillus oryzae morphology*[157]

Sclerotinia sclerotiorum

[156] Greenberger, P. A. Allergic bronchopulmonary aspergillosis. *J. Allergy Clin. Immunol.* 2002, 110, p. 685–692.
[157] S.S. Tzean and J. L. Chen Mycobank.

Sclerotinia sclerotiorum, known by different names such as: blossom blight, watery soft rot, stem rot, crown rot and commonly - white mold, is one of the most devastating plant pathogen.[158] The fungus is able to infect more than 400 species of plants a large part being crops and numerous weeds. Its predilection is towards dicotyledonous crops such as sunflower, oilseed rape, chickpea, peanut, soybean, lentils, edible dry bean, and various vegetables, but also monocotyledonous species such as tulip and onion.[159] In United States only, the annual losses are exceeding 200 million dollars, representing also an economical problem. Because of the extensive crop damage and the development of fungus resistance, research on this pathogen concerning the biology and control of it appears of great importance.

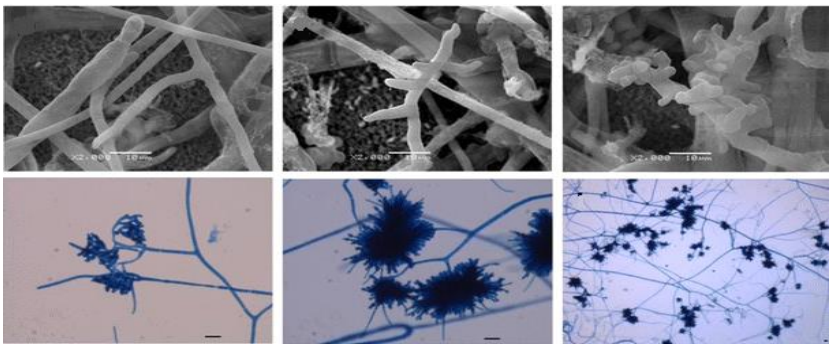


Figure 44 *A Sclerotinia sclerotiorum morphology*[160]

Botrytis cinerea

Botrytis cinerea, known to cause the gray mold in more than 500 plant species, is one of the most extensively studied necrotrophic fungal pathogens.[161] Each year, this pathogen has a disastrous economic impact on various economically important crops including grape, strawberry, and tomato being able to survive inside stems, leaves, flowers, fruits, and seeds.[162] The annual economic losses of *B. cinerea* easily exceed $10 billion worldwide.[163] Controlling

[158] Purdy L.H. Sclerotinia sclerotiorum: history, diseases and symptomatology, host range, geographic distribution, and impact, Conference Proceedings, Phytopathology, 1979.

[159] Boland G.J., Hall R. Index of plant hosts of Sclerotinia sclerotiorum. *J. Plant Pathol*, 1994, p. 93-108.

[160] Ordóñez-Valencia C., Ferrera-Cerrato R., Quintanar-Zúñiga R.E., Flores-Ortiz C.M., Márquez Guzmán G.J., Alarcón A., Larsen J., García-Barradas O. Morphological development of sclerotia by Sclerotinia sclerotiorum: a view from light and scanning electron microscopy. *Ann. Microbiol.*, 2015, 65, 2, p. 765-770.

[161] Williamson B., Tudzynski B., Tudzynski P., van Kan J.A. Botrytis cinerea: the cause of grey mould disease. *Mol. Plant Pathol.* 2007, 8, p. 561–580.

[162] Dean R., Van Kan J.A., Pretorius Z.A., Hammond-Kosack K.E., Di Pietro A., Spanu P.D., Rudd J.J., Dickman M., Kahmann R., Ellis J., Foster G.D. The top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol*. 2012, 13, p. 414–430.

[163] Weiberg A.1., Wang M., Lin F.M., Zhao H., Zhang Z., Kaloshian I., Huang H.D., Jin H. Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science*, 2013, 342, p. 118–123.

B. cinerea is difficult because it has a broad range of hosts, various attack modes and also can survive in favorable or unfavorable conditions.[164]

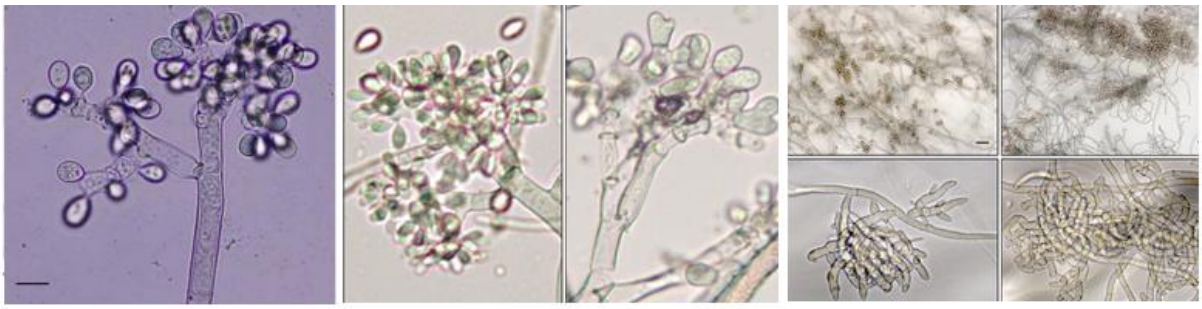


Figure 45 *Botrytis cinerea morphology*[165]

Fusarium solani

Fusarium solani is a frequently isolated fungi from soil and plant debris being associated with serious invasive mycoses in immunocompromised and immunosuppressed patients.[166] The *Fusarium solani* complex species (FSSC), is a diverse complex of over 45 phylogenetic and/or biological species.[167] These species are relatively morphologically similar generally identified under the name *F. solani*. They are ubiquitous in soil and plant material, where they act as decomposers, but they are also hosting pathogens of agriculturally important plants such as peas, beans, potatoes and cucurbits.

Figure 46 *Fusarium solani morphology*[168]

[164] Fillinger S., Elad Y. Botrytis-the Fungus, the Pathogen and its Management in Agricultural Systems. *Pub. Springer*, New York, 2016.
[165] Antal Z., Rascle C., Cimerman A., Viaud M., Billon-Grand G., Choquer M., Bruel C. The Homeobox BcHOX8 Gene in *Botrytis Cinerea* Regulates Vegetative Growth and Morphology. *PLoS ONE*, 2012, 7, 10, p. 48134.
[166] Booth C. The Genus Fusarium. Commonwealth Mycological Institute, Surrey, 1971, England.
[167] O'Donnell K. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. *Mycologia*, 2000, 92, p. 919-938.
[168] Chehri K., Salleh B., Yli-Mattila T., Reddy K.R., Abbasi S. Molecular characterization of pathogenic *Fusarium* species in cucurbit plants from Kermanshah province, Iran. *Saudi J. Biol. Sci*. 2011, 18, p. 341–351.

Besides this, they have been increasingly reported to cause opportunistic infections in humans and animals, such as systemic infections with a high mortality rate, as well as eyes and skin infections.[169] Patients suffering from neutropenia, a particular category of strongly immunocompromised patients, are susceptible to spreading the infection from superficial or subcutaneous initiation, dissemination which is usually fatal.[170]

Alternaria alternata

Alternaria alternata, the most common species of genus *Alternaria*, is a well-known fungus, occurring cosmopolitically in various environments. During the year, the spores can be found in the organic constituents of soil. Between spring and autumn, they become airborne and are therefore even more spread.[171] Though usually considered saprophytic contaminants, *Alternaria* is now held to be responsible for a number of diseases.

The first mention of an allergy caused by molds dates back to the very end of the seventeenth century. In 1698, Sir John Floyer was the one who noted the phenomenon of an adverse asthmatic reaction to mold. *Alternaria* is now known as an important allergen, which in 1930s was linked for the first time with allergic asthma.[172, 173] Now, the role of allergies to *Alternaria* in the development and exacerbation of asthma gains more and more recognition in medicine. As a parasitic species, *Alternaria* attacks plants, animals, and humans, causing also alternariosis - a type of mycosis especially prevalent in immunocompromised patients.[174]

Figure 47 *Alternaria alternata morphology*[175]

[169] Gugnani H.C., Talwar R.S., Njoku-Obi A.N. Mycotic keratitis in Nigeria. A study of 21 cases. *Br. J. Ophthalmol.* 1976, 60, 9, p. 607-613.
[170] Gupta A.K., Baran R., Summerbell R.C. Fusarium infections of the skin. *Curr. Opin. Infect Dis.* 2000, 13, 2, p. 121-128.
[171] Misaghi I.J., Grogan R.G., Duniway J.M., Kimble K.A. Influence of environment and culture media on spore morphology of Alternaria alternata. *Phytopathology*, 1977, 68, p. 29–34.
[172] Pavon Moreno M.A., Gonzalez Alonso I., de Martin Santos R., García Lacarra T. The importance of genus Alternaria in mycotoxins production and human diseases. *Nutr. Hosp.* 27, p. 1772–1781.
[173] Hopkins J.G., Benham R.W., Kesten B.M. Asthma due to a fungus Alternaria. *JAMA*, 1930, 94, p. 6–10
[174] Bush R.K., Prochnau J.J. Case study: Alternaria-induced asthma. *J. Allergy Clin. Immunol.* 113, p. 227–234.
[175] McKenzie E. Alternaria alternata *(Alternaria alternata).* PaDIL - http.p.//www.padil.gov.au.

Cladosporium cladosporioides

Cladosporium species are one of the most common type of fungi worldwide, being isolated from almost any environmental source, being found in almost every geographic location.[176] The *Cladosporium* species are mostly isolated from soil and plant material, where they are frequently encountered or secondary invaders on follicular lesions, concomitant with other plant-pathogenic fungi. Several species are important pathogens of plants and some are also able to affect animals, including humans.[177] *Cladosporium* is both a common and important allergen,[178] which together with *Alternaria* is one of the most important fungal airway having the ability to sporulate easily, the two being the main fungal causes of asthma and hay fever in the Western Hemisphere, as well as some type III allergies (hypersensitivity pneumonitis). *Cladosporium* sp. has also been found to cause chronic rhinosinusitis being found in nasal mucus of patients suffering from such health problem.[179] Though it may not seem, this mold is considered to be non-pathogenic.

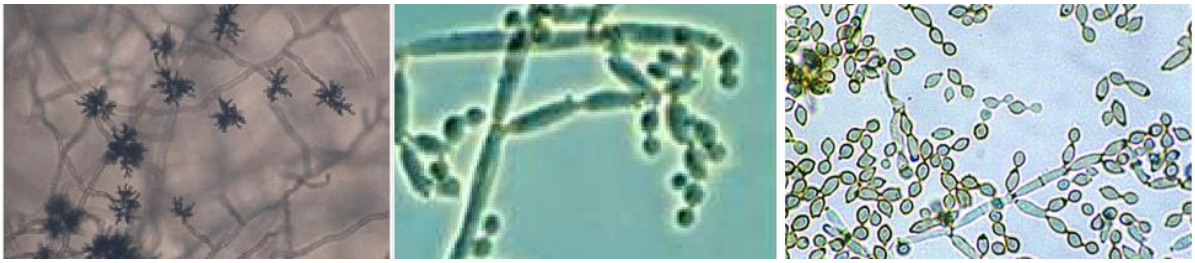


Figure 48 *Cladosporium cladosporioides morphology*[180]

Paecilomyces variotii

Paecilomyces species is a cosmopolitan filamentous that exists in air, inhabits also the soil, decaying plants, and food products. The fungus resembles *Penicillium* fungi from which can be easily differentiated by its branched conidiophores and cylindrical conidiogenous cells with tapering tips. *Paecilomyces variotii* is usually considered a contaminant but it has been also reported as a causative agent of human mycoses, at the moment being of clinical interest

[176] Bensch K., Braun U., Groenewald J.Z., Crous P.W. The genus *Cladosporium*. *Stud. Mycol.* 2012, 72, 1, p. 401.
[177] Ma X., Gu Y., Liu X., Desheng L., Shanshan L., Jiafa H., Chengdong W., Sanjie C., Xiaobo H., Xintian W., Jiaxue R., Cao D., Changcheng L., Yufei T. Phaeohyphomycotic dermatitis in a giant panda (Ailuropoda melanoleuca) caused by *Cladosporium cladosporioides*. *Med. Mycol. Case Rep.*2013, 2, p. 119–121.
[178] David J.C. A contribution to the systematics of Cladosporium. Revision of the fungi previously referred to Heterosporium. *Mycological papers*, 1997, 172, CAB International, Wallingford, United Kingdom.
[179] Sellart-Altisent M., Torres-Rodríguez J.M., Gómez de Ana S., Alvarado-Ramírez E. Nasal fungal microbiota in allergic and healthy subjects. *Rev. Iberoam. Micol.* 2007, 24, p. 125–130.
[180] Mushimiyimana I., Tallapragada P. Optimization of Process Parameters for Biosynthesis of Cellulase by Cladosporium Cladosporioides Using Agro Wastes, 2013.

because of its pathogenicity and resistance to antifungal agents. *Paecilomyces variotii* is known to produce serious infections in immunocompromised patients such as fungemia,[181] pyelonephritis,[182] sinusitis,[183] endocarditis,[184] and peritonitis[185].

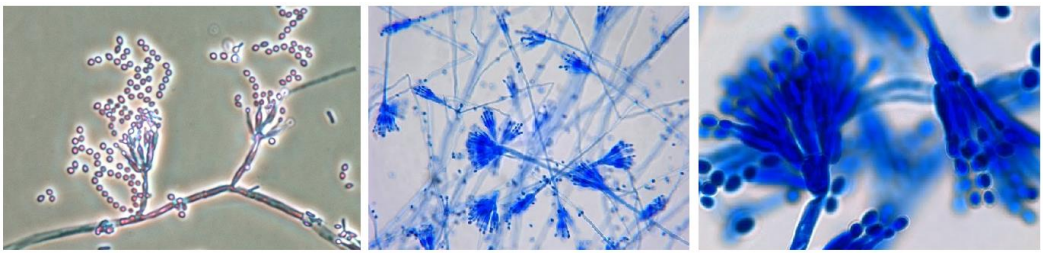


Figure 49 *Paecilomyces variotii morphology*

Penicillium ochrochloron

Penicillium is an anamorph genus which phylogenetically belongs to *Trichocomaceae*. The name *Penicillium* was introduced by Link in 1809 and is derived from *penicillus*, which means "little brush" coming from the resemblance of the spore producing structures (conidiophores). *Penicillium* is found in soil, decaying vegetation, air, causes food contamination, colonizes leather objects and is an indicator organism for dampness indoors. Some *Penicillium* species produce toxic compounds (mycotoxins). *Penicillium ochrochloron* spores can trigger allergic reactions in individuals sensitive to mold. Therefore, the health of occupants may be adversely affected in an environment that has an amplification of *Penicillium*.

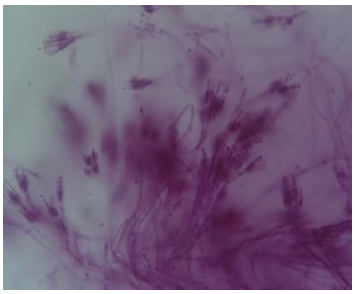


Figure 50 *Penicillium morphology*

Geotrichum candidum

[181] Shing M.M., Ip M., Li C.K. *Paecilomyces variotii* fungemia in a bone marrow transplant patient. *Bone marrow Transplant*, 1996, 17, p. 281-283.
[182] Sherwood J.A., Dansky A.S. *Paecilomyces pyelonephritis* complicating nephrolithiasis and review of Paecilomyces infections. *J. Urol.*, 1983, 130, p. 326-8.
[183] Thompson R.F., Bode R.B., Rhodes J.C. *Paecilomyces variotii*: an unusual case of isolated sphenoid sinusitis. *Arch. Otolaryngol. Head Neck Surg*. 1988, 114, p. 489-496.
[184] Kalish S. B., Goldschmidt R., Li C., Knop R., Cook F.V., Wilner G., Victor T.A. Infective endocarditis caused by *Paecilomyces variotii*. *Am. J. Clin. Pathol*. 1982, 78, 2, p. 249–252.
[185] Marzec A., Heron L.G., Pritchard R.C., Butcher R.H., Powell H.R., Disney A.P., Tosolini F.A. *Paecilomyces peritonitis* in peritoneal dialysate. *J. Clin. Microbiol.* 1993, 31, p. 2392-2395.

Geotrichum is a species of fungi that can be found in every environment in nature, in soil, in water and in air. *G. candidum* is usually sensitive to systemic antifungal agents.[186] As it can be found in milk products, plants and cereals, it has also been shown to be found endogenous normal in the human flora (mucus and faeces).[187] *G. candidum*, mostly saprophytic, can cause clinical picture called Geotrichosis, but fortunately *G. candidum* is usually sensitive to systemic antifungal agents. Infection caused by this fungus may be either bronchial or pulmonary. Normally, bronchial infection requires no special treatment other than routine therapy. However, pulmonary invasion, if not treated with the specific treatment, can be fatal. In the case of serious infections, the treatment response becomes difficult in immunosuppressive cases.[188]

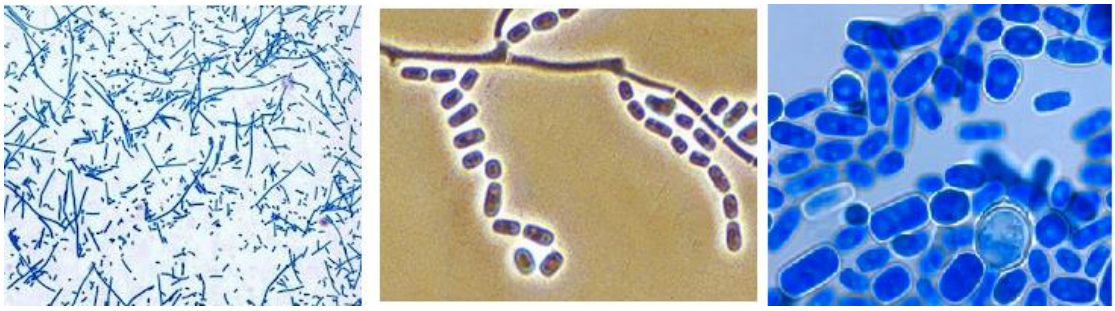


Figure 51 *Geotrichum candidum morphology*

[186] Alvarez-Lerma F., Palomar M., Leo´ n C., Olaechea P., Cerdá E., Bermejo B. Fungal colonization and/or infection in intensive care units. Multicenter study of 1,562 patients. *Med Clin (Barc)*, 2003, 121, p. 161–166.
[187] Walsh T.J., Groll A., Hiemenz J., Roilides E., Anaissie E. Infections due to emerging and uncommon medically important fungal pathogens. *Clin. Microbiol. and Infect.* 2004, 10, p. 48–66.
[188] Chitasombat M.N., Kofteridis D.P., Jiang Y., Tarrand J., Lewis R.E., Kontoyiannis D.P. Rare opportunistic (non-Candida, non-Cryptococcus) yeast bloodstream infections in patients with cancer. *J. Infect.*, 2012, 64, p. 68-75.

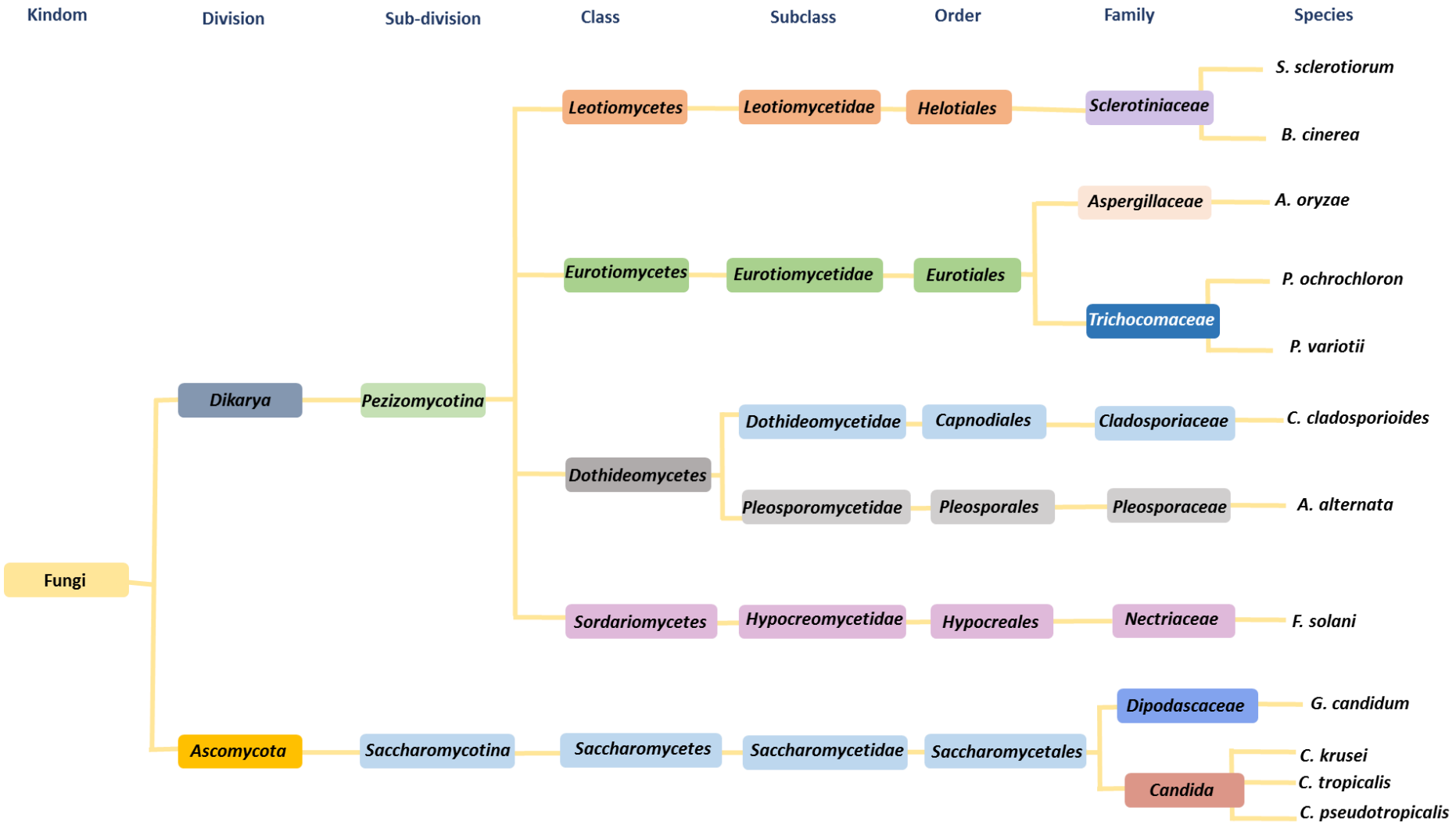


Figure 52 *Phylogenetic tree of the tested fungi*

II.3.2 Pyrrolidones - antifungal results and properties

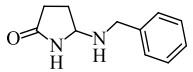
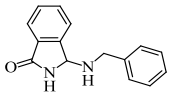
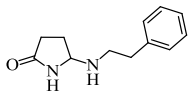
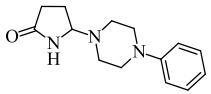
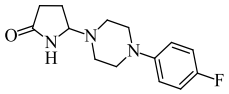
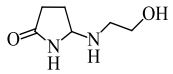
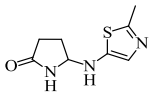
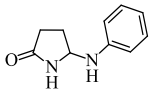
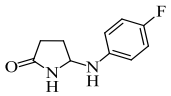
In the frame of this project we designed and synthesized a series of multi-substituted ɤ-lactam derivatives starting from the pterolactam through environmentally friendly and mild methods as previously described. In order to study the biological impact of different linkers, simple aniline, alkoxy, hydrazine and hydrazides derivatives were first tested, the results being presented in **Tables 26-29**.

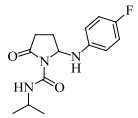
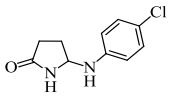
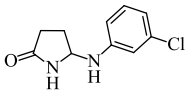
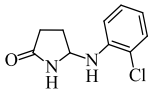
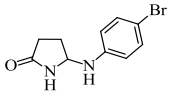
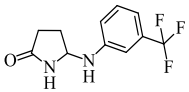
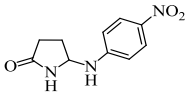
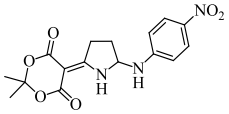
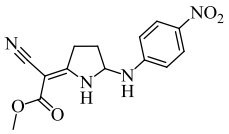
Analyzing the preliminary screenings at a concentration of 100µg/mL, the comparison of the activities and structures of the compounds in **Tables 26-29** showed that aliphatic chain structures are decreasing the antifungal properties of the studied compounds, meanwhile substitution of the benzene ring and linker type between ɤ-lactam and aromatic moiety, significantly influenced the activity of the pyrrolidone derivatives.

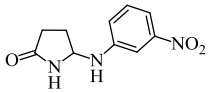
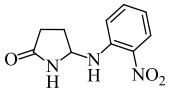
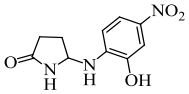
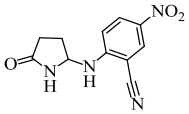
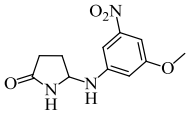
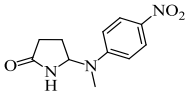
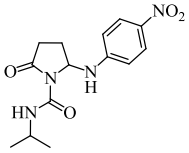
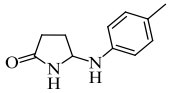
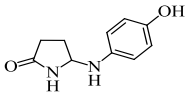
As a general overview, from the antifungal tests, it can be stated that amino compounds showed significantly higher activity, compared to the corresponding alcohol derivatives. Besides this, among the nitrogen linkage molecules, amines and hydrazines families showed an enhanced antifungal inhibitory rate.

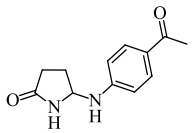
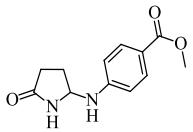
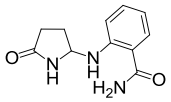
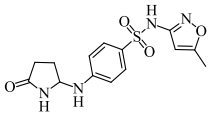
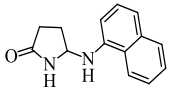
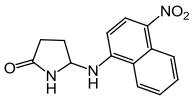
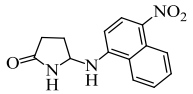
Focusing on 5-aminopyrrolidin-2-ones, we can notice a certain selectivity towards *Fusarium solani*, most of the compounds being active with an inhibitory rate higher than 50%. Though the simple aniline derivative **12** completely lacks notable activity, even on *F. solani*, its inhibition rate being 10%, the benzylamine derivative **4** and the 5-(phenethylamino)pyrrolidin-2-one **5** have 33% respectively 95% inhibition rate, meaning there could be a link between lipophilicity and activity. On the other part, though structurally similar, phenylpiperazin-1-yl compound **6** is lacking any activity while the 4-fluoro- derivative presents 100% antifungal inhibition on *F. solani* at 100 µg/mL.

Going further with other tested 5-aminopyrrolidin-2-ones, among the halogenated aniline derivative, 4-fluoro- and 4-bromo- compounds **13** and **17** had the same inhibition rate of 70%, while the chlorinated derivatives **14**-**16** are lacking any antifungal activity. Considering the *N*-substituted 4-fluoro- derivative, **86**, while the *F. solani* activity is kept, we can see that *P. ochrochloron* and *S. sclerotiorum* activities of **13** are now lost.

N° compound	N°HEI	Structure	BC	SS	AO	PV	PO	AA	CC	FS	GC	CK	CP	CT
4	2907		0	0	8	0	0	0	0	33	20	12	35	0
83	3161		0	0	14	8	5	0	0	23	36	0	2	0
5	2971		0	15	24	12	26	0	0	95	0	32	0	5
6	2948		36	6	12	19	30	0	21	18	31	0	0	5
7	2951		0	0	9	15	0	15	31	100	3	0	20	22
10	2908		0	30	15	12	19	0	5	65	53	0	0	5
11	2996		0	12	14	9	19	0	0	3	31	0	0	0
12	2906		0	28	37	2	31	0	26	10	0	29	0	17
13	2910		0	32	0	15	39	0	8	70	0	35	32	22

86	3018		0	0	23	22	0	15	8	68	0	23	8	12
14	2913		10	0	10	23	0	0	15	45	20	19	8	31
15	2918		0	10	0	17	21	43	0	2	18	28	40	32
16	2966		0	11	0	13	32	0	24	29	21	0	25	33
17	2917		0	15	0	23	6	0	24	71	0	5	5	28
18	2911		4	0	14	15	0	0	5	28	0	8	0	23
19	2912		26	0	0	17	16	40	62	100	1	42	10	25
84	3214		0	0	0	41	26	0	34	0	0	62	20	22
85	3221		33	0	0	24	40	12	33	10	0	82	28	0

20	3222		0	6	12	3	0	25	28	43	0	0	12	21
21	3228		0	0	22	53	7	0	0	49	0	25	5	31
22	3215		0	11	44	46	33	37	36	0	8	39	43	40
23	3224		0	8	10	0	0	0	40	52	0	32	33	34
24	2991		18	0	39	96	98	75	15	57	24	38	97	73
25	3220		0	11	34	0	0	22	2	84	36	30	18	51
87	3018		15	18	21	3	0	10	38	65	41	10	18	38
26	2915		0	2	6	51	1	0	15	36	0	25	0	35
27	2990		0	29	0	30	11	0	0	100	10	5	51	31

28	2916		0	0	16	31	2	20	0	35	0	2	28	32
29	2914		14	0	8	1	15	0	18	35	32	0	0	0
30	2987		0	4	0	19	44	0	7	55	0	23	18	33
33	3272		26	12	0	52	76	86	26	64	41	70	58	38
34	2902		0	66	0	16	0	34	0	0	0	0	2	0
35	3223		43	6	84	54	83	0	29	98	43	70	21	48
36	2997		0	79	0	40	47	3	0	42	72	43	17	

B. cynerea - BC, *S. sclerotiorum* - SS, *A. oryzae* - AO, *P. variotii* - PV, *P. ochrochloron* - PO, *A. alternata* - AA, *C. cladosporioides* – CC, *F. solani* - FS, *G. candidum* - GC, *C. krusei* - CK, *C. tropicalis* – CT and *C. pseudotropicalis* - CP.

Table 25 *Inhibition of growth (%) of some aniline derivatives at 100µg/mL*

90-100	80-89	70-79	60-69	50-59

A notable molecule which's structure-activity relationship was extended is **19**, the 4-nitro-aniline derivative, that apparently targeted the same fungus *F. solani*, having an inhibition rate of 100% at 100 µg/mL. Besides this, the same molecule has a slight antifungal activity towards *C. cladosporioides* growth. The modification of the carbonyl group results in a loss of activity towards *F. solani* but increases the anti-*C. krusei* potency, compounds **84** and **85** exhibiting 62, respectively 82% of inhibition at 100 µg/mL on the last one species.

Considering the modulation of nitro- group position, we can notice that only the *para*-position molecule **19** presented some inhibitory activity. Surprisingly, with the introduction of substituents, though 2-hydroxy-4-nitrophenyl compound **22** completely lost its antifungal activity, 2-cyano-4-nitrophenyl derivative **23** kept one fold. On the other hand, 3-methoxy-5-nitroaniline derivative **24**, though it also presented 57% inhibition of *F. solani*, it gained 5 more activities, on *A. alternata*, *C. pseudotropicalis*, *C. tropicalis*, *P. variotii* and *P. ochrochloron*, making this molecule a broad spectrum antifungal. Further, we tested molecules **25** and **87** which both have in common the *N*-substitution and which did nott seem to be influencing so much the inhibitory rate, both of them continuing to be active on *F. solani*.

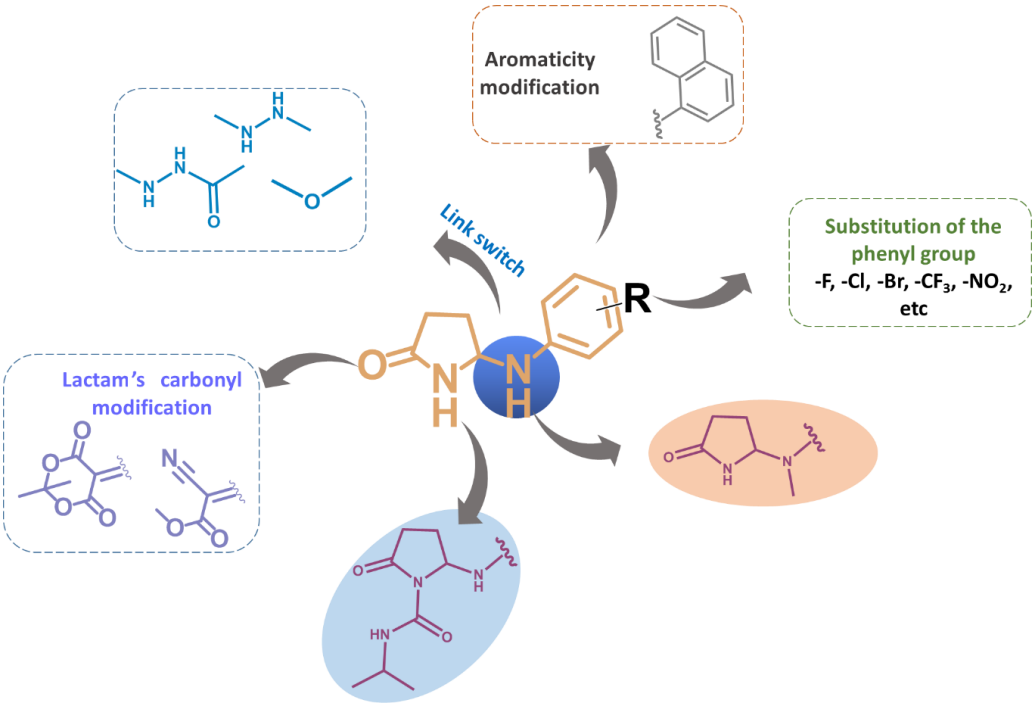


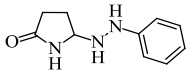
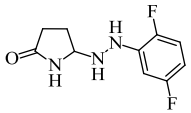
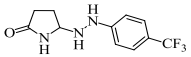
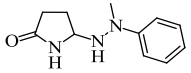
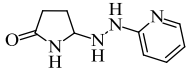
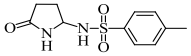
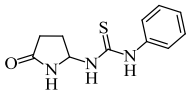
Figure 53 *Structure modifications around the lactam ring*

The introduction of electron-donating groups on the phenyl moiety such as methyl-, resulted mostly in an activity loss, rendering compound **26** unactive. On the other part, 4-hydroxy- derivative, **27** had a very good inhibition rate on the same fungus, *F. solani*, at 100 µg/mL.

Further, we were delighted to see that sulfamethoxazole pterolactam derivative **33** has a good broad activity profile, being active towards *F. solani*, *A. alternata*, *C. krusei*, *P. variotii* and *P. ochrochloron*, representing the second most active compound belonging to this series, with *A. alternata* antifungal activity. When the phenyl moiety was replaced by a naphthyl-group, (compound **34**) there were not any important changes in term of biological activity, except some moderate activity towards *C. cladosporioides*. However, compared to it, the 4-nitro-naphthyl derivative, compound **35**, at 100 µg/mL presented activity on *F. solani*, and except that, it has also some good activity toward 3 fungi species from the same order of *Eurotiale*: *A. oryzae*, *P. variotii* and *P. ochrochloron* and also *C. krusei*. 2-Aminoquinolin-8-ol derivative **36** is keeping the simple naphthyl derivative activity towards *C. cladosporioides* and besides it, being the first one from this series, presented anti-*G. candidum* activity.

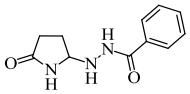
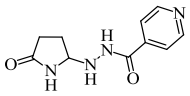
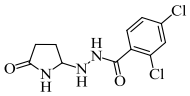
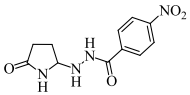
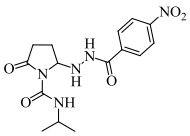
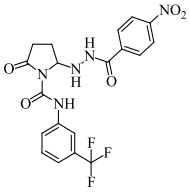
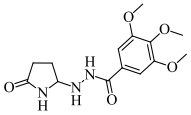
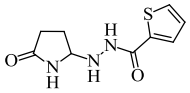
Switching to other link types, such as hydrazine derivatives, we were surprised to discover that compound **37**, the simple phenylhydrazine molecule, had a good inhibition on 9 from the 12 tested strains, and also compared to anilines derivatives, is active on fungi belonging to the *Sclerotiniaceae* family as well (**Table 27**). Analyzing further the results, we observed that the substitution of the phenyl ring results in the narrowing of the antifungal spectrum, compared with **37** which is targeting nine fungal strains. It's noteworthy that fluorinated molecules **38** and **39**, present both anti-*Fusarium solani* activity. Trifluoromethylated derivative **38** is the only compound being active towards *C. cladosporioides*. The *N*-substitution of the 5-(2-phenylhydrazinyl)pyrrolidin-2-one (compound **40**), results also in the sensitivity loss towards species such as *C. tropicalis*, *A. oryzae*, and *Scloriniaceae* tested fungi. However, the molecule kept the *F. solani* activity and exhibited decreased inhibition rates toward *C. pseudotropicalis*, *P. variotii* and *P. ochrochloron* activities. Replacing the phenyl- by a 2-pyridyl- group caused a complete activity loss**,** compound **41** presenting only some moderate *F. solani* activity.

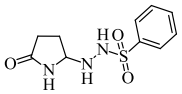
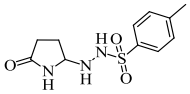
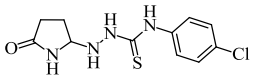
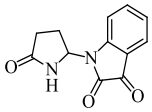
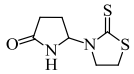
The next tested 5-pyrrolidin-2-ones were the hydrazides derivatives. Having an extra carbonyl group on the link between the γ–lactam and the aromatic ring, did not influence positively the antifungal potency. What was interesting was that some of these molecules continue to be active towards *F. solani*, but unfortunately, no particular candidate seemed to be making a big difference (**Table 28**). While the simple benzoic hydrazide **46**, is unactive, the substitution of the ring, seems to improve a little the activity.

N° compound	N°HEI	Structure	BC	SS	AO	PV	PO	AA	CC	FS	GC	CK	CP	CT
37	2909		62	76	71	78	90	82	44	100	0	35	96	89
38	2943		0	0	0	0	45	0	16	95	8	0	20	56
39	3192		39	40	37	82	35	2	81	100	22	51	38	51
40	3180		20	46	45	69	56	0	42	100	18	20	61	20
41	3179		0	0	23	11	21	0	0	64	32	0	0	8
42	3042		0	0	0	14	25	0	14	0	0	40	0	0
45	2956		0	0	0	0	0	0	18	72	15	28	15	25

B. cynerea - BC, *S. sclerotiorum* - SS, *A. oryzae* - AO, *P. variotii* - PV, *P. ochrochloron* - PO, *A. alternata* - AA, *C. cladosporioides* – CC, *F. solani* - FS, *G. candidum* - GC, *C. krusei* - CK, *C. tropicalis* – CT and *C. pseudotropicalis* - CP.

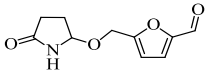
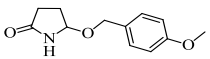
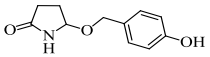
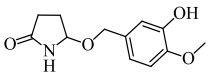
Table 26 *Inhibition of growth (%) of some hydrazine derivatives at 100µg/mL*

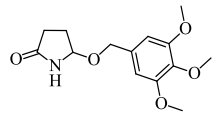
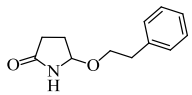
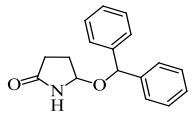
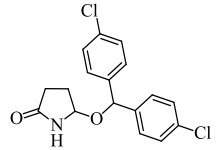
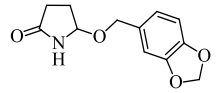
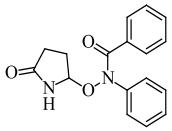
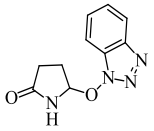
N° compound	N°HEI	Structure	BC	SS	AO	PV	PO	AA	CC	FS	GC	CK	CP	CT
46	2970		2	22	35	7	0	0	30	30	52	0	22	25
47	2947		11	0	20	0	0	0	10	10	50	0	25	19
50	3008		0	0	1	15	33	25	32	32	0	72	5	0
51	2940		0	29	10	13	1	20	21	65	0	20	30	41
88	3013		0	14	23	0	29	18	20	50	0	0	95	40
89	3016		0	0	0	16	26	26	19	0	53	53	0	0
52	2941		0	0	2	7	1	0	5	70	0	27	30	20
53	2944		0	0	0	0	4	0	34	58	0	0	45	44

55	3040		0	17	32	0	0	17	25	69	1	0	51	25
56	3035		10	7	40	0	41	0	0	43	0	0	0	0
57	2946		3	0	8	29	38	26	46	0	11	15	5	0
58	2972		0	9	22	36	0	0	0	62	0	18	47	32
60	2995		0	17	10	26	32	0	16	48	31	28	0	45

B. cynerea - BC, *S. sclerotiorum* - SS, *A. oryzae* - AO, *P. variotii* - PV, *P. ochrochloron* - PO, *A. alternata* - AA, *C. cladosporioides* – CC, *F. solani* - FS, *G. candidum* - GC, *C. krusei* - CK, *C. tropicalis* – CT and *C. pseudotropicalis* - CP.

Table 27 *Inhibition of growth (%) of some hydrazide derivatives at 100µg/mL*

N° compound	N°HEI	Structure	BC	SS	AO	PV	PO	AA	CC	FS	GC	CK	CP	CT
62	2965		11	0	0	31	37	8	36	0	27	0	33	0
66	2992		0	0	0	12	29	0	18	0	0	50	0	5
67	2969		0	0	0	26	0	0	0	0	0	0	12	0
68	2974		0	0	9	14	30	18	26	0	0	42	12	0

69	2964		28	0	10	33	41	22	33	10	48	0	13	0
71	2982		0	23	9	7	0	0	11	10	0	0	32	0
73	2968		0	0	0	43	34	8	0	30	64	0	23	8
74	2977		6	10	64	78	0	8	60	73	79	92	8	38
75	2989		0	0	12	30	80	2	0	0	0	2	0	0
78	2983		20	0	34	42	30	25	51	20	33	6	0	0
79	2984		0	0	0	28	11	21	2	0	58	10	32	18

B. cynerea - BC, *S. sclerotiorum* - SS, *A. oryzae* - AO, *P. variotii* - PV, *P. ochrochloron* - PO, *A. alternata* - AA, *C. cladosporioides* – CC, *F. solani* - FS, *G. candidum* - GC, *C. krusei* - CK, *C. tropicalis* – CT and *C. pseudotropicalis* - CP.

Table 28 *Inhibition of growth (%) of some oxygenated derivatives at 100µg/mL*

90-100	80-89	70-79	60-69	50-59

Besides this, the replacement of the phenyl moiety did not change anything, the 4-pyridyl- and the thiophene derivatives **47** and **53** having a profile similar to those of derivative **46**. Chlorinated and nitro- derivatives seemed to lack activity, while trimethoxy hydrazide is moderately inhibiting *F. solani* growth. Sulphonylhydrazide derivative **55** seems to have an improved anti-*F. solani* profile, presenting 69% growth inhibition, while 4-methyl- derivative caused the complete loss of activity. Within the oxygenated derivatives, we can state that we did not observe any outstanding antifungal activities. Among the 11 tested compounds, only 3 were moderately active (**Table 29**). It is noteworthy mentioning **73**, which seemed selective for *F. solani*, while the chlorinated analogue **74** was acting like a broad spectrum antifungal, covering 2 yeasts, *G. candidum*, *C. krusei* and 4 sporulating fungi: *F. solani*, *P. variotii*, *A. oryzae* and *C. cladosporioides*. Compound **75**, the last active derivative from the series, is the only one which is targeting *P. ochrochloron*, inhibiting 80% of its growth at 100 µg/mL.

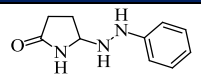
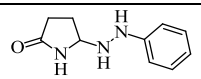
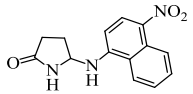
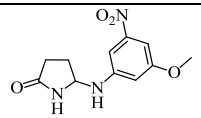
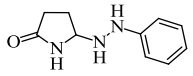
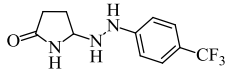
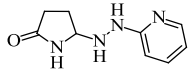
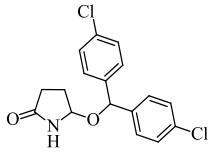
II.3.2.1 IC_{50} results discussion

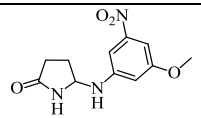
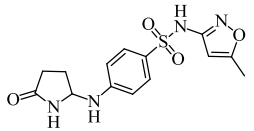
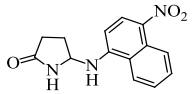
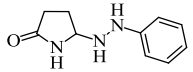
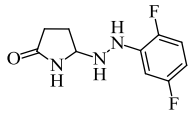
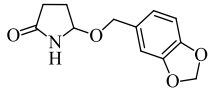
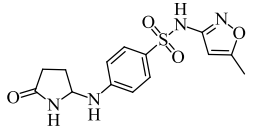
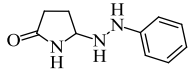
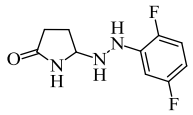
In order to explore the antifungal potential, the molecules presenting an inhibition rate higher the 70% at 100 mg/L were chosen for further screenings and the IC_{50} values indicating the linear relationship between drug concentration and the inhibition rate were summarized in **Table 30**.

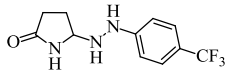
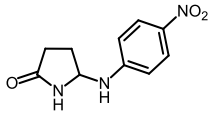
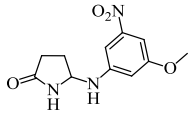
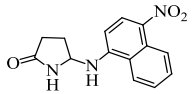
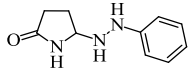
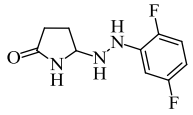
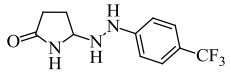
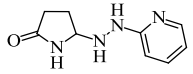
Analyzing the data from the table, we can easily notice which are the most resistant strains and also the most sensitive towards 5-pyrrolidones derivatives. From the *Sclerotiniaceae* family, *S. sclerotiorum* seemed to be slightly inhibited by **37**, with an IC_{50} value of 23.1 µg/mL, while for *B. cinerea* no molecules were selected for further dilutions.

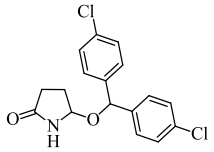
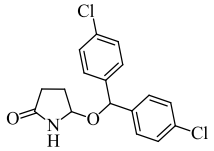
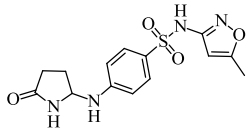
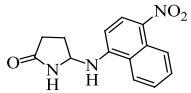
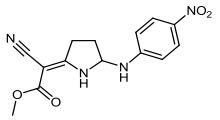
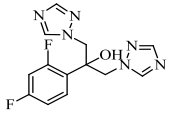
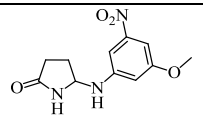
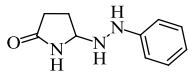
Among the *Eurotiales* order fungi, the most sensitive fungi is *P. ochrochloron* being targeted by 6 derivatives (namely compounds **24**, **33**, **35**, **37**, **38** and **75**), followed by *P. variotii* with 5 molecules exhibiting inhibition towards it and *A. oryzae*, the last being the less susceptible, and belongs to a different family, the *Aspergillaceae* while the first 2 belong to the same family of *Trichocomaceae*. Within this order, derivative **37** was the only one targeting all the three strains, having very good antifungal activity on *P. ochrochloron*, with an IC_{50} of 4µg/mL, being the most active compound targeting this strain. A lower activity was observed against *P. variotii* (IC_{50} of 7 µg/mL), and a weak one on *A. oryzae* of 43 µg/mL. However, the hydrazine derivative acts better

as an antifungal agent compared to the Hymexazole used as standard (IC_{50}= 62 µg/mL for *P. ochrochloron*, IC_{50}=57 µg/mL for *P. variotii* and IC_{50}=45 µg/mL for *A. oryzae*).

Order	Family	Strain	N° compound	N° HEI	Structure	IC_{50} (µg/mL)
Helotiales	***Sclerotiniaceae***	***Sclerotinia sclerotiorum***	37	2909		23
Eurotiales	***Aspergillaceae***	***Aspergillus oryzae***	37	2909		43
			35	3223		51
			Hymexazole			45
	Trichocomaceae	***Paecilomyces variotii***	24	2991		0.0125
			37	2909		88
			39	3192		2
			41	3179		14
			74	2977		43
			Hymexazole			57

		Penicillium ochrochloron	24	2991		29
			33	3272		79
			35	3223		21
			37	2909		4
			38	2943		21
			75	2989		31
			Hymexazole			62
Pleosporales	***Pleosporaceae***	***Alternaria alternata***	33	3272		1
			37	2909		4
			38	2943		19

			Hymexazole			38
Capnodiales	***Cladosporiaceae***	***Cladosporium cladosporioides***	39	3192		3
			Hymexazole			29
Hypocreales	***Nectriaceae***	***Fusarium solani***	19	2912		58
			24	2991		71
			35	3223		13
			37	2909		19
			38	2943		24
			39	3192		12
			41	3179		29
			Hymexazole			16

Saccharomycetales	***Dipodascaceae***	***Geotrichum candidum***	74	2977		1.7
				Ketoconazole		1.6
	Candida	***Candida krusei***	74	2977		13
			33	3272		55
			35	3223		44
			85	3221		49
			Fluconazole			14
		Candida tropicalis	24	2991		65
			37	2909		58
				Ketoconazole		16

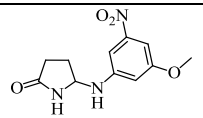
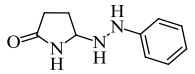
Candida pseudotropicalis	24	2991		51
	37	2909		0.57
		Ketoconazole		10

Table 29 *IC_{50} results of selected molecules*

Considering the *Trichocomaceae* family fungi, an excellent inhibition was shown by 5-((3-methoxy-5-nitrophenyl)amino)pyrrolidin-2-one **24 – a nitro derivative -** having a great IC_{50} of 0.0125 µg/mL on *P. variotii*, the fungi being extremely sensitive towards it, while on *P. ochrochloron* the same molecule exhibited a moderate activity of 29 µg/mL. Interestingly, the trifluoromethylhydrazine derivative **39** proved to be the most efficient antifungal molecule against *P. variotii* with an IC_{50} of 2 µg/mL, 25 folds better that the Hymexazol and approximately 3 folds compared to **37**, the simple hydrazine derivative. Picolinylhydrazine derivative **41** proved to inhibit the same strain, however less efficiently compared to the previously mentioned pyrrolidone derivatives. Considering the fungi strains belonging to the *Trichocomaceae* order, only two particular oxy-pyrrolodinone derivatives were active against it, one of them presenting an excellent activity. 5-(Bis(4-chlorophenyl)methoxy)pyrrolidin-2-one **74** exhibited an IC_{50} of 4 µg/mL. Meanwhile, the piperonyl alcohol derivative **75** was found to be active on *P. ochrochloron*, with a moderate IC_{50} of 31 µg/mL, being selective towards this strain.

Going further on the *Dothideomycetes* class of fungi, the most active derivatives were diluted for the test of *A. alternata* and *C. cladosporioides.* However, few compounds were showing an activity when diluted, *A. alternata* being targeted by three possible antifungals while for *C. cladosporioides*, another sporulating fungi, there is only one possible candidate. Among the three pyrrolidones which exhibited activity at dilution against *A. alternata*, the best is represented by the sulfamethoxazole derivative **33** with an IC_{50} of 1 µg/mL, followed by the broad spectrum antifungal **37** having an IC_{50} egal to 4 µg/mL. At 6 folds of difference the 2,5-difluorohydrazine derivative, compound **38** has an IC_{50} of 19 µg/mL, showing that the introduction of deactivating fluorine atoms was not efficient, lowering the activity, but even so, the value is higher compared to the one of Hymexazol (IC_{50}=38 µg/mL).

The fourth class of fungi which we explored is the *Sordariomycetes* one, from which one representative was tested, as previously seen from the 100 µg/mL screening, *F. solani*. Considering this fungus, seven compounds were found to present antifungal activities with IC_{50} ranging from 12 to 71 µg/mL, among which two were aniline derivatives and five were hydrazine derivatives, however two being better than the standard Hymexazol. The best candidate found targeting this strain is the 5-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)pyrrolidin-2-one **39** with an IC_{50} of 12 µg/mL followed by the 4-nitronaphthylamino- derivative **35** with an IC_{50} of 13 µg/mL. The other

hydrazine derivatives, respectively **37**, **38** and **41**, though have shown activity towards *F. solani* growth, it was moderate.

Considering the yeasts we have tested, three were *non albicans candida* strains (NAC species) and one yeast belonging to the *Dipodascaceae* family. Regarding the last mentioned yeast, only one compound was found to show very good antifungal activity, equaling ketoconazole activity, with an IC_{50} of 1.7 µg/mL. Regarding *C. krusei*, several pyrrolidone derivatives have shown moderate activity, but the lowest IC_{50} of 44 µg/mL belonging to compound **35** that is still approximately four times bigger that fluconazole´s value. Worth mentioning was the broad spectrum derivative **37** towards which *C. tropicalis* and *C. pseudotropicalis* were found to be also susceptible to, the last one being more sensitive with an IC_{50} of 0.57 µg/mL.

II.3.2.2 Cell toxicity

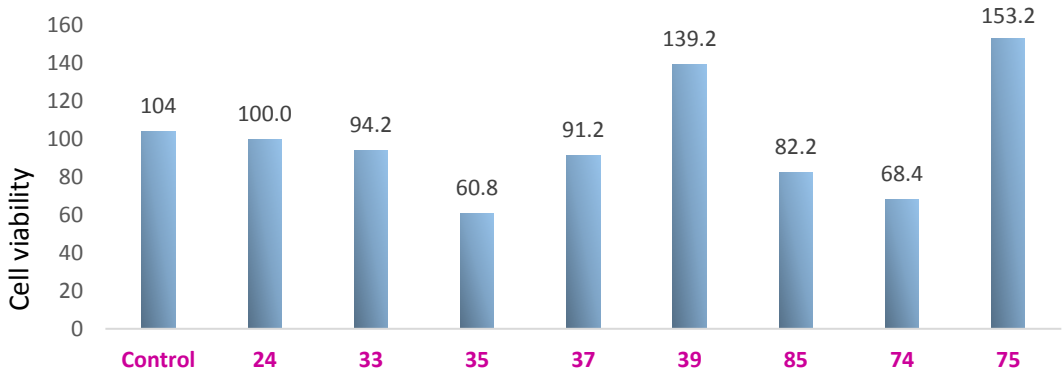


Figure 54 *HEK293 cell line (TPP culture plates) cell viability of selected molecules molecules tested at 100 µM concentration*

In order to check the general mammalian cell toxicity of the tested compounds presented herein, selected compounds were screened against human embryonic kidney cells (HEK293). The arylhydrazine derivatives showed a slight toxicity in viable kidney cells, the viability of cells dropping down around 10% at 100 µM for **37** as example. Aniline derivatives such as **24** and **33** lead to a minimal reduction in viable kidney cells, less than 10% where the only marked cell death was observed at a concentration of 100µM. However, for two derivatives which had shown good antifungal activity such as **35** and **74**, the cell viability dropped to around 60 but not concerning cell viability decrease. In conclusion, the molecules proved to be safe, having "fungicide-like" profile and could be employed as such.

II.3.2.3 Conclusions

With regard to the structure-activity relationship, there are few aspects to be mentioned:

(1) While most of the alkoxy derivatives failed to deliver significant antifungal activity, the amino link has improved the biological activity.

(2) Moreover, as we switched further the link, we have identified hydrazine derivatives to be the most active.

(3) The benefit of having electron withdrawing groups on the benzene ring has been well noticed within the amino-pyrrolidinone derivatives, representing a positive contribution to the fungicidal activity, nitro- derivatives being among the most active molecules.

(4) *N*-substitutions of the link, resulted in a weaker antifungal activity, possible explanation of why some alcohol derivatives are not active.

(5) Replacing the phenyl group by a naphthyl one can bring an improvement, as seen in the case of the *para*-nitro derivatives, **35** having a 4 folds lower IC_{50} compared to **19** against *F. solani*.

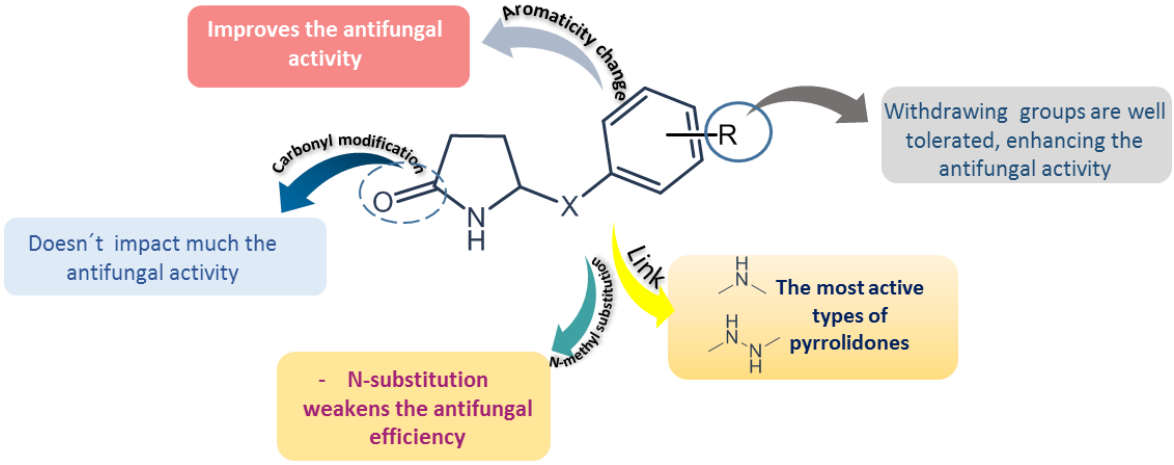


Figure 55 *SAR of pyrrolidones series*

To summarize, approximately all derivatives which were obtained through CsF protocol were tested for their antifungal activity. Regarding the results of these series, the most susceptible strains from the list we tested, were *B. cinerea*, *S. sclerotiorum*, *C. cladosporium* and *G. candidum*. Towards 5-amino-pyrrolidin-2-ones, we can notice an important sensitivity of *F. solani* followed by *P. variotii*. The best candidates found within these series were derivatives **24** and **37**, having broad spectrum activities and proved to be lacking cytotoxicity (**Figure 53**). As a consequence,

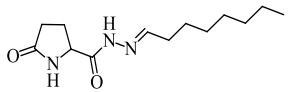
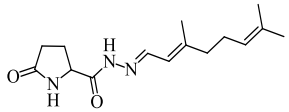
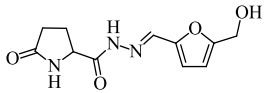
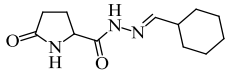
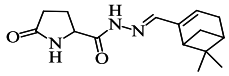
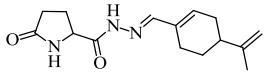
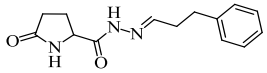
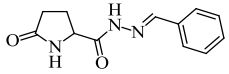
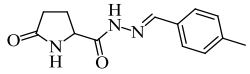
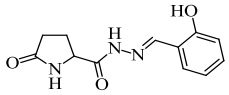
from these results some selected antifungal agents will be more studied in Chapter III for their physico-chemical properties as additives in paints.

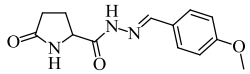
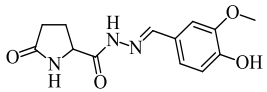
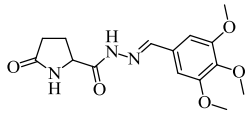
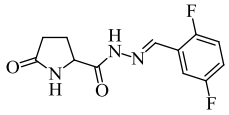
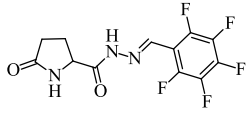
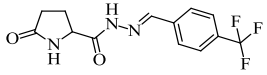
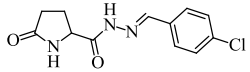
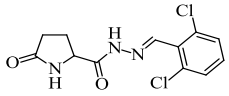
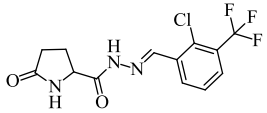
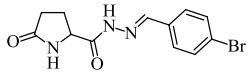
II.3.3 Pyroglutamic acid hydrazones antifungal results and properties

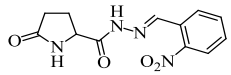
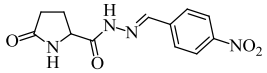
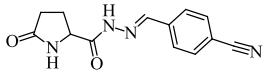
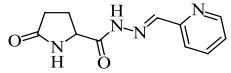
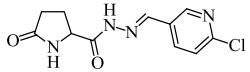
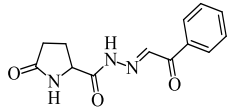
A series of novel pyroglutamic acid *N-acylhydrazone* derivatives (NAH) series was synthesized through an environmentally friendly method and subsequently tested for their antifungal activity against the same panel of fungi and molds. To the best of our knowledge, this is the first report of pyroglutamic hydrazones derivatives with potent controlling effects against different fungi. However, as mentioned in the *N*-acylhydrazones synthesis subchapter, hydrazone derivatives have also emerged as compounds with the ability to potentiate antifungal activities *in vitro*.

The results of the *in vitro* antifungal activity of compounds **123- 148**, against 9 fungi and 3 yeasts were listed in **Table 31**, in which the antifungal activity was expressed as inhibition percentage at a concentration of 100 µg/L. The results showed that target compounds were active to various extents. According to this preliminary screening, most of the synthesized compounds exhibited a certain degree of antifungal activities, especially against *F. solani* and *G. candidum.* The strains which were not susceptible at all to the tested compounds were *Sclerotiniaceae* family strains (*B. cinerea* and *S. sclerotiorum)* and *Trichocomaceae* family (represented by *P. variotii* and *P. ochrochloron)*. Besides the mentioned one, *A. alternata* and *A. oryzae* were not sensitive neither towards the screened molecules. Considering structural patterns, alkyl chains NAHs such as **123** and **124** are lacking antifungal activity. The same behavior could be noticed for the cyclohexane hydrazone **126**, which except for a slight inhibition of 62% of *G. candidum* growth, did not present any other activity. In the same context of antifungal activity lack entered the perilllaldehyde as well as the myrtenal derivatives, **127** and **128**.

Using the optimum substitution of aromatic ring, we explored the requirement of the substituent for an antifungal activity. Overall, compounds having aromatic nuclei showed more promising antifungal profiles. While 5-oxo-*N*'-(3-phenylpropylidene)pyrrolidine-2-carbohydrazide showed a moderate activity only on *G. candidum* (derivative **129**), the simple phenyl- hydrazone **130** has shown a better antifungal activity, being also active against *F. solani*. Among the electron donating substituents on the *N*-aryl groups, the methyl- group derivative **131** causes a *G. candidum* activity loss while the *F. solani* inhibitory rate is improved, compared to **130**.

N° Compound	N°HEI	Structure	BC	SS	AO	PV	PO	AA	CC	FS	GC	CK	CP	CT
123	**3140**		0	0	0	38	54	21	5	0	35	0	0	17
124	**3163**		27	15	17	0	0	0	27	43	5	0	49	15
125	**3164**		0	0	0	45	28	22	0	21	75	18	26	22
126	**3138**		8	0	8	36	23	8	1	0	62	0	30	0
127	**3165**		0	0	4	39	0	0	0	0	0	0	18	0
128	**3166**		13	0	0	31	45	0	56	0	39	5	18	0
129	**3141**		6	0	0	39	29	0	53	0	68	0	12	17
130	**261**		0	0	0	46	25	0	12	73	63	17	9	0
131	**3146**		0	0	11	35	0	0	5	94	28	28	32	32
132	**3162**		0	21	7	14	30	38	0	40	70	38	18	7

133	**3160**		0	38	24	36	23	3	35	0	25	40	40	49
134	**3147**		0	0	0	22	0	20	0	0	0	0	37	0
135	**3145**		0	0	0	26	32	29	0	0	0	50	18	0
136	**3159**		6	0	13	37	26	0	34	58	53	0	8	15
137	**3158**		17	5	4	0	21	0	11	40	36	0	0	0
138	**3157**		0	0	0	44	44	21	11	55	85	21	21	17
139	**3142**		34	58	0	27	92	20	98	61	92	26	50	47
140	**3144**		13	27	27	26	20	0	32	61	73	0	32	16
141	**3207**		0	0	4	19	44	12	36	0	62	47	18	0
142	**3143**		40	37	43	0	77	0	72	100	100	0	2	92

143	**3155**		27	21	32	3	13	0	0	100	50	0	35	19
144	**3154**		0	9	0	20	14	30	40	100	30	22	38	42
145	**3139**		0	0	0	33	20	42	0	0	0	10	0	0
146	**3149**		0	58	12	13	22	32	12	0	0	22	8	12
147	**3206**		0	0	0	42	62	0	33	23	72	2	10	0
148	**3156**		0	36	9	1	12	0	7	0	0	50	8	15

B. cynerea - BC, *S. sclerotiorum* - SS, *A. oryzae* - AO, *P. variotii* - PV, *P. ochrochloron* - PO, *A. alternata* - AA, *C. cladosporioides* – CC, *F. solani* - FS, *G. candidum* - GC, *C. krusei* - CK, *C. tropicalis* – CT and *C. pseudotropicalis* - CP.

Table 30 *Inhibition of growth (%) of N-acylhydrazone derivatives at 100 µg/mL*

90-100	80-89	70-79	60-69	50-59

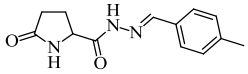
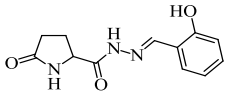
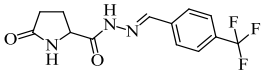
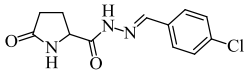
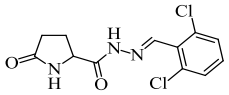
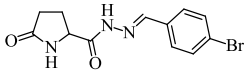
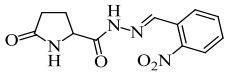
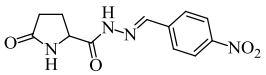
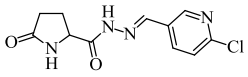
While the salicyl hydrazone **132** kept 70% of *G. candidum* inhibition growth at a concentration of 100 µg/mL, the rest of electron withdrawing phenylhydrazones **133** and **134**, are completely lacking antifungal activity.

Considering the deactivating groups such as halogens, the corresponding hydrazones possess the best spectrum of activity against various strains and the most potent activity as judged by their inhibitory rates. To our surprise, among the fluorinated NAHs, only 4-trifluoromethylphenyl derivative **138** exhibits a good antifungal activity at 100 µg/mL. The most tolerated group with a broad antifungal spectrum at 100 µg/mL is the 4-chlorophenylhydrazone derivative **139** which exhibited 4 good activity rates on *P. ochrochloron*, *C. cladosporoides*, *F. solani*, *G. candidum* and a moderate activity on *S. sclerotiorum* and *C. pseudotropicalis*. The 2,6-dichlorinated NAH **140** on the other hand, presented a completely different behavior, its activity being narrowed to *F. solani* and *G. candidum*. Another interesting antifungal candidate is represented by 4-bromophenylhydrazone **142** which has a very good profile on approximately the same strains as **139**, in plus being the only derivative which presented a good inhibition growth of *C. tropicalis* which was lost in the dilution test. Hydrazones with stronger deactivating groups as nitro- and cyano- substituents were also evaluated. Switching the position of the nitro- group, from *ortho*- (derivative **143**) to *para*- position (derivative **144**) on the phenyl ring, we can not observe any activity changement, and the selective activity against *F. solani* is kept. For other strong deactivating groups such as cyano-, we can observe an activity loss, derivative **145** having no particular inhibitory activity. Other hydrazones such as pyridine hydrazones were tested as well. We could notice that **146** had no activity, while *N'*-((6-chloropyridin-3-yl)methylene)-5-oxopyrrolidine-2-carbohydrazide **147**, was slightly active on *P. ochrochloron* and *G. candidum*. Within this series, the last modification was realized on the linker. From the *in vitro* screening we observed that having an extra carbonyl between the hydrazone and the phenyl ring is causing a lack of activity for **148** compared to simple aromatic NAH **130**.

II.3.3.1 IC_{50} results discussion

Next, *in vitro* antifungal activity of molecules presenting an inhibition rate higher than 70% was done by tube dilution method against tested microorganisms. The results of antifungal activity showed that few of synthesized compounds exhibited considerable antifungal activity while others showed moderate antifungal activity. The IC_{50} values indicating the linear relationship between drug concentration and the inhibition rate were summarized in **Table 32**.

Analyzing the data from the table, we can easily notice the most active molecules which are confirmed in the dilution test, **139** and **142**, however targeting fungi in different manner and potency. While the chloro-derivative **139** was found the most active against *C. cladosporioides* and *G. candidum* with IC_{50} values of 23.51, respectively 1.85 µg/mL, the bromo- hydrazone **142** had most potent activity against *P. ochrochloron* and *F. solani* of 5.27 respectively 6.51 µg/mL. These compounds may be taken as leads to discover novel antifungal agents, however multiple improvements could be done.

N° Compound	N°HEI	Structure	PO	CC	FS	GC
131	**3146**		/	/	101.54	/
132	**3162**		/	/	/	62.05
138	**3157**		/	/	/	63.74
139	**3142**		15.41	23.91	/	1.85
140	**3144**		/	/	/	64.78
142	**3143**		5.27	53.03	6.51	5.89
143	**3155**		/	/	59.11	/
144	**3154**		/	/	51.65	/
147	**3206**		/	/	/	38.40
	Hymexazole		62.17	28.90	16.53	/
	Fluconazole					1.59

P. ochrochloron - PO, *C. cladosporioides* – CC, *F. solani* - FS, *G. candidum* - GC

Table 31 *IC_{50} results of selected molecules*

Considering the structure activity relationship study (**Figure 55**), an aromatic ring is necessary to maintain an antifungal activity. The presence of electron-releasing groups is not so well tolerated furnishing low to moderate antifungal activities while *para* substitution of the

phenyl ring by a deactivating atom like chloride or bromine is resulting in broad spectrum antifungals, with general better activities than the positive control. Interestingly, in order to obtain *F. solani* selective antifungals, nitro-groups are needed, *para*-position derivative being slightly more potent than the *ortho*-derivative.

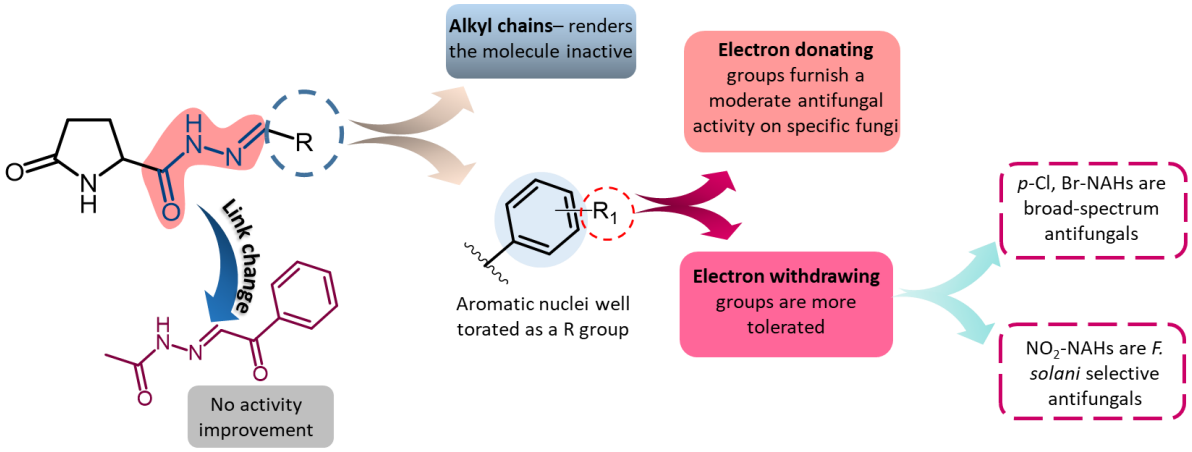


Figure 56 *SAR study regarding pyroglutamic acid N-acylhydrazones*

In conclusion, 26 *N*-acylhydrazones were submitted to antifungal screening. The spectrum of activities of these products is generally narrow, most of them being active on two strains, and all compounds display no activity against *B. cinerea, S. sclerotiorum, P. variotii, P. ochrochloron. P. variotii, A. alternata* and *A. oryzae.*

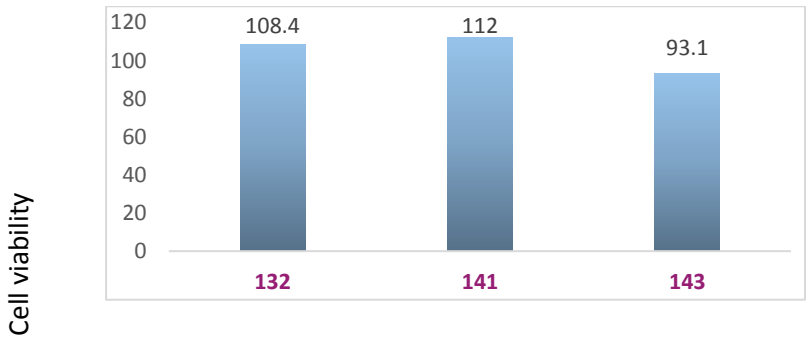


Figure 57 *HEK293 cell line (TPP culture plates) cell viability of selected molecules at a 100 µM concentration*

The two most potent, broad spectrum hydrazones **139** and **142** and the nitro- derivative **144** were also tested for their cytotoxicity and showed a safe to employ profile (**Figure 57**). These encouraging results may lead for the development of novel therapeutic antifungals, however different structural modulations are required for biological improvement.

II.3.4 Conclusions and perspectives considering the global antifungal results

Within this biological results subchapter, the antifungal activity of two main series, of pyrrolidones and pyrroglutamic acid hydrazones, was studied and discussed. Among the studied molecules, there are 3 antifungal candidates that worth to be mentioned, which are targeting a large panel of fungi known to cause health problems, being suitable preservation additives for the formulation of anti-mold paints and coatings.

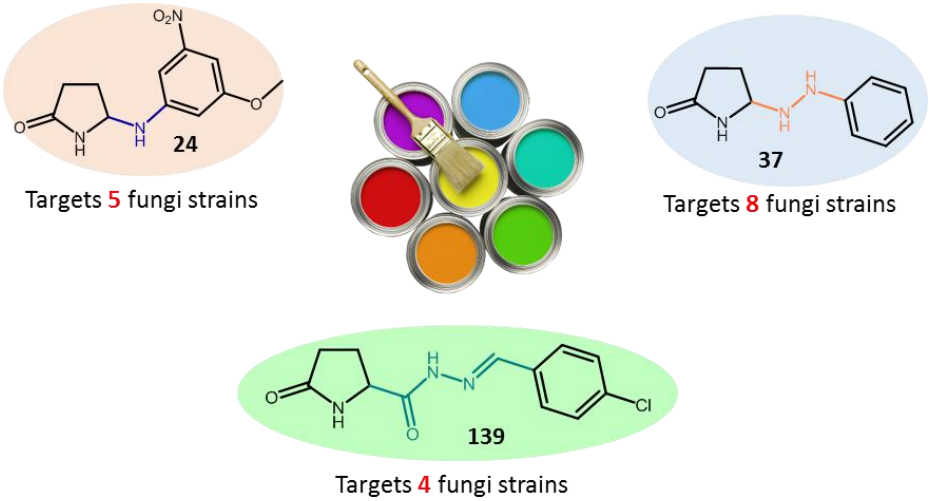


Figure 58 *Suitable candidates for the formulation as antifungal additives*

In the frame of the developing of smart paints as anti-fungal and anti-mildew paint, which work through the action of an additive which inhibits the growth of fungi, mold and mildew, some of these molecules have been tested for their thermic stability as well, to verify the possibility of formulating them. The tests which are discussed in the analysis chapter, proved that the compounds are stable at high temperatures and do not decompose in air.

Things considered, their broad activity, lack of cytotoxicity, and good thermal stability, are making these identified molecules, suitable for formulation.

II.4 Other biological targets: Farnesyltransferase

Farnesyltransferase enzyme (FTase) is responsible for post-translational addition of isoprenoid lipids to proteins and is considered to be a critical therapeutic target in many diseases. FTase catalyzes the addition of a C_{15}-farnesyl lipid group to the cysteine residue located in the COOH-terminal tetrapeptide motif of a variety of important substrate proteins, including well-known Ras protein superfamily. Starting with the fact that inhibition of farnesylation was proved to slow the growth of cancer cells, by blocking the Ras activation, a number of farnesylation inhibitors were investigated, and a few are in preclinical and clinical trials. However, though in preclinical models, the farnesyl transferase inhibitors showed great potency against tumor cells, in clinical studies, their activity was far less.

II.4.1 Farnesyltransferase: a responsible enzyme within important pathologies

Farnesyltransferase inhibitors and cancer

Cancer is one of the most important disease of this century, characterized by self-sufficiency in growth signals, sustained angiogenesis, tissue invasion, and metastasis.[189]

Among the proteins that undergo farnesylation, Ras proteins are found mutated in around 30% of human cancers, leading to sustained activation and the subsequently stimulation of growth.[190] Considering this, in the last twenty years and more, farnesyltransferase inhibitors have been developed for cancer treatment. So far, many preclinical studies demonstrated that FTIs can stop tumor growth, being antitumoral agents with little toxicity. From the long list of scaffold which have been developed as FTIs, we can remind pyridine analogues, piperidines, imidazoles, polycyclics thiazoles, imidazoles, phenothiazine derivatives, indolizine-chalcones andbenzophenones.[191,192,193,194] By this date five compounds have entered into clinical

[189] Khabar K. S. Hallmarks of cancer and AU-rich elements. *Wiley Interdiscip. Rev.RNA*, 2017, 8, 1368.
[190] Zhou B., Der C. J., Cox A. D. The role of wild type RAS isoforms in cancer. *Cell Dev. Biol.* 2016, 58, p. 60–69.
[191] Shen Y., Qiang S., Ma S. The Recent Development of Farnesyltransferase Inhibitors as Anticancer and Antimalarial Agents. *Mini-Rev. Med. Chem.* 2015, 15, p. 837–857.
[192] Belei D., Dumea C., Samson A., Farce A., Dubois J., Bîcu E., Ghinet A. New farnesyltransferase inhibitors in the phenothiazine series. *Bioorg. Med. Chem. Lett.* 2012, 22, p. 4517–4522.
[193] Moise I. M., Ghinet A., Belei D., Dubois J., Farce A., Bîcu E. New indolizine–chalcones as potent inhibitors of human farnesyltransferase: Design, synthesis and biological evaluation. *Bioorg. Med. Chem. Lett.* 2016, 26, p. 3730–3734.
[194] Moorthy N., Sousa S. F., Ramos M. J., Fernandes P.A. Farnesyltransferase Inhibitors: A Comprehensive Review Based on Quantitative Structural Analysis. *Curr. Med. Chem.* 2013, 20, p. 4888–4923.

investigation, from which we have lonafarnib, tipifarnib, antroquinonol, L778123 and BMS-214662.[195]

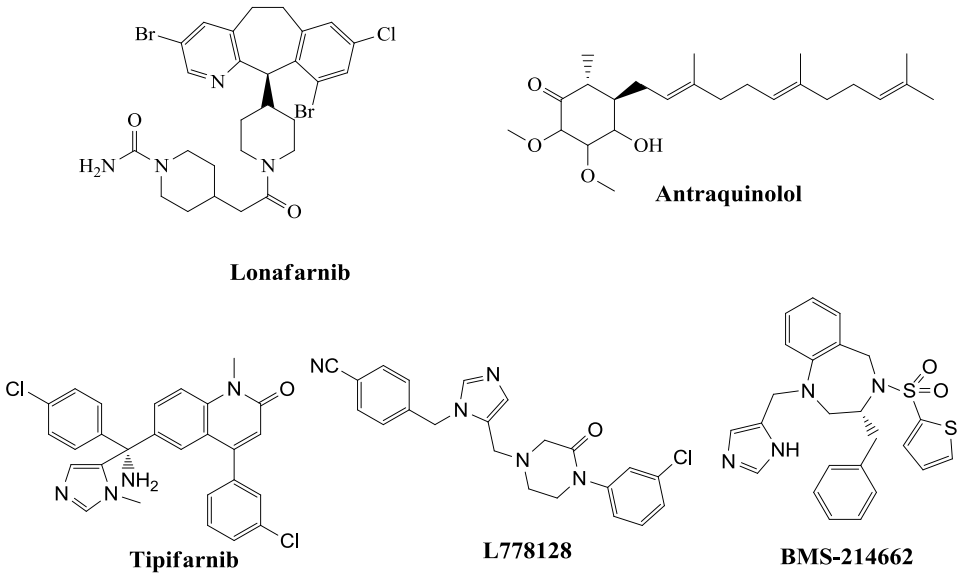


Figure 59 *FTIs which entered into clinical investigations as anti-cancerous drugs*

Farnesyltransferase inhibitors and hepatitis D

Chronic delta hepatitis is a serious form of chronic liver, affecting 20 million HBV-infected people worldwide, and the causative agent is hepatitis D virus (HDV), a small RNA virus that requires farnesylation of its major structural protein (HDV antigen) in order to replicate.[196]

By inhibiting the FTase, a large amount of hepatitis delta antigen (LHDAg) prenylation is therefore suppressed, preventing this way the formation of virus-like particles (VLPs) with the HBV surface antigen (HBsAg) in HBV-infected cells, which are spreading the infection.[197] Things considered, there is a major interest for the use of FTIs to interfere the further infection. In 2015 successful phase IIA trail was completed, and lonafarnib demonstrated to significantly

[195] Wang J., Yao X., Huang J. New tricks for human farnesyltransferase inhibitor: cancer and beyond. *Med. Chem. Commun.* 2017, 8, p. 841-854.
[196] Chi-Ruei H., Szecheng J. L. Hepatitis D virus infection, replication and cross-talk with the hepatitis B virus. *World J. Gastroenterol.* 2014, 28, 20, 40, p. 14589–14597.
[197] Bordier B.B., Marion P.L., Ohashi K., Kay M.K., Greenberg H.B., Casey J.L., Glenn J.S. A Prenylation Inhibitor Prevents Production of Infectious Hepatitis Delta Virus Particles. *J. Virol.* 2002, 76, p. 10465–10472.

reduces virus levels.[198] At this moment, there are several outgoing clinical trials, which may provide further evidence for the efficacy of FTIs in chronic HDV.

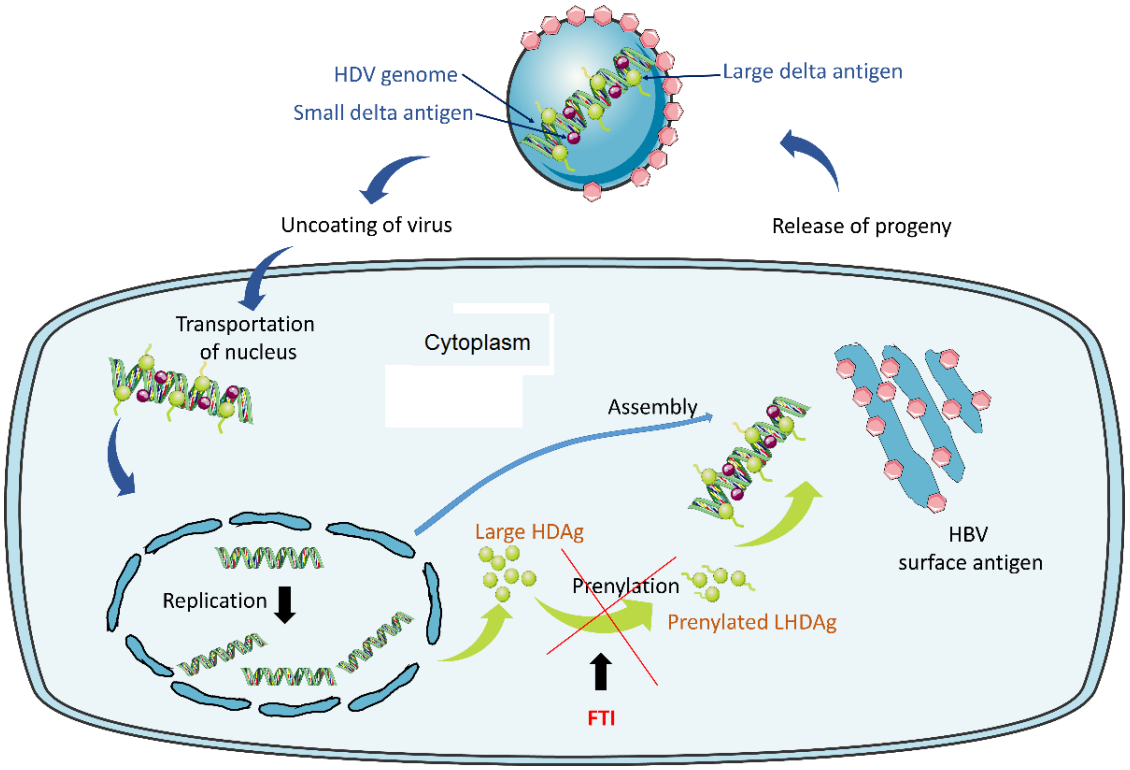


Figure 60 *The inhibition of the prenylation of the Hepatitis D virus's large delta antigen by FTIs*

Farnesyltransferase inhibitors and rare diseases

By far one of the most intriguing discovery in past few years is that mutations within A-type lamins can cause distinct heritable and *de novo* multisystem degenerative disorders, in humans, laminopathies. It has been proved that when prelamin A maturation process is altered, it affects entire sets of tissues, leading to progeroid syndromes, rare genetic disorders mimicking clinical and molecular features of ageing, lipodystrophies and muscular dystrophies. Such disorders include Hutchinson–Gilford progeria (HGPS), Werner syndrome, restrictive dermopathy, familial partial lipodystrophy (FPLD2) and mandibuloacral dysplasia (MADA).[199]

[198] Koh C., Canini L., Dahari H., Zhao X., Uprichard S.L., Haynes-Williams V., Winters M.A., Subramanya G., Cooper S.L., Pinto P., Wolff E.F., Bishop R., Ai Thanda Han M., Cotler S.J., Kleiner D.E., Keskin O., Idilman R., Yurdaydin C., Glenn J.S., Heller T.. Oral prenylation inhibition with lonafarnib in chronic hepatitis D infection: a proof-of-concept randomised, double-blind, placebo-controlled phase 2A trial. *Lancet Infect. Dis.* 2015, 15, p. 1167–1174.

[199] Gonzalo S., Kreienkamp R., Askjaer P. Hutchinson-Gilford Progeria Syndrome: a premature aging disease caused by LMNA gene mutations. *Ageing Res. Rev.* 2017, 33, p. 18–29.

In Hutchinson–Gilford progeria (HGPS), it has been proved that the toxicity of farnesylated prelamin A (progerin) results from its lack of proteolytic cleavage site required for the removal of post-translationally attached farnesyl moiety. Consequently, progerin remains associated with the inner nuclear membrane, unable to be released for degradation because of persistent farnesylation. The protein attached to the nuclear membrane, weakens cell growth, cells which now lack nuclear plasticity, the nuclei being stiffer and fragile (**Figure 61**).[200,201]

In agreement with this central hypothesis, drugs impairing protein farnesylation have been shown to ameliorate the nuclear morphological abnormalities in laminopathic cells accumulating prelamin A and clinical trials are now being initiated with some compounds to determine their efficacy in human patients. The findings reported showed variable rates of improvement in vascular function and reduced number of rib fractures.

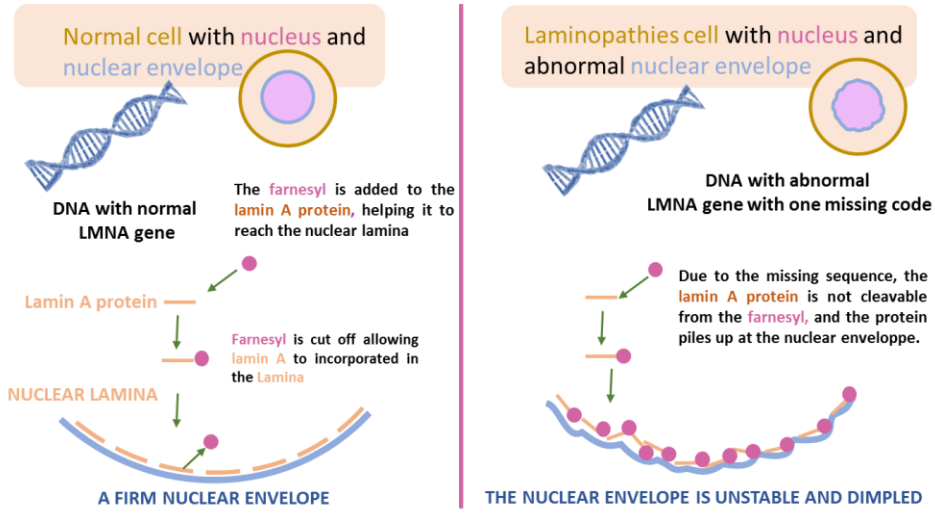


Figure 61 *Lamin A processing within a normal cell and a laminopathy disorder cell*

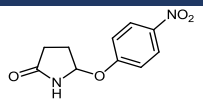
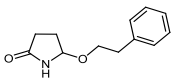
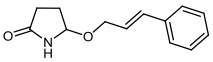
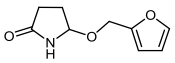
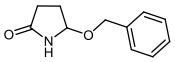
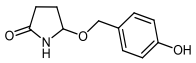
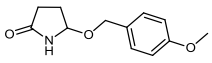
Considering the *in vitro* and *in vivo* results, clinical trials of FTIs in progeria were started, and results from the phase II clinical trial for children with HGPS proved that lonafarnib may improve vascular stiffness, bone structure, and sensorineural hearing.[202]

[200] Eriksson M., Brown W.T., Gordon L.B., Glynn M.W., Singer J., Scott L., Erdos M.R., Robbins C.M., Moses T.Y., Berglund P., Dutra A., Pak E., Durkin S., Csoka A.B., Boehnke M., Glover T.W., Collins F.S. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature*, 2003, 423, p. 293–298.
[201] Glynn M.W., Glover T.W. Incomplete processing of mutant lamin A in Hutchinson-Gilford progeria leads to nuclear abnormalities, which are reversed by farnesyltransferase inhibition. *Hum. Mol. Genet.* 2005, 14, p. 2959–2969.
[202] Gordon L.B., Kleinman M.E., Miller D.T., Neuberg D.S., Giobbie-Hurder A., Gerhard-Herman M., Smoot L.B., Gordon C.M., Cleveland R., Snyder B.D., Fligor B., Bishop W.R., Statkevich P., Regen A., Sonis A., Riley S., Ploski C., Correia A., Quinn N., Ullrich N.J., Nazarian A., Liang M.G., Huh S.Y., Schwartzman A., Kieran M.W. Clinical trial of a farnesyltransferase inhibitor in children with Hutchinson-Gilford progeria syndrome. *Proc. Natl. Acad. Sci.* 2012, 109, 41, p. 16666–16671.

Regarding the importance of this target, and its implications in multiple biological pathways, our team has extensively screened the library and pyro-derivatives were found to be inhibiting this enzyme.[203] Prenylated proteins were also discovered in some pathogenic protozoa, such as *Giardia lamblia*, *Schistosoma mansonii*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania mexicanan*, *Toxoplasma gondii* and *Plasmodium falciparum*,[204] reason why FTIs found its use as the treatments for the malaria and African sleeping sickness caused by *Plasmodium falciparum* and *Trypanosoma brucei*, respectively. Things considered, we thought useful to screen some novel series obtained within the project, farnesyltransferase being a key target in the development of novel antipathogenic tratements, but also as a cytoxicity test.

II.4.2 Farnesyltransferase screening results and discussion considering 5-pyrrolidones series

FTase enzyme inhibition was determined by *in vitro* inhibition of farnesylation of human FTase. As a first step, several selected molecules were screened at a concentration of 100 µM, and following the results, those compounds inhibiting FTase > 60% were summited for IC_{50} test. Initial structure–activity relationship (SAR) studies focused on assessing the different effect of having a *N,N'*-aminal or a *N,O*-acetal, the following results leading us to choose amino-pyrrolidones as target family (**Table 33**), oxy-derivatives lacking important activity (**Table 32**).

N° compound	HEI N°	Structure	Inhibition % at 100 µM
64	3225		0
71	2982		36
72	2986		55
3	2976		24
65	3001		44
67	2969		37
66	2992		42

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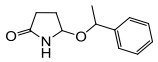
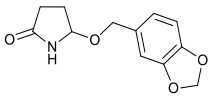
70	2985		0
75	2989		39

Table 32 *Inhibition % of oxy-pyrrolidones derivatives*

Considering the amino-pyrrolidones, though the alkyl chain molecule **10** showed increased potency with an IC_{50} of 0.237 µM compared to some aniline containing molecules, as the aromatic derivatives showed greater potency and possibility to modulate as well, we continued further with the last ones. Adding a phenyl group to a short alkyl chain completely removed the activity, rendering molecules **4** and **5** completely unactive. On the other hand if the aromatic ring was directly linked to amino, the activity was boosted and 5-(phenylamino)pyrrolidin-2-one **12** exhibited the lowest IC_{50} from the tested compounds, of 0.24 nM.

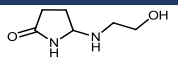
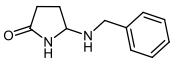
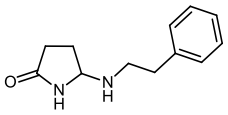
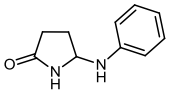
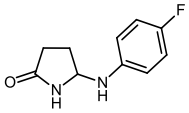
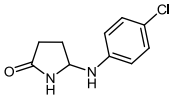
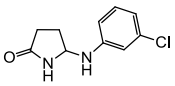
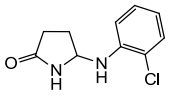
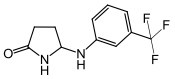
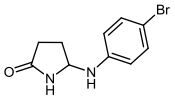
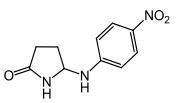
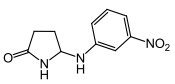
However, ring substitutions were not well tolerated at all. Starting with withdrawing substituents such as halogen atom: F-, Cl-, Br-, or group as trifluoromethyl- on the ring, loss of activity was impressive. The position of the atom is not influencing much the activity of the halogenated aniline derivatives, as we can observe in the case of chloro-phenyl substituted compounds **14**-**16**, though it seemed that *ortho*-position is more tolerated, however the IC_{50} stayed above 100 µM.

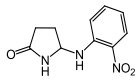
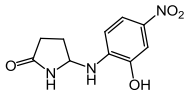
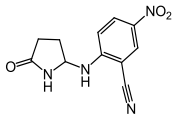
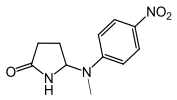
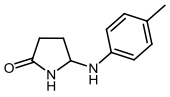
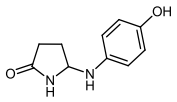
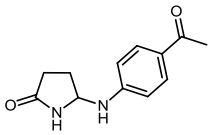
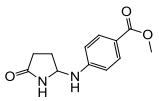
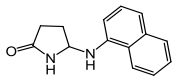
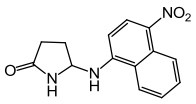
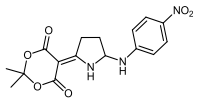
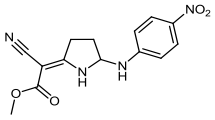
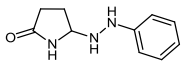
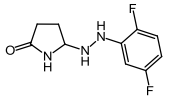
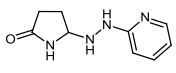
Analyzing the other deactivating groups substituted pyrrolidones, for the nitro-derivatives, the activity was partially recovered, but in the micromolar range. While *para*-nitrophenyl derivative **19** showed a good IC_{50} of 3.58 µM, having a nitro group in *ortho*-position led to the partial loss of activity, derivative **21** having an IC_{50} of 41.45 µM, while if the nitro-group was in the *meta* position, the FTase activity was completely lost.

In our efforts to improve the activity, derivatives **19**, **22** and **25** were designed as well. As the *ortho*-position was partially tolerated and some activity was kept, while the nitro- group was fixed in the *para*-position, two groups were added in the *ortho*-position, either an activating one, as hydroxyl- or a strongly deactivating one as cyano-. Having a cyano- group in *ortho*-position of the 5-((4-nitrophenyl)amino)pyrrolidin-2-one rendered **23** inactive, the hydroxyl-one was folding on the activity of **19**, compound **22** having an IC_{50} of 7.58 µM. Going further with the modulation of derivative **19**, an interesting fact was observed considering the *N*-methyl substitution of the link. If the hydrogen was substituted by a simple methyl group, the activity was completely lost, pyrrolidone **25** presenting any inhibitory activity. The possible explanation

could be that the methyl was blocking a former hydrogen bonding existant between **19** and the enzyme, this could also explain the fact why the oxy-link derivative **64** was not active.

By changing the aromaticity of the molecule **19**, we have observed a biological activity improvement, the bulkier nitro-naphthyl moiety molecule **35**, presenting an IC_{50} of 0.151 µM. Furthermore, additional efforts were made, through the modification of the carbonyl group of 4-nitro- derivative **19**, resulting in derivatives **84** and **85**. However, the *in vitro* test results have shown a folded activity by 2, respectively 10 times, derivative **84** having an IC_{50} of 6.81 µM while **85** has an IC_{50} of 33.22 µM.

N° compound	HEI N°	Structure	Inhibition % at 100 µM	IC_{50} (µM)
10	2908		89	0.237
4	2907		22	/
5	2971		37	/
12	2906		72	0.0002365
13	2910		68	ND
14	2913		31	/
15	2918		54	ND
16	2966		61	/
18	2911		17	/
17	2917		39	/
19	2912		92	3.58
20	3222		26	/

21	3228		91	41.45
22	3215		70	7.58
23	3224		29	/
25	3220		10	/
26	2915		36	/
27	2990		32	/
28	2916		36	/
29	2914		55	/
34	2902		Fluorescence problem	NT
35	3223		76	0.151
84	3214		90	6.815
85	3221		82	33.22
37	2909		18	/
38	2943		22	/
41	3179		47	/

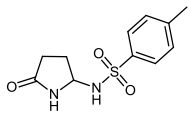
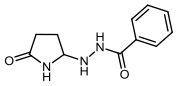
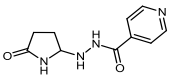
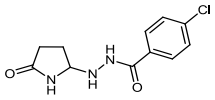
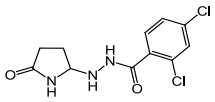
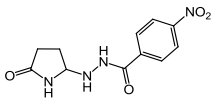
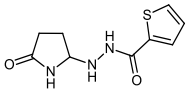
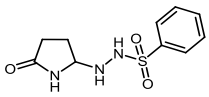
42	3042		48	/
46	2970		36	/
47	2947		26	/
49	2939		16	/
50	3008		45	/
51	2940		29	/
53	2944		24	/
55	3040		93% (Fluorescence problem)	ND

Table 33 *Inhibition percentages and IC_{50} result of the most active molecules on FTase*

ND - not determinated

The addition of electron-donating groups on the phenyl group was generating a complete loss of activity, as seen in the case of halogenated pyrrolidones (**Table 34**, entries 18-19). Unfortunately, the simple naphtyl derivative could not have been tested properly because of fluorescence false positive.

The current study established a new SAR:

(1) the amino link is important in the structure of pyrrolidone farnesyltransferase inhibitors.

(2) the ring substitution is not well tolerated, the only group that maintains the activity being nitro-group.

(3) the *para*-position of the nitro group is important.

(4) The *N*-substitution lead to the complete loss of activity.

(5) The presence of a bulky aromatic group improves the activity.

(6) The carbonyl modifications are not resulting in a complete loss of activity and probably the carbonyl does not have a big activity influence, but the lactam ring is important for the biological efficacy (**Figure 62**).

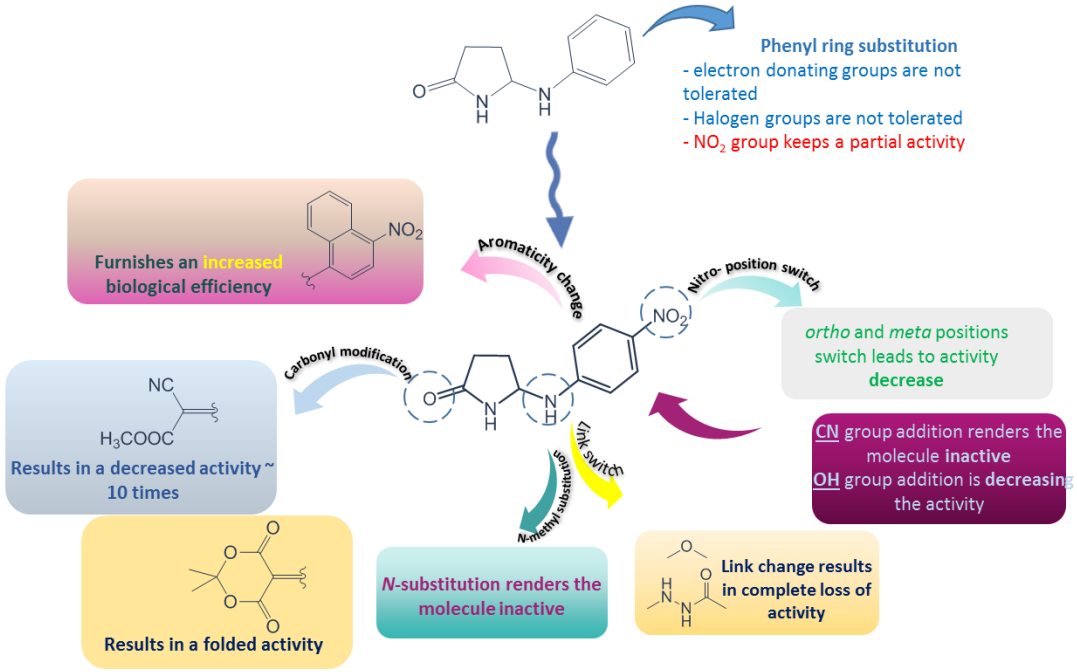


Figure 62 *SAR of pyrrolidone FTase inhibitors*

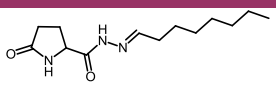
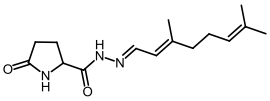
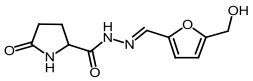
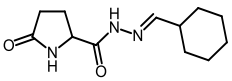
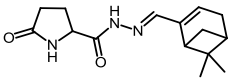
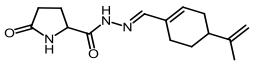
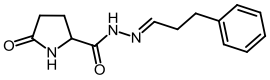
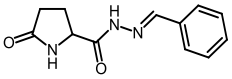
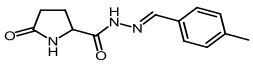
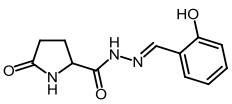
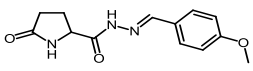
In conclusion, selected pyrrolidone derivatives were tested for their FTase inhibitory capacity, and several compounds were discovered to have average to excellent biological activities. 5-(Phenylamino)pyrrolidin-2-one **12** is the most potent molecule which was screened, with an IC_{50} value in the order of a subnanomolar range (0.2365 nM). The substitution of this molecule failed to provide more active molecules or at least maintain the activity order, possibly because of a more efficient positioning of the molecule in active sites, which may lead to different interactions, responsible for its high potency in this assay, while the weak activity of substituted anilines might be simply because there are situations when "less is more".

II.4.3 Farnesyltransferase screening results and discussion of pyroglutamic NAHs series

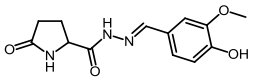
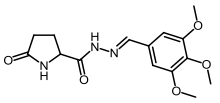
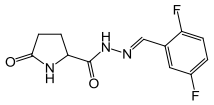
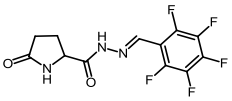
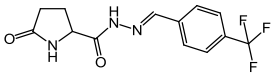
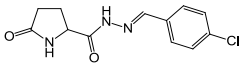
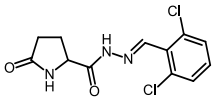
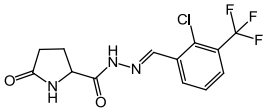
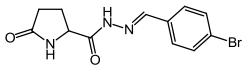
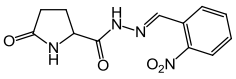
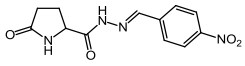
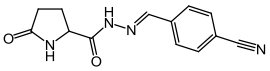
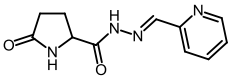
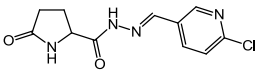
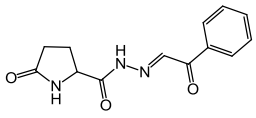
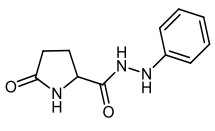
As mentioned in the *N*-acylhydrazones sub-chapter, besides the main activity that is the antifungal activity of hydrazones in which we were particularly interested in, such molecules present other pharmacological properties as well. One of such properties is as potential anticancer drugs. In the context of the laboratory´s continuous interest in developing potent farnesyltransferase inhibitors, the *N*-acylhydrazone compounds were tested as potential

farnesyltransferase inhibitors. Considering the fact that NAH derivatives are able to form stable complexes with metallic cations such as zinc, aluminium or iron,[205] we thought such properties of metal chelators should be explored, under the presumption that they could inhibit zinc proteins such as farnesyltransferase.

A first screening at a high single dose (100 µM) was realized to identify compounds that exhibit strong inhibition of the protein among the hydrazone series and in order to establish early structure-activity relationships regarding *N*-acylhydrazone moiety, different links between the pyrrolidone ring and the aromatic moiety were tested as well (**Table 35**, entries 26, 27). Only candidates inhibiting more than 60% of the protein were selected for IC_{50} determination by sequential dilutions at eight decreasing concentrations.

Entry	N° Compound	N°HEI	Structure	Inhibition % at 100 µM	IC_{50} (µM)
1	123	3140		58	/
2	124	3163		77	0.341
3	125	3164		51	/
4	126	3138		48	/
5	127	3165		23	/
6	128	3166		42	/
7	129	3141		41	/
8	130	261		22	/
9	131	3146		18	/
10	132	3162		84	0.493
11	133	3160		65	2.29

[205] Repich H., Orysyk S.I., Orysyk V.V.,.Zborovskiib Y.L., Pekhnyoa V.I., Vovk M.V. Synthesis, crystal structure and spectral characterization of the first Ag+ complex compounds involving *O,N,O*-coordinated *N*-acylhydrazones of salicylaldehyde. *Molecular Structure* 2017, 1144, p. 225-236.

12	134	3147		92	/
13	135	3145		0	/
14	136	3159		48	/
15	137	3158		27	/
16	138	3157		54	/
17	139	3142		60	/
18	140	3144		37	/
19	141	3207		26	/
20	142	3143		71	0.090
21	143	3155		45	/
22	144	3154		86	17.6
23	145	3139		53	/
24	146	3149		62	0.164
25	147	3206		49	/
26	148	3156		62	0.0011
27	92	2840		12	/

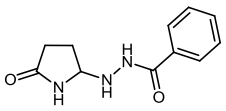
28	46	2970		36

Table 34 *Inhibition percentages and IC_{50} results of the most active NAH on FTase*

The results of the FTase inhibition assays revealed some very promising compounds with activity in the nanomolar range. Saturated aliphatic moieties NAH were first tested. Compound **123**, with a linear octyl chain, revealed a moderate 58% of inhibition at 100 µM whereas compound **124** which resulted from the coupling of citral with the pyroglutamic precursor, strongly inhibited the human FTase with an IC_{50} value of 0.341 µM. Its activity could maybe be due to the isoprenyl hydrophobic chain, which is quite similar to that of the farnesyl group, even if it is shorter. Compound **124** could represent an exciting platform molecule for the discovery of new FTase inhibitors. Other biosourced derivatives were screened as well. Compounds **125**, **127** and **128** coming respectively from hydroxymethyl furfural, myrtenal and perillaldehyde, did not reveal interesting inhibitory activities (**Table 35**, entries 3, 5, 6). Considering cyclohexyl NAH, derivative **126** had a slightly lower activity (51% of inhibition at 100 µM).

Going further with the aromatic NAHs, we can observe that the substitution of the aromatic moiety had a substantial impact on the biological activity. While the simple NAH model compound **130** revealed no activity against the protein (**Table 35**, entry 8) the addition of electron-donating groups led to different effects with regard to the FTase inhibition. Compounds having a methyl- group in *para* position, or a trimethoxyphenyl hydrazone moiety present no improved profile (**Table 35**, entries 9, 13). However, the salicyl derivative **132**, bearing in *ortho*-position hydroxyl presented a very good profile, with an IC_{50} of 0.493 µM. Also, introducing a methoxy- donating group in *para*-position improved the activity and led to a good inhibitor (**Table 35**, entry 11, compound **133**, IC_{50} (FTase) = 2.29 µM).

When halides are introduced on the aromatic ring, a general activity is observed, with an excellent activity for the 4-bromated compound **142** that displayed an IC_{50} of 90 nM (**Table 35**, entry 20). The *p*-bromo substitution has already been highlighted by our group as beneficial for inhibitory potential against FTase in different series of compounds.[206] Electro-withdrawing groups (-CF_3, -NO_2 and -CN) introduced in the *para* position resulted in poor results. Only the nitrated compound **144** was able to inhibit the FTase with an acceptable IC_{50} value of 17.6 µM

[206] Moise I.M., Ghinet A., Belei D. New indolizine–chalcones as potent inhibitors of human farnesyltransferase: Design, synthesis and biological evaluation. *Bioorg. Med. Chem. Lett*. 2016, 26, 15, p. 3730-3734.

(**Table 35**, entry 22), proving the importance of the *para*-position, as the *ortho*-derivative **143** had none (**Table 35**, entry 21).

Replacing the phenyl moiety by a 2-pyridinyl ring allowed to obtain the potent FTI **146** with an IC_{50} value of 0.164 µM (**Table 35**, entry 24). However, using a 4-chloro-3-pyridinyl ring decreased the inhibitory activity (**Table 35**, entry 25). As the elongation of the carbon chain between the carbohydrazide and the phenyl part seemed to increase the activity, compound **129** revealing a higher inhibitory activity than compound **130** at 100 µM (**Table 35**, compare entry 7 and entry 8) we decided to test also derivative **126**. To our delight, the latter compound, bearing an additional carbonyl group between the carbohydrazide and the aromatic ring was found to be highly active against FTase with an IC_{50} value of 1.1 nM (**Table 35**, entry 26). While the 5-atoms linker in **126** allowed us to reach a very strong farnesyltransferase activity with an IC_{50} of 1.1 nM (**Table 35**, entry 2), we wanted to see if there was any interest in reducing the link and the carbazide **92** was also tested. Seemed that reducing the linker to 3-atoms did not lead to any potent activity (**Table 35**, entries 27).

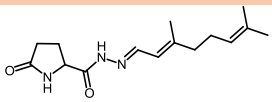
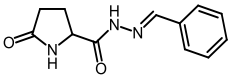
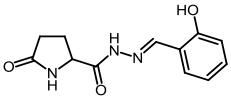
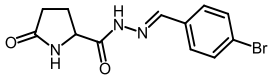
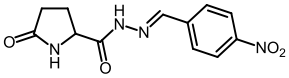
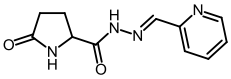
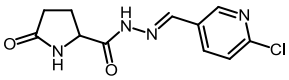
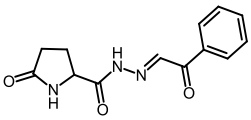
Entry	N° Compound	N°HEI	Structure	Zn^{2+}	Mg^{2+}	IC_{50} (µM)
1	124	3163		yes	no	0.341
2	130	261		no	no	-
3	132	3162		yes	yes	0.493
4	142	3143		no	no	0.090
5	144	3154		no	no	17.6
6	146	3149		yes	no	0.164
7	147	3206		no	no	-
8	148	3156		yes	no	0.0011

***Table 35** Chelation results of selected NAHs*

As farnesyltransferase is known as a zinc enzyme, some experiments were conducted by UV-Vis to examine the ability of the targeted compounds to chelate zinc cations. The molar ratio method was used with compounds **124**, **130**, **132**, **142**, **144**, **146**, **147**, **148**, in order to bring to light stable complexes. In a typical experiment, a 10^{-4} M solution of the designed FTI

was placed in the measurement UV quartz cell and increasing amounts of a concentrated zinc acetate solution were sequentially added. The spectra of the complexes were compared with those obtained in presence of sodium acetate in the same concentration of acetate, in order to eliminate the sole influence of the pH variation. The same experiments were also realized in presence of magnesium acetate to check the selectivity of the chelation **Figure 63** shows the results obtained for compounds **130**, **124** and **148.** Analyzing the data, it is clear that compound **130** is unable to form a stable complex with Zn^{2+} whereas compounds **124** and **148** strongly chelated zinc cations. For compound **148**, the species with the maximum peak at 280 nm represents the free product and the increasing species at 364 nm is the complexed structure.

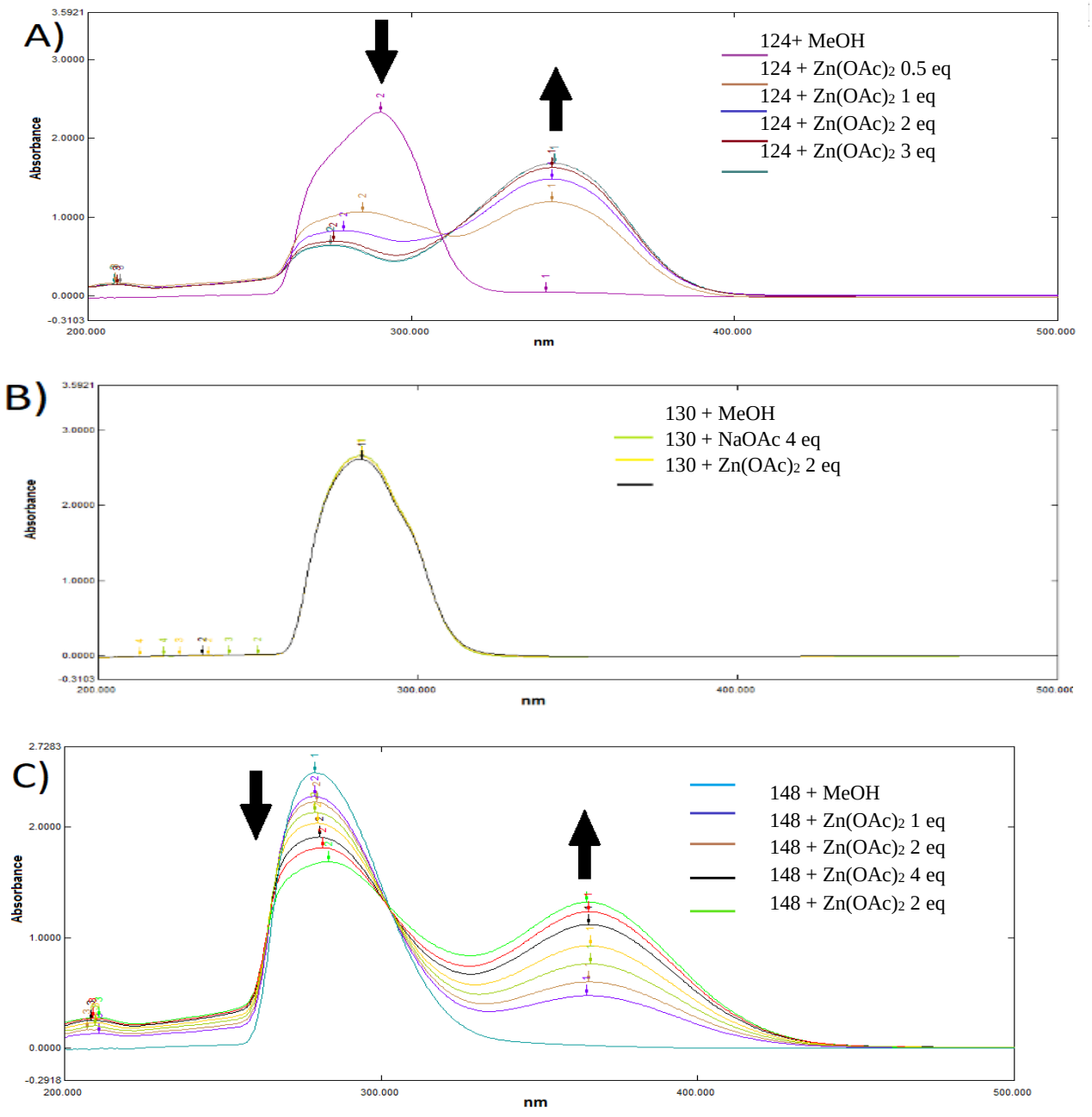


Figure 63 *UV-Vis spectra obtained for compounds 124, 130 and 148.*

To summarize the results and underline the main structure-activity relationship aspects, we can say the following:

(1) Alkyl moieties are not well tolerated, excepting the citral derivative which activity might be linked to its similarity with the farnesyl group;

(2) Aromatic substitution of the phenyl ring is better tolerated;

(3) Stronger electron-donating groups are well tolerated, while methyl- derivatives do not present the seeking activity;

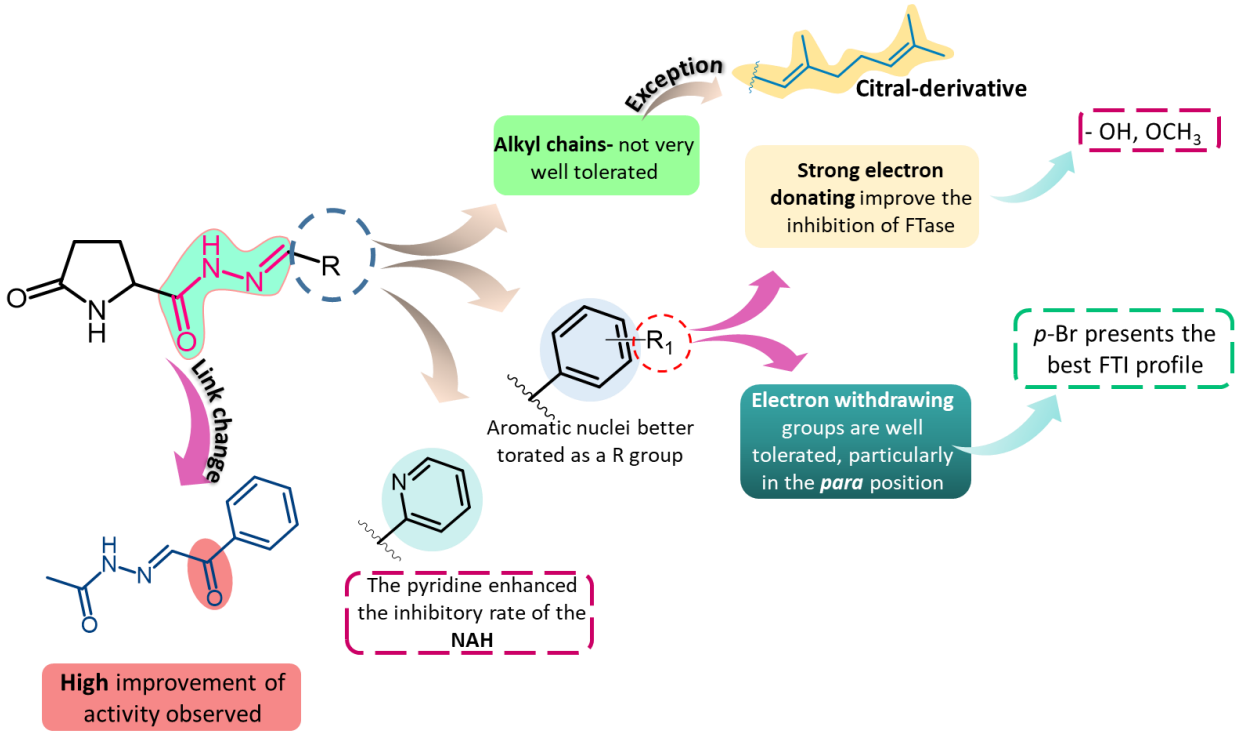


Figure 64 *SAR of NAHs as FTIs*

(4) Among the halogen substituted NAH derivatives, the brominated derivative was the best candidate;

(5) The lipophilicity of the pyridine group was more tolerated compared to the phenyl analogue;

(6) The additional carbonyl group is essential for the activity furnishing **126** with an IC_{50} equal to 1.1 nM, whereas compound **130**, exhibits only a low activity.

As mentioned before in the antifungal subchapter 2.5.1, such NAHs present little to no cytotoxicity, having a safe profile, proving they are good candidates for further development as FTI inhibitors. Moreover, extended research will be needed especially for the modulation of derivative **126** and elucidation of its mode of action, while so far we speculate that the

impressive biological activity could be attributed to its strong ability to chelate the zinc metallic cation.

II.4.4 Conclusion and perspectives considering the global biological results

Within this sub-chapter, two series of molecules were screened for their farnesyltransferase inhibitory efficiency, as an additional test which is frequently done within our team. The test revealed that derivatives **12** and **148** have shown the greatest inhibitory rate, with very low values of IC_{50} (**Figure 65**).

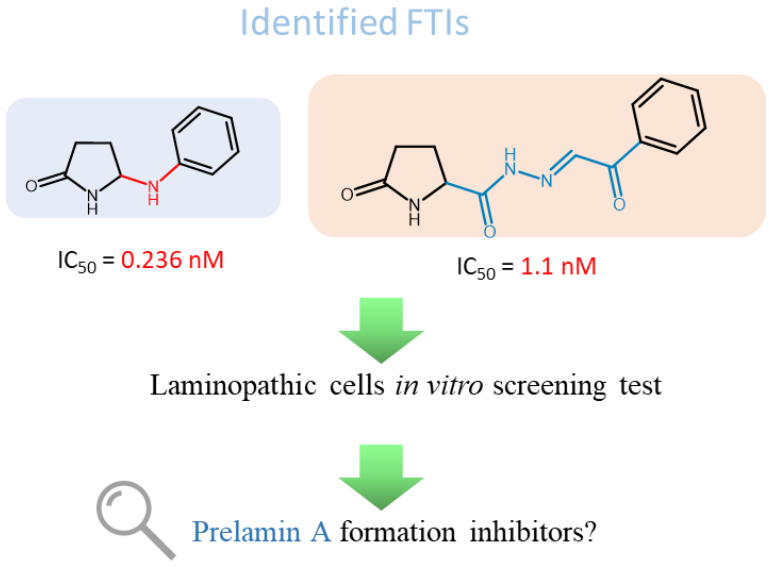


***Figure 65** FTIs identified molecules*

Considering future perspectives, the identified molecules will be further tested for their efficiency of preventing prelamin A formation. This enzyme which was briefly previously discussed, is known to cause disorders laminopathies such as Hutchinson–Gilford progeria, and other aging-related disorders, which at the moment are continuously investigated and for which there are no efficient treatments.

CHAPTER III

ANALYSIS TECHNIQUES

AND STABILITY STUDIES

III.1 Chirality

In the 18th and 19th centuries, the tartaric acid which was discovered during the winemaking as it was building up the vat walls, was very much used after by chemists in dyes and also in medicinal drugs synthesis. Because of a technical mistake, in 1819 the wine was boiled too much and by hazard the paratartaric acid was produced. At that time, the only way to solve the mistery of how a molecule is built, was by studying its crystal structure. The way crystal interacted with the light, was a hint about the molecule properties. In 1832 the French chemist Jean Baptiste Biot observed that tartaric acid obtained from tartar was optically active, rotating the plane of polarized light clockwise (dextrorotatory).

Its unique properties have intrigued scientists, including young Louis Pasteur, who a little more than a decade later, conducted a careful study of the crystalline forms assumed by various salts of these acids. Pasteur observed the existence of two crystals being mirror images in tartaric acid and also discovered that mirror-image crystals, as a 50/50 mix in the solution, canceled out each other's ability to rotate polarized light. He managed to separate the different crystals apart with a tweezer, discovering that one part was the known dextrorotatory tartaric acid measured by Biot while the second led to a previously unknown levorotatory tartaric acid. Today, Louis Pasteur is recognized as being the first who showed the existence of chiral molecules and achieved the first resolution of a racemic mixture, and laid the foundation of what we now call stereochemistry. The word "chiral" comes from the ancient Greek "cheir" which means hand. Chemistry changed forever. The relationship between isomers, which are different compounds with the same molecular formula but different chemical structures is depicted in **Figure 66**.

Every living body contains amino acids, sugars, proteins and nucleic acids, essential for a living body and many of them are of chiral molecules. An interesting feature of these chiral biomolecules is that in nature they usually exist in only one of the two possible enantiomeric forms. Most often, enantiopure drugs are desired, as they will be used in living systems. Drug molecules can be considered as to tiny keys that fit into locks in the body and express a particular biological response. Since the 'locks' in living organisms are chiral, and exist in only one of the two possible enantiomeric forms, only one enantiomer of the 'key' molecule should be used.[207]

[207] Lien A. N., Hua H., Chuong P.H. Chiral Drugs. An Overview. *Int. J. Biomed. Sci*. 2, 2, p. 85-100.

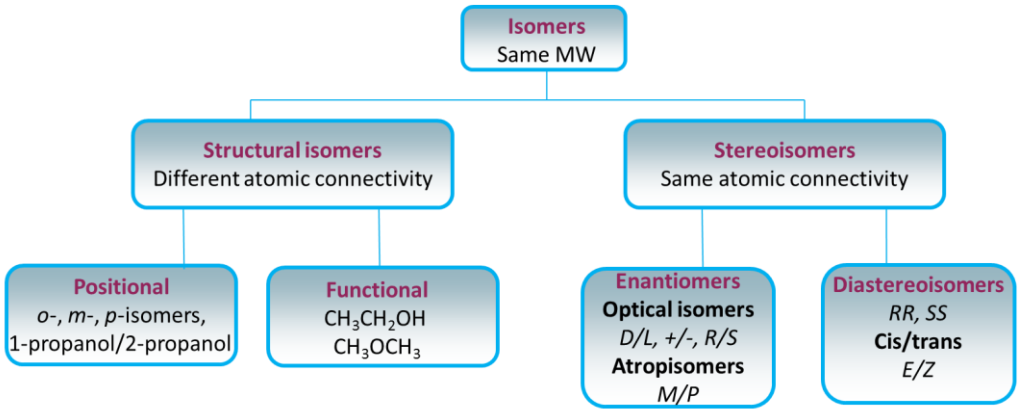


Figure 66 *Relationship between isomers*

In 1992 the FDA issued a policy statement regarding the development of new stereoisomeric drugs. The new policy stated that the stereoisomeric composition of any drug containing a chiral center must be known and also characterized in pharmacological, toxicological and clinical studies. Things considered, this would require the physical separation of the two enantiomers. At that moment, the field of enantiomeric separations become an important tool in the analytical separation science arsenal. The importance of chirality has been appreciated and addressed by the pharmaceutical industry for decades. As a consequence, technologies for measuring and making enantiopure materials have improved, and the simple production of enantiopure pharmaceuticals has become commonplace, the top selling drugs in the world now being sold as a single enantiomeric form.

In general, the stereogenic center responsible for chirality is an asymmetric carbon. But chirality does not lie solely on the presence or absence of asymmetric carbons. There are chiral metal complexes that involve three bidentate ligands and show an axial chirality or planar chirality. It is the same for alleles, diphenyls and spiro compounds.

Throughout life, our body is subjected to many chemical reactions to prevent enzymes folding, or proteins and peptides composed of amino acids. Except for glycine, all amino acids are chiral. Fortunately, the vast majority of them exist naturally in the L form. However, there are low levels in the body in the form of D. This racemization of the L into form D was detected in tissues and bones of dead organisms. The rate of racemization D/L of certain amino acids, (which should not be confused with the absolute configurations R/S), can thus play the role of indicator of fossil dating but also as a food controller in the food industry.

In the last 30 years, chiral separations have evolved. Now commercially available chiral columns, chiral derivatizing reagents, and chiral selectors for approaches that include all of the analytical separation techniques as high-performance liquid chromatography (HPLC), gas

chromatography (GC), supercritical fluid chromatography (SFC), and capillary electrophoresis (CE) exist.

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a “stationary phase”, which separates the analyte from other compounds in the mixture based on differential partitioning between the mobile and stationary phases.

Chromatography can be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use and also a form of purification technique. Analytical chromatography is done usually on small scale and is measuring the relative proportions of analytes in a mixture. Chiral chromatography involves the separation of stereoisomers. Considering the enantiomers, particularly, the conventional chromatography or other methods of separation processes are not efficient in separating them. In order to separate enantiomers, the mobile phase or the stationary phase must be chiral, giving different affinities between the analytes. Chiral entity will preferentially interact with one of the enantiomers more than the other and thus separate the two. In the case of a chiral agent such as a stationary phase for example, the enantiomers form short-lived diastereomeric adsorbates that can be separated on an achiral column. This interaction, or chiral recognition, has been called the three point interaction model, a minimum of three simultaneous interactions being needed between the CSP and the enantiomers. At least one of these interactions will be dependent on the enantiomer configuration, as it will be present for one enantiomer but weaker or completely absent for the other one.

III.1.1 Supercritical phase chromatography

Supercritical phase chromatography was introduced in 1962, when Klesper and *al*. demonstrated that working at a pressure above the critical point of the mobile phase was able to work at lower temperatures than in gas phase (GC) and thus ensured a better stability of the studied compounds. The mobile phase was then constituted from a halogenated alkane and the stationary phase of a polyethylene glycol. The authors described it as being a chromatography using a dense gas phase that allows the study of thermo-labile compounds. At this time, the name HPGC (high pressure gas chromatography) was proposed. In the 1960s, other groups became interested in HPGC and demonstrated the superiority of this technique in separating

thermally unstable compounds in GC.[208] However, the SFC was only seen as a variant of the GC and not as a new innovative separation method. This technique does not have managed to win for the GC, despite the commercialization of a first device of SFC in the 1970s. It was actually a liquid chromatography system (LC) equipped with a pressure regulator. The ability to adjust the pressure with this system has allowed to separate polycyclic aromatic compounds by using a pressure gradient in a shorter time than in liquid chromatography. For the first time, SFC became competitive with CG.

SFC, then seen as an approach intermediary between GC and LC since its inception, failed to establish itself as chromatographic method. At the same time, Gere *et al*. developed a system dedicated to the SFC, which largely takes the elements of a LC system, with the exception of a back pressure regulator used to maintain fluid in the supercritical state and allowed to use a binary mobile phase consisting of CO_2 and co-solvent in a small amount.[209] This system, marketed by Hewlett-Packard, used for the first time packed columns for an application in SFC. Gere *et al.* showed that an increase of the efficiency was obtained by decreasing the particle size of the columns used (3, 5 and 10 μm).

They also showed that for an identical particle size, SFC and LC offer similar efficiency but with greater optimal linear velocity for SFC, which means that high efficiencies are achieved in SFC even at high flow-rates. Despite these advances, the SFC remained at the end of the 1980s a not so used method because of instrumental instabilities, which made this technique unreliable, sensitive and robust in the eyes of chromatographists.

In the 1990s, few laboratories continued to believe in SFC. At this time, Berger, considered by many chromatographers as the father of the Modern SFC, worked extensively on the analysis of polar compounds[210] in SFC and the use of acidic and basic additives in the mobile phase to improve the peaks. In 1995, employed by Hewlett-Packard, he founded Berger Instruments Inc with some of his colleagues and then begin to modify the first instrument marketed by Hewlett-Packard to make it compatible with the use of packed columns. This system was mainly used in pharmaceutical industry.

[208] Giddings J.C., Myers M.N., McLaren L., Keller R.A. High pressure gas chromatography of nonvolatile species. Compressed gas is used to cause migration of intractable solutes. *Science*, 1968, 162, p. 67–73.
[209] Gere D.R., Board R., McManigill D. Supercritical fluid chromatography with small particle diameter packed columns. *Anal. Chem.* 1982, 54, p. 736–740.
[210] Berger T.A. Separation of polar solutes by packed column supercritical fluid chromatography. *J. Chromatogr. A.* 1997, 785, p. 3–33.

For another two decades, the use of SFC at analytical scale was very weak and the technique survived only thanks to the recognized advantages for the enantiomeric purification on the preparative scale. Today, the SFC dominates, however a minority of applications still being done on a capillary column, typically in the oil field. The SFC is a reference in the field of chiral separation,[211] both analytical rather than preparative scale, and many pharmaceutical companies have today adopted for the benefit of it or in addition to HPLC.[212] Indeed, the SFC has many advantages over HPLC for separation and purification of chiral compounds: (i) the separations carried out are rapid, (ii) the used CO_2 commonly as a supercritical fluid is non-toxic, (iii) the evaporation of the solvent at the preparative scale is facilitated by the return of CO_2 to the gaseous state when it is no longer maintained in the supercritical state as well as by the low organic solvent content of collected fractions, which represents a significant economic advantage.

III.1.1.1 The use of packed columns

The packed SFC consists of the use of packed columns and reflects the fact that the SFC can also be considered to be the extension of liquid chromatography. This system is inspired directly from a HPLC system. Using packed columns increases interaction surfaces with the stationary phase and decrease dead volumes. Just like in LC, the mobile phase can consist of a binary mixture: a co-solvent (or polar modifier) is added to CO_2 in order to modulate the eluting power of the mobile phase. This strategy allowing the study of more polar compounds and widens the field application of the SFC, in particular to that of the pharmaceutical compounds.[213] Given the low viscosity of supercritical fluids, pressure drops in the system are lower than in LC. It is therefore technically possible, though a packed column, to increase the flow of the phase mobile without the risk of reaching instrumental limits. Moreover, considering the great value of diffusion coefficient, this increase in throughput will have no impact on the quality of the observed separations. These properties lead to flows that are often 3 times higher in SFC compared to the LC, but at the same time, a lower solvent consumption since the largest part of the mobile phase is CO_2.

[211] Anton K., Eppinger J., Frederiksen L.,Francotte E., Berger T.A., Wilson W.H. Chiral separations by packed-column super- and subcritical fluid chromatography. *J. Chromatogr. A.* 1994, 666, p. 395–401.
[212] Welch C.J., Biba M., Gouker J.R., Kath G., Augustine P., Hosek P. Solving multicomponent chiral separation challenges using a new SFC tandem column screening tool. *Chirality*, 2017, 19, p. 184–189.
[213] Pinkston J., Wen D., Morand K.L., Tirey D.A., Stanton D.T. Comparison of LC/MS and SFC/MS for Screening of a Large and Diverse Library of Pharmaceutically Relevant Compounds. *Anal. Chem.* 2006, 78, p. 7467-7472.

III.1.1.2 SFC analytics today

Currently, the range of commercial equipment is quite limited. One of today's systems include the ACQUITY UPC², a dedicated SFC device marketed by Waters Corporation in 2012. In 2011, Agilent commercialized an LC / SFC hybrid system called the 1260 Infinity SFC System, which simultaneously perform UHPLC analysis and switch to SFC through the addition of a CO_2 pumping system and a back-pressure regulator. These instruments have been designed to reduce dead volumes and gradient delay and thus improve analytical performance, as for UHPLC systems. The term UHPSFC (for "Ultra-high performance supercritical fluid chromatography") is now emerging, and refers to the combined use of an optimized SFC system and sub-2 µm columns.[214]

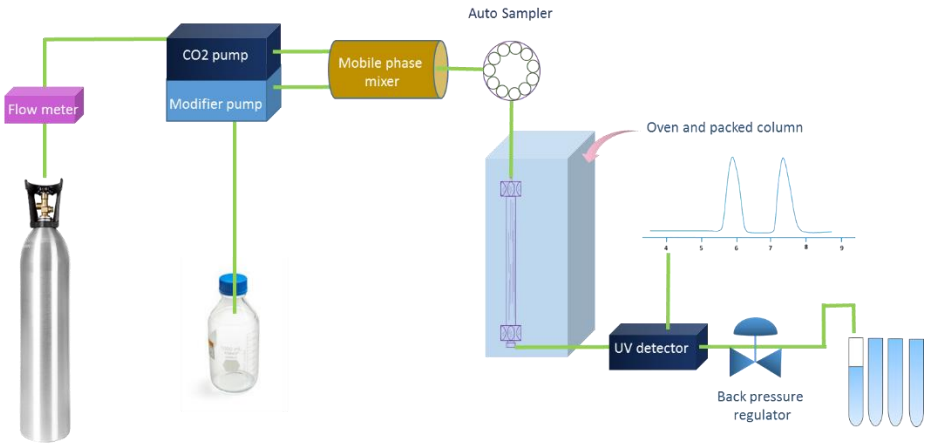


Figure 67 *General scheme of a SFC device*

It should be noted that other manufacturers market analytical SFC systems (Shimadzu, Pic Solution or Jasco). However, these still seem not so often used in the academic world, in view of their weak presence in the literature. All of these systems use chromatographic columns of geometries similar to the liquid chromatography.

III.1.1.3 Supercritical fluids

Each compound can exist under three different states: solid, liquid or gaseous. The state in which the compound is found is in function of pressure and temperature (**Figure 68**).

[214] Berger T.A. Demonstration of high speeds with low pressure drops using 1.8 µm particles in SFC *Chromatographia*, 2010, 72, p. 597–602.

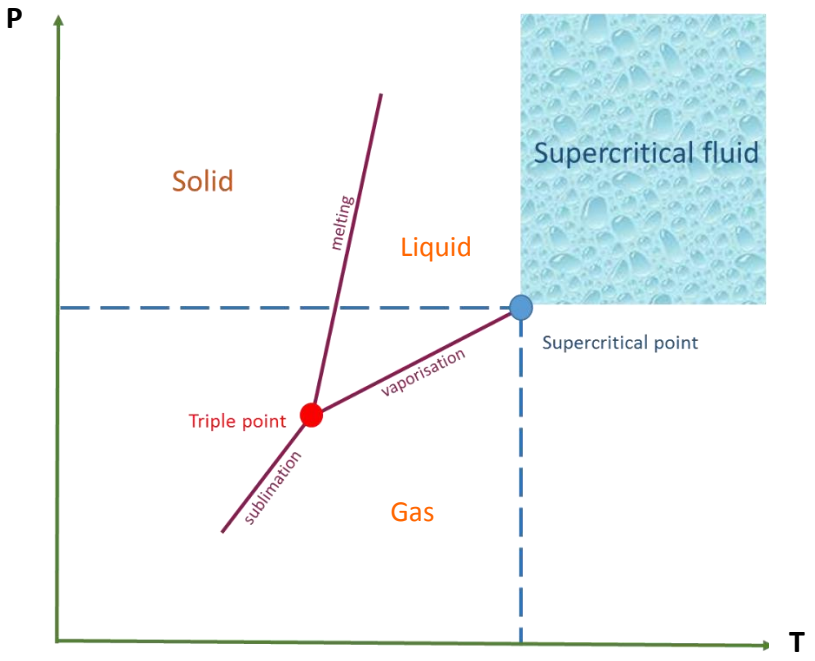


Figure 68 *Diagram of state of a pure substance*

The domains of these different states are delimited by the curves of state change, reflecting phase transitions (melting for the transition from solid to liquid state, vaporization for the transition from liquid to gaseous state and sublimation for transition from solid to gaseous state). Each pure substance will therefore exist under a state of matter, except from the curves of change of state where the two states coexist and at the triple point where the three states of matter are in equilibrium.

The vaporization curve is interrupted at the critical point, with coordinates (Tc, Pc). The supercritical state is obtained when a pure substance is subjected to a pressure and a temperature higher than the critical pressure (P_C) and the critical temperature (T_C). At the liquid/gas interface, on the vaporization curve, the separation of the two phases is clear (**Figure 68**). At the critical point, the density of the liquid and gaseous phases tends towards an equilibrium and the change of state curve is interrupted, which guarantees a continuum of the physicochemical properties during the transition to the supercritical state. It is then more difficult to distinguish the separation of the two phases. While in supercritical state, the two phases merge. This homogeneous phase is one of a supercritical fluid.

III.1.1.4 Mobile phases

Several types of fluids have been used in the supercritical state in chromatography,[215] depending on the desired application: reaction, purification, extraction, chromatography (**Table 37**).

Substance	T_c (°C)	P_c (bar)
Carbon dioxide	31	74
Nitrous oxide	37	73
Freon	96	49
Propane	97	43
Ammonia	132	113
Methanol	240	80
Water	374	221

Table 36 *P_C and T_C of some fluids*

Among these fluids, some are not used in chromatography because it is difficult to reach the conditions of P_C and T_C. This is the case of water (T_C = 374°C and P_C = 221°C), methanol (T_C = 240°C) and ammonia (high T_C and P_C) which is also extremely corrosive.

Current supercritical phase chromatography systems as well as liquid chromatography, use a binary pump to create the mobile phase flow. This pump allows the mixing of two different solvent routes in a mixing chamber. One of the pathways consists of compressed CO_2 and the other of an organic solvent which has the role to modulate the elution force of the mobile phase. This solvent is called polar modifier or co-solvent. In order to be used as a polar modifier, the solvent must in particular be miscible with the compressed CO_2 and be available in HPLC quality. In time, SFC became an universal tool for the separation of a broad range of analytes, though the simple adjustment of the modifier (**Figure 69**).

In practice, the most commonly encountered co-solvent is methanol as it can modulate

[215] Guiochon G., Tarafder A. Fundamental challenges and opportunities for preparative supercritical fluid chromatography. *J. Chromatogr. A*. 2011, 1218, p. 1037– 1114.

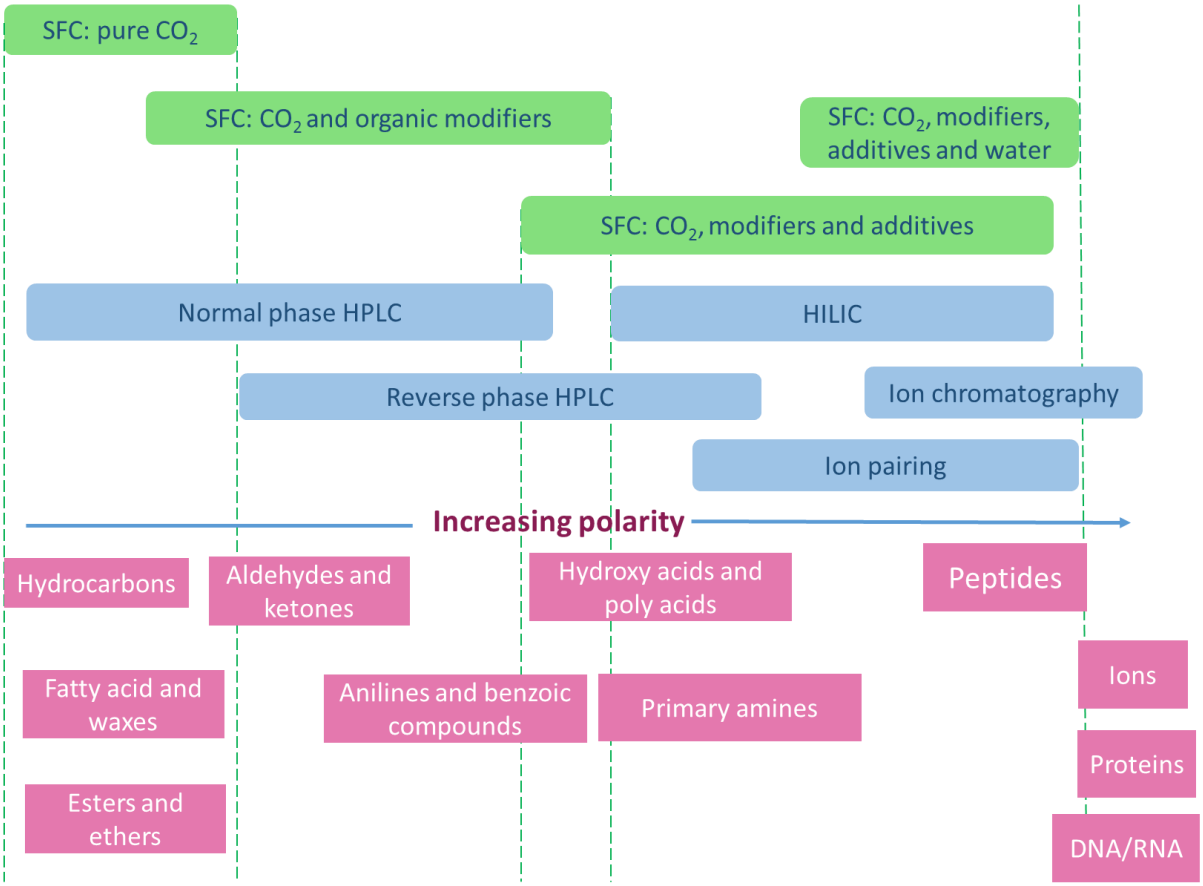


Figure 69 *Type of mobile phases to be used depending on the polarity of the targeted analytes*[216]

the elution strength, it is available in analytical quality, inexpensive and has relatively low toxicity.

Other solvents such as ethanol, isopropanol and less frequently acetonitrile are also used. The polarity (P') is one important characteristic of a solvent, which has an impact on the elution strength, respectively on the selectivity. The values obtained for the four most used modifiers are reported in **Table 38**.

Solvent	Polarity index (P')
Methanol	6.6
Acetonitrile	6.2
Ethanol	5.2
Isopropanol	3.9

[216] Berger T. A., Supercritical Fluid Chromatography. Primer. Agilent Technologies, 2015.

Table 37 Polarity of the main co-solvents used in SFC

It is recognized that when a co-solvent is used in the mobile phase, the analytes are found within an aggregate consisting of co-solvent molecules. This theory is in agreement with the increase of the diffusion coefficient of the analytes when a co-solvent is added. In fact, the formation of aggregates is leading to an increase in the collision cross section and therefore a decrease in diffusivity.

III.1.1.5 Stationary phases in SFC enantioseparation

In modern SFC, most of the separations are mainly performed on packed columns, though capillary columns also exist. The column as a parameter, determines if a method has the potential to succeed or not being absolutely vital for the separation. Many of the chiral CSPs used in SFC are well known from HPLC, being originally designed and marketed for chiral HPLC. Among the most known columns which have been transferred to SFC library of columns as well, are: polysaccharide, cyclodextrin, and (glyco)protein based, as well as Pirkle-type CSPs. Some of the general structure are depicted in **Figure 70**.

Among these, polysaccharides-based stationary phases have taken a dominant position in chiral SFC because of their easy accessibility and broad enantioselectivity.[217] Many publications have underlined the important role of amylose and cellulose based CSPs, which can be found either immobilized or coated on silica gel, the last one having a limited solvent compatibility and solvents that dissolve the selector coating are therefore incompatible such as acetone or tetrahydrofuran.[218,219]

[217] Dispas A., Jambo H., Andre S., Tyteca E., Hubert P. Supercritical fluid chromatography: a promising alternative to current bioanalytical techniques. *Bioanalysis*, 2018, 10, 2, p. 107–124.
[218] Svan A., Hedeland M., Arvidsson T., Jasper J.T., Sedlak D.L., Pettersson C.E. Rapid chiral separation of atenolol, metoprolol, propranolol and the zwitterionic metoprolol acid using supercritical fluid chromatography-tandem mass spectrometry - Application to wetland microcosms. *J. Chromatogr. A*, 2015, 1409, p. 251-258.
[219] Yang Z., Xu X., Sun L., Wang H., Fawcett J.P., Yang Y., Gu J. Development and validation of an enantioselective SFC-MS/MS method for simultaneous separation and quantification of oxcarbazepine and its chiral metabolites in beagle dog plasma. *J. Chromatogr. B.* 2016, 1020, p. 36–42.

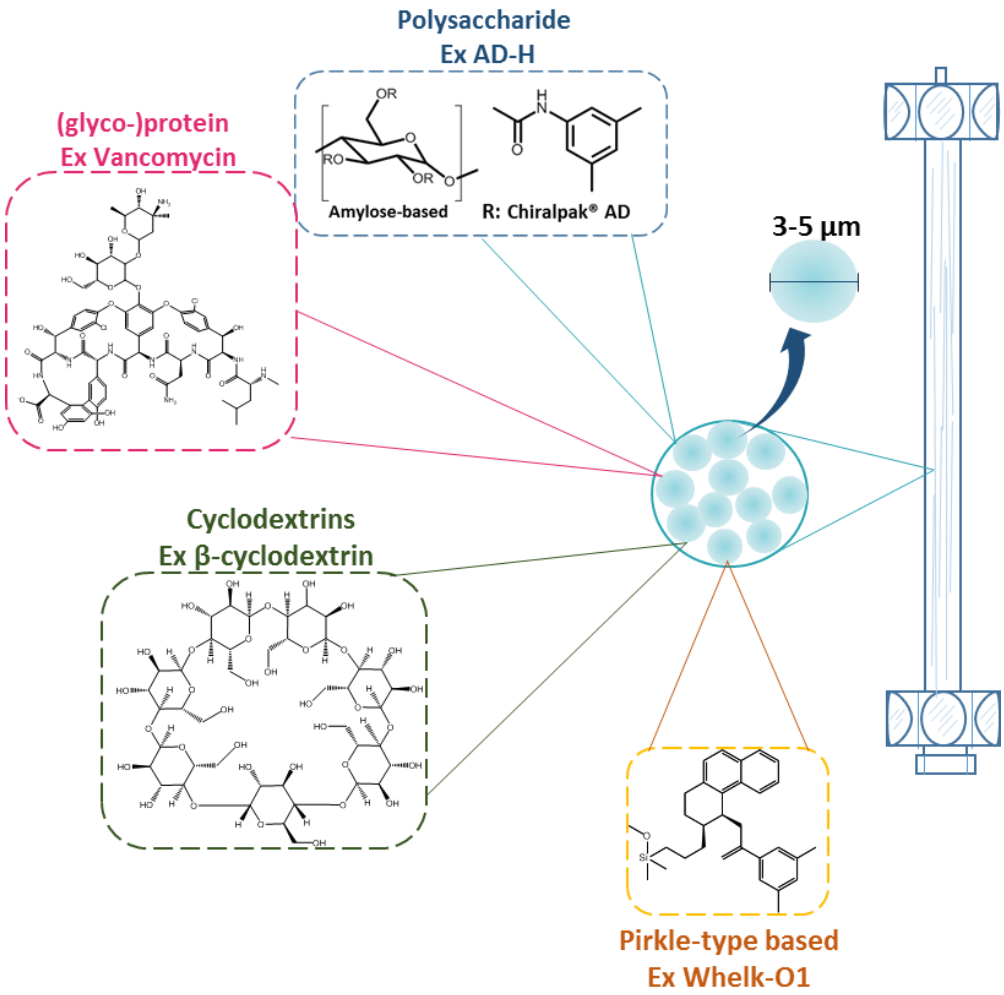


Figure 70 *Different structure of the most used chiral stationary phases (CSPs)*

III.1.1.6 Chiral separation mechanism

Because of their structures, polysaccharides present multiple binding sites, the reason why the chiral recognition mechanism can be quite complex and so far not completely elucidated. However, it is assumed that the chiral separation comes from the inclusion interactions inside the polysaccharide helical structure, aromatic functional groups which interacts with the selector along with the hydrogen bindings all adding to enantioselectivity. The important enantiodiscrimination model on which the chiral separation is based on is the three-point interaction between the chiral selector and the analyte (**Figure 71**).

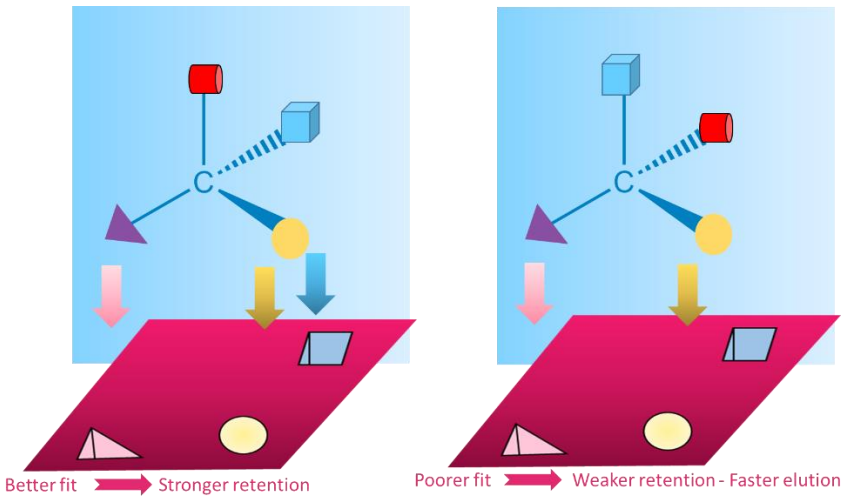


Figure 71 *Three-point interaction model, where one enantiomer matches the three inclusion sites, whereas its mirror enantiomers matches maximum two sites.*

III.1.1.7 Study results and discussion for the performance of chlorinated chiral stationary phases or separation of selected pyrrolidone derivatives

It is known that the introduction of electron-withdrawing groups such as halogens groups, or electron-donating substituents like alkyl groups are modifying the electron density on the phenyl ring by their inductive effect, leading to weaker or stronger π–π interactions, consequently the structure enhances the enantioselective interactions, the resolving capacity being improved. Based on this fact, Chankvetadze *et al.* developed some chlorinated and methylated polysaccharide derivatives as chiral selectors as for example cellulose *tris*(3-chloro-4-methylphenylcarbamate).[220] However, these halogenated polysaccharides based CSPs originally developed for HPLC have not reached the market until 2005.

So far, the coated chlorinated carbamate phases were deeply characterized and have found applications as their immobilized versions. However, in 2018, there were only few

[220] Chankvetadze B., Yashima E., Okamoto Y., Chloromethylphenylcarbamate derivatives of cellulose as chiral stationary phases for high-performance liquid chromatography. *J. Chromatogr. A*, 1994, 670, p. 39-49.

studies[221,222] concerning the use of cellulose *tris*(3-chloro-4-methylphenylcarbamate) chiral column or articles dealing with cellulose *tris*(3,5-dichlorophenylcarbamate)[223,224,225] in SFC.

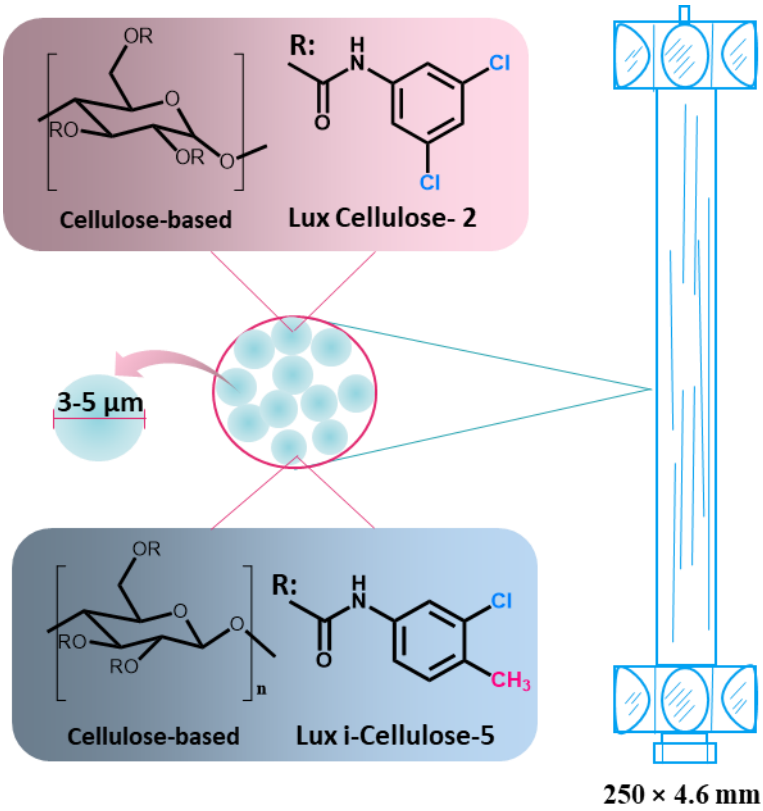


***Figure 72** Chlorinated stationary phases for the separation of 5-pyrrolidones*

Considering this aspect, in order to enrich our knowledge around the resolving capacity of these columns along with enantiopurity check and separation optimization of several selected molecules which have been synthesized within this project, we decided to investigate the two column mentioned before: cellulose *tris*(3-chloro-4-methylphenylcarbamate) and cellulose *tris*(3,5-dichlorophenylcarbamate) (**Figure 72**).

[221] Alvarenga N., Porto A.L.M., Barreiro J.C. Enantioselective separation of (±)-β-hydroxy-1,2,3-triazoles by supercritical fluid chromatography and high-performance liquid chromatography. *Chirality*, 2018, 30, p. 890-899.
[222] Zhao L., Xie J., Guo F., Liu K. Enantioseparation of napropamide by supercritical fluid chromatography: Effects of the chromatographic conditions and separation mechanism. *Chirality*, 2018, 30, p. 661-669.
[223] Pirrone G. F., Mathew R. M., Makarov A., Bernardoni F., Klapars A., Hartman R., Limanto J., Regalado E.L. Supercritical fluid chromatography-photodiode array detection-electrospray ionization mass spectrometry as a framework for impurity fate mapping in the development and manufacture of drug substances. *J. Chromatogr. B*, 2018, 1080, p. 42-49.
[224] Hegade R.S, Lynen F. Chiral stationary phase optimized selectivity supercritical fluid chromatography: a strategy for the separation of mixtures of chiral isomers. *J. Chromatogr. A*, 2018, 1586, p. 116-127.
[225] Lipka E., Dascalu A-E., Messara Y., Tsutsqiridze E., Farkas T., Chankvetadze B. Separation of enantiomers of native amino acids with polysaccharide-based chiral columns in supercritical fluid chromatography. *J. Chromatogr. A*, 2019, 1585, p. 207-212.

The molecules which were used in this study, are pyrrolidin-2-one derivatives, obtained from pterolactam as racemates. As it is known, each enantiomer presents its own pharmacological properties and to be able to separate a racemic mixture is important, in the perpective of biological testing. The pyrrolidin-2-one scaffold is already known as a privileged synthon showing high potential to afford small molecules with interesting biological activities as was reminded in Chapter I.

Six molecules having overall different links have been selected for this study (**Figure 73**), the goal being to compare the separation performances of each chiral selector along taking into consideration the influence of the flow-rate and percentage of methanol on parameters such as retention and resolution.

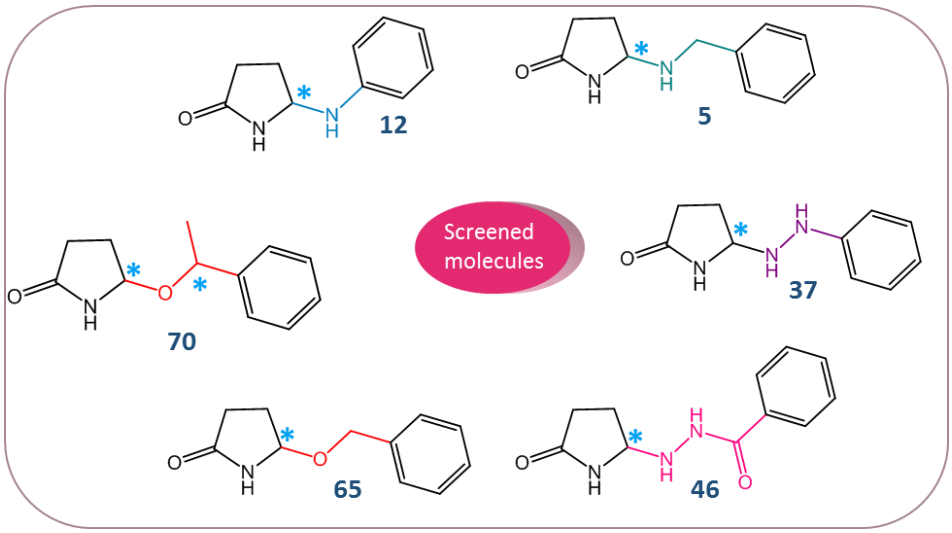


Figure 73 *Screened molecules for enantioseparation*

III.1.1.7.1 Studied chromatographic parameters

The most important criteria in SFC is to obtain the optimum resolution in the minimum time with a baseline separation.

$$R_s = \frac{2(t_{R2} - t_{R1})}{w_1 + w_2}$$

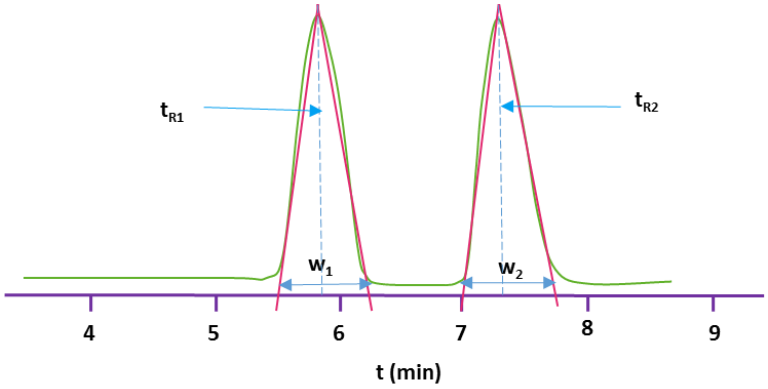


Figure 74 *Resolution calculation*

The resolution equation is as depicted in the **Figure 74**, where t_{R1} and t_{R2} are the retention times of the peaks of enantiomers and ω_1 and ω_2 are the peak widths measured at the baseline between tangents drawn to the peak sides.

The Fundamental Resolution Equation (Purnell equation):

$$R_s = \frac{1}{4\sqrt{N}} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{k+1}$$

Efficiency Selectivity Retention

Which indicates that resolution is influenced by three important parameters:

- Efficiency
- Selectivity (Separation factor)
- Retention (Capacity factor)

Efficiency

The efficiency of a chromatographic peak measures the dispersion of the analyte band as it travels through the SFC system and column. N is the plate number, measuring the peak dispersion on the SFC column, reflecting its performance.

$$N = 16\left(\frac{t_R}{w}\right)^2$$

Retention

The retention (or capacity) factor (k) is a mean of measuring the retention of an analyte on the chromatographic column for each enantiomer, k_1 and k_2 by:

$$k = \frac{t_R - t_0}{t_0}$$

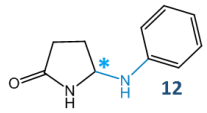
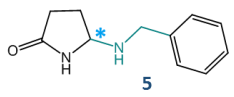
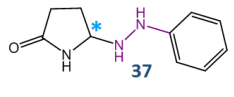
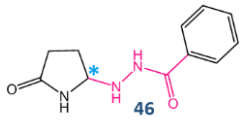
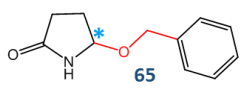
Selectivity

The selectivity (or separation) factor α is the ability of the chromatographic system to 'chemically' distinguish between sample components. This parameter is measured as ratio of the retention (capacity) factors (k) of two successive peaks:

$$\alpha = \frac{k_2}{k_1}$$

III.1.1.7.2 Selection of stationary and mobile phases

The packed chosen columns with two types of chiral selectors Lux Cellulose-2 and Lux i-Cellulose-5, were employed for their separation of the six molecules mentioned in **Figure 73**. The mobile phase is an important criterium both in HPLC and SFC. In order to make our choice considering the co-solvent, we firstly tested 10% of either acetonitrile, *iso*propanol, ethanol or methanol, at a flow-rate of 4 mL/min. Unfortunately, aprotic solvent such as acetonitrile led to the higher retention times, which is followed by *iso*propanol and then ethanol. While in ethanol we did not succeed to resolve all the racemates, methanol seemed a better option, all the molecules being separated. Considering the obtained resolutions and analysis times, the best results were observed with methanol and are included in **Tables 38** and **39**.

Compound	Flow-rate (mL/min)	t_{r1} (min)	t_{r2} (min)	k_1	k_2	a	R_s	N
	2	29.06	32.19	18.76	20.90	1.11	1.50	3770
	2.5	22.62	25.23	17.85	20.03	1.12	1.30	2516
	3	18.49	20.56	17.07	19.09	1.12	1.14	2057
	3.5	15.57	17.15	16.49	18.27	1.11	0.97	1764
	4	13.34	14.80	20.52	22.87	1.11	0.96	1521
	2	24.34	27.89	15.61	18.04	1.16	1.80	3183
	2.5	19.15	21.92	14.96	17.27	1.15	1.46	2133
	3	15.61	17.98	14.26	16.58	1.16	1.31	1577
	3.5	13.04	14.86	13.66	15.70	1.15	1.10	1297
	4	11.21	12.83	17.08	19.69	1.15	1.05	1109
	2	11.93	13.81	7.14	8.42	1.18	1.84	2934
	2.5	9.38	10.82	6.81	8.02	1.18	1.68	2509
	3	7.65	8.85	6.48	7.65	1.18	1.59	2211
	3.5	6.56	7.56	6.37	7.49	1.18	1.41	1828
	4	5.64	6.49	8.09	9.46	1.17	1.24	1434
	2	59.53	84.85	39.63	56.92	1.44	4.79	4120
	2.5	45.82	67.78	37.18	55.48	1.49	4.68	3338
	3	37.06	54.68	35.22	52.45	1.49	4.12	2614
	3.5	30.53	45.21	33.29	49.80	1.50	3.76	2142
	4	26.28	39.15	41.38	62.14	1.50	3.43	1741
	2	7.42	-	4.07	-	-	-	-
	2.5	5.93	-	3.94	-	-	-	-
	3	4.88	-	3.76	-	-	-	-
	3.5	4.09	-	3.59	-	-	-	-

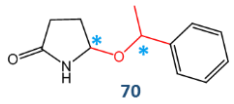
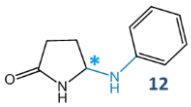
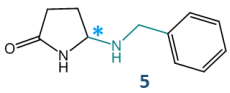
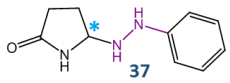
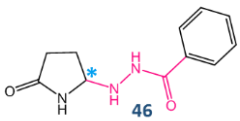
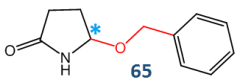
	4	3.56	-	4.74	-	-	-	-
 70	2	11.15	-	6.61	-	-	-	-
	2.5	8.79	-	6.32	-	-	-	-
	3	7.21	-	6.04	-	-	-	-
	3.5	6.05	-	5.79	-	-	-	-
	4	5.28	-	7.51	-	-	-	-

Table 38 *Screening results and obtained parameters under different flow-rates with 10% of MeOH on Lux i-Cellulose-5, 5 µm*

As can be observed from the table results, oxy-pyrrolidones **65** and **70** were not resolved by Lux i-cellulose 5 CSP, but only by Lux cellulose-2. Moreover, derivatives **12**, **5** and **37** were only partially separated on Lux-cellulose 5 which furnished separation values R<1.5, compared to Lux-cellulose 2 which proved to be more efficient than the first one, affording baseline resolved racemates even on 5 µm particles diameter CSP columns. From the preliminary screening we are able to say that the dichlorinated carbamate stationary phase Lux Cellulose-2 seemed to be a better choice. However, more investigations were made, next step being the study of flow-rate decrease on the resolution.

Compound	Flow-rate (mL/min)	t_{r1} (min)	t_{r2} (min)	k_1	k_2	α	R_s	N
 12	2	28.42	31.5	17.90	19.94	1.11	1.47	3643
	2.5	24.25	28.41	20.29	23.94	1.18	1.97	2899
	3	19.93	23.44	19.76	23.42	1.19	1.88	2517
	3.5	16.85	19.79	19.06	22.57	1.18	1.67	2012
	4	15.32	17.22	19.27	21.79	1.13	1.15	1731
 5	2	24.88	29.79	15.54	18.81	1.21	2.83	4703
	2.5	19.36	23.26	16.00	19.42	1.21	2.72	4220
	3	16.05	19.329	15.72	19.13	1.22	2.44	3320
	3.5	13.58	16.35	15.17	18.47	1.22	2.16	2599
	4	11.80	14.19	14.62	17.78	1.22	1.87	1976
 37	2	11.04	12.53	6.34	7.33	1.16	1.71	3312
	2.5	8.69	9.84	6.63	7.64	1.15	1.51	2670
	3	7.21	8.19	6.51	7.53	1.16	1.42	2256
	3.5	6.03	6.89	6.19	7.20	1.16	1.34	1894
	4	5.39	6.09	6.14	7.07	1.15	1.15	1593
 46	2	50.31	68.64	32.46	44.64	1.38	3.59	2895
	2.5	38.42	52.24	32.73	44.86	1.37	3.47	2754
	3	31.50	43.22	31.82	44.02	1.38	3.32	2402
	3.5	26.53	36.31	30.58	42.23	1.38	3.05	2049
	4	22.73	31.34	29.08	40.46	1.39	2.88	1761
 65	2	8.15	8.92	4.42	4.93	1.12	1.19	3063
	2.5	6.39	7.01	4.61	5.15	1.12	1.16	2793
	3	5.33	5.84	4.55	5.08	1.12	1.04	2277
	3.5	4.54	4.97	4.40	4.91	1.12	0.89	1702

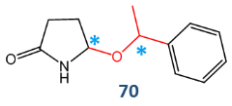
	4	3.93	4.29	4.20	4.68	1.11	0.79	1398
	2	11.36	12.5	6.55	7.31	1.12	1.00	1913
	2.5	8.92	9.92	6.84	7.71	1.13	0.95	1447
	3	7.44	8.26	6.75	7.60	1.13	1.01	1671
70	3.5	6.31	6.99	6.52	7.33	1.12	1.08	1974
	4	5.49	6.05	6.26	7.01	1.12	0.97	1717

Table 39 *Screening results and obtained parameters under different flow-rates with 10% of MeOH on Lux Cellulose-2, 5 µm*

III.1.1.7.3 Flow-rate selection

Things considered, with a 0.5 mL step, the flow-rate was decreased down to 2 mL/mL, the corresponding results on the two columns being summarized in **Tables 38** and **39**.

As a general feature, the separation on the Lux cellulose-2 CSP of mostly all the compounds benefited from this decrease of flow-rate leading to higher resolution values. The first screened molecule, **12** while at 4 mL/min presented a 1.15, at 2 mL/min reaches 1.47 after a strange decrease from a resolution value of 1.97, corresponding to a 2.5 mL/min flow-rate.

The next screened molecule was the benzylamine **5** for which the resolution value constantly increased with the decrease of the flow-rate, jumping with one unit from 1.87 to 2.83. The same thing could be said about phenyl hydrazine-pyrrolidone **37**, however the last's separation resolution value being less influenced. While a weak improvement could be seen in the case of **46**, the flow-rate decrease led to important retention times, probably due to supplementary dipole-dipole interactions and hydrogen bonding with the CSP. Moreover, this compound makes the exception for which the resolution values obtained on Lux cellulose-2 are lower than on Lux cellulose-5.

Considering the study of the last 2 oxy-derivatives **65** and **70**, no improvements were seen, the flow-rate decrease producing just a slight change from 0.79 to 1.19 for the first compound, respectively 0.97 to 1.00 for the second molecule **70**.

In order to further improve the resolution, especially for derivatives **65** and **80**, the influence of the percentage of methanol with a constant flow-rate of 2 mL/min was explored on the column which showed the best separation performance, Lux cellulose-2.

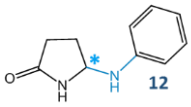
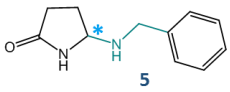
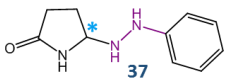
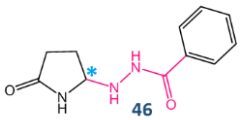
III.1.1.7.4 Percentage of methanol in the mobile phase

In a general manner, in SFC the modifier plays a key role and its effects are numerous: i) the organic solvent changes the polarity of the mobile phase, ii) it changes the density of the mobile phase, particularly when pressure and temperature conditions are such that fluid

compressibility is high that is to say close to the critical point and when the fluid is more gas-like, iii) the solvent changes the polarity and possibly the three dimensional structure of the CSP through its extensive adsorption on the CSP surface.[226]

In SFC, the percentage of co-solvent affects very strongly the retention time and then the resolution. In order to investigate the percentage of methanol influence, with a step of 2.5 the percentage of co-solvant was varied between 7.5 and 15% the results being comprised in **Table 40**.

As expected, with the decrease of co-solvent amount, an increase of the retention factor was obtained. This behavior was observed in **Table 40**. While for derivatives such as **65** and **75** the retention behavior was less significant, as we obtained variations from 4.51 to 12.56, respectively 1.75 to 8.36 for **75** taking as example k_1 corresponding to 15% and 7.5%. The reason could be attributed to the diminishing of the number of H bonds. If we compared molecules such as **12** where having an NH link as donor site, with **65** or **70** where instead of an –O- link as acceptor site, we observed that in case of the first one the retention factors were highly increasing with the decrease of the co-solvent %. The same behavior could be seen as well in the case of other donor accepting links pyrrolidones, such as **5**, **37** and **46**

Compound	MeOH	t_{r1} (min)	t_{r2} (min)	k_1	k_2	α	R_s
	7.5%	50.44	59.51	34.77	41.21	1.18	1.89
	10%	28.421	31.5	19.30	21.50	1.11	1.47
	12.5%	21.67	25.42	14.70	17.42	1.18	1.91
	15%	16.26	18.81	10.87	12.73	1.17	2.83
	7.5%	41.75	50.77	28.61	35.01	1.22	1.87
	10%	24.88	29.79	16.77	20.28	1.21	2.83
	12.5%	17.53	20.96	11.70	14.19	1.21	1.90
	15%	13.30	15.684	8.71	10.45	1.20	1.69
	7.5%	16.03	18.7	10.37	12.26	1.18	1.72
	10%	11.04	12.53	6.89	7.95	1.15	1.61
	12.5%	8.26	9.36	4.99	5.78	1.16	1.10
	15%	6.67	7.54	3.87	4.50	1.16	1.09
	7.5%	-	-	-	-	-	-
	10%	50.317	68.64	34.94	48.03	1.37	3.59
	12.5%	31.44	42.71	21.78	29.95	1.37	2.97

[226] West C. Enantioselective separation with supercritical fluids. *Curr. Anal. Chem.* 2014, 10, p. 99-120.

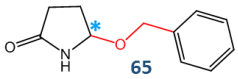
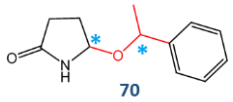
	15%	21.42	28.81	14.64	20.03	1.37	2.50
 65	7.5%	17.01	19.12	11.06	12.56	1.14	1.02
	10%	11.36	12.5	7.11	7.93	1.11	1.00
	12.5%	8.66	9.48	5.28	5.87	1.11	0.98
	15%	6.96	7.55	4.08	4.51	1.11	1.06
 70	7.5%	11.74	13.2	7.33	8.36	1.14	1.79
	10%	8.20	8.96	4.86	5.40	1.11	1.17
	12.5%	6.38	7.03	3.62	4.09	1.13	1.10
	15%	5.20	5.64	2.80	1.75	0.63	0.90

Table 40 *Screening results and obtained parameters under different modifier percentages on Lux Cellulose-2, 5 µm*

The most important influence of the percentage decrease could be seen for derivative **46**, where we did not have a 7.5% screening as the analysis time as it was too long and inefficient. However, considering the Rs/t_{R2} ratio, this would depend on the compound. Taking for example compounds such as **12**, **5**, and **65**, the impact of the modifier percentage was not relevant, the resolution values being slightly improved. On the other hand, for derivatives as **37**, **46** and **70**, a real improvement could be observed considering resolutions values, which were increasing with the decrease of the modifier. For derivatives **12**, **5** and **46**, the best resolutions in shorter analysis time were achieved with 15% of methanol and for **37** and **70** under 7.5% of co-solvent. All the five optimized chromatograms were represented on the **Figure 75**. For the last derivative **65**, as we did not managed to get a baseline separation, we considered to go further with the study, and changed the particle dimension of the chiral selector which worked best, Lux cellulose-2.

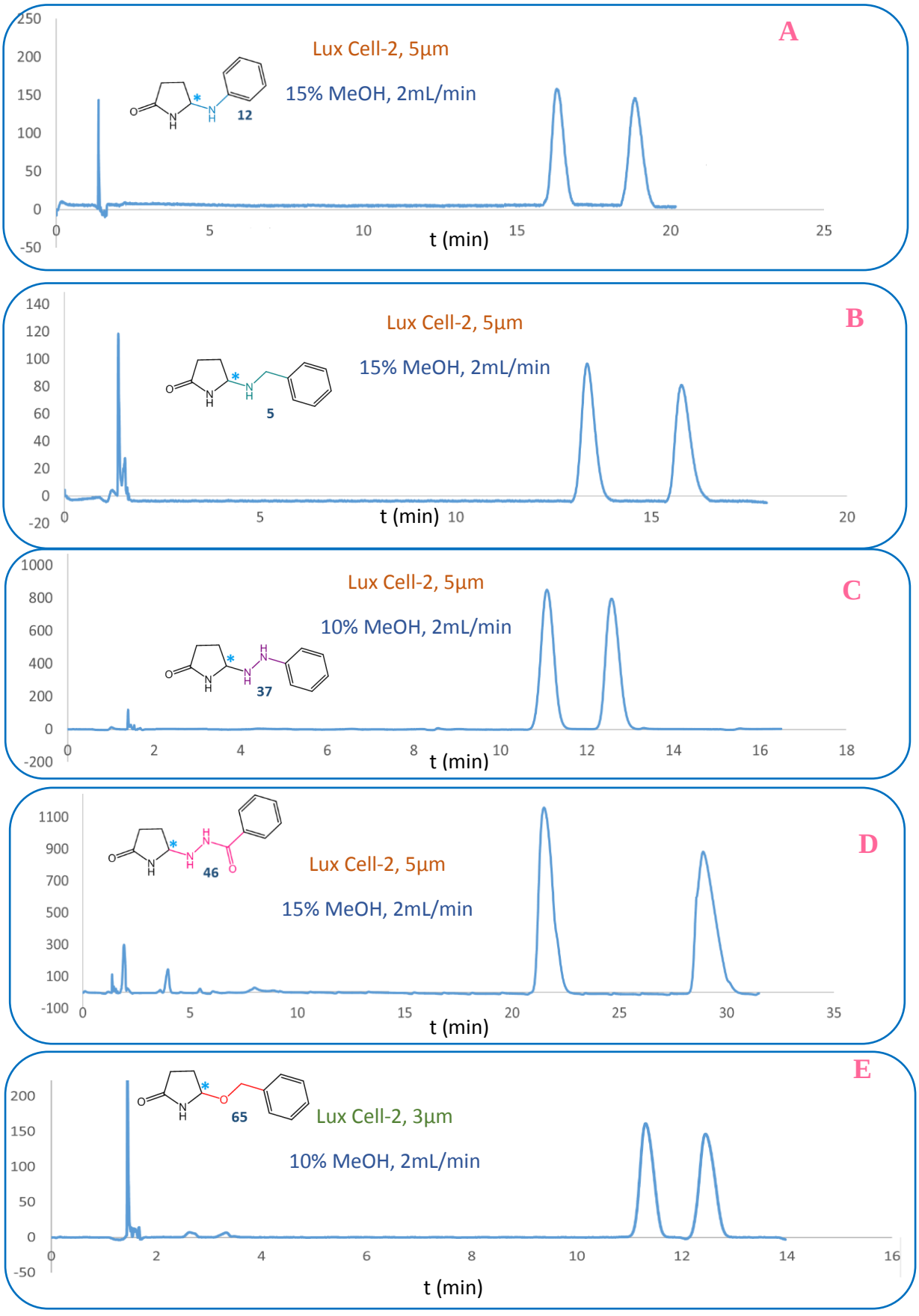
III.1.1.7.5 Reduction of the particle size effect on resolution

In the last decade, a trend of packed chiral columns with smaller particles was observed.[227,228] The mean diameter or particle size (d_p), of the spherical supports used for the stationary phase of a column, is a physical dimension that has a significant impact on the performance of the column.

[227] Hamman C., Wong M., Hayes M., Gibbons P. A high throughput approach to purifying chiral molecules using 3 µm analytical chiral stationary phases via supercritical fluid chromatography. *J. Chromatogr. A*, 2011, 1218, p. 3529-3536.

[228] Hegstad S., Havnen H., Helland A., Falch B.M.H., Spigset O. Enantiomeric separation and quantification of citalopram in serum by ultrahigh performance supercritical fluid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2017, 1061-1062, p. 103-109.

Cellulose *tris*(3-chloro-4-methylphenylcarbamate) columns with 3µm particle are now commercially available from different suppliers. As we were keen to separate molecule **65** as



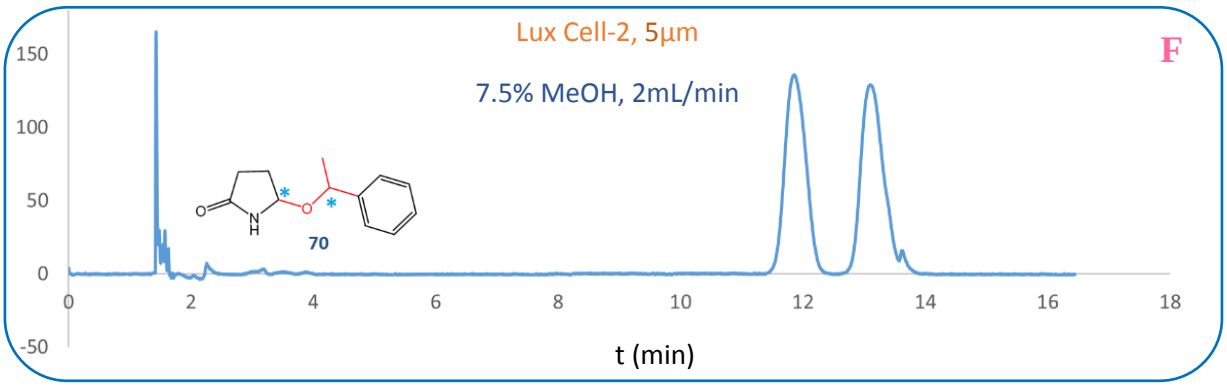


Figure 75 *Best separation conditions for the six pyrrolidones derivatives at 210 nm absorbance*

well, the Lux cellulose-2, 3µm particles was used successfully to separate it, as it can be seen in **Figure 75** - E.

III.1.1.8.5 Conclusive remarks

Within this subchapter we have optimized the chiral separation of 6 pyrrolidones derivatives having 5 types of links, serving as a model for future possible separations. Moreover, the performance of Lux i-Cellulose-5 (5µm) and Lux Cellulose-2 (5 µm), 250 × 4.6 mm columns with respectively an immobilized and a coated chlorinated polysaccharide stationary phases were evaluated towards six pyrrolidone derivatives, with respect to flow-rate and methanol concentration using supercritical fluid chromatography.

The optimum linear velocity corresponded to a low flow-rate equal to 2 mL/min. Big differences in terms of separation, was seen for Lux cellulose-2 (5µm) CSP which was found to separate the enantiomers of five out of six racemates thanks to various percentages of methanol as a co-solvent. Concerning the sixth derivative **65**, decreasing the particle size from 5 to 3 µm, led to the improvement of efficiency resulting in better chiral separation. However, only 2 peaks were seen in all the screenings regarding derivative **70**, which has 2 chiral centers, probably because of the unefficiency of the column to resolve the 4 diastereoisomers.

This study result is of practical significance for future separation of similar analytes, as no study was made on racemic 5-pyrrolidones being also described in a recent article.[229]

[229] Dascalu A.E., Ghinet A., Billamboz M., Lipka E. Performance comparison of chlorinated chiral stationary phases in supercritical fluid chromatography for separation of selected pyrrolidone derivatives. *JPA*, 2019.

III.1.1.9 Preparative enantioseparations of the novel FTI – **12**

As described in Chapter II, the most active molecule found to inhibiting human farnesyltransferase was HEI 2906, derivative **12** which was used previously in the chlorinated CPS performance study.

It is known that in early phases of drug development, the candidates are undergoing sophisticated analytical methods suitable for full separations. In the light of preparative chiral separation, in this sub-chapter, the study to find the perfect system conditions, in order to isolate pure enantiomers of derivative **12**, with sufficient amount to support extended biological investigation, is presented.

In the moment, we have only few preparative columns dimensions, from which CHIRALPAK®AD-H has the best separation performances (**Figure 76**). Things considered, we decided to do an optimization study to find the best conditions in order to obtain a good separation of molecule **12**. For the analytical screening, the samples solutions were prepared in methanol, 2 mg/mL, and for the preliminary study a loop of 20 µL was used.

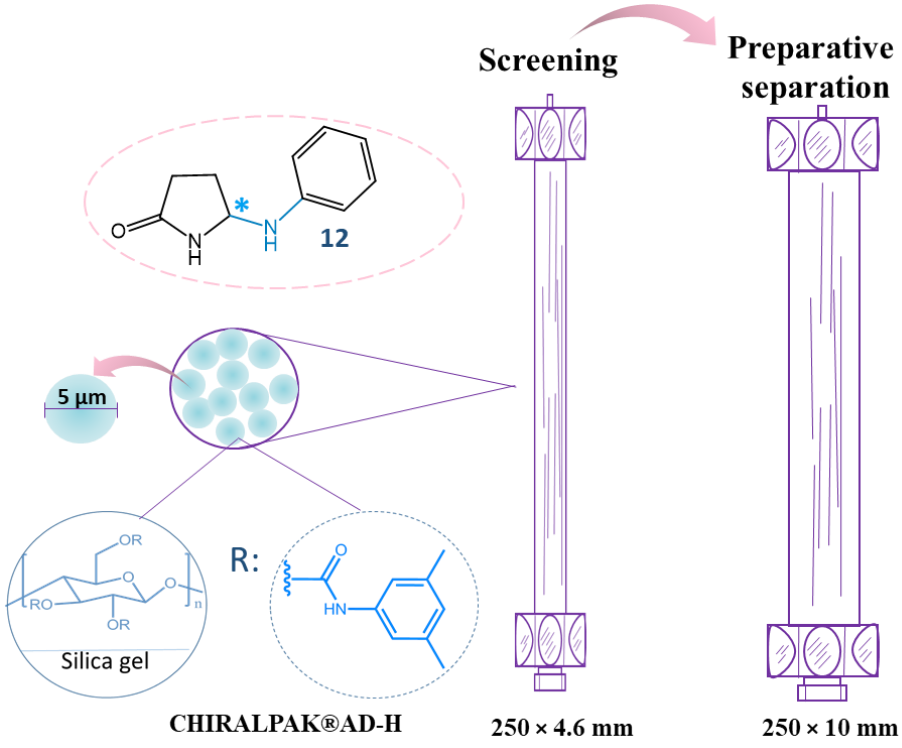


Figure 76 *Chiral selector used for the study*

Modifier nature influence

As seen in the previous study, such molecules are separating well in polar solvents such as MeOH or even EtOH. However, must be underlined the fact that this molecule is unstable in either one, degradating within hours.

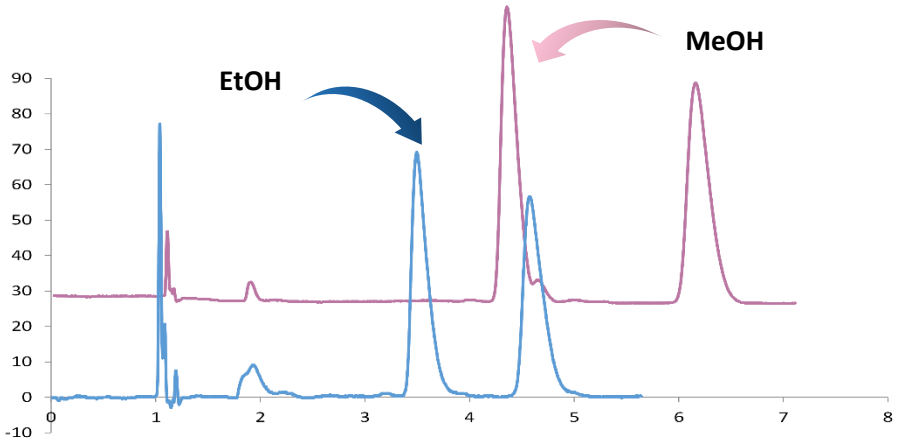


Figure 77 *Screening of* ***12*** *in 30% MeOH/EtOH, 3mL/min, AD-H*

To our surprise, employing ethanol as co-solvant led to a faster elution, while in methanol, the compound is more retained, but better resolved. In the present conditions, while for ethanol separation the resolution is 1.6, in methanol at the same modifier percentage, one unit of resolution is gained (**Table 41**). In these circumstances, we chose methanol as modifier, due to the higher resolution value which was obtained.

Solvent/Co-solvent	Flow rate (mL/min)	t_{r1}(min)	t_{r2}(min)	k_1	k_2	R_s
CO_2/MeOH 70 : 30	3	4.1	5.8	2.98	4.63	2.63
CO_2/MeOH 70 : 30	4	2.87	4.02	2.72	4.22	2.27
CO_2/MeOH 60 : 40	3	3.02	4.14	1.82	2.86	2.055
CO_2/EtOH 70 : 30	3	3.48	4.55	2.41	3.46	1.6

Table 41 *Screening analysis results of* ***12*** *considering modifier choice and percentage*

Modifier percentage and flow-rate influence

Next step in order to find the optimum conditions, was to modify the co-solvent percentage. However, in order to remain in the "green frame", but also to keep a fast analysis time, the percentage of co-solvant was not increased more than 40%, neither decreased more that 30%, as the resolution was satisfactory for the last case.

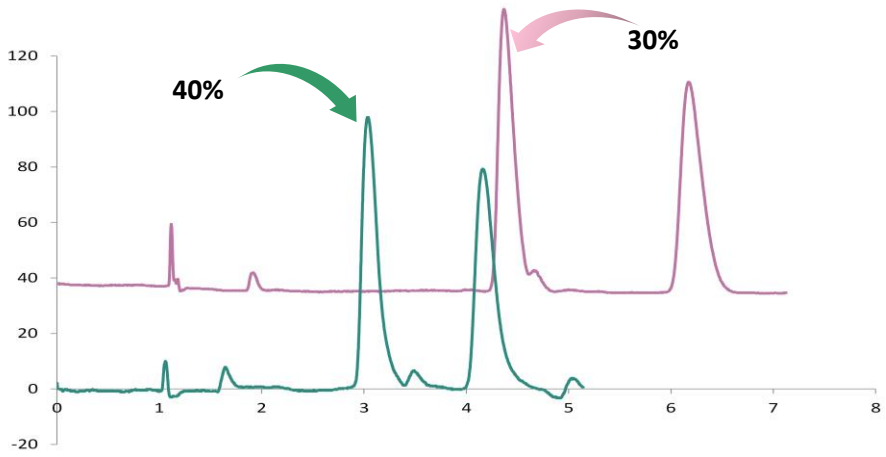


Figure 78 *Screening of* ***12*** *in 30/40% MeOH, 3mL/min, AD-H*

As it was observed from the table above, increasing the modifier percentage led to a resolution decrease from 2.63 corresponding to 30% to 2.05 corresponding to 40%.

Considering the flow-rate, we decided to increase it from 3 to 4 mL/min. Seemed that the flow-rate was impacting less the resolution compared to co-solvant pourcentage, the first one decreasing the resolution from 2.63 to 2.27. In this context, the flowrate was fixed at 3 mL/min.

Injection volume influence

Keeping the best conditions of separation of 30% MeOH, 3 mL/min, we decided to go further with loop injection switch and increased it but keeping a baseline separation. Things considered, as the previous loop was the 20 µL, we decided to use a 50, 100, respectively 250 µL loop, the last still affording a good separation (**Figure 79**).

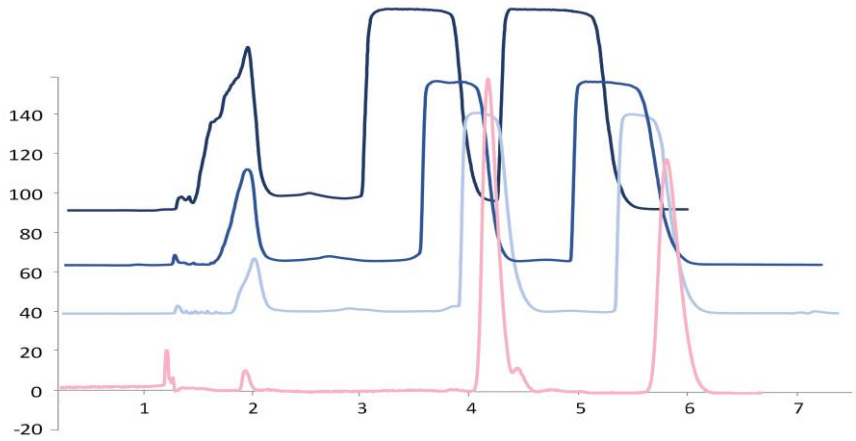


Figure 79 *Screening of* ***12*** *(2mg/mL) using different injection loops in 30% MeOH, 3mL/min, AD-H*

Preparative separation results

Further for our preparative separation, we decided to use a flow rate of 3 mL/min, 30% of methanol as modifier, on the same screening column of 250x4.6 mm, with a 250 µL loop, however, carefully cutting before the ending of each peak. 5 mL of each enantiomer has been separated for further analysis. As it is common at the end, after we collect each enantiomer sample, we reinjected them in the same conditions, for the enantio-purity test. Unfortunately, we were astonished to find out that this molecule is racemizing immediately after the separation.

Considering such result, all the family of pyrrolidones was questioned whether it was possible of having always a racemization, or the lack of substituents is leading to it. However, more investigations will be needed to elucidate this aspect.

Conclusive remarks

Within this short study, we tried to separate HEI2906 in order to test the two enantiomers. Two solvents were chosen as modifiers: ethanol and methanol, the last one proving its higher efficiency. The compound had a low solubility, of 2 mg/mL which made the study challenging, besides the fact it represented a big disadvantage when performing separation in a larger scale. Regarding the maximum injecting volume which is 250 µL/min, this would have yielded in maximum around 5 mg/h of compound, respectively 2.5 mg/h pof each enantiomer. Considering the post-separation racemization result, all the family of pyrrolidones was questioned whether it was possible of having always a racemization, or if the lack of substituents is leading to it. However, more investigations will be needed to elucidate this aspect.

III.1.2 Capillary electrophoresis as a chiral separation technique

Capillary electrophoresis (CE) represents a separation technique, complementary to chromatographic methods, which mainly allows the separation of charged species under an electric field. Initially it was developed for the analysis of biological molecules, considered today as a fast and very efficient method of separation, covering both small and medium molecules as biological macromolecules.

The first CE analysis was introduced by Hjerten[230] in 1967 who used quartz capillaries of relatively large internal diameter (0.3 mm). Since then, and especially thanks to the work of Jorgenson and Lukacs[231] which used glass capillaries of small internal diameter (75µm), the CE experienced a real development with the marketing of powerful and automatable instruments. The introduction of small internal diameter (<100µm) decreased the heating produced by joule effect. Through this particular advantage, degradation of the compounds inside the capillary is now avoided, and also the intensity of the electric field can now be increased and modulated, giving acces to faster, more resolved separations and higher peak efficiency.

Thanks to scientific advances, considerable instrumental progress has been made on the CE especially in terms of injection methods, modification of the internal wall of the capillaries in order to avoid possible absorption phenomena as well as detection mode. These improvements paved the way for development of several electrophoretic separation modes and many chemical applications,[232] pharmaceutical,[233] agricultural, food industry,[234,235] and environmental. The scope of the CE has considerably expanded, from the analysis of small molecules, organic ions or chiral molecules, proteins and also DNA.

Considering the separation modes, the CE combines: capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC), capillary gel electrophoresis (CGE), isotachophoresis (ITP) and capillary isoelectric focusing (cIEF). The choice of the electrophoretic separation mode naturally depend on the physicochemical properties of the compounds to be separated.

III.1.2.1 Capillary zone electrophoresis principle

Capillary zone electrophoresis (CZE), also called free solution capillary electrophoresis, represents today the simplest and most used electrophoretic separation mode. It allows the separation of charged species according to their sizes and their electric charges at a given pH. In other words, the separation of species is based on a difference in migration speed

[230] Hjerten S. Free zone electrophoresis. *Chromatographic reviews*, 1967, 9, p. 122-219.
[231] Jorgenson J. W., Lukacs K. D. Free-zone electrophoresis in glass capillaries. *Clinical chemistry*, 1981, 27, p. 1551-1553.
[232] Timerbaev A. R. Element speciation analysis by capillary electrophoresis. *Talanta*, 2000, 52, p. 573-606.
[233] Bowman, J., Tang L., Silverman C.E. Analysis of small amines in pharmaceuticals by capillary ion electrophoresis with conductivity detection. *J. Pharm. Biomed. Anal.* 2000, 23, p. 663-669.
[234] Garcia-Canas V. Recent advances in the application of capillary electromigration methods for food analysis and Foodomics. *Electrophoresis*, 2014, *35*, p. 147-169.
[235] Simo C. Capillary electrophoresis-mass spectrometry in food analysis. *Electrophoresis*, 2005, 26, p. 1306-1318.

of electrically charged species under action of an electric field. This separation is possible thanks to two transport mechanisms: electromigration and electroosmosis (**Figure 80**).

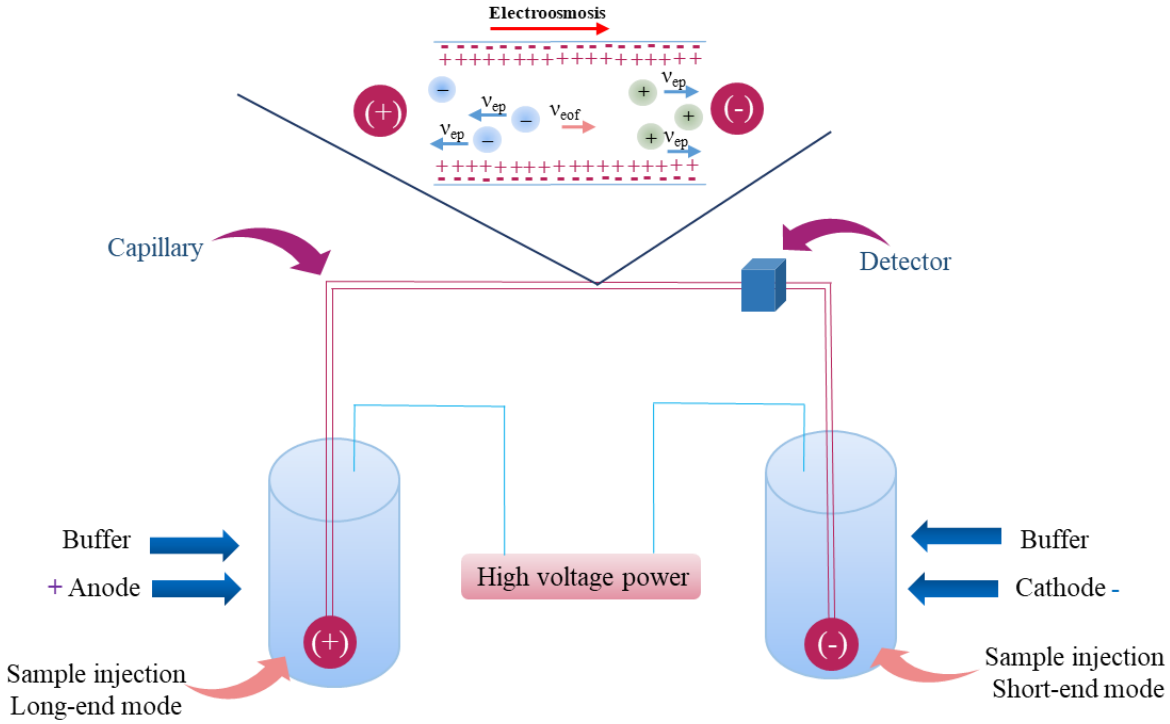


Figure 80 *Capillary electrophoresis zone separation principle*

The electromigration is the movement of a charged species when subjected to an electric field. In CZE, the cations migrate towards the cathode while the anions are attracted to the anode. The species move with a speed that is a function of the intensity of the applied electric field, the charge of the species and their size. Thus, species migrate faster when they are small and with a high charge density. The migration speed of a charged species, called the electrophoretic velocity, is defined according to the following relationship:

$$v_{ep} = \mu_{ep} E = \mu_{ep} \frac{V}{L}$$

Moreover, a molecule's total velocity, v_{tot} is the sum of its electrophoretic velocity and the electroosmotic flow velocity:

$$v_{tot} = v_{ep} + v_{eof}$$

Where

$$v_{eof} = \mu_{eof} E$$

With ν_{ep} electrophoretic velocity ($cm.s^{-1}$); μ_{ep} electrophoretic mobility ($cm^2.V^{-1}.s^{-1}$); ν_{eof} electroosmotic flow velocity; μ_{eof} electroosmotic flow mobility; E the electric field ($V.cm^{-1}$); L is the length of the capillary and V the potential difference applied at its ends.

Another way to express ν_{tot} is to divide the distance it travels by the elapsed time:

$$v_{tot} = l.t_m$$

With *l* being the distance between the point of injection and the detector, *L* is the length of the capillary tube, while t_m is the solute's migration time.

Electroosmosis is defined as the flow of a liquid inside a capillary whose internal wall has a charge surface when subjected to an electric field. The inner wall of the silica capillary is covered with silanol groups that ionize when the pH of the background electrolyte (BGE) is greater than 2 in order to form a negatively charged inner wall. In order to respect the conditions of electroneutrality, the cations present in the BGE are therefore attracted by the negative charges of the wall of the capillary.

The electrophoretic velocity, as well as the electrophoretic mobility, depends on the length of the capillary of separation (20 to 100 cm), its internal diameter (20 to 100 µm), the applied potential difference (10 to 40 kV), pH, BGE composition (ionic strength, viscosity, addition of a complexing agent or organic solvent) and temperature. In order to optimize the separation in CZE, these parameters are crucial.

III.1.2.2 Quantitative parameters in CZE

Efficiency

Capillary electrophoresis as technique allows you to obtain high separation efficiencies thanks to the absence of a stationary phase and the flat profile of the electroosmotic flow. The efficiency of a separation is given by the number of theoretical plates, *N* which in capillary electrophoresis is:

$$N = \frac{l^2}{2Dt_m} = \left(\frac{\mu_{eof} + \mu_{ep}}{2DL}\right).Vl$$

Where: L is the length of the capillary tube, t_m and *D* is the solute's diffusion coefficient.

Considering this equation, we can say that the effectiveness of a separation increases when the applied voltage is high. However, high voltages cause heating through Joule effect, which can influence the separation or even degrade the analytes.

Resolution

The resolution between two species is:

$$R = \frac{1}{4}.\frac{\mu_{ep2} - \mu_{ep1}}{\mu_{eof} + \overline{\mu_{ep}}}.\sqrt{N}$$

Considering the equation, in order to increase the resolution between two species, their electrophoretic mobilities and theoretical plates have to be increased. These amounts to increase the tension or to change the nature of the BGE. Moreover, this equation ilustrates that a very high electro-osmotic flow may decrease the resolution between two species.

Selectivity

The selectivity between two analytes is defined such as the ratio of their retention factors. In capillary electrophoresis the analogous expression for selectivity would be as following:

$$\alpha = \frac{\mu_{ep1}}{\mu_{ep2}}$$

III.1.2.3 The optimization of a separation

In order to optimize a separation, there are different parameters which should be taken into account when it comes to. These parameters directly affect electroosmotic mobility and electrophoretic species to separate.

- ✓ The pH of the BGE is one of the most important parameters because it conditions the ionization state.
- ✓ The nature of the chiral selector.
- ✓ The addition of additives or organic solvent can improve the efficiency and resolution of peaks.
- ✓ The ionic strength of BGE influences the apparent mobility of each species. Indeed the increase in strength ionic increases the resolution of analytes.
- ✓ The applied voltage and the temperature affect the analysis time and to a lesser extent the resolution.

- ✓ The length and internal diameter of the capillary play a major role in efficiency, resolution, time of analysis and heat emissions by Joule effect. Small internal diameter of a capillary leading to a more effective and resolved separation.
- ✓ The nature of the surface charge of the capillary (positive, negative or neutral) determines the flow direction electroosmotic. As a general rule, the electroosmotic flow always flows towards the electrode whose sign is the same as that of the charges present on the walls of the capillary.

III.1.2.4 The enantio-separation of selected molecules through CE

Separations of enantiomeric compounds have been achieved by different chromatographic techniques mainly due to the preparative scale possibility. Besides these, capillary electrophoresis also emerged as excellent alternative for stereoisomeric purity analysis, additional to high performance liquid chromatography (HPLC) and super critical fluid chromatography (SFC) as an orthogonal method for enantiomeric purity verification.[236] Among the advantages of this technique, there are: short migration times, a low consumption of analyte and chemicals, and a wealth of available chiral selector types. Among these, the most popular are cyclodextrins.[237]

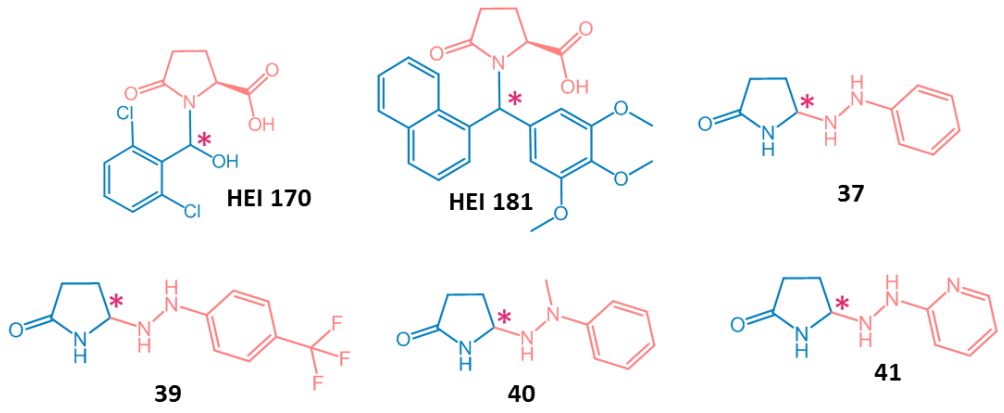


Figure 81 *Selected derivatives for CE enatiomers separation*

A large variety of these cyclodextrins are now commercially available, which can be used either alone or in combinations of two different types ("dual-cyclodextrin system"), as

[236] Zhu, Q., Scriba, G. Analysis of small molecule drugs, excipients and counter ions in pharmaceuticals by capillary electromigration methods – recent developments. *J. Pharm. Biomed. Anal*. 2018, 147, p. 425-438.
[237] Řezanka P., Navrátilová K., Řezanka M., Král V. Application of cyclodextrins in chiral capillary electrophoresis. *Electrophoresis*, 2014, 35, p. 2701-2721.

witnessed by an abundance of publications on the topic and very recent review by B. Chankvetadze.[238]

Within this chapter, a recent CD study is presented, which was carried out for the chiral separation of two compounds from the HEI library[239] bearing two asymmetric carbons (with one blocked center) and four compounds (**37**, **39**, **40**, **41**) bearing one asymmetric carbon (**Figure 81**). From our interest to find the best conditions in order to separate molecules bearing L-pGlu or γ-lactam moieties, we have developed an electrophoretic orthogonal separation method of minimal cost and environmental impact for the optimization of these drug candidates.

III.1.2.4.1 Cyclodextrins as chiral selectors

Cyclodextrins are the most popular chiral selectors used in CE.[240] For academic research usually single isomers CDs are used, but for practical purposes the random substituted CDs give often suitable results especially when CD derivatives produced industrially are used. Based on our experience and considering the dimensions of the molecules, we were interested to test only SBE-β-CD Captisol® and HS-γ-CD.

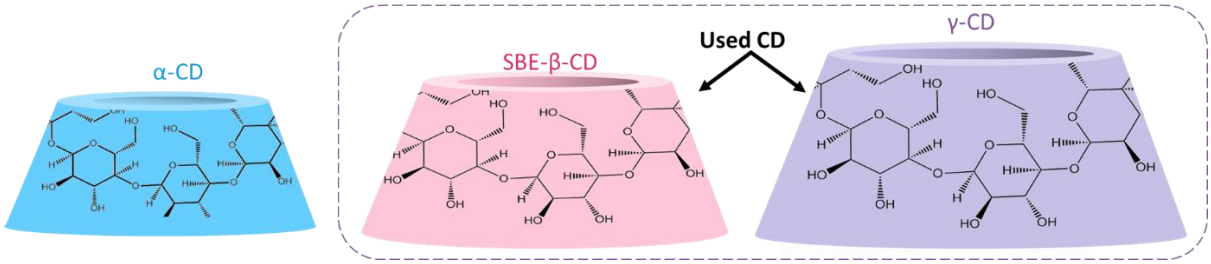


Figure 82 *Used CD within the study*

Based on the experience acquired in our laboratory, the first tested conditions were the following: a BGE consisting of 25 mM phosphate buffer at pH 2.5, in a fused-silica capillary coated with PEO. At this pH, the EOF being considered negligible. According to **Figure 81**, among the analytes, the compounds **HEI 170** and **HEI 181** are neutral (having no electrophoretic mobility) and the compounds **37** to **41** are positively ionized. Therefore, since some of the compounds are uncharged, it was decided to test only charged CDs, and particularly

[238] Chankvetadze B. Contemporary theory of enantioseparations in capillary electrophoresis. *J. Chromatogr.* A, 2018, 1567, p. 2-25.
[239] Legrand A., Rigo B., Henichart J.P., Norberg B., Camus F., Durant F., Couturier D. Studies on pyrrolidinones. Synthesis and cyclization of *N*-[α-naphthyl-(3,4,5-trimethoxyphenyl)methyl]pyroglutamic acid. *J. Heterocycl. Chem.* 2000, 37, p. 215-227.
[240] Szente L., Szemán J. Cyclodextrins in analytical chemistry: host–guest type molecular recognition. *Anal. Chem.* 2013, 85, p. 8024–8030.

negatively charged CDs, because the most commonly studied pharmaceutical compounds are basic. Since these anionic CDs have a self-mobility turned towards the anode and analogues **HEI 170** and **HEI 181** remained uncharged at pH 2.5, only the cathodic injection permitted their detection. Cathodic injection was also implemented for the positively charged derivatives **37** to **41**, since the mobility of the complex formed between the stereoisomer and the CD were found to be anodic. Both the short-end (SE – capillary effective length of 10 cm) and the long-end (LE – capillary effective length of 40.1 cm) modes were tested with reversing the polarity of the electrodes.

III.1.2.4.2 SBE-β-CD

Sulfobutylether beta-cyclodextrin (SBE-β-CD) is a pharmaceutical excipient in US Pharmacopoeia as solubilizing agent and is a component of several drug formulations in the market.[241] Its composition is strictly regulated, therefore, the average degree of substitution (DS) and the distribution of the components of various DS fall always in a narrow range. The popularity of this cyclodextrin comes from the standard quality results in high reproducibility for analytical applications.

One of the goals of this work was to develop an economic method of separation for pyro-derivatives. In this context, the SBE-β-CD was chosen as this selector being given as free testing sample from the purchaser. This CD's efficiency was previously described for the separation of a wide range of chiral compounds showing a relevant resolving power even at a very low concentration.[242,243] In acid buffer, SBE possesses four sulfonic groups bonded at position 6 of 4 of the CD glucopyranoses through the butyl chain and it is negatively charged at any commonly used pH in CE.[244]

[241] Puskás I., Varga E., Tuza K., Szemán J. Sulfobutylethercyclodextrins: structure, degree of substitution and functional performance. Cyclodextrins, 2015, Chapter 10, Nova Science Publishers, Inc.
[242] Delplanques T., Boulahjar R., Charton J., Houze C., Howsam M., Servais A.C., Fillet M., Lipka E. Single and dual cyclodextrins systems for the enantiomeric and diastereoisomeric separations of structurally related dihydropyridone analogues. *Electrophoresis,* 2017, 38, p. 1922-1931.
[243] Baudelet D., Ghinet A., Furman C., Dezitter X, Gautret P., Rigo B., Millet R., Vaccher C., Lipka E. Antagonists of the P2X7 receptor: Mechanism of enantioselective recognition using highly sulfated and sulfobutylether cyclodextrins by capillary electrokinetic chromatography. *Electrophoresis*, 2014, 35, p. 2892–2899.
[244] Fanali S. Identification of chiral drug isomers by capillary electrophoresis. *J. Chromatogr. A*, 1996, 735, p. 77-121.

III.1.2.4.3 SBE-β-CD in acid buffer

For starters, the SBE-β-CD was tested in different concentrations comprised between 5 and 22.5 mM (*i.e* 5; 10; 15; 17.5; 20; 22.5 mM) both in SE and LE modes. Considering derivative **HEI 170**, to our surprise this CD proved to be very efficient. In SE mode, this selector led to a total enantiomeric resolution of compound **HEI 170**, whatever the concentration was with migration times which increase from 5 to 15 mM and then decreasing from 15 to 22.5 mM with a resolution value which varied in an opposite way (**Figure 83**).

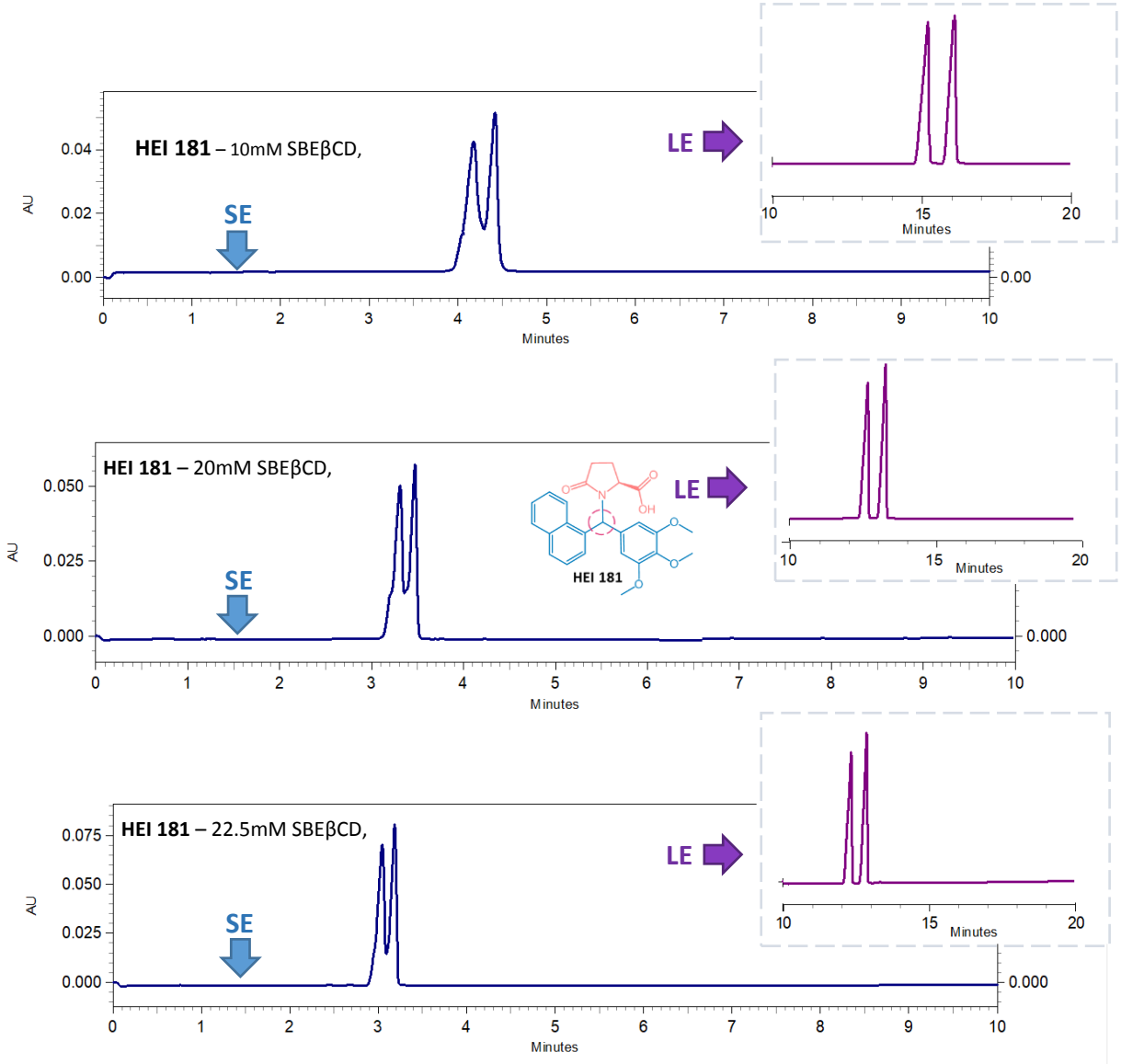


Figure 83 *Concentration influence of SBEβCD on the separation and migration of HEI181*

More specifically, at 5 mM the migration times of the two peaks were 3.94 and 4.59, with a resolution of 4.97, while at 15 mM retention values were 5.17, 5.65 with a lower resolution of 2.81. On the other hand, at a higher concentration of 22.5 mM, the retention times

are decreasing with 3.39 and 3.92, for each peak, with a resolution value which increased again reaching 4.29. For example at 5 mM the migration times and resolution were 3.94, 4.59 and 4.97 respectively, at 15 mM those values were 5.17, 5.65 and 2.81 and were equal to 3.39, 3.92 and 4.29 at 22.5 mM. However, in LE mode, no reproducible results were obtained, in some cases this aspect might be due to the fact that everytime the capillary was breaking the system was changing leading to different results.

Going futher with **HEI 181**, in SE mode the two peaks were not baseline resolved whereas in LE mode the resolution was complete, as seen on **Figure 83** for 10, 20 and 22.5 mM of SBE-β-CD. γ-Lactam derivative **37** followed also this trend. Considering the last of the remaining derivatives, employing the same separation conditions, we have not obtained the same results as previously seen with **HEI 170**, **HEI 181** and **37** and we decided to change the pH of the buffer in order to obtain a separation for the last unseparated molecules as well.

III.1.2.4.4 SBE-β-CD in basic buffer

We chose to continue our study by using a basic BGE consisted of a 50 mM borate buffer adjusted to a pH of 10. At this pH, an electroosmotic flow is naturally induced from anode to cathode, in an untreated fused-silica capillary. Moreover, in a pH of 10 buffer, compounds **39**, **40** and **41** are neutral.

Unfortunately, considering the next derivative to be resolved, **39**, the chiral selector failed to separate it, only one peak being observed both in LE and SE whatever the employed concentration.

Under those conditions the analogue **40** was completely resolved both in SE and LE. However, in SE the migration time was shorter and the resolution as the efficiency were superior. For instance at 17.5 mM the migration times were 3.55 and 4.12 with a low resolution of 1.74, while in LE the encountered migration times were 12.35 and 15.82, but the resolution goes up to 5.60.

Considering the next compound **41**, the obtained electrophoregrams were not satisfactory. We further decided to switch the type of anionic CD in order to obtain a separation of the racemic mixtures of **39** and **41** left unresolved.

To summarize the study involving SBE-β-CD (**Table 42**), this chiral selector successfully resolved derivatives **HEI 170**, **HEI 181**, **37** in acidic buffer while **40** was resolved in basic buffer (**Table 42**). The best resolved derivative was **HEI 170** with the highest resolution of 2.82, with short migration times. The next separated molecule, **HEI 181**, even if the structure seems to be bulky, this intermediary cavity cyclodextrin successfully provided an acceptable separation, in LE. The last molecule separated in acid buffer with the help of SBE-β-CD as chiral selector was **37**, which in LE mode presented a resolution value of 1.96. The only which was notseparated in acid buffer was molecule **40**, which however, was successfully separated in alkaline buffer, providing a resolution of 1.92.

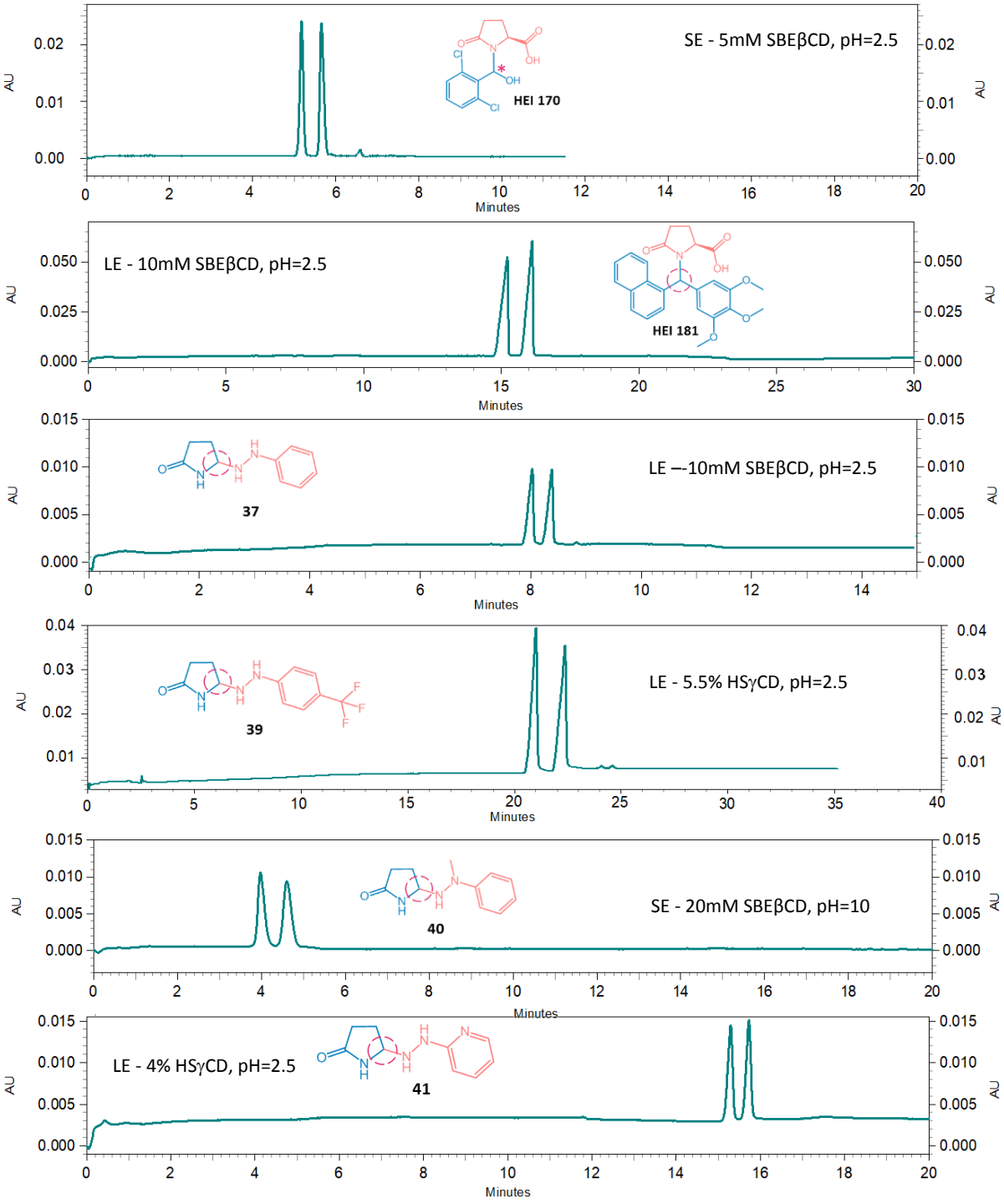
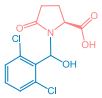
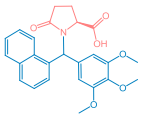
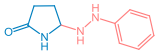
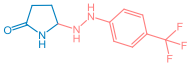
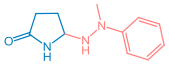
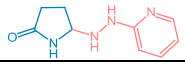


Figure 84 *Electropherograms of the six screened derivatives in the optimized BGEs*

Within this screening, SBE-β-CD was used in so called "carrier mode" meaning that CD was used not only for separation of enantiomers but also for their transport to the detection window. The proof of this statement was that the migration time of the analyte decreased with increasing of CD concentration, while the selectivity and resolution may have reached the optimum at the certain CD concentration. However, at higher CD concentration the effect of

CD on the viscosity of the BGE must have been also considered as increasing, thus leading to an increase of the migration time.

Compound	Sample concentration	Buffer nature and concentration	Voltage	Chiral selector	Injection mode	Migration time (min)	Resolution
HEI 170 	0.1 mM	Phosphate 25 mM pH 2.5	20 kV	SBE-β-CD 5 mM	SE	5.17 5.65	2.82
HEI 181 	0.1 mM	Phosphate 25 mM pH 2.5	20 kV	SBE-β-CD 10 mM	LE	15.22 16.12	2.02
37 	0.1 mM	Phosphate 25 mM pH 2.5	20 kV	SBE-β-CD 10 mM	LE	8.05 8.41	1.96
39 	0.1 mM	Phosphate 25 mM pH 2.5	20 kV	HS-γ-CD 5.5%	LE	21.05 22.50	1.65
40 	0.1 mM	Borate 50 mM pH 10	20 kV	SBE-β-CD 20 mM	SE	3.98 4.61	1.92
41 	0.1 mM	Phosphate 25 mM pH 2.5	20 kV	HS-γ-CD 4%	LE	15.30 15.83	1.97

Conditions: fused-silica capillary (coated with PEO or not) 50.1 cm (effective length 10 or 40.1 cm) x 50 µm id; UV detection at 210 nm; cathodic injection, 1 psi pressure for 5 s of 0.100 mM solution; temperature 25°C.

***Table 42** Conditions of optimal separation of the six analytes*

III.1.2.4.5 Highly sulfated-γ-CD

Despite of their high cost, the good enantioseparation abilities of highly sulfated CDs have been demonstrated in numerous previous papers, including for neutral pharmaceutical compounds. [245,246,247]

HS-γ-CD in acidic phosphate buffer

[245] Sohajda T., Skakacs Z., Szente L., Noszal B., Béni S. Chiral recognition of imperanene enantiomers by various cyclodextrins: A capillary electrophoresis and NMR spectroscopy study. *Electrophoresis*, 2012, 33, p. 1458-1464.
[246] Fejos I., Urbancsock Z., Zhou W., Sohajda T., Hu W., Szente L., Béni S. Separation of alogliptin enantiomers in cyclodextrin-modified capillary electrophoresis: A validated method. *Electrophoresis*, 2014, 35, p. 2885–2891.
[247] Lipka E., Landagaray E., Ettaoussi M., Yous S., Vaccher C. Enhanced detection for determination of enantiomeric purity of novel agomelatine analogs by EKC using single and dual cyclodextrin systems. *Electrophoresis*, 2014, 35, p. 2785–2792.

HS-γ-CDs in solution were then tested and were prepared at *ca.*2 mM (0.5% (*w/v*)), *ca.*4 mM (1% (*w/v*)), *ca.*16 mM (4% (*w/v*)), *ca.*20 mM (5% (*w/v*)) and *ca.*24 mM (6% (*w/v*)) in an acidic 25 mM phosphate buffer a pH 2.5.

Considering analogue **40**, the changement of CD did not improve the resolution. This phenomenon could be explained through the bulky structure of the molecule which was bearing a trifluoromethyl group which we assumed to be stopping the inclusion in the cyclodextrin cavity. However, other efforts were made in order to obtain a separation and the racemic mixture **40**, was lastly tested under LE with an intermediary concentration of 22 mM (5.5% (w/v)). Surprisingly, this concentration led to a correct resolution of 1.65 in 22 min of migration times (**Figure 84**). However, in SE mode the resolution was lost.

The last racemic mixture we wanted to separate was that of **41**, and to our delight, the HS-γ-CD furnished that separation in LE as well as a partial separation in SE. This behavior can be seen for all the concentrations except the lowest one at 2 mM (0.5%) when only one peak was observed. The best concentration that furnished a good baseline separation was 4%, with retention times of 15.30, respectively 15.83, with a resolution of 1.97 in LE mode.

III.1.2.5 Conclusions considering the CE study

To sum up this study, the separation of a series of six γ-lactam derivatives with one or two stereogenic centers was successfully achieved by capillary electrophoresis using mainly SBE-β-CD as chiral selectors and HS-γ-CD in a complementary way, the best conditions being summarized in **Table 42**. Overall, it can be stated that the analysis times were mostly short (less than 8 min) for three compounds and around 20 minutes for the two others, with moderate resolution values (comprised between 1.92 and 2.82). As we noticed ourselves as well as it can be seen in many of the published works, chiral separations can be unpredictable this topic remaining challenging. The use of CDs in the background electrolyte brought not only enantioselectivity but also chemoselectivity, which was the main factor responsible for the separation of stereoisomers.[248]

It is worth noting that the chiral separation occured through a chiral selector which recognized both enantiomers stereoselectively with different binding constants, provided that

[248] Chankvetadze B. Contemporary theory of enantioseparations in capillary electrophoresis. *J. Chromatogr. A,* 2018, 1567, p. 2–25.

there was a difference in the mobilities between the free analyte and the complexed one. In comparison, other techniques such as HPLC, the methods developed here were more economical with a low environmental impact, avoiding the use of organic solvents. The diversity of the cyclodextrin types, the broad range of concentrations which can be employed makes this technique highly valuable for the enantiopurity check. Considering the study from this sub-chapter, which has been recently published,[249] it can be concluded that the versatile SBE-β-CD would be the first choice as a chiral selector for future enantiomeric purity determination of pyrroglutamic acid or pyrrolidone derivatives.

III.1.3 Conclusion and perspectives

Within this sub-chapter, some selected molecules have been analyzed in the frame of a preparative separation in SFC, while through capillary electrophoresis we have searched the optimum conditions for the enantio-separation. Besides this, we have proved that in SFC, chlorinated CSP overall improved enantiomeric separation of the selected pyrrolidone-scaffold which were used as models, however, a separation mechanism would be needed to explain this behavior. In CE, we found that sulfobutylether beta-cyclodextrin (SBE-β-CD) was a good choice as a chiral selector for the tested racemates and it will most-likely remain first option when screening molecules around the same scaffold.

[249] Dascalu A.E., Ghinet A., Billamboz M., Lipka E. Separations of antifungal compounds in capillary electrophoresis with two anionic cyclodextrins. *Electrophoresis*, 2019, p. 1-6.

III.2 Physical characterization of selected compounds for formulation consideration

In the perspective of the formulation in paints and coatings as antifungal additives, several stability tests were done around some selected molecules, from different chemical families which were synthesized for this project. The used solid state analysis techniques are TGA, DSC and DVS, which are going to be briefly discussed along with the general obtained results.

III.2.1 Generalities about the used techniques: TGA, DSC, DVS

The term "thermal analysis" is frequently used to describe analytical techniques which study the behavior of a sample as a function of temperature (ΔT). Measurements are based on dynamic relationships between temperature and Mass, Volume, or Heat of reaction.[250]

The two used thermal analytical techniques within this chapter are:

- Thermo Gravimetry Analysis (TGA)
- Differential Scanning Calorimetry (DSC)

The DSC and TGA are used in a wide variety of fields, such as medicine, biocompatibility testing, classical thermodynamics studies, materials science, in chemistry and pharmaceutical industry, representing excellent methods for solid products kinetics study or stability tests, being essential for the formulation of new active ingredients.

Thermo Gravimetry Analysis (TGA)

TGA is a thermal analysis technique that consists in measuring the mass of a sample when it is subjected to temperature variations, at a controlled rate, being recorded as a function of time or temperature. The TGA device has a high accuracy scale. The decomposition upon heating of a sample to observe weight changes is the underlying principle of TGA.

TGA allows to observe the effects of thermal decomposition, evaporation, reduction, desorption, sublimation, oxidation, absorption, etc. TGA qualifies the thermal stability of compounds. It also makes it possible to determine the temperatures at which chemical reactions take place as well as to dose certain volatile compounds (solvents) in a solid sample.

[250] Soni N. Thermal Methods of Analysis. *Mod. Appl. Pharm. Pharmacol*, 2017.

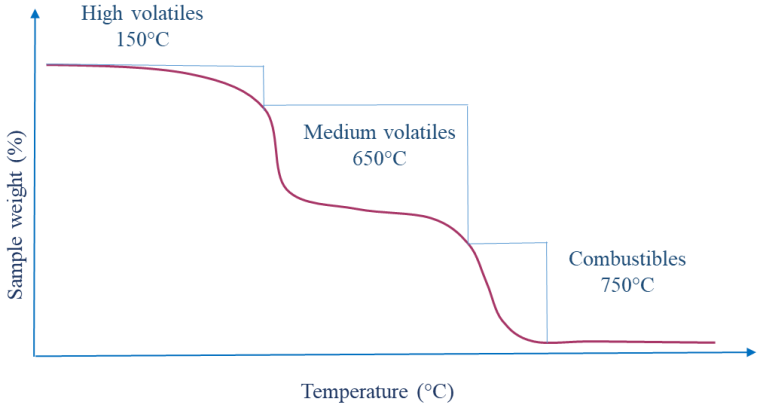


Figure 85 *TGA curve model*

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is a technique for measuring the energy necessary to establish a nearly zero temperature difference between a substance and an inert reference material. The device has 2 measurement cells: 1 reference cell (usually empty) and a cell in which the sample to be analyzed is placed. Each cell has its own heating system and temperature control. An identical temperature program is applied to the 2 cells.

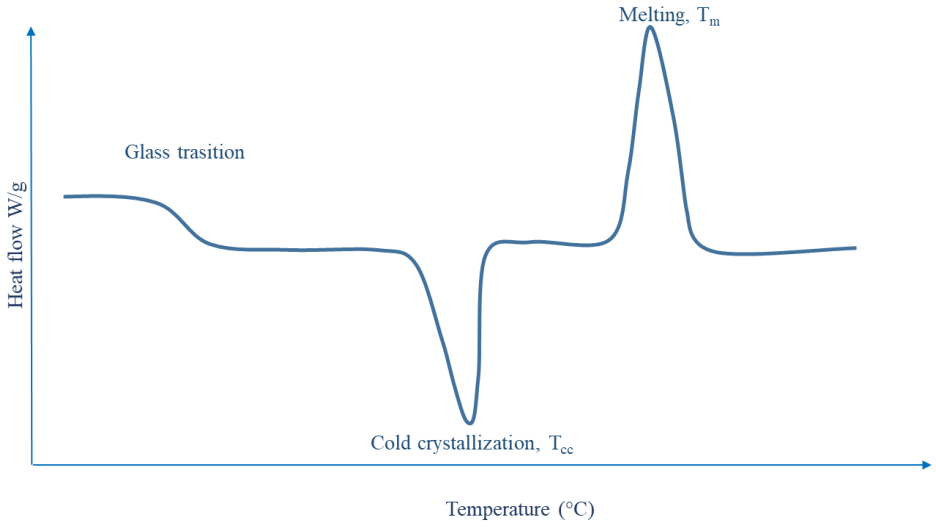


Figure 86 *Features of a DSC curve*

DSC is the perfect method for determining thermal quantities, studying thermal processes and characterizing or comparing materials. This technique is extensively used in the areas of quality control, research and materials development. DSC also measures its thermal properties: heat capacities, change of state enthalpies, melting temperature, glass transition, crystallization or degradation (**Figure 86**).

II.2.2 Thermal analysis discussion

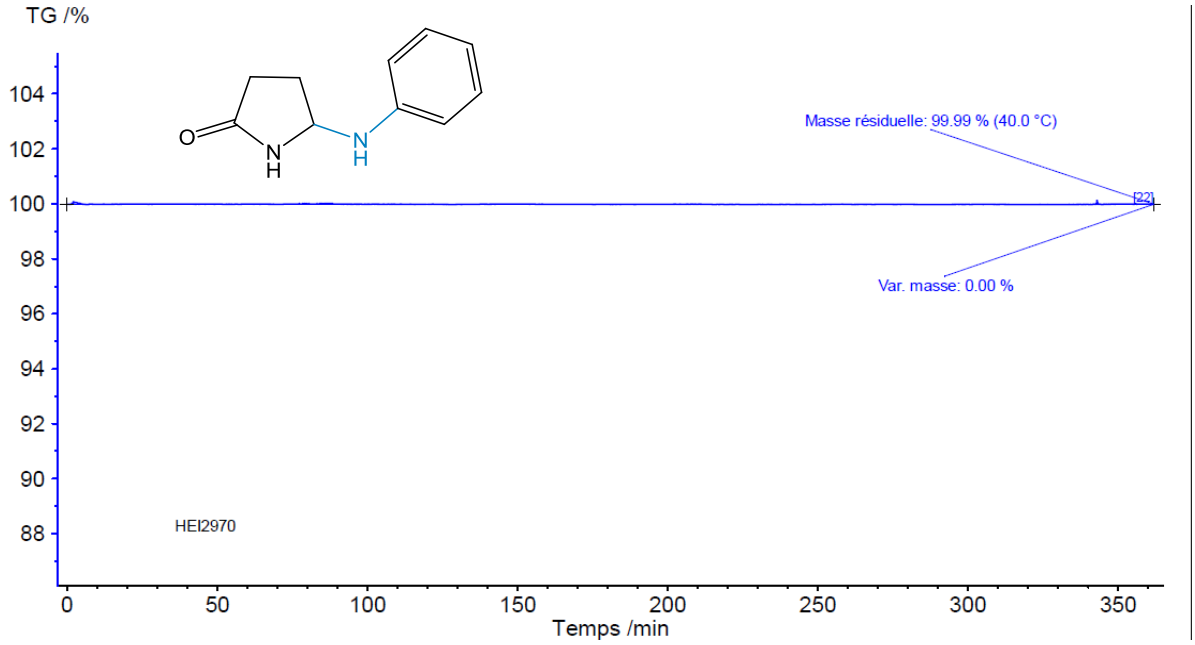


Figure 87 *The thermo-gravimetric analysis (TGA) of HEI 2906, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is perfectly stable, without any mass variation.*

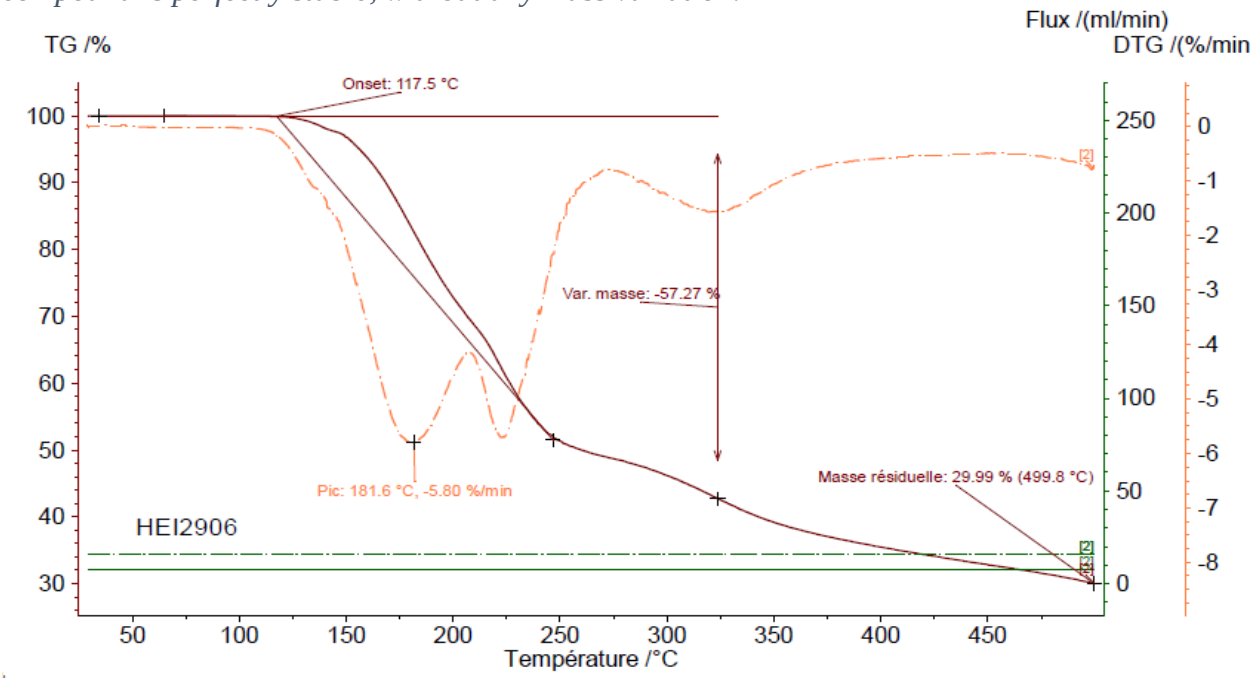


Figure 88 *TGA of the same compound HEI 2906, up to 500°C. On the TGA profile, the initial step shows weight loss of -5.80% in the temperature range of 24°C to 182°C, which might have resulted from the release of moisture and volatile matter. The second step shows steep weight loss of -57.27 % at the temperature range from 250°C to 500°C due to advanced decomposition of the compound*

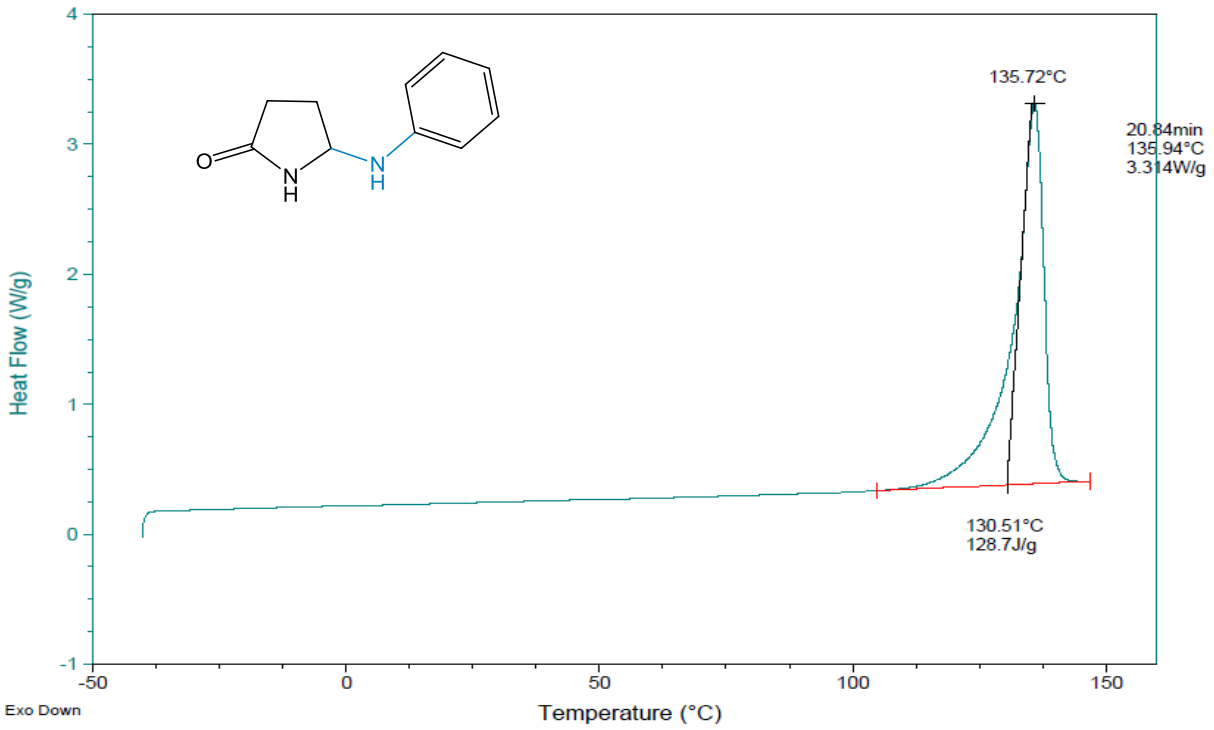


Figure 89 *The differential scanning calorimetry (DSC) of HEI 2906 heated at constant flow. As it can be observed, at 135.72°C the peak temperature is defining the melting point, having an enthalpy of 128.7 J/g.*

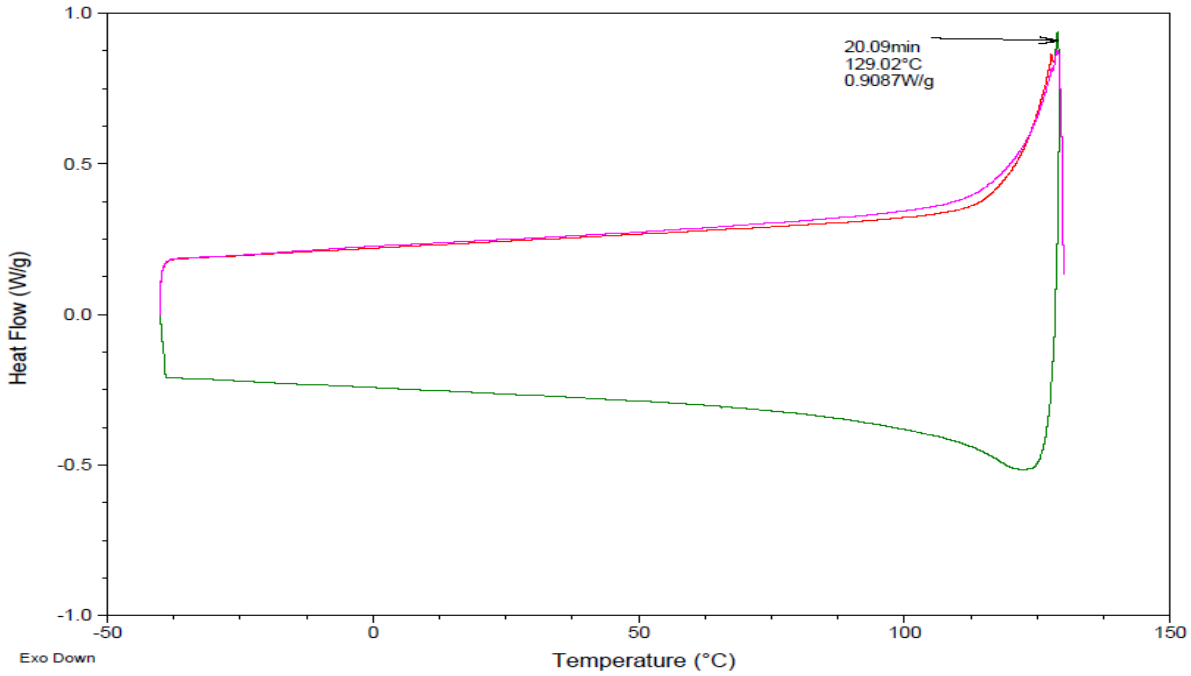


Figure 90 *DSC of HEI 2906 - two heating cycles. It can be observed that the two heating cycles are reproducible, though a temperature shift of the melting point between the two, can be observed. In the cold cycle, a crystallization can be seen as well.*

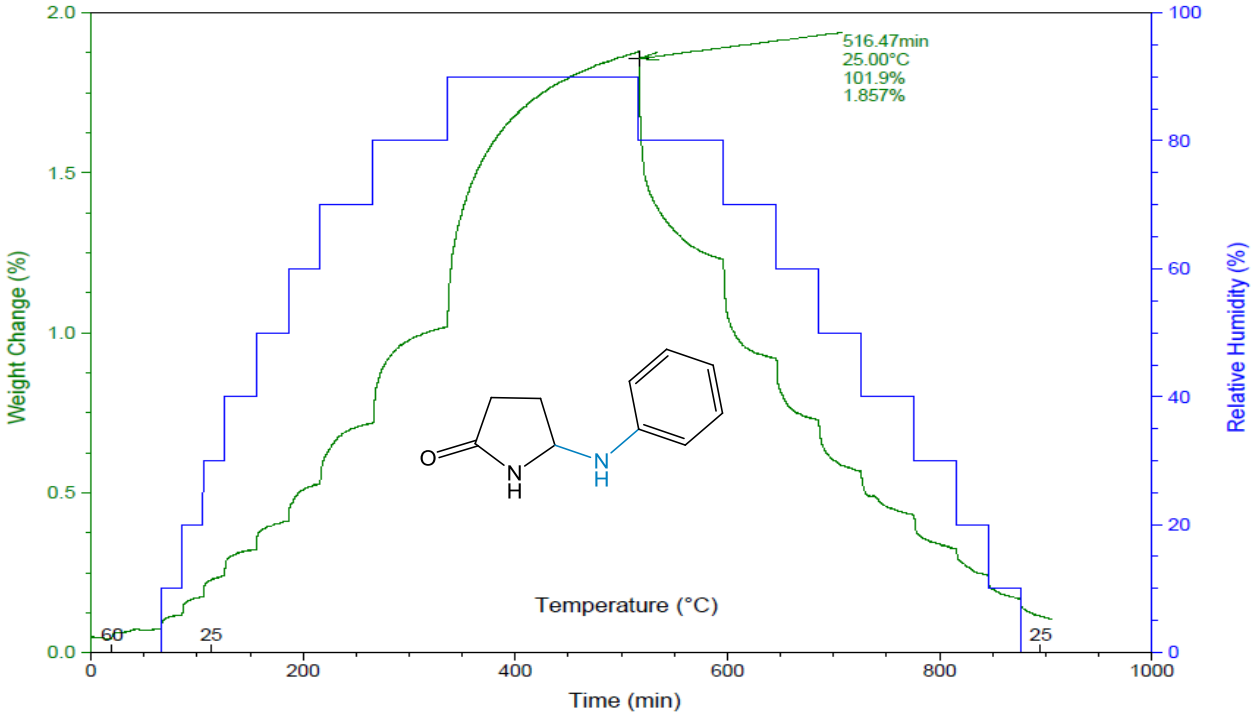


Figure 91 *Dynamic vapor sorption (DVS) change in mass plot and water sorption limit measurement of HEI 2906. As it can be seen from the plot, the compound is slightly hygroscopic, the water mass gain being 1.857%.*

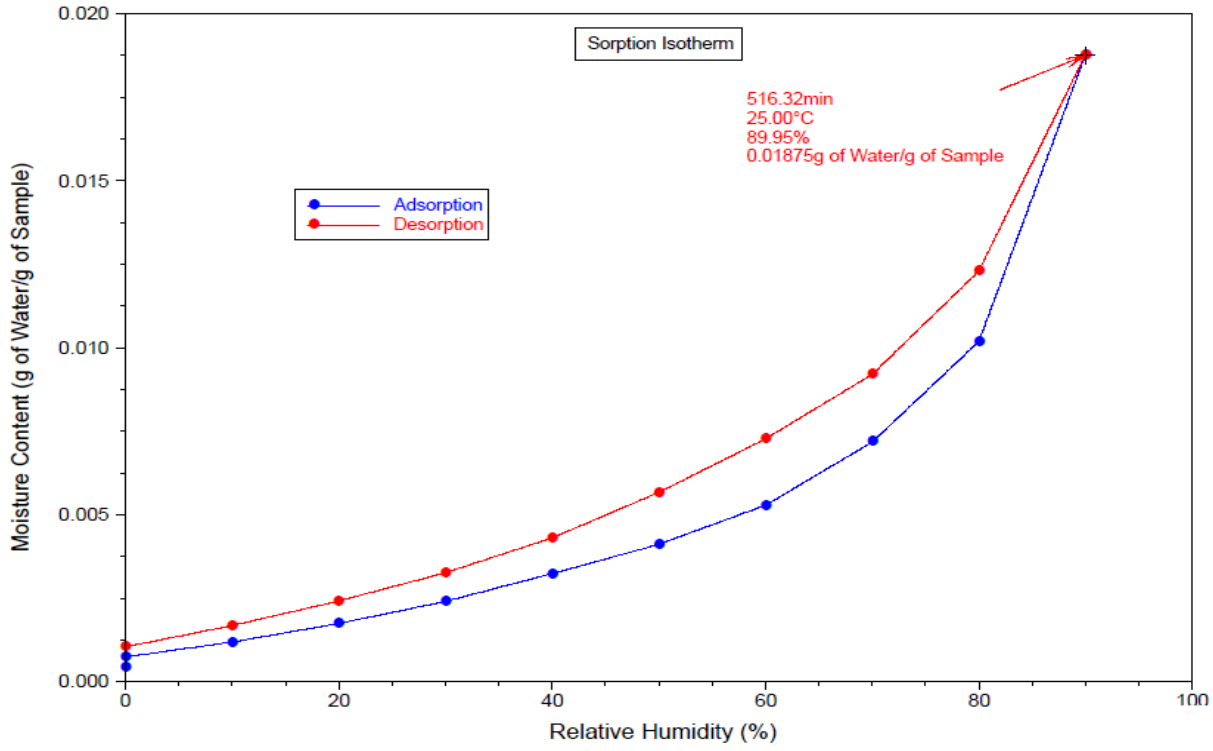


Figure 92 *DVS isotherm plot of HEI 2906. From the isotherm we can see clearly that there wasn´t any tendency of bulk gain of water and the absorption and desorption have the same mechanism.*

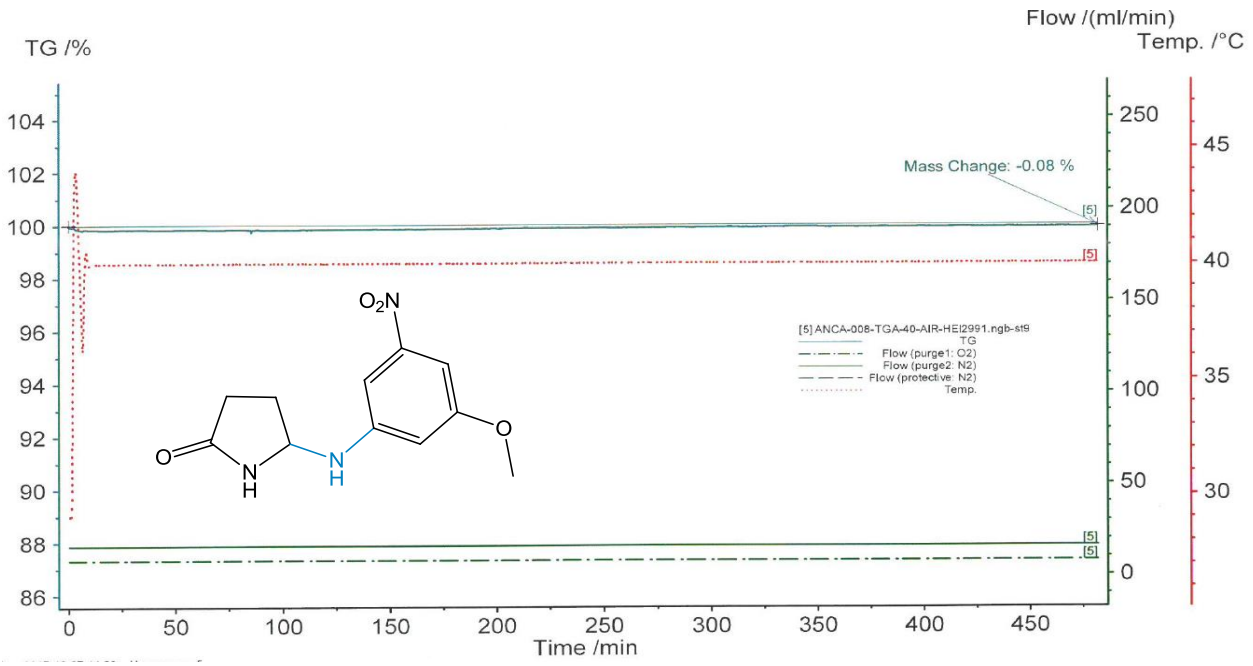


Figure 93 *The thermo-gravimetric analysis (TGA) of HEI 2991, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is stable, without any important mass variation, that 0.08% could be due to solvent traces.*

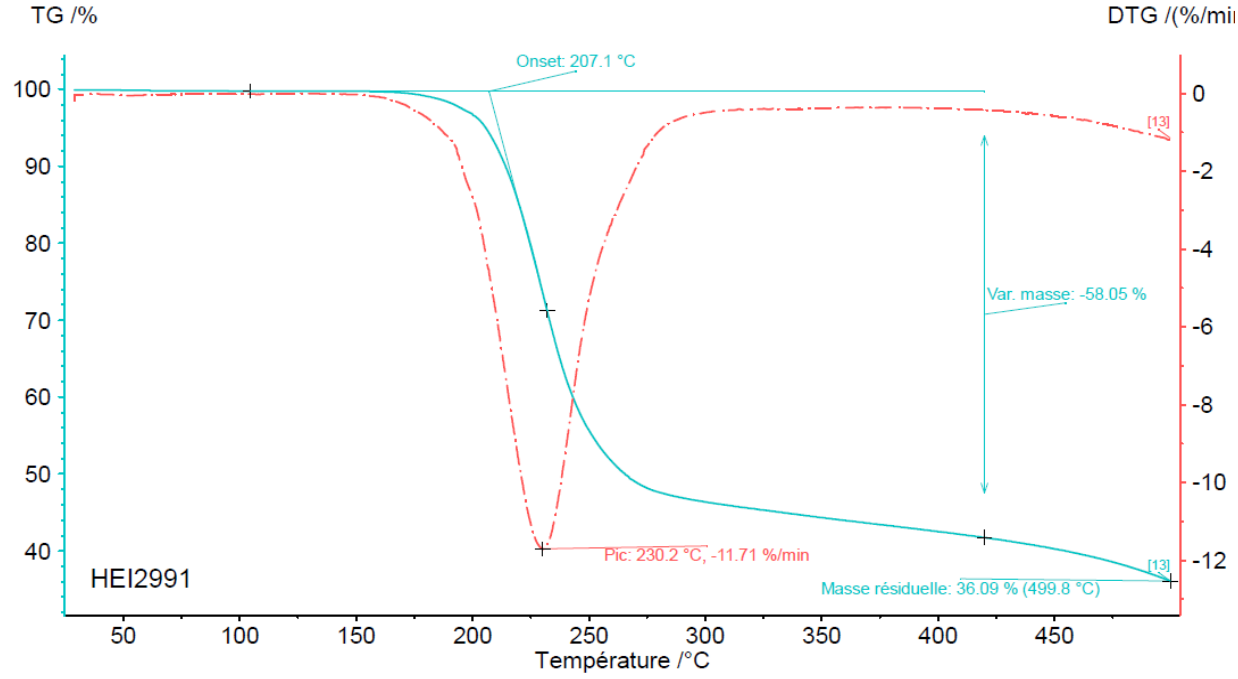


Figure 94 *TGA of the same compound HEI 2991, up to 500°C. On the TGA profile, which correspond to a more stable compound, between 25-250°C we can see an important mass variation of -11.71% resulting from possible moisture. The second step shows another weight loss due to decomposition, but however to be remarked, there is an important remaining mass of 36.09%.*

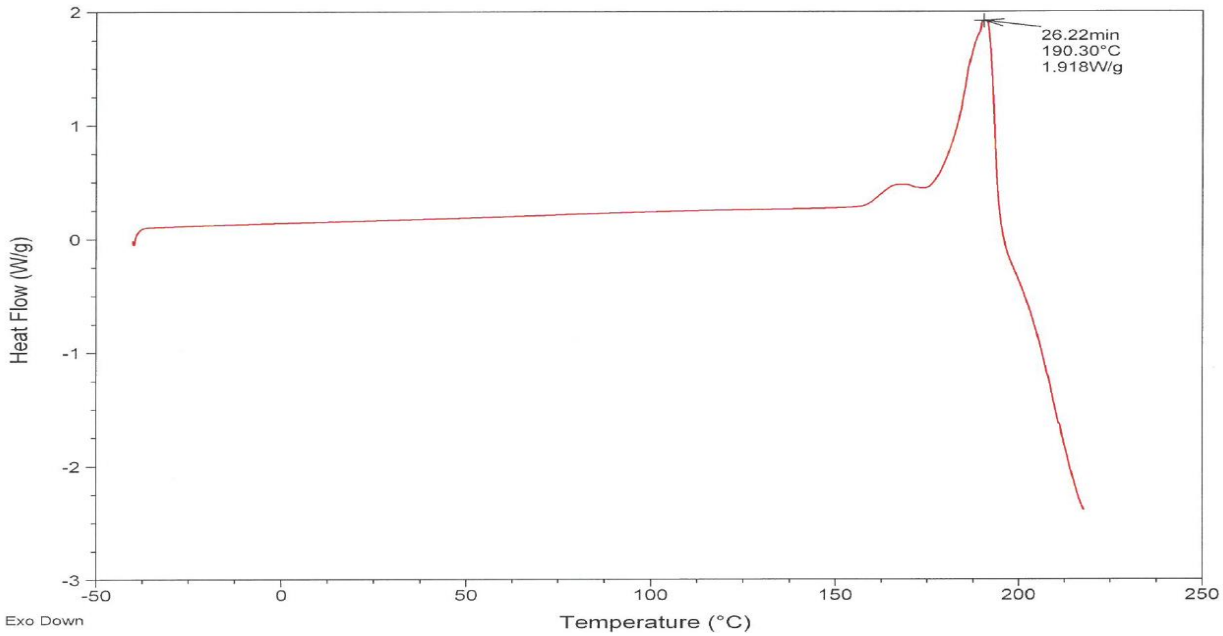


Figure 95 *DSC of HEI 2991 heated at constant flow. The first small peak might be because a possible evaporation, further at 190.30°C we can see a peak temperature defining the melting point.*

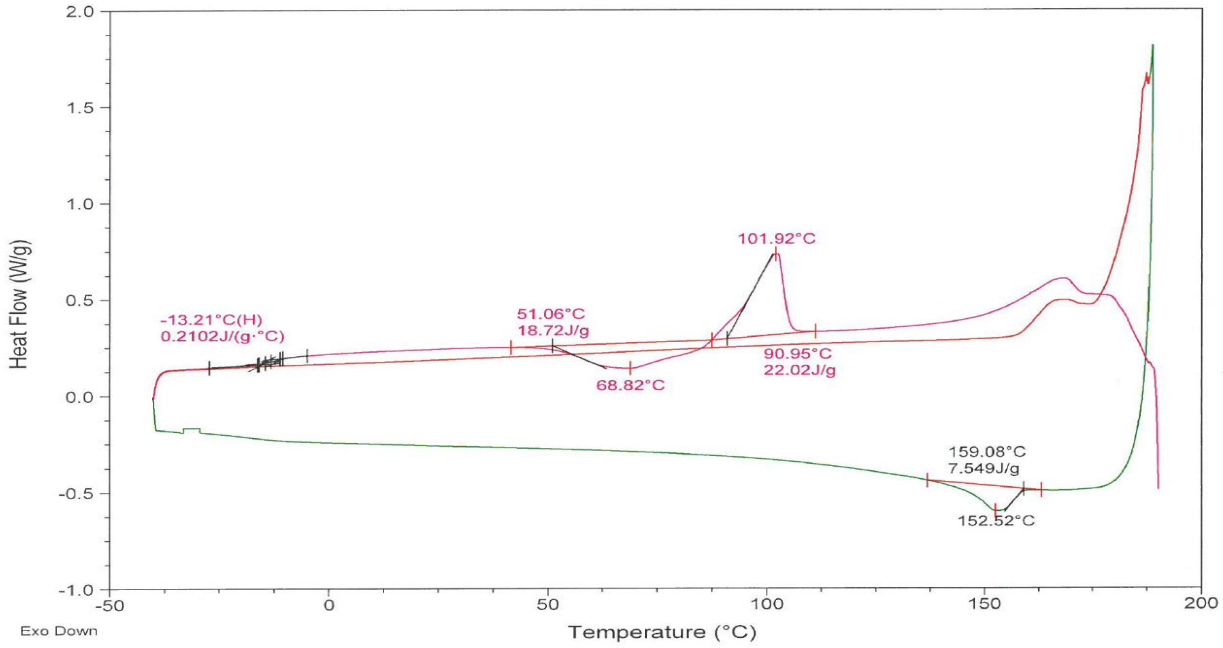


Figure 96 *DSC of HEI 2991 - two heating cycles. It can be observed the first heating cycle is as seen before. The cold cycle present a crystallization at 152.52°. At the beginning of the second heating cycle at -13.21 we can observe a glass transition. Next, at 51.06°C we can notice a crystallization followed by a melting having almost the same enthalpy and we can presume is the same compound. Further we can see the same evaporation as in the first cycle which however is not preceding a melting, the compound seeming to be degradated.*

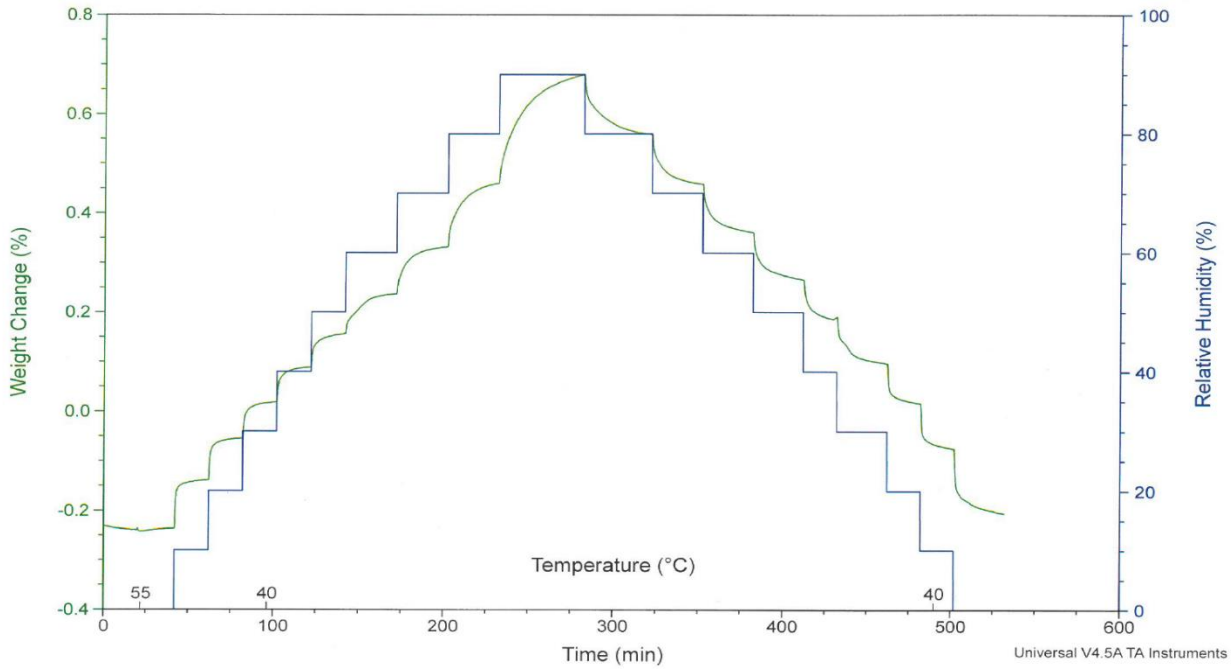


Figure 97 *Dynamic vapor sorption (DVS) change in mass plot and water sorption limit measurement of HEI 2991. As it can be seen from the plot, the compound is gaining water in a small quantity in defined steps.*

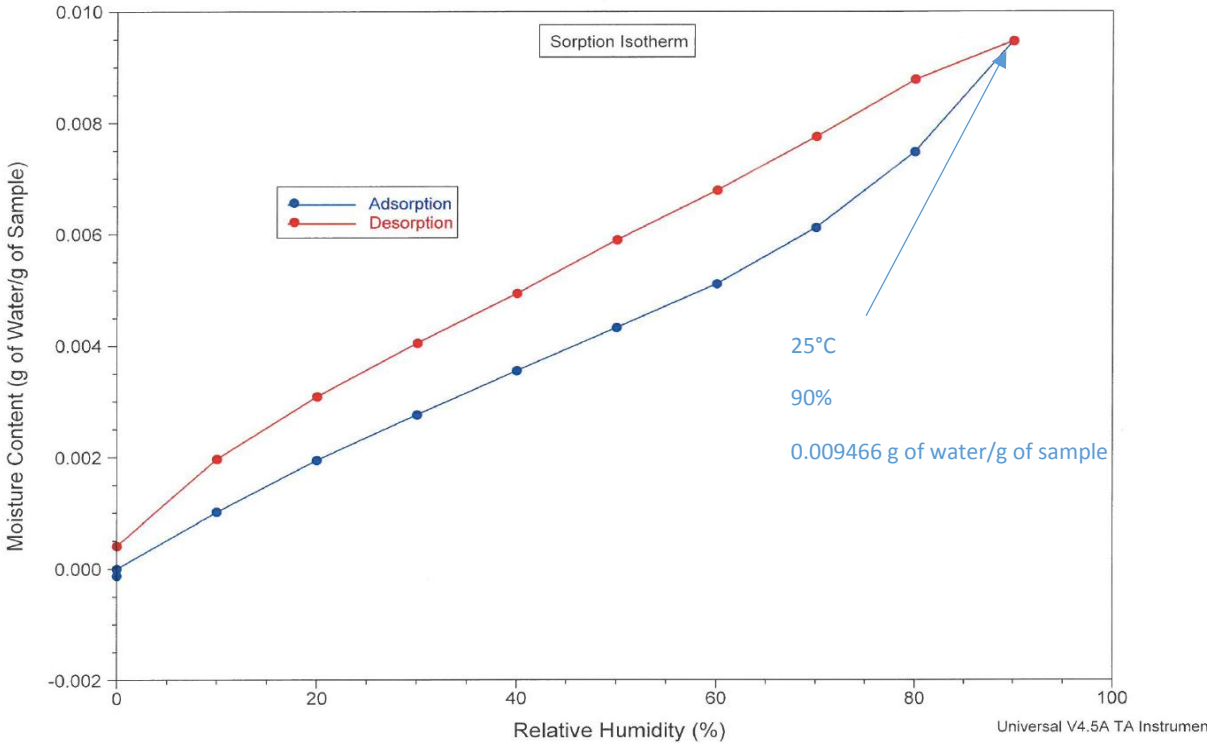


Figure 98 *DVS isotherm plot of HEI 2991. From the isotherm we can see clearly that there wasn´t any tendency of bulk gain of water and the absorption and desorption have the same mechanism. The compound is clearly not hygroscopic.*

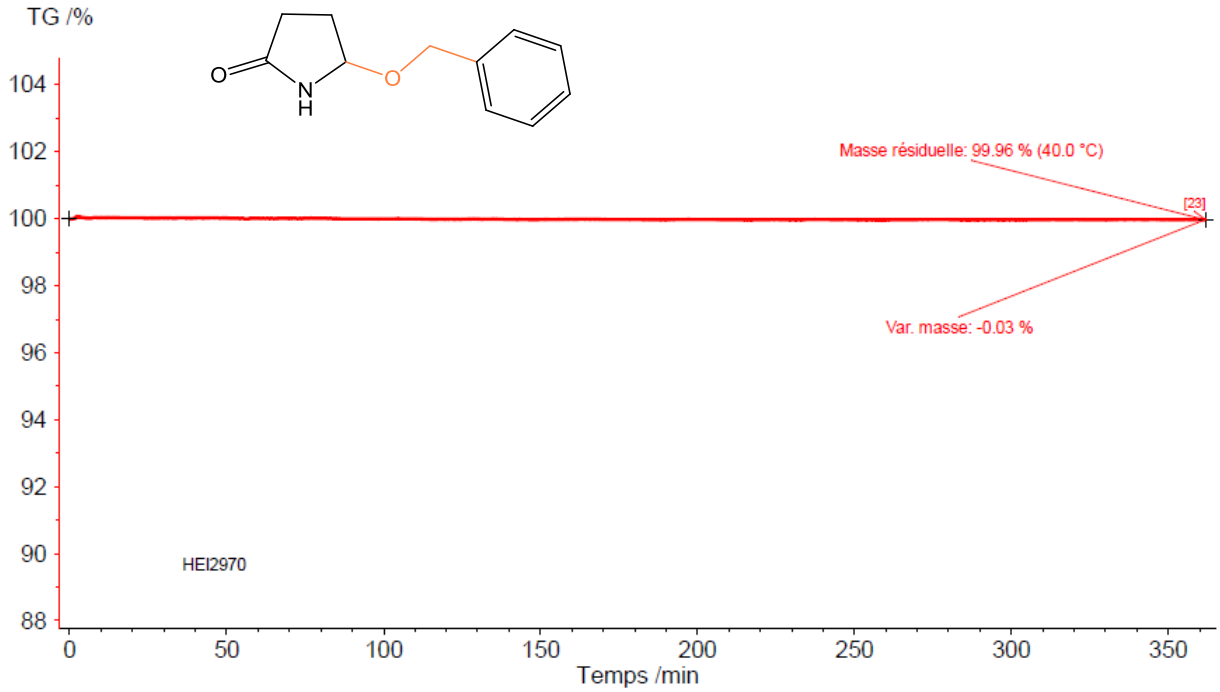


Figure 99 *TGA of HEI 3001, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is very stable, having a 0.03% mass variation.*

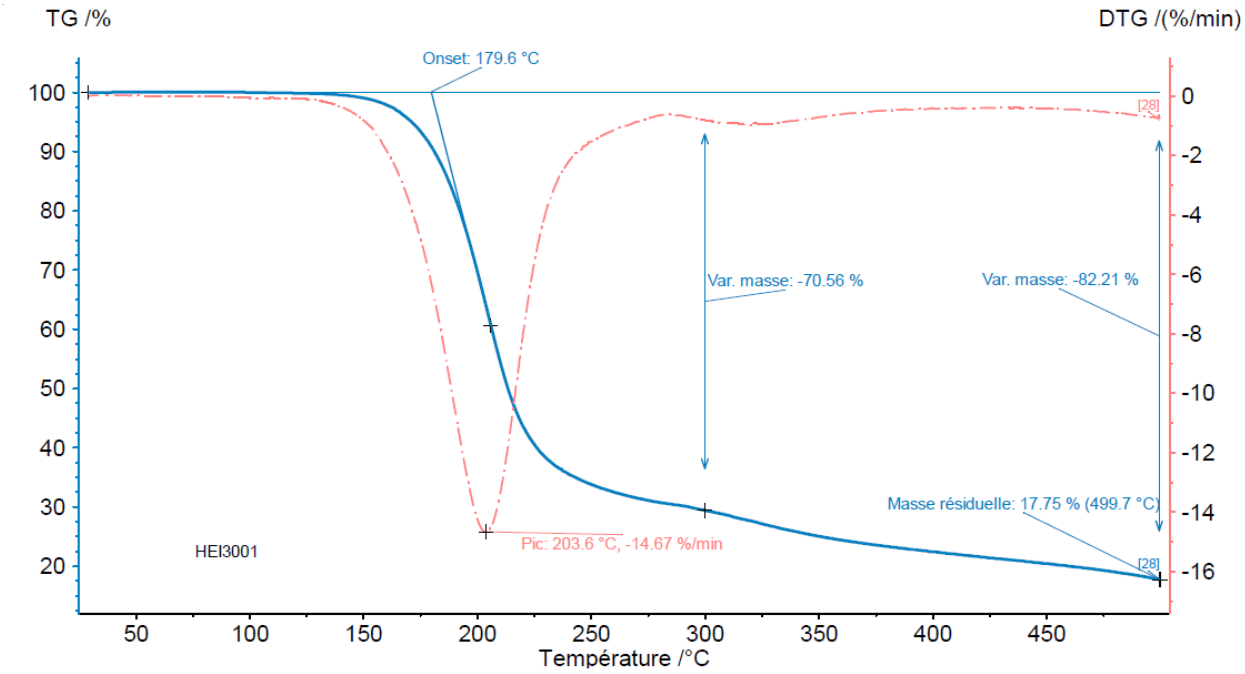


Figure 100 *TGA of the same compound HEI 3001, up to 500°C. On the TGA profile, which is quite clean, in a first step between in the range 25-250°C we can notice an important mass variation of -14.67% resulting from possible moisture or degradation. However the most important mass loss is between 200 and 400°C, 500°C, the residual mass being 17.75%.*

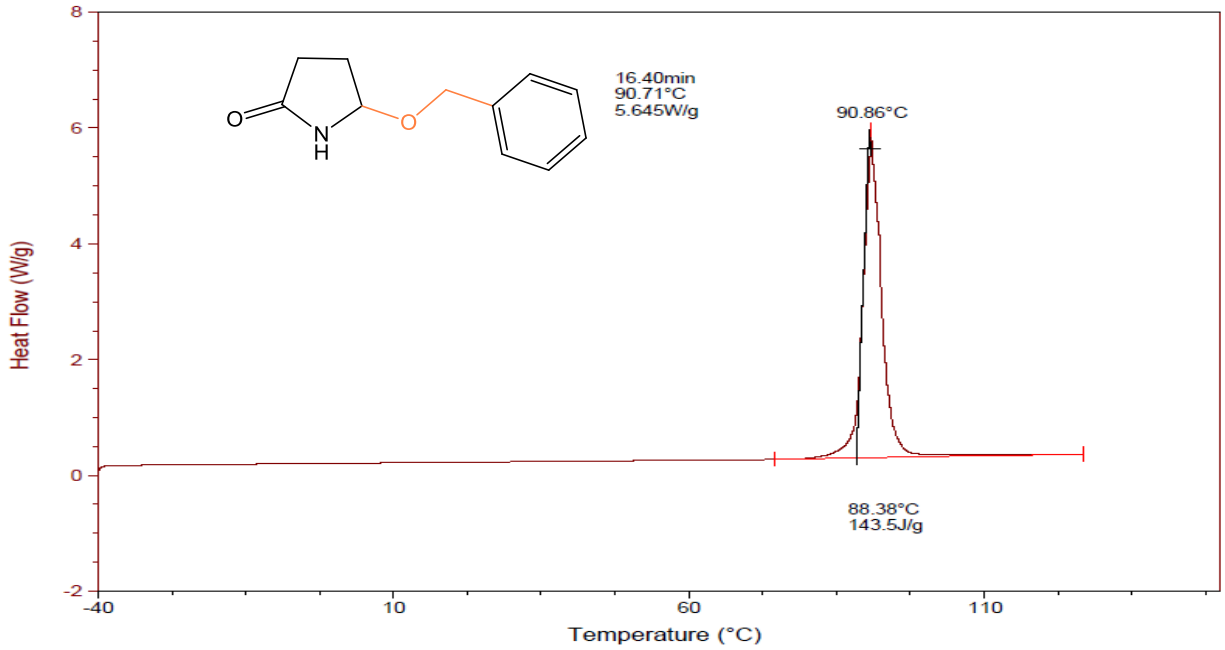


Figure 101 *DSC of HEI 3001 heated at constant flow. As it can be observed, at 90.86°C a peak temperature defining the melting point, having an enthalpy of 143.5 J/g.*

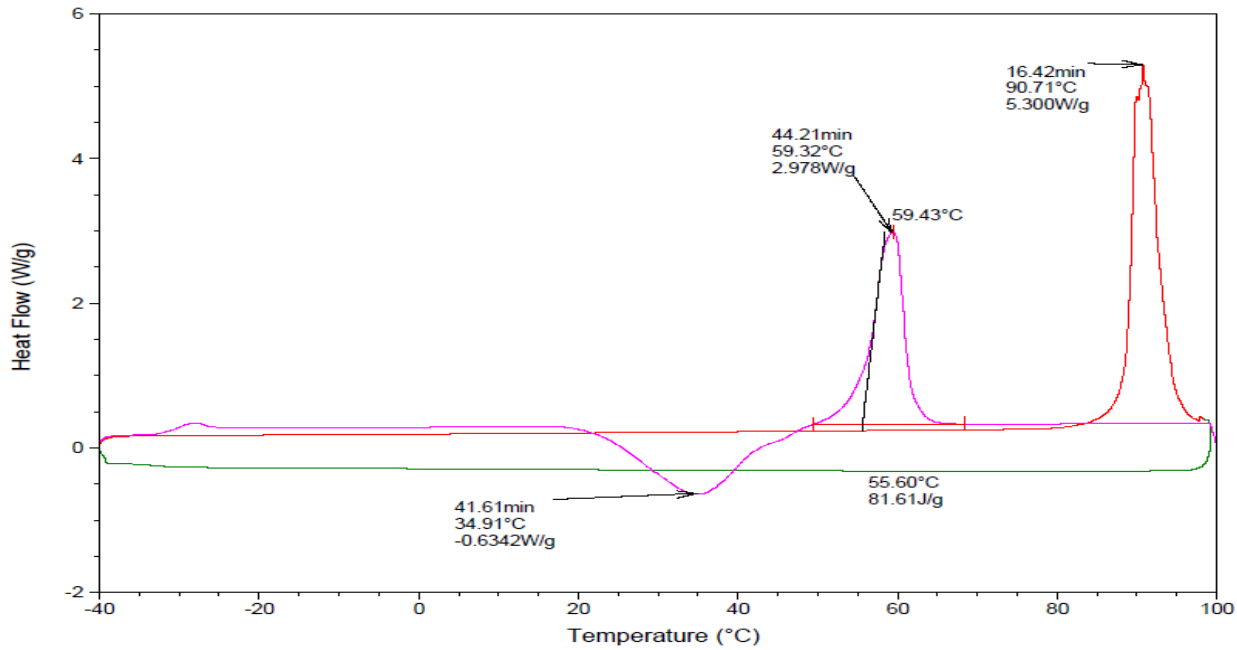


Figure 102 *DSC of HEI 3001 - two heating cycles. It can be observed that the two heating cycles are not reproducible possibly because of a decomposition, as the second cycle presents two melting points 90.71 respectively 59.32. During the second heating cycle a glass transition can be observed at the beginning of it. Also, an interesting behavior of such compounds is the crystallization observed before the melting; thing being said, the second cycle m.p. might describe the same compound but as in a different crystalline state.*

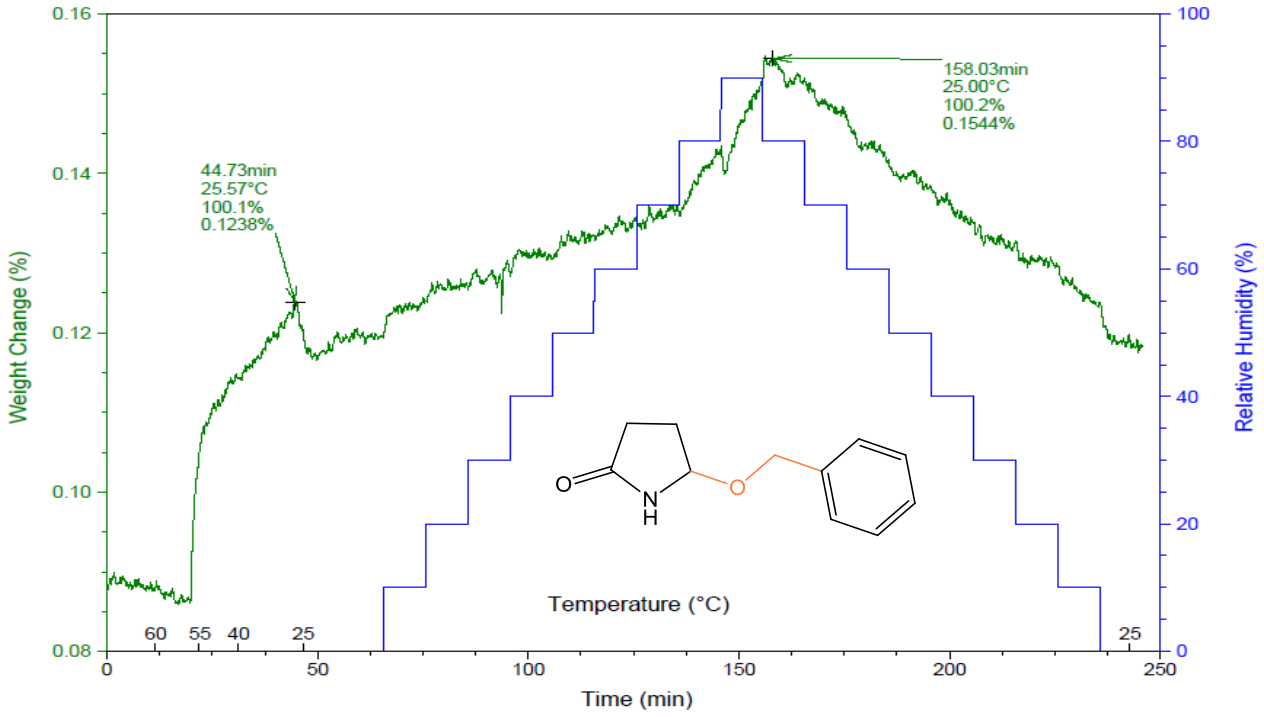


Figure 103 *DVS change in mass plot and water sorption limit measurement of HEI 3001. As it can be seen from the plot, the compound is not hygroscopic, the water mass gain being only 0.154%. However, the water intake kinetics is not normal.*

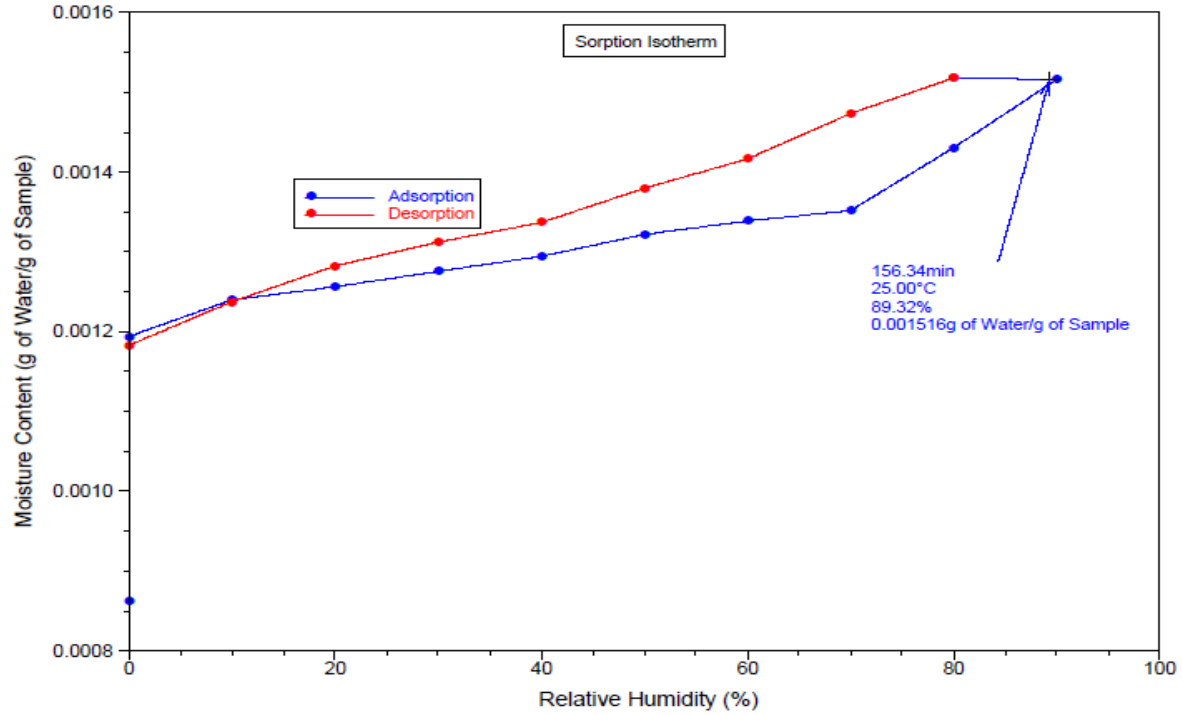


Figure 104 *DVS isotherm plot of HEI 3001. From the sorption isotherm we can see clearly that the compound is not hygroscopic at all.*

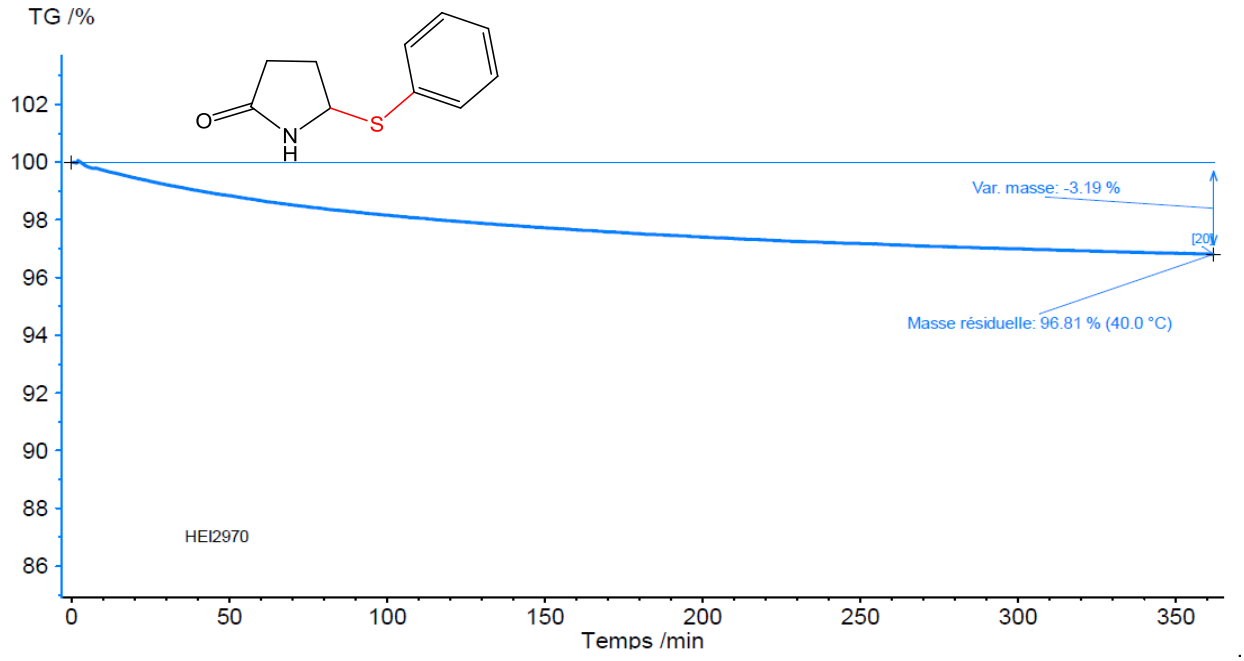


Figure 105 *TGA of HEI 2957, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is unstable, having a -3.19% mass variation which can be due to eventual release of volatile matter.*

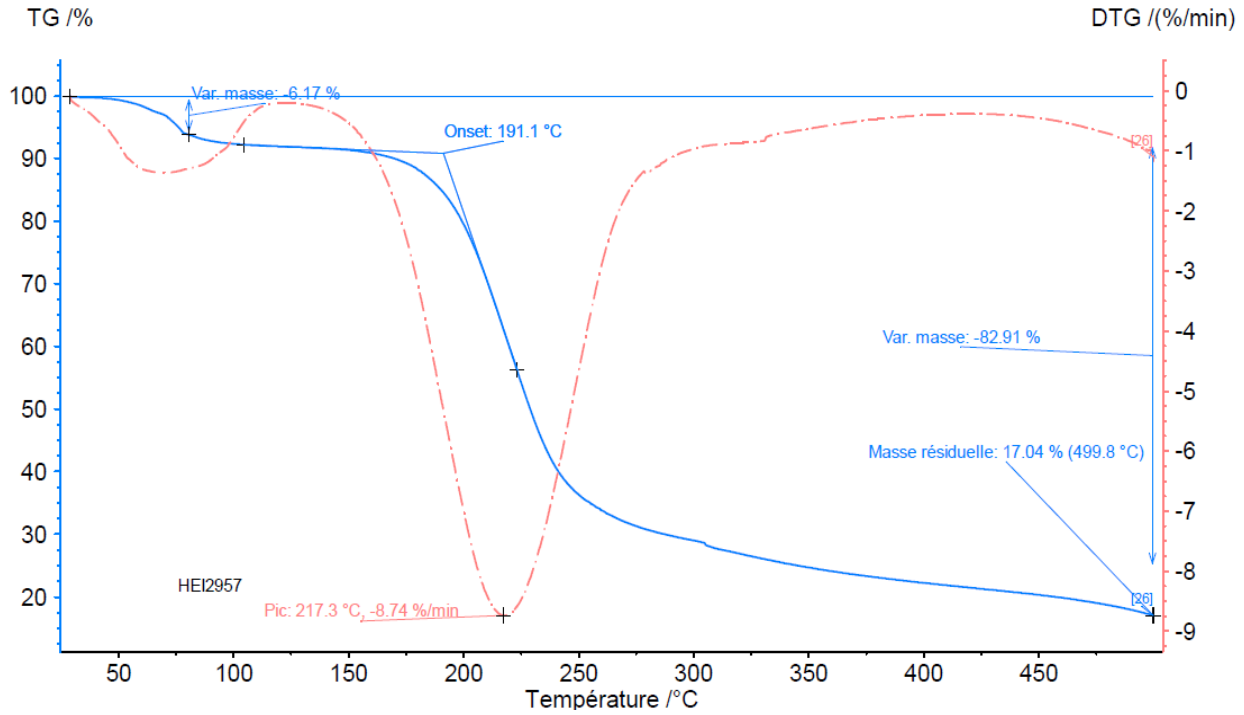


Figure 106 *TGA of the same compound HEI 2957, up to 500°C. On the TGA profile, in the same range 25-100°C we can see the same mass variation which could be observed in the first TGA, now of -6.17%. The second step shows another weight loss in the temperature range of 100°C to 182°C, which might have resulted in partial decomposition. Between 250 and 500°C total degradation can be seen, though the residual mass is 17.04%.*

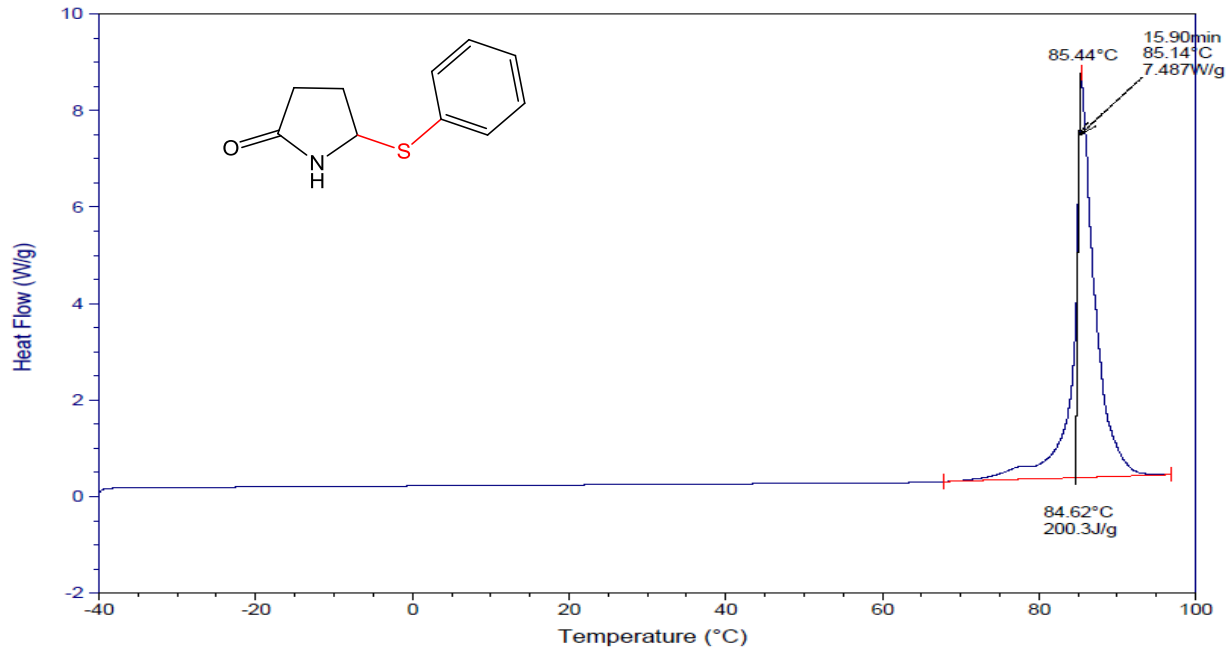


Figure 107 *DSC of HEI 2957 heated at constant flow. As it can be observed, at 85.44°C the peak temperature is defining the melting point, having an enthalpy of 200.3 J/g. Just before the melting, a slight degradation can be seen which can explain the mass variation seen in the first TGA.*

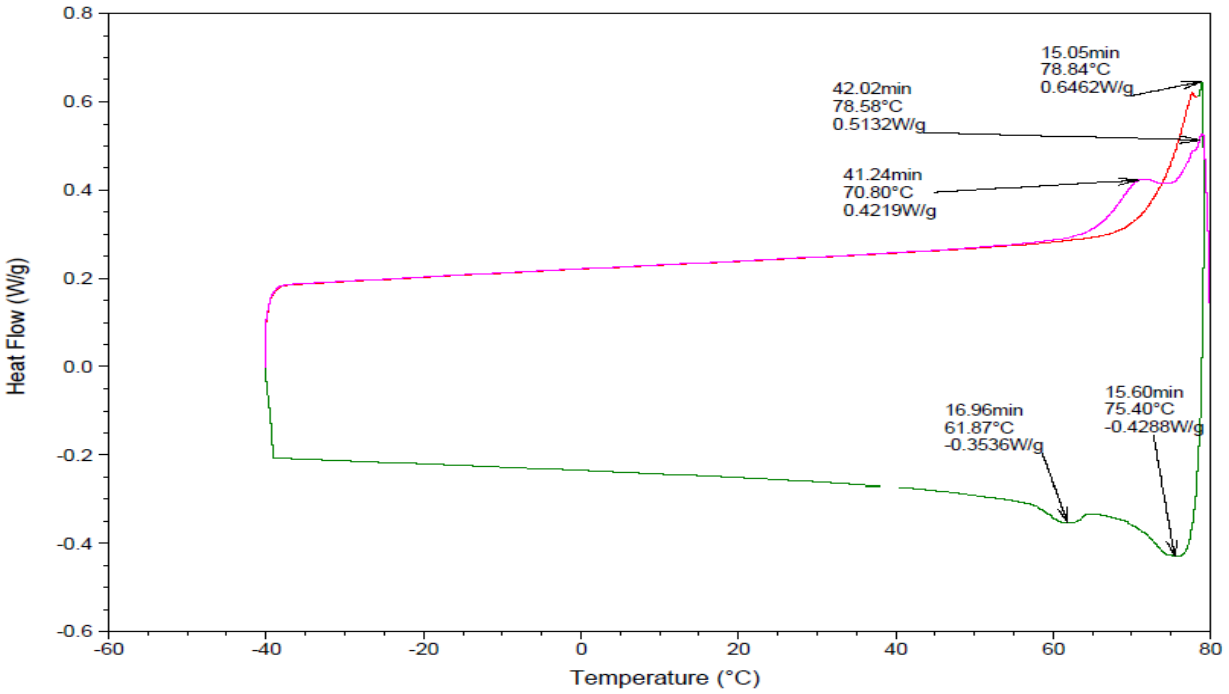


Figure 108 *DSC of HEI 2957 - two heating cycles. It can be observed that the two heating cycles are not reproducible possibly because of a decomposition, seen in the first DSC and confirmed by the 2nd heating cycle of this DSC. In the cold cycle, two crystallizations can be seen, corresponding to the melted initial compound and the second degradated compound.*

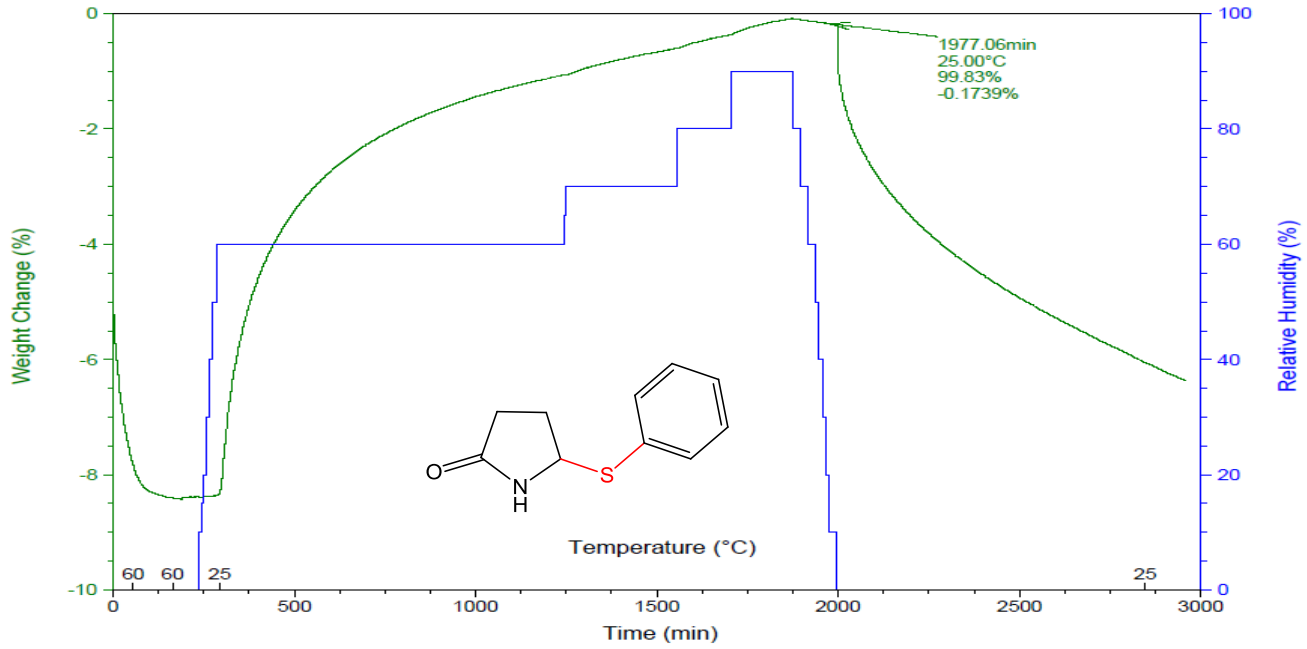


Figure 109 *DVS change in mass plot and water sorption limit measurement of HEI 2957. As it can be seen from the plot, the compound doesn´t seem to be hygroscopic, but it eliminates water, mass eliminated being 0.17% so the compound initially might have been as a hydrate form.*

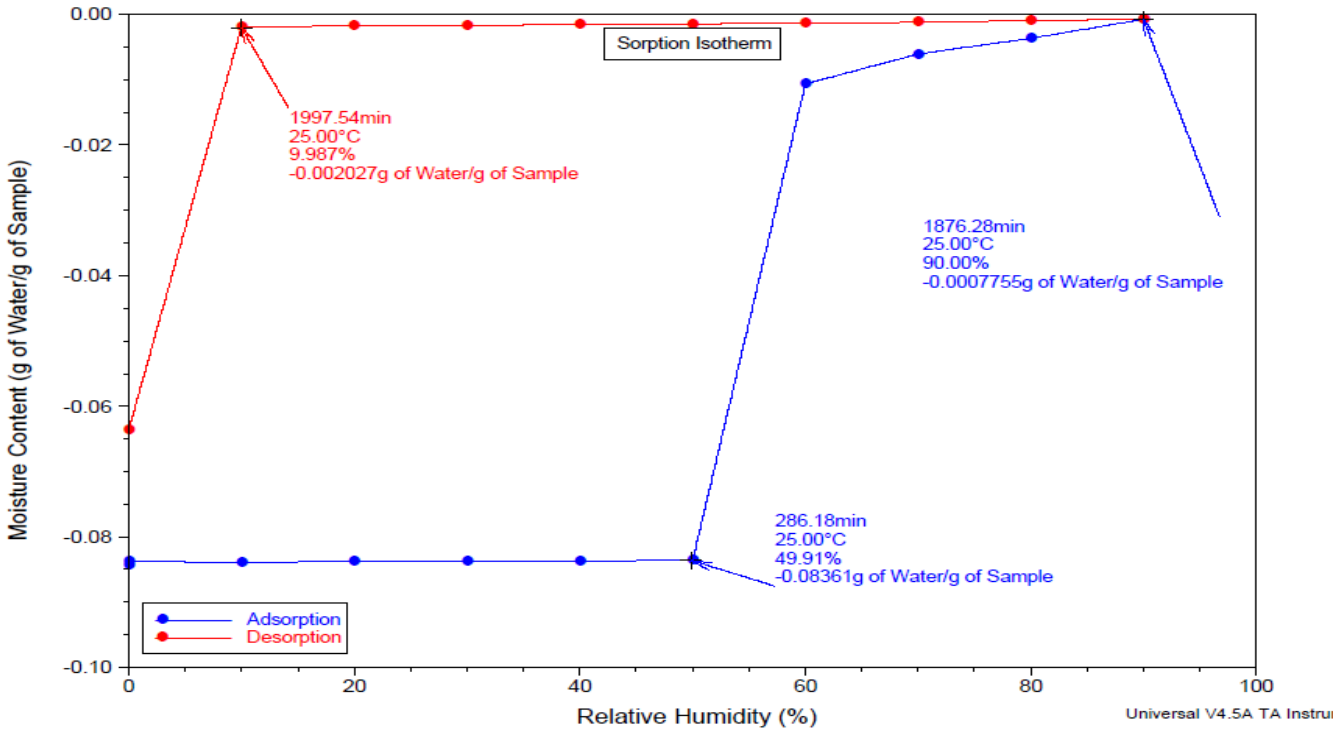


Figure 110 *DVS isotherm plot of HEI 2957. From the isotherm we can see a change upon the hydratation, the molecule eliminating water in a first step, and bulk gaining in the second step, overall it´s profile resting hygroscopic.*

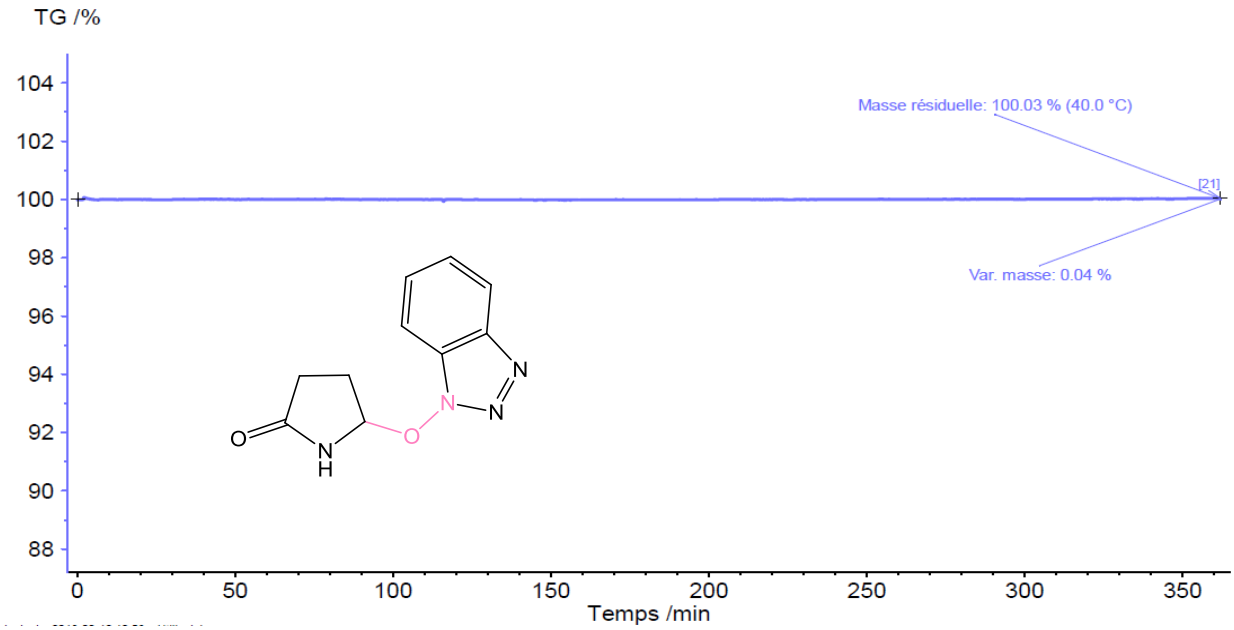


Figure 111 *TGA of HEI 2984, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is very stable, having a little gain of 0.04% mass due to probably solvent traces evaporation.*

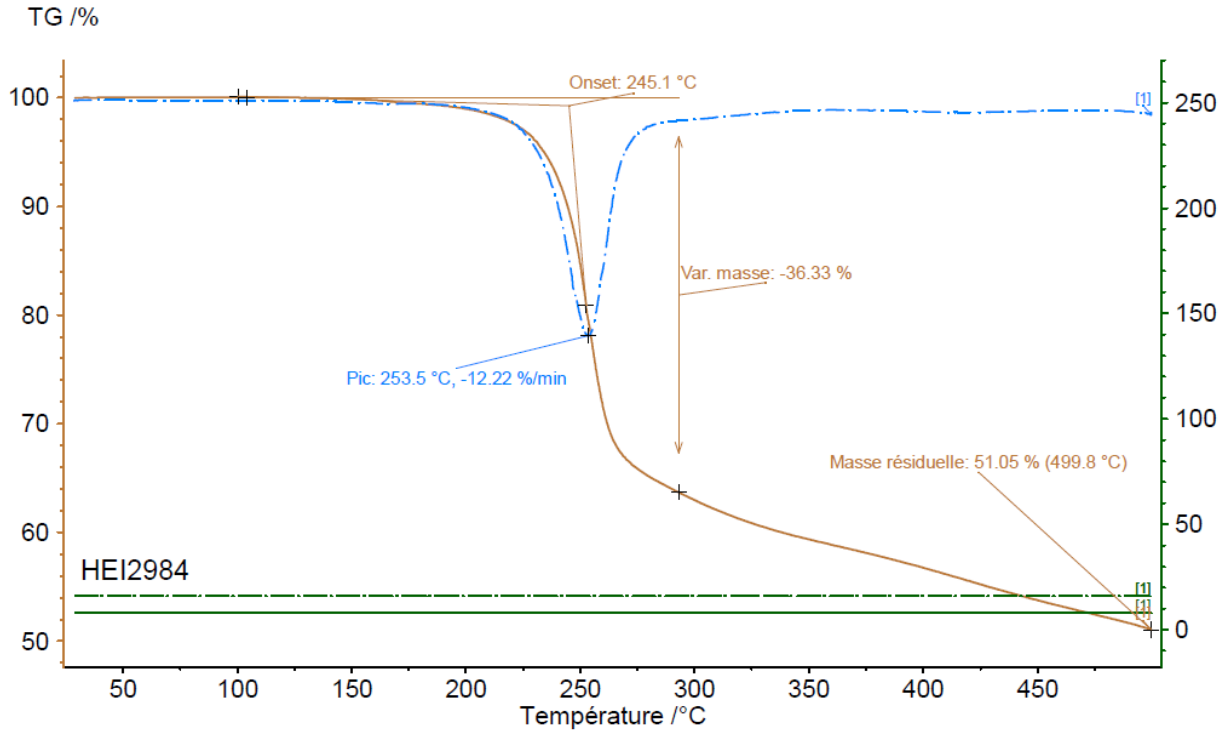


Figure 112 *TGA of the same compound HEI 2984, up to 500°C. On the TGA profile, in a first step in the range 25-300°C we can notice an important mass variation of -12.22% resulting from possible moisture or degradation. What´s notable considering this particular compound is the important residual mass of 51.05%.*

§

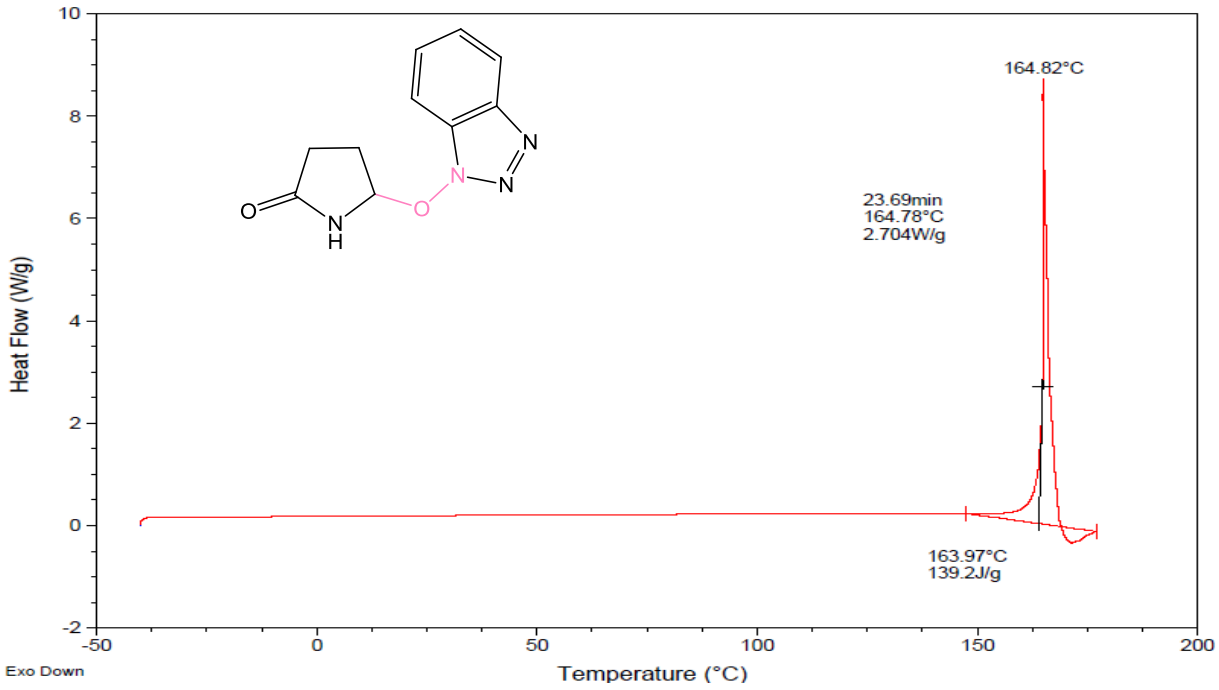


Figure 113 *DSC of HEI 2984 heated at constant flow. As it can be observed, at 164.82°C a peak temperature defining the melting point, with a corresponding enthalpy of 132.2 J/g followed by a crystallization.*

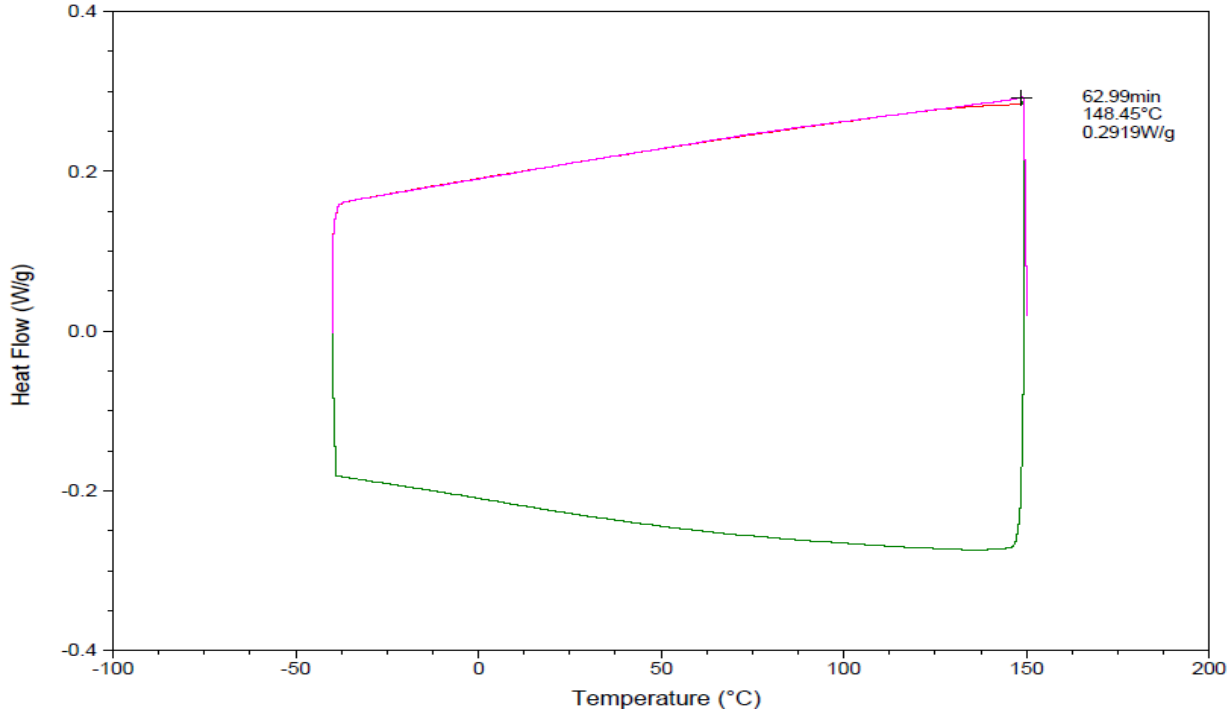


Figure 114 *DSC of HEI 2984 - two heating cycles. It can be observed that the compound is very stable and the two heating cycles are perfectly reproducible at a temperature of 10 degrees below the m.p.*

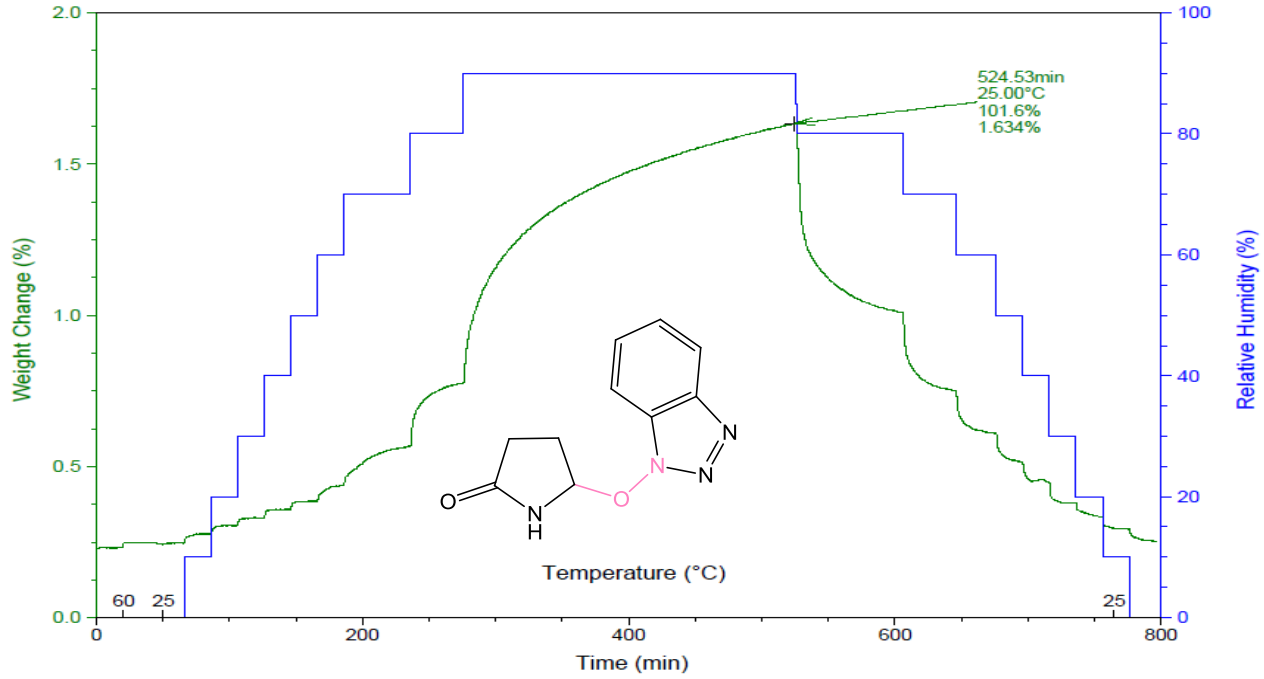


Figure 115 *DVS change in mass plot and water sorption limit measurement of HEI 2984. As it can be seen from the plot, between 40% and 80 % RH there´s a bulk water intake, the compound seems a little hygroscopic, uptaking 1.634% water.*

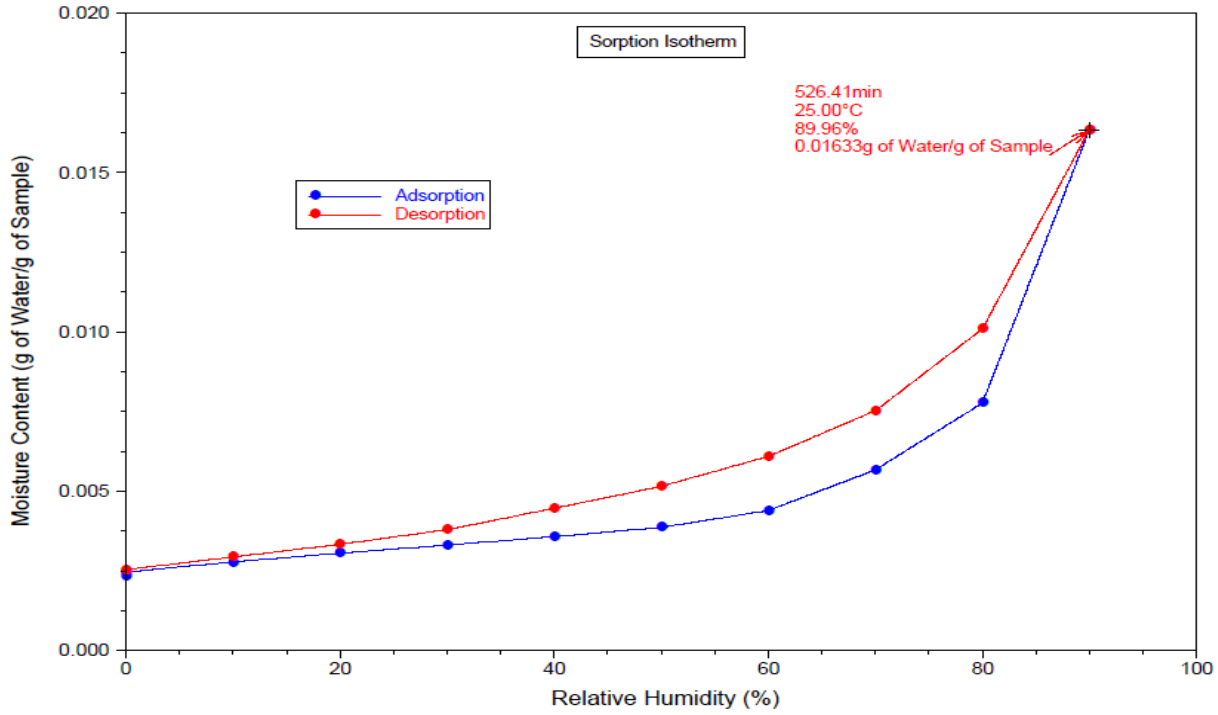


Figure 116 *DVS isotherm plot of HEI 2984. From the isotherm it can be observed that the compound possibly forms a hydrate during the adsorption.*

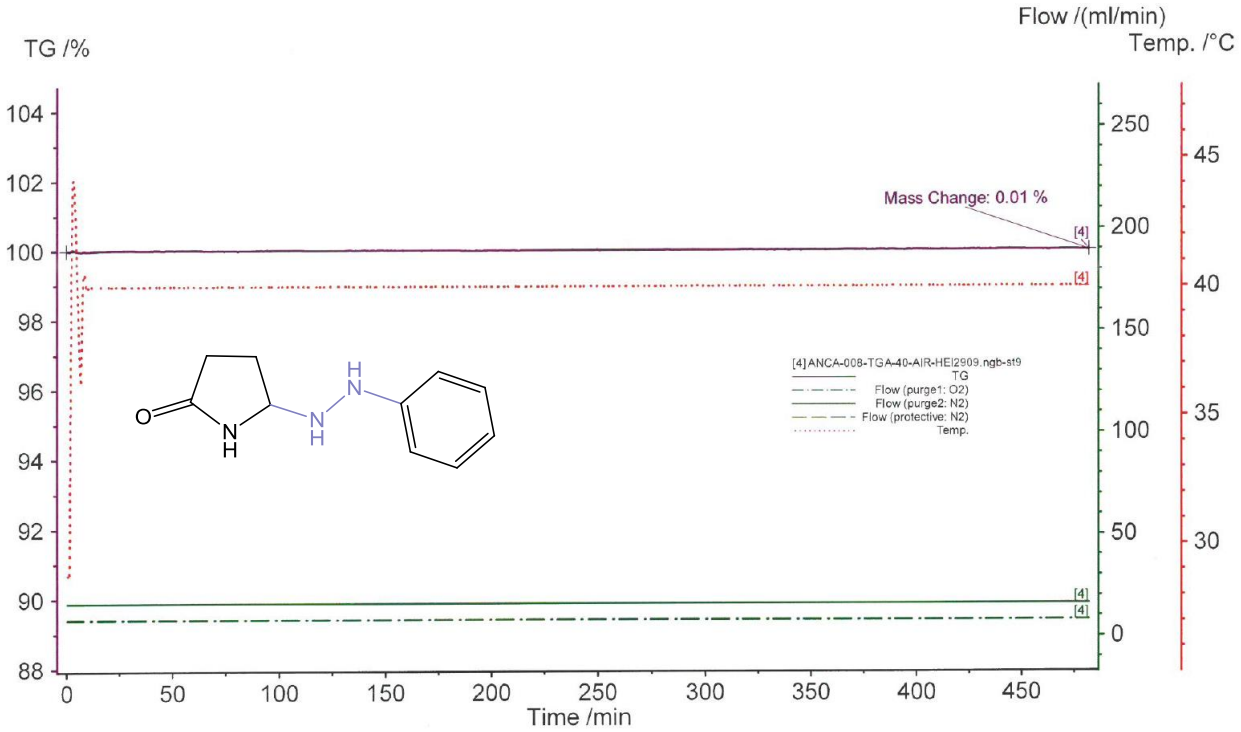


Figure 117 *TGA of HEI 2909, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is very stable, having only 0.01% mass change.*

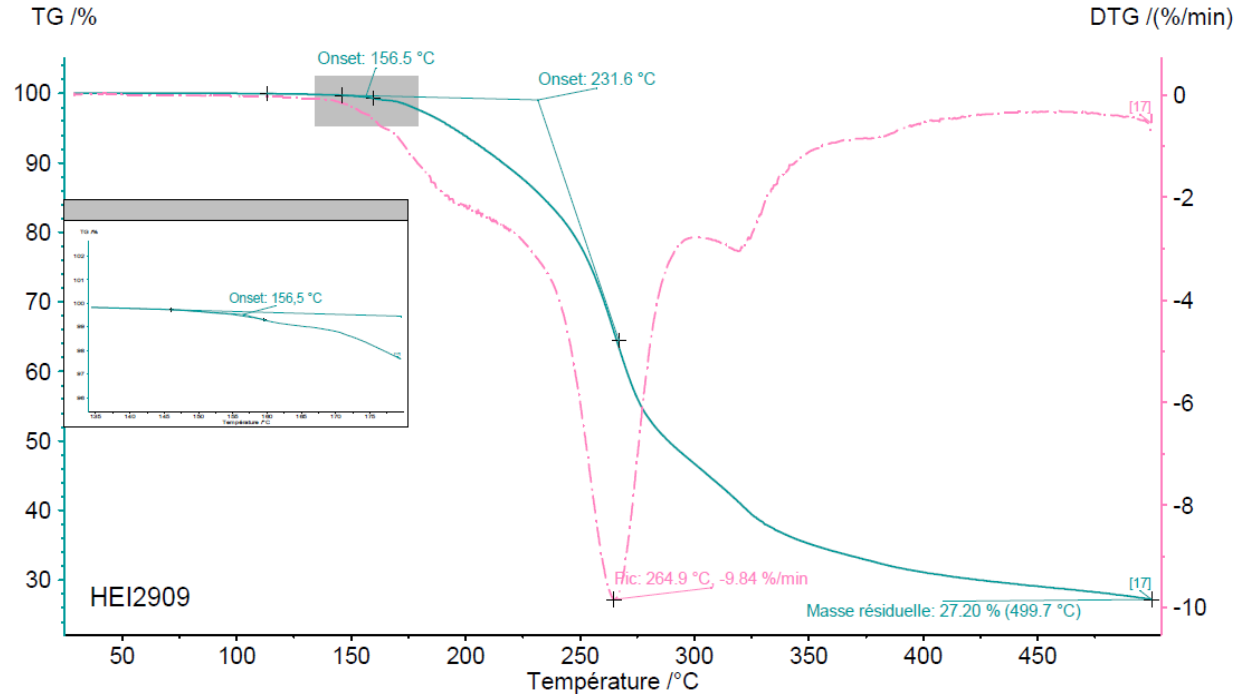


Figure 118 *TGA of the same compound HEI 2909, up to 500°C. On the TGA profile, in a first step in the range 25-300°C we can notice an important mass variation of -9.84% resulting from possible moisture or degradation. Its residual mass is quite important, being 27.20%.*

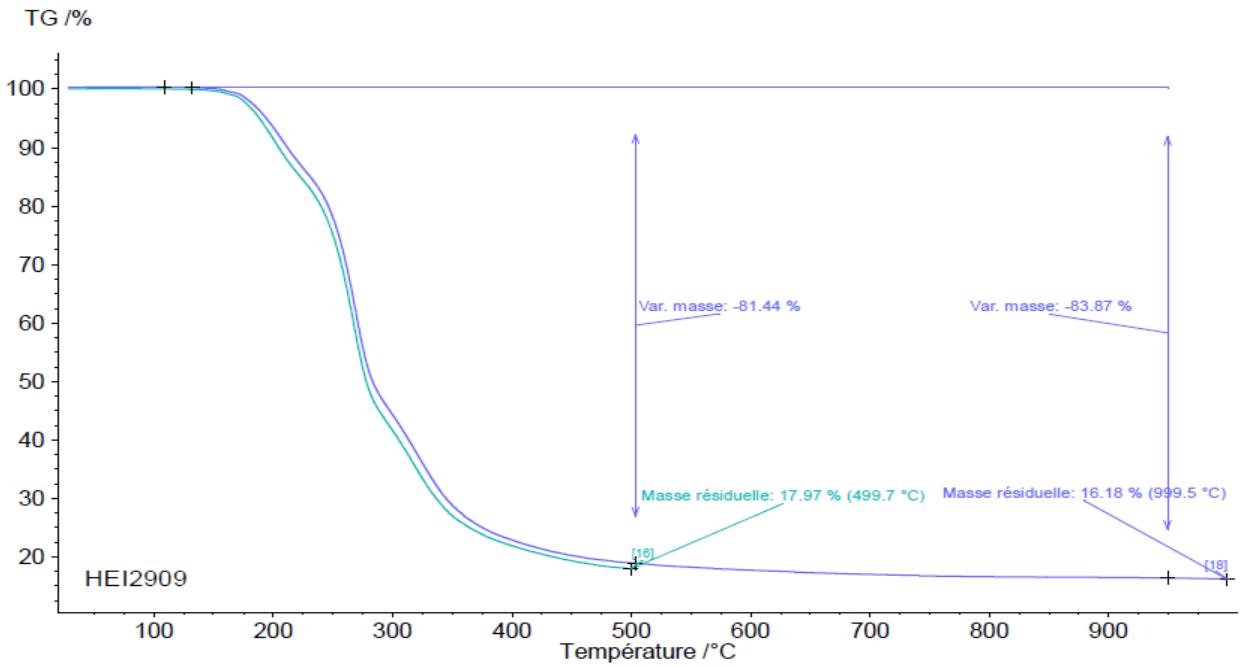


Figure 119 *TGA of the same compound HEI 2909, up to 1000°C. On the TGA profile, which correspond to a less stable compound, between 25-250°C we can see the important mass variation of -9.84% resulting from possible moisture or degradation. Notable is the residual mass diffrences between two TGA (16 and 17) of 16.97 respectively 27.20% at 500°C. And as it can be seen, at 1000°C still, the residual mass is important, 16.18%.*

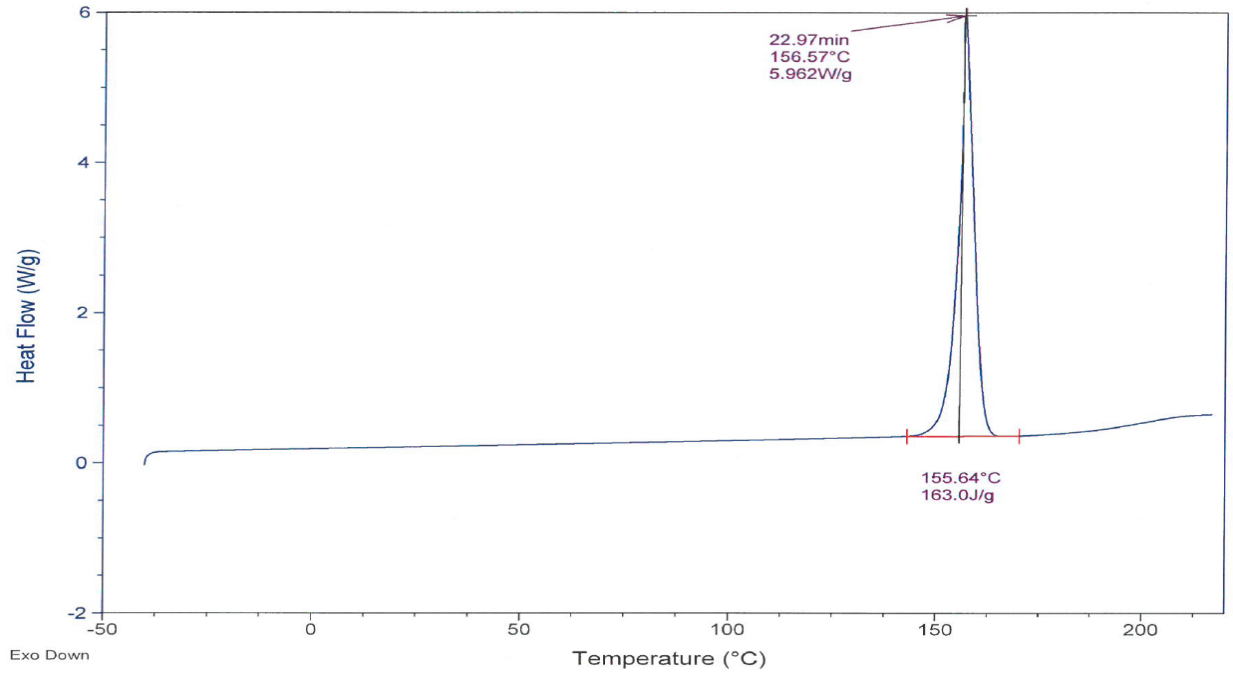


Figure 120 *DSC of HEI 2909 heated at constant flow. As it can be observed, at 156.57°C we have a peak temperature defining the melting point, with a corresponding enthalpy of 156.57 J/g followed by a possible further evaporation.*

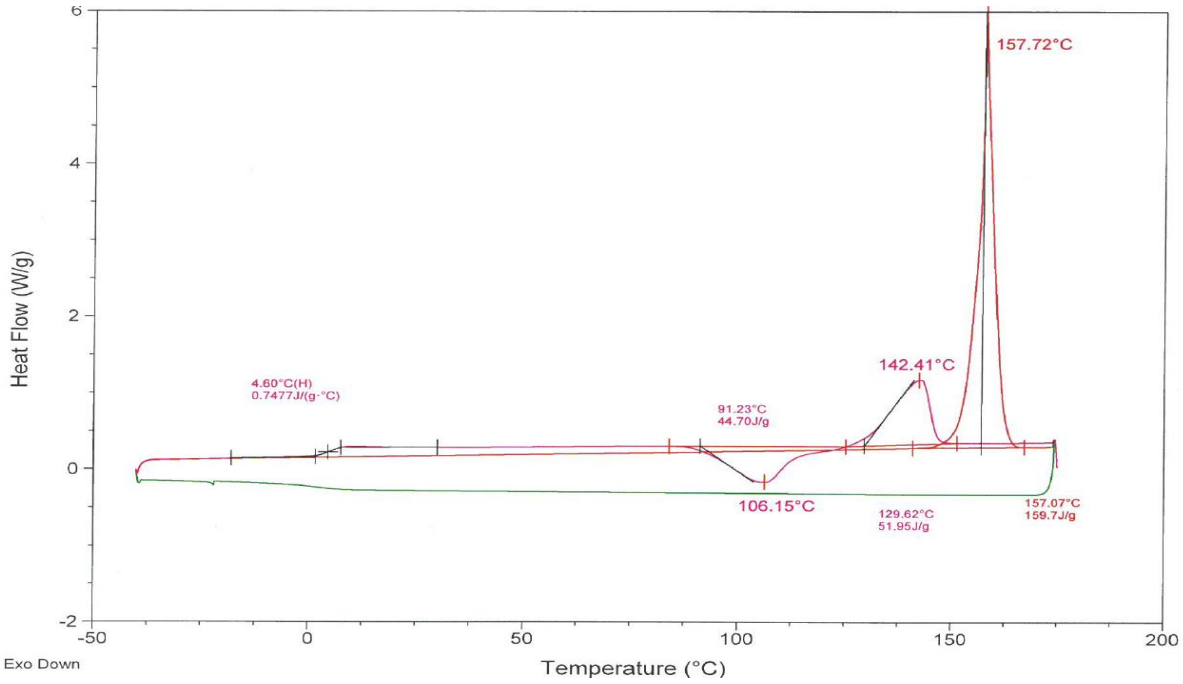


Figure 121 *DSC of HEI 2909 - two heating cycles. As it can be noticed, during the second heating cycle, the compounds show different behavior, at 4.6°C we can observe a glass transition, followed by a hot crystallization at 106.5°C and the melting which now shifted probably because of crystallization change.*

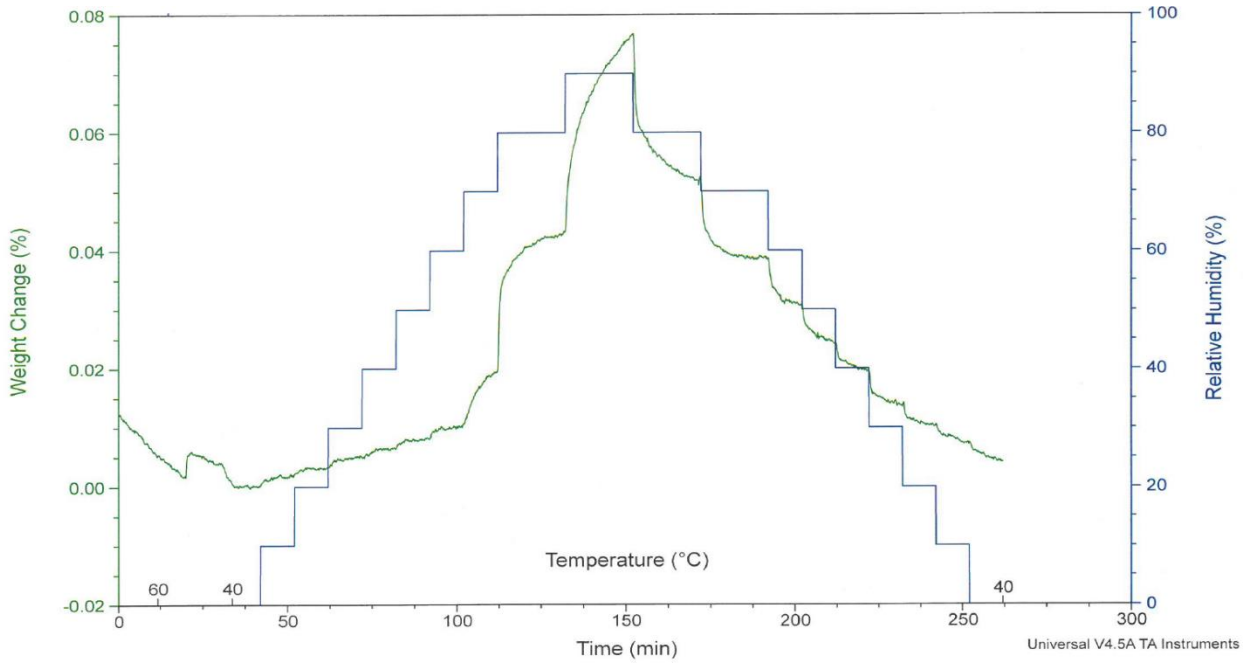


Figure 122 *DVS change in mass plot and water sorption limit measurement of HEI 2909. As it can be seen from the plot, between 40% and 60 % RH there´s a bulk water intake however insignificant, the compound not being hygroscopic.*

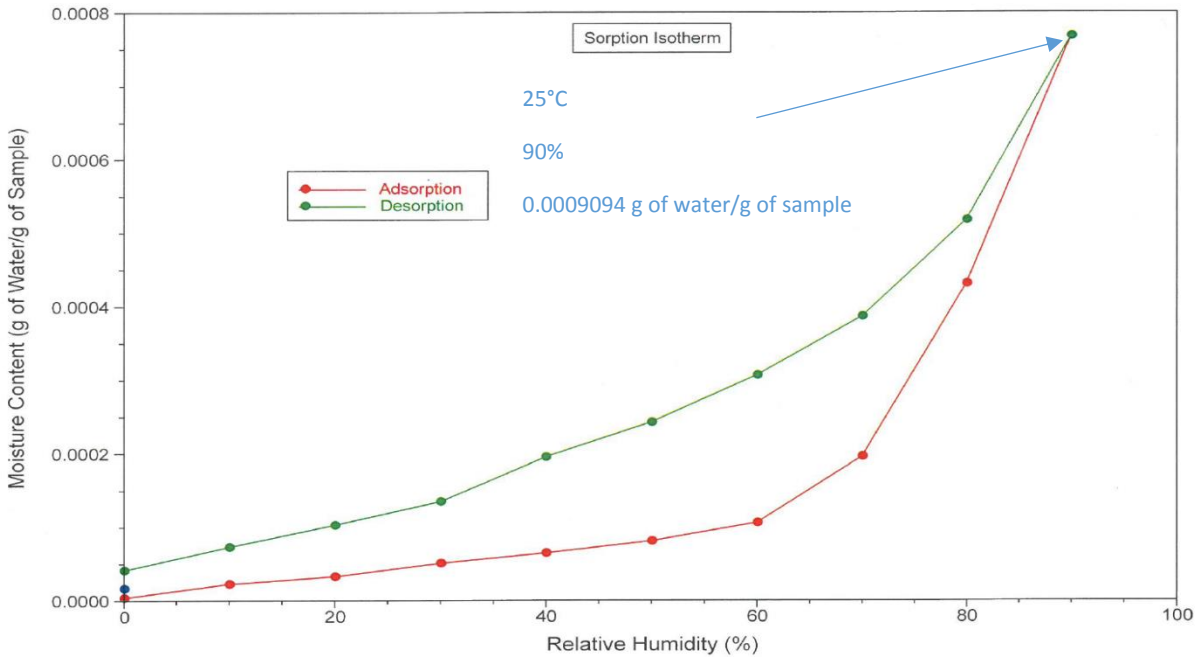


Figure 123 *DVS isotherm plot of HEI 2909. From the sorption isotherm we can see a tendency of bulk gain of water, while de desorption happens smoothly.*

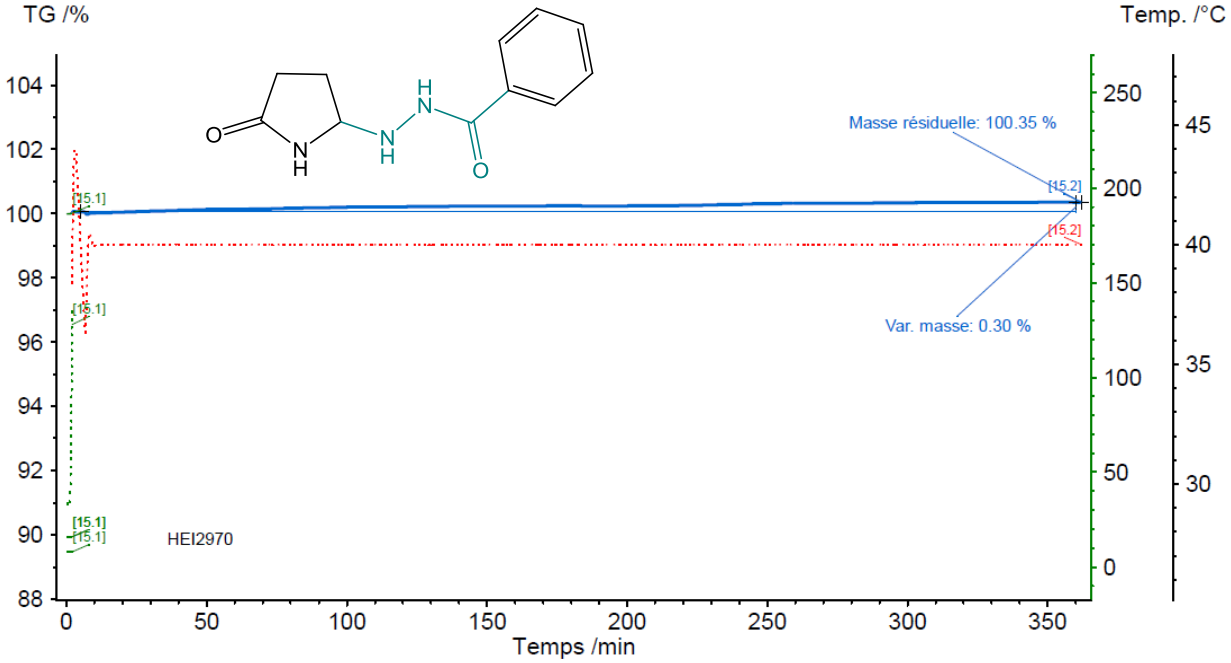


Figure 124 *TGA of HEI 2970, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is quite stable, having a 0.30% mass variation which can be due to eventual oxidation or solvent evaporation, however insignificant.*

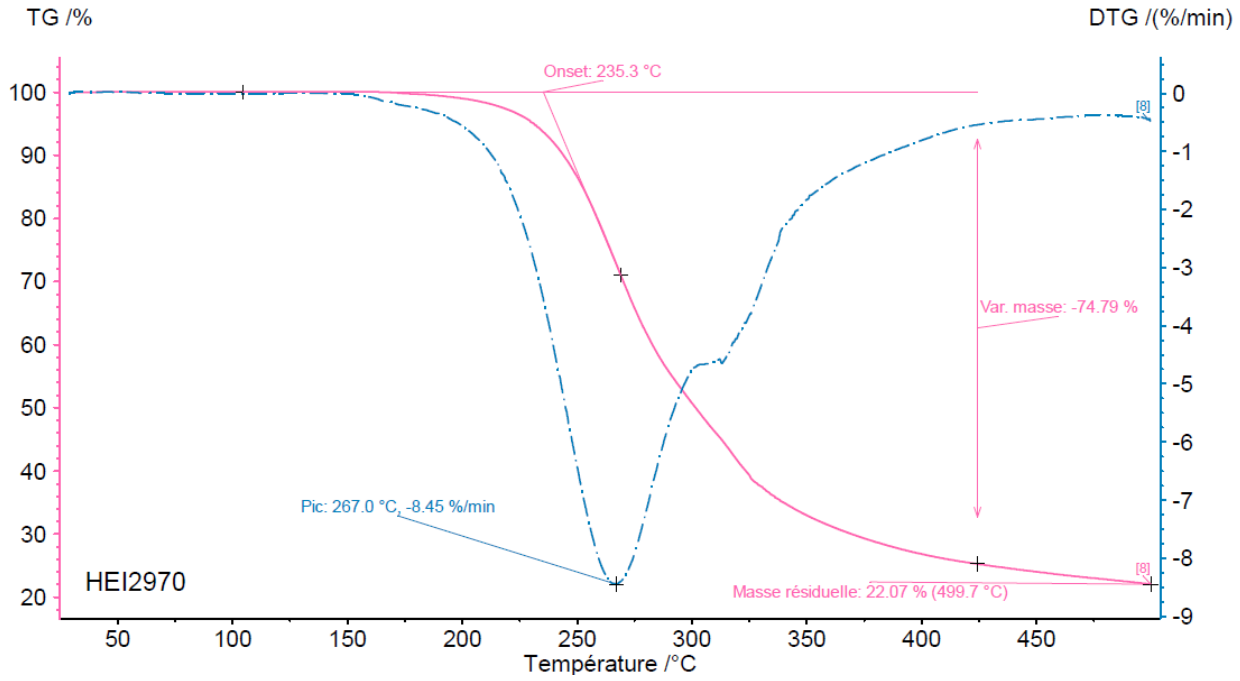


Figure 125 *TGA of the same compound HEI 2970, up to 500°C. On the TGA profile, in a first step between in the range 25-250°C we can notice a mass variation of -8.45% resulting from possible moisture or degradation and also a pretty high melting point. At 500 °C an important residual mass is observed, of 22.07%.*

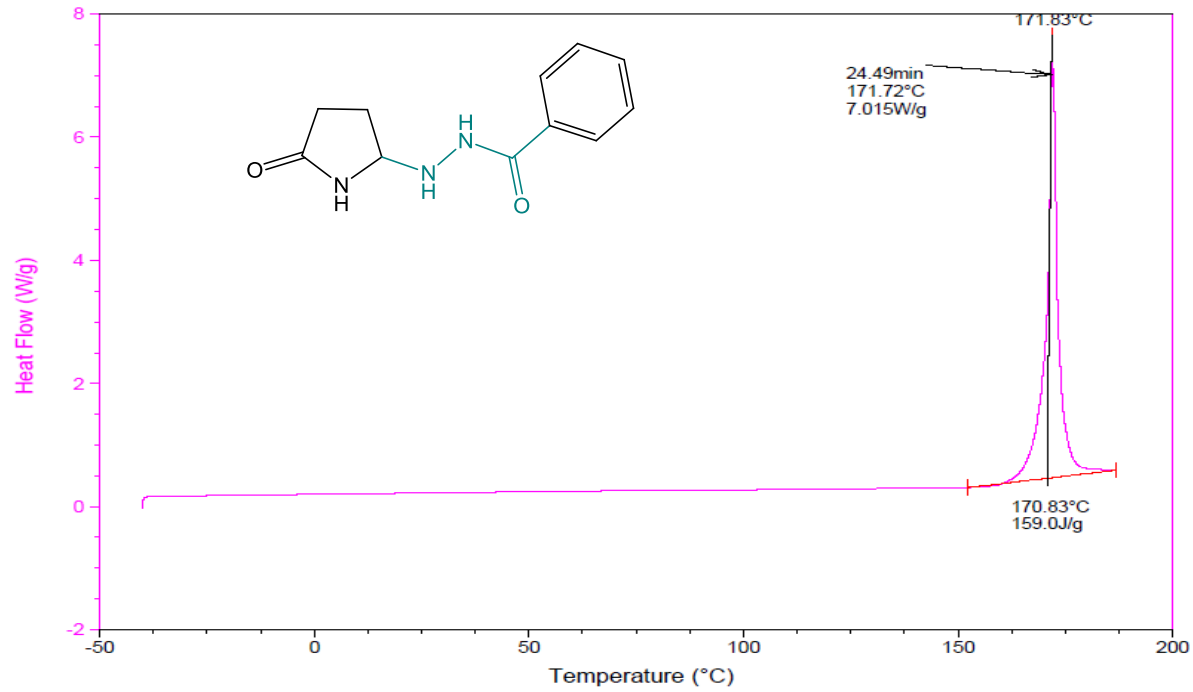


Figure 126 *DSC of HEI 2970 heated at constant flow. As it can be observed, at 171.83°C a peak temperature is defining the melting point, having an enthalpy of 159.0 J/g.*

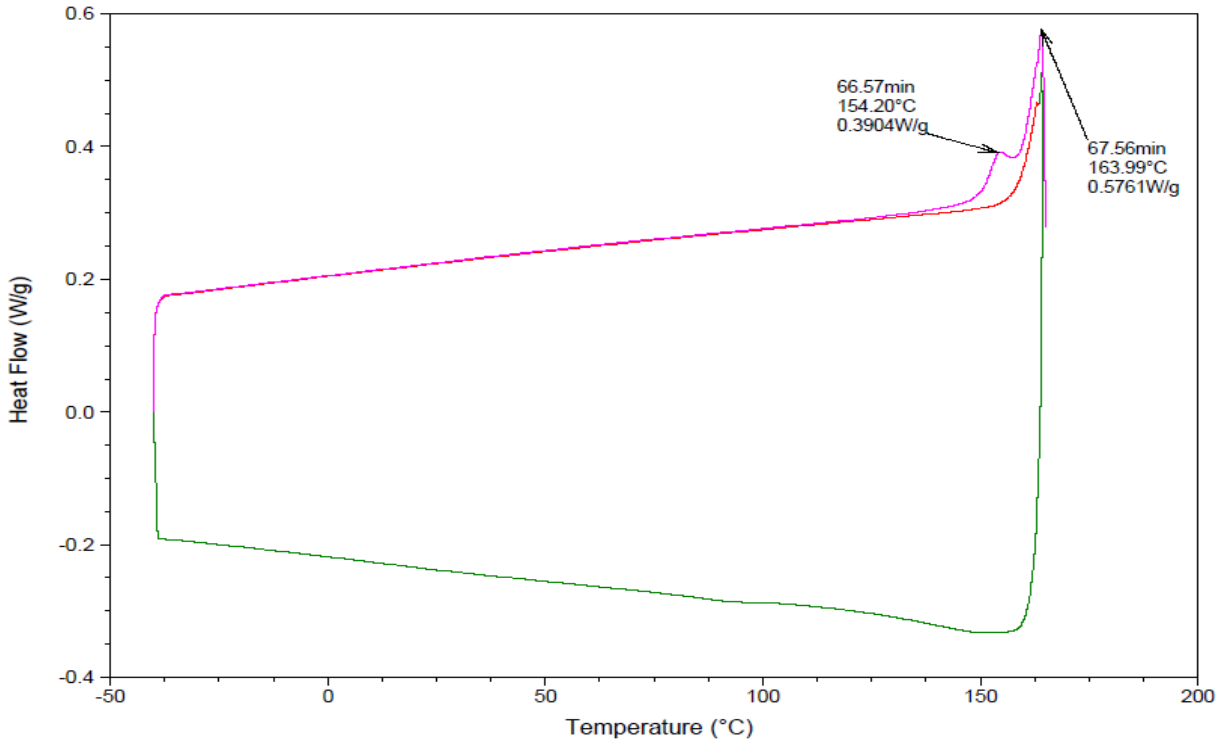


Figure 127 *DSC of HEI 2970 - two heating cycles. It can be observed that the two heating cycles are not reproducible possibly because of a decomposition, as the second cycle presents two melting points. In the cold cycle, a rather weak tendency of crystallization is observed.*

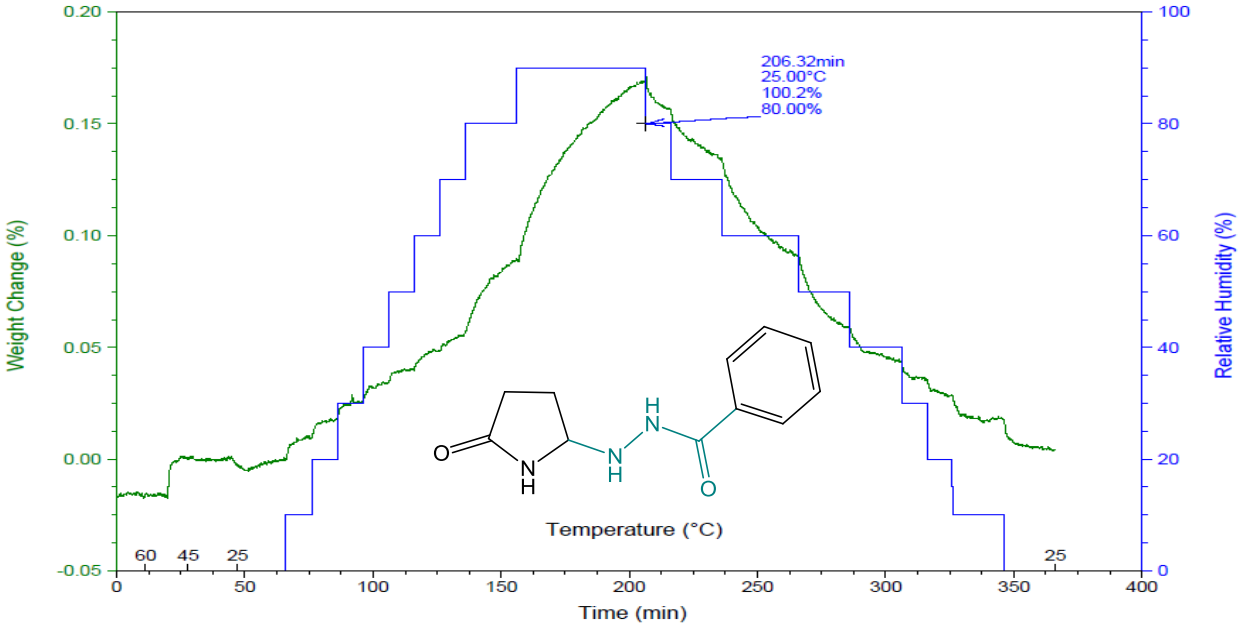


Figure 128 *DVS change in mass plot and water sorption limit measurement of HEI 2970. As it can be seen from the plot, the compound is not hygroscopic, the water mass gain being only 0.2%. The little quantity of water seems to be bulk, with no defined steps of RH.*

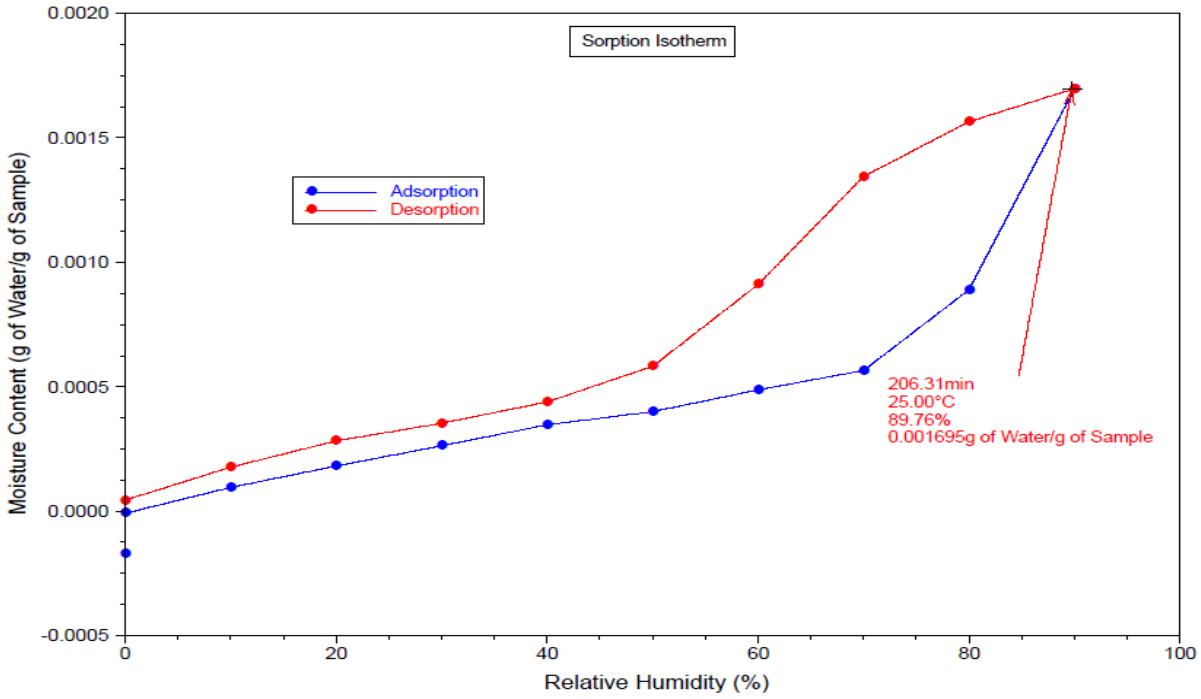


Figure 129 *DVS isotherm plot of HEI 2970. From the sorption isotherm we can see clearly that there wasn´t any tendency of bulk gain of water and the absorption and desorption have the same mechanism and it´s happening mainly between 50 and 90 RH%.*

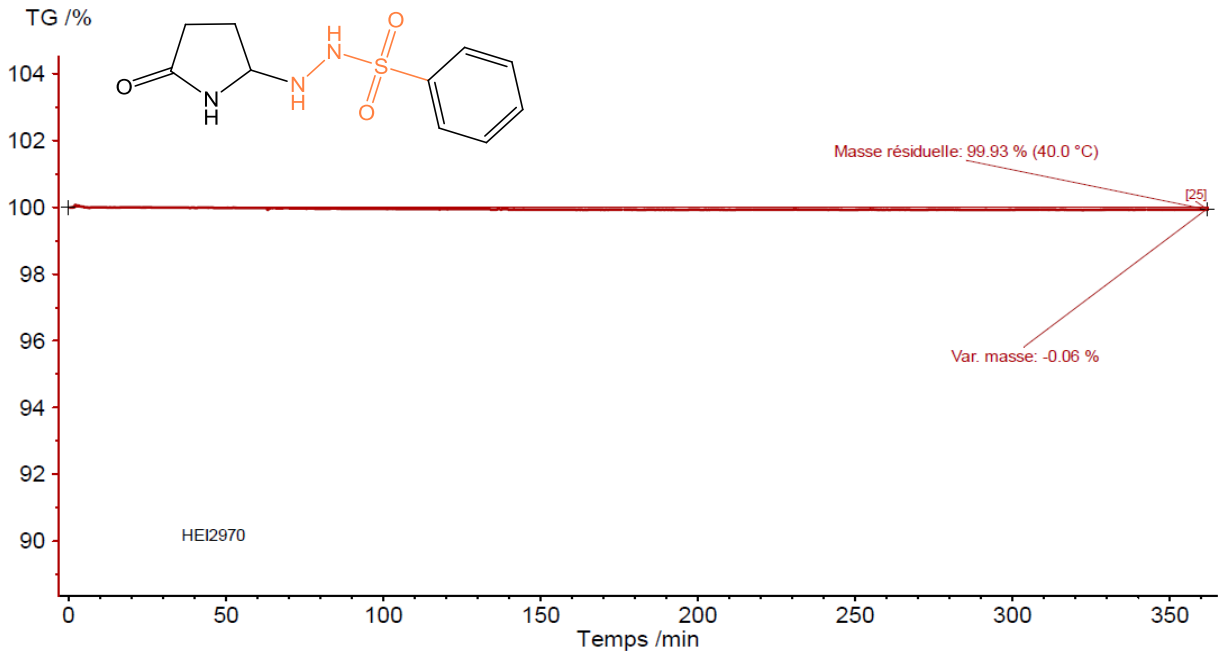


Figure 130 *TGA of HEI 3040, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound very stable, having a -0.06% mass variation.*

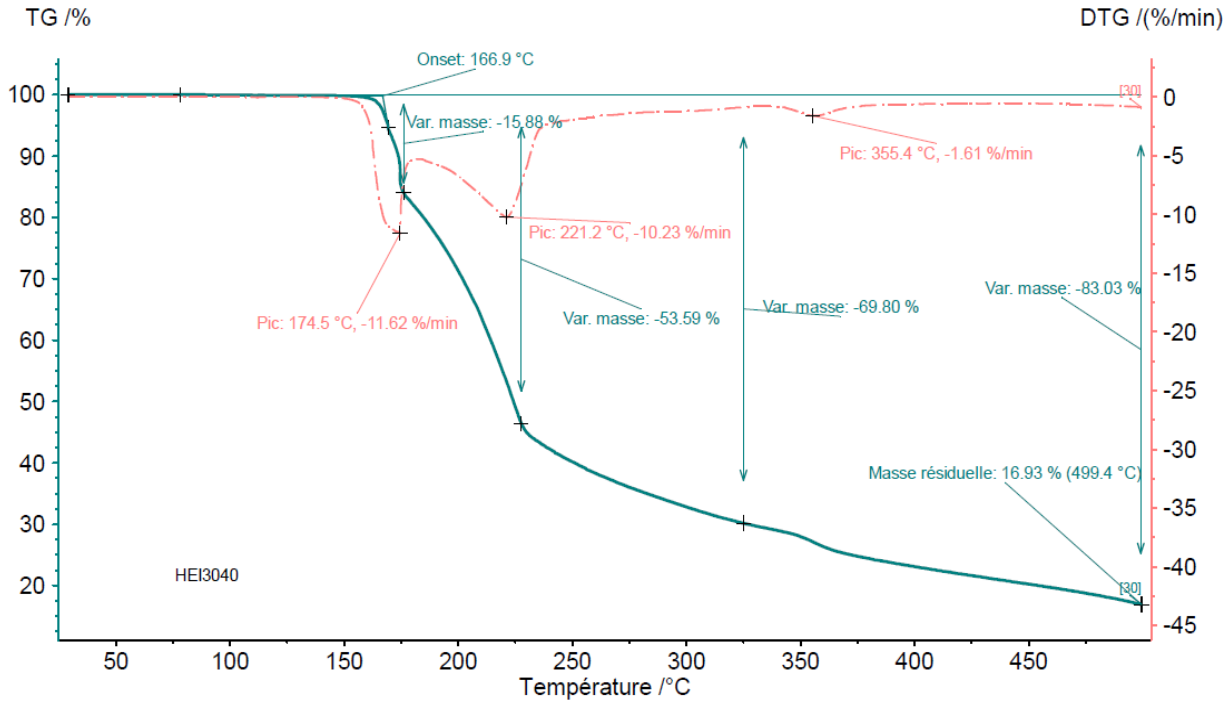


Figure 131 *TGA of the same compound HEI 3040, up to 500°C. On the TGA profile, which has multiple degradation steps, in a first range 25-200°C we can notice an important mass variation of -15.88%, possibly because of hydrazine elimination followed by another -10.23% between 200-250°C, all leading to a final 83.03% mass loss.*

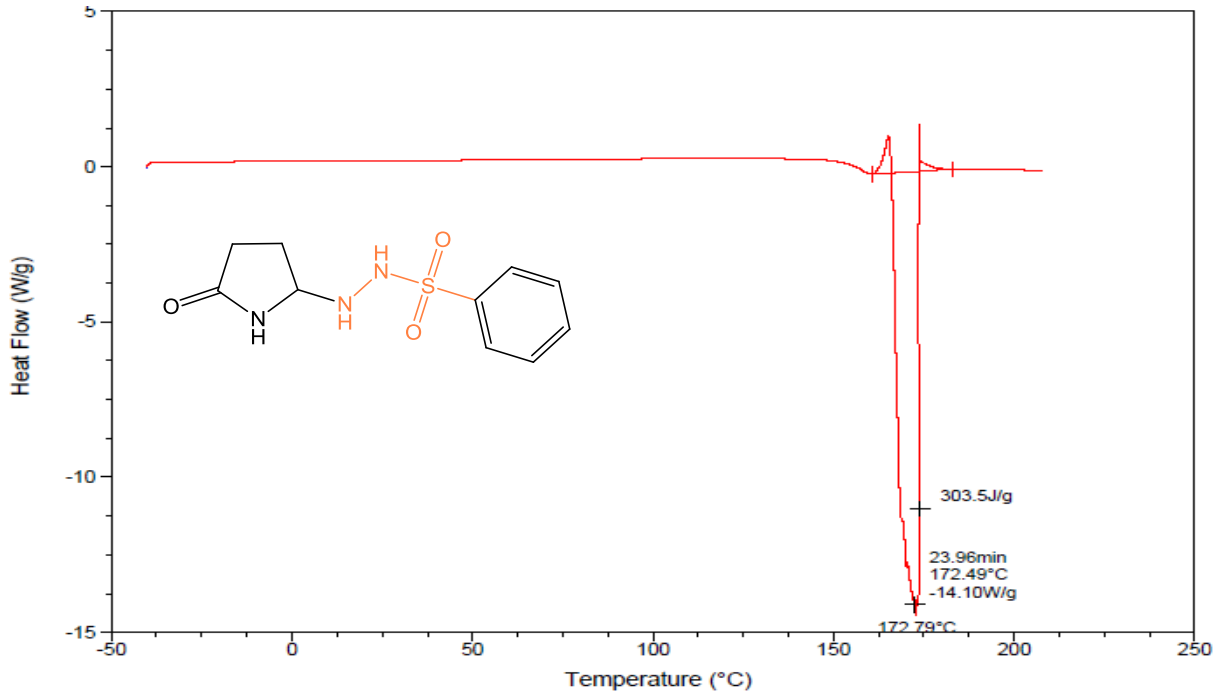


Figure 132 *DSC of HEI 3040 heated at constant flow. As it can be observed, at 172.79°C a peak temperature is defining the melting point, followed by a sudden crystallization.*

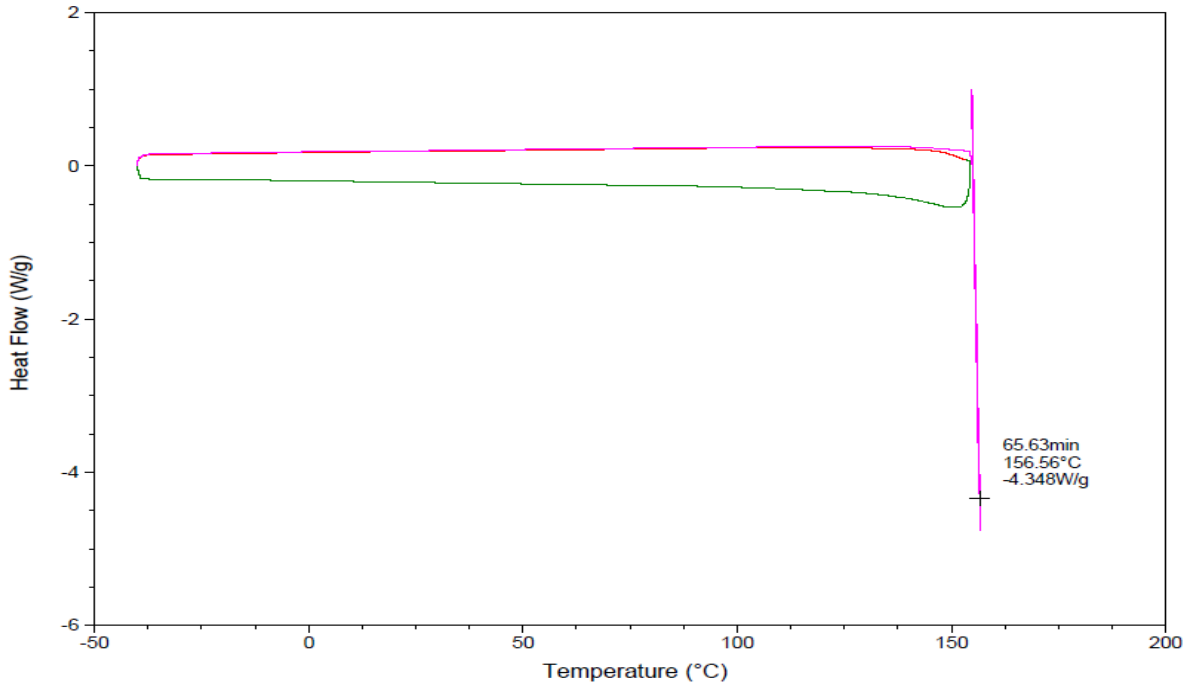


Figure 133 *DSC of HEI 3040 - two heating cycles. It can be observed that the compound is quite stable at a cycle below its melting point. Though, the second heating cycle present a peak which might be due to crystallization as seen before.*

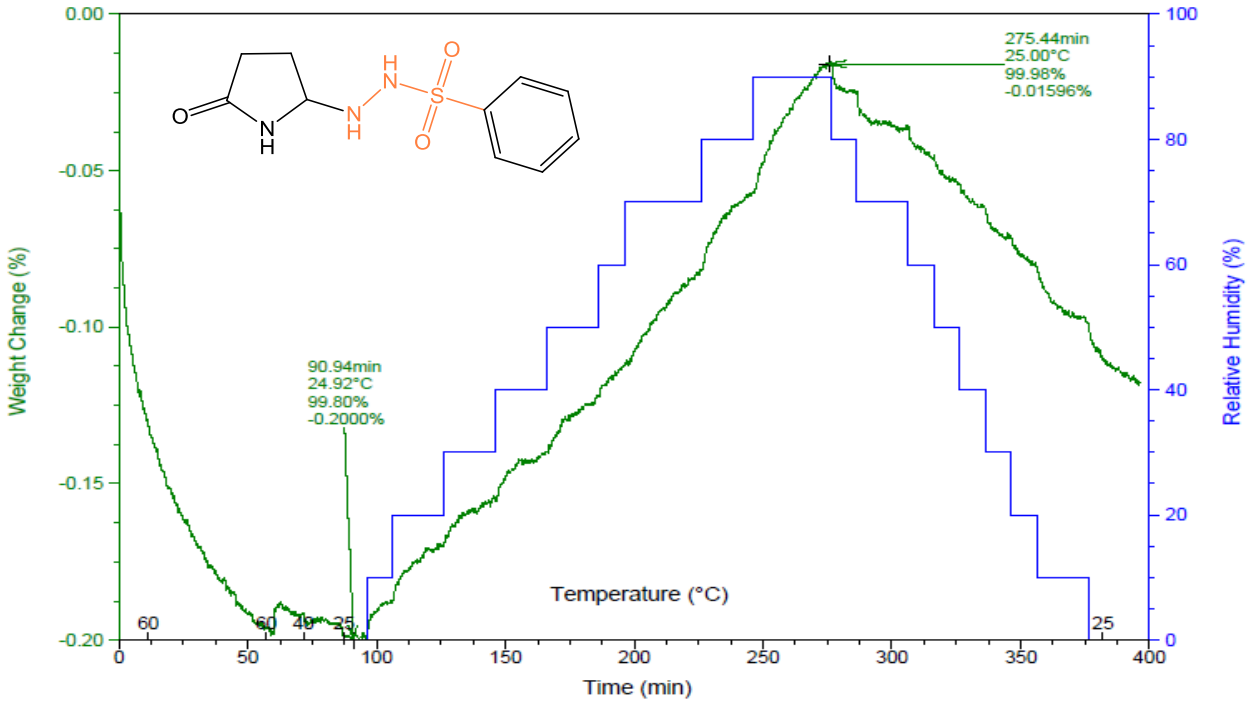


Figure 134 *DVS change in mass plot and water sorption limit measurement of HEI 3040. As it can be seen from the plot, the compound is not hygroscopic, but its eliminating water during the analysis, the mass loss being -0.0159%.*

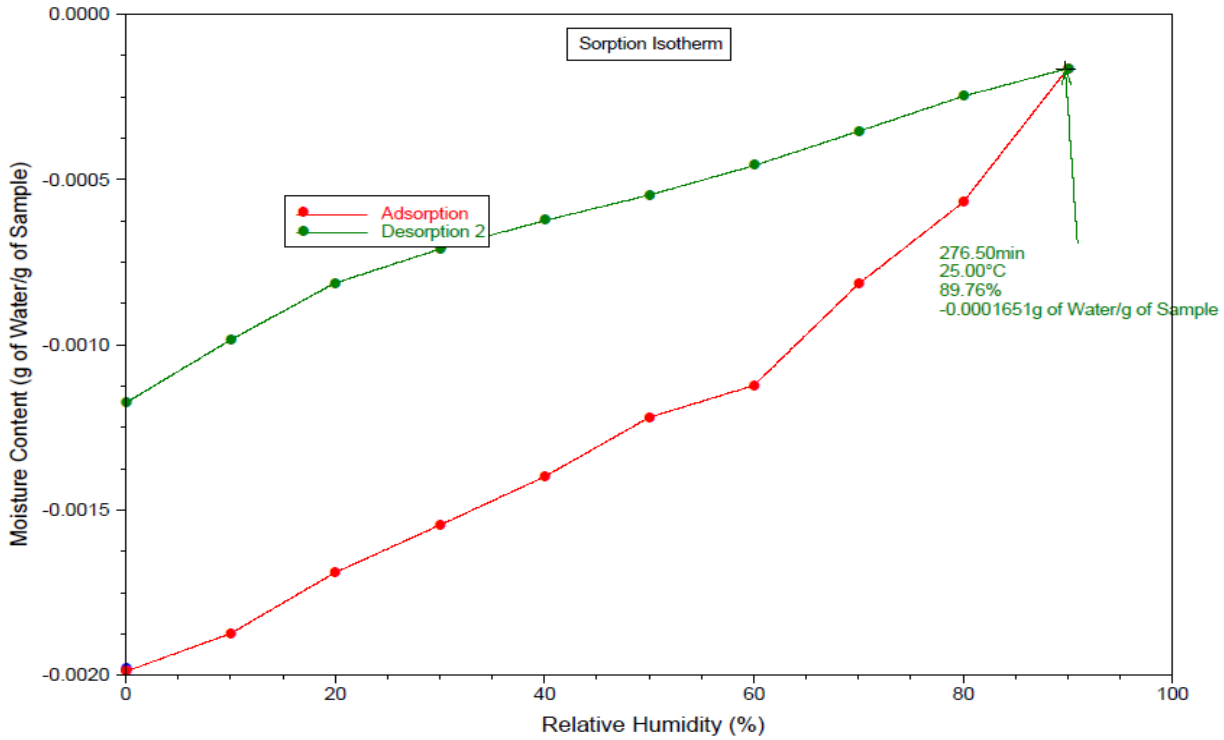


Figure 135 *DVS isotherm plot of HEI 3040. From the desorption isotherm it can be seen a little water elimination, but insignificant.*

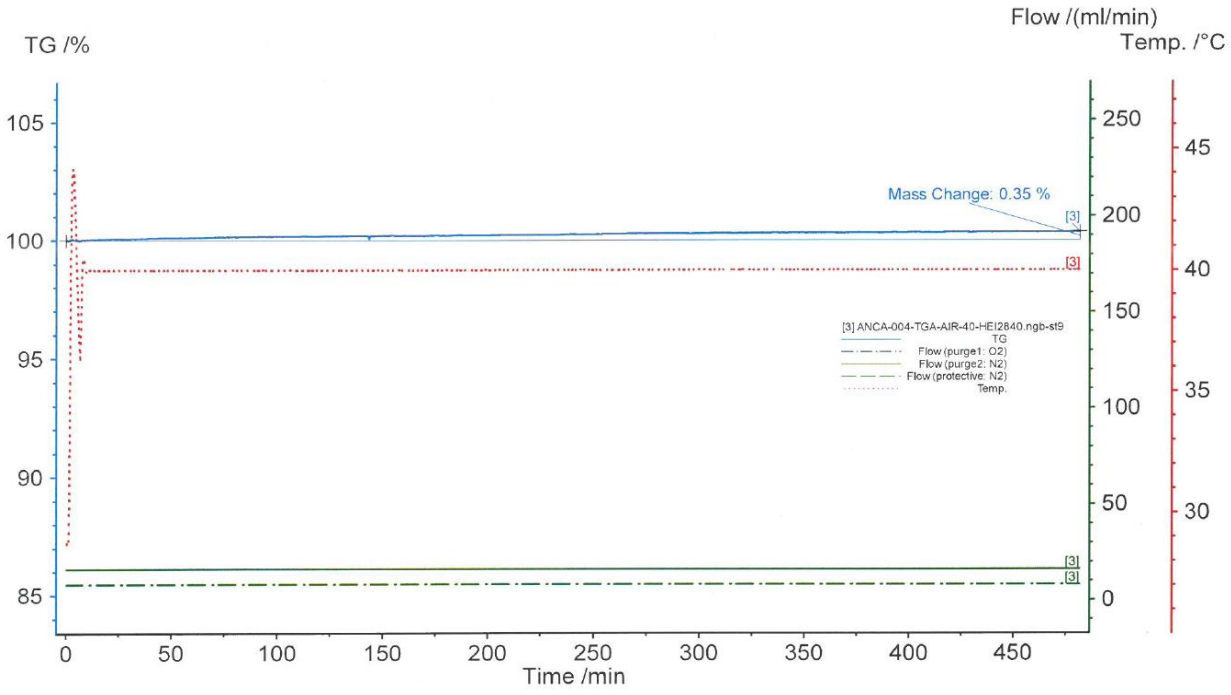


Figure 136 *TGA of HEI 2840, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is a little unstable having 0.35% mass variation, which can come from solvent evaporation or possible air oxidation.*

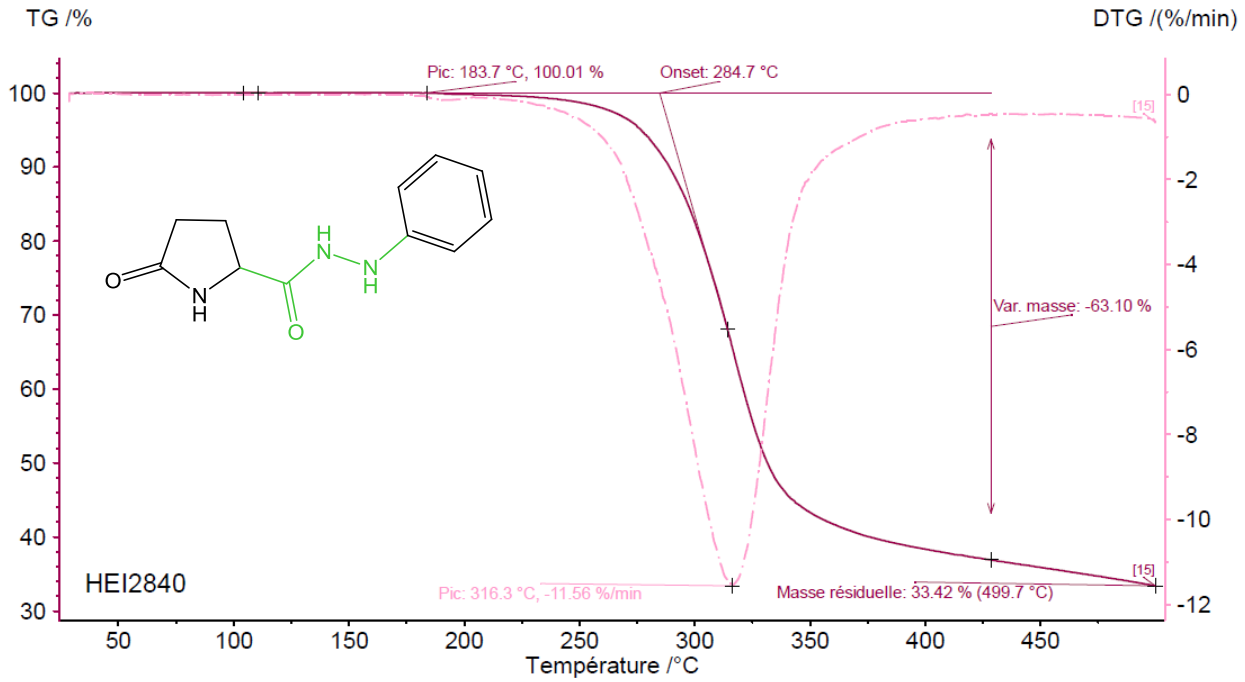


Figure 137 *TGA of the same compound HEI 2840, up to 500°C. From the TGA profile we can observe the stability of the compound at high temperatures. For instance, at 316.3°C the mass loss is low - 11.56%, and the residual mass at 500°C is also notable, of 33.42%.*

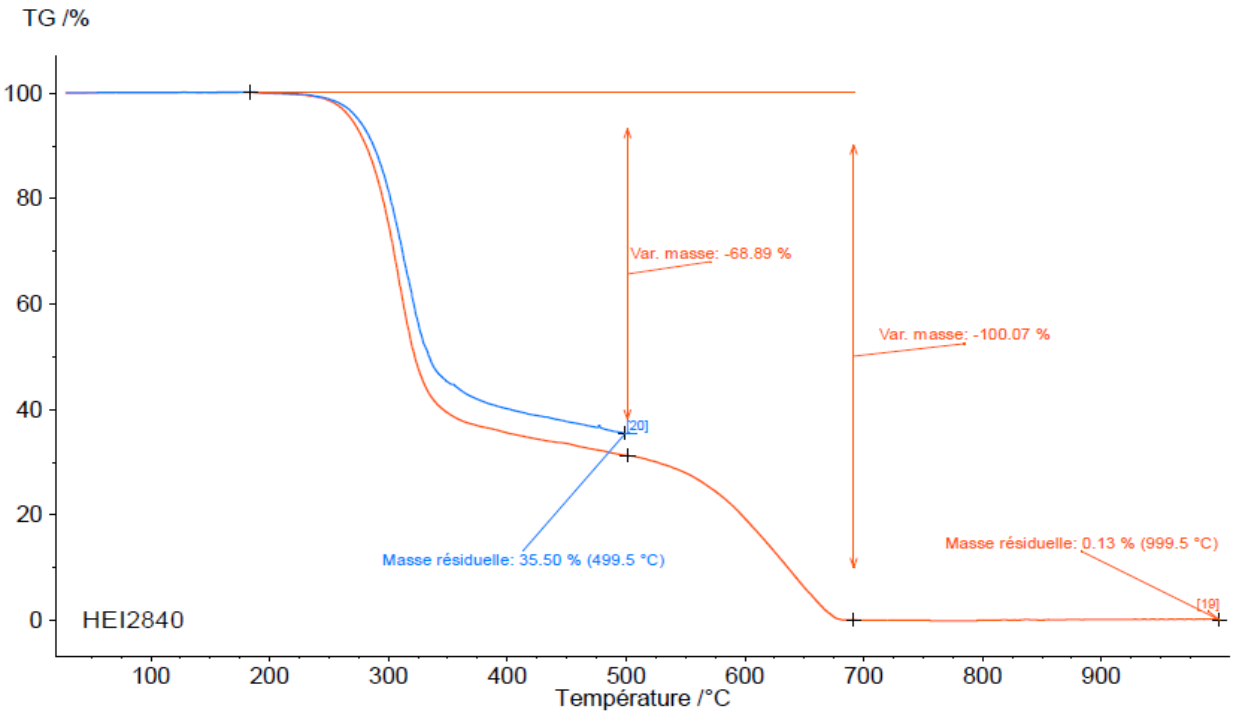


Figure 138 *Superposed TGAs of the same compound HEI 2840. Interesting fact is that the final step from 500 to 1000°C results with the total mass loss, compared to other compounds where nor 1000°C succeeded to completely degradate it.*

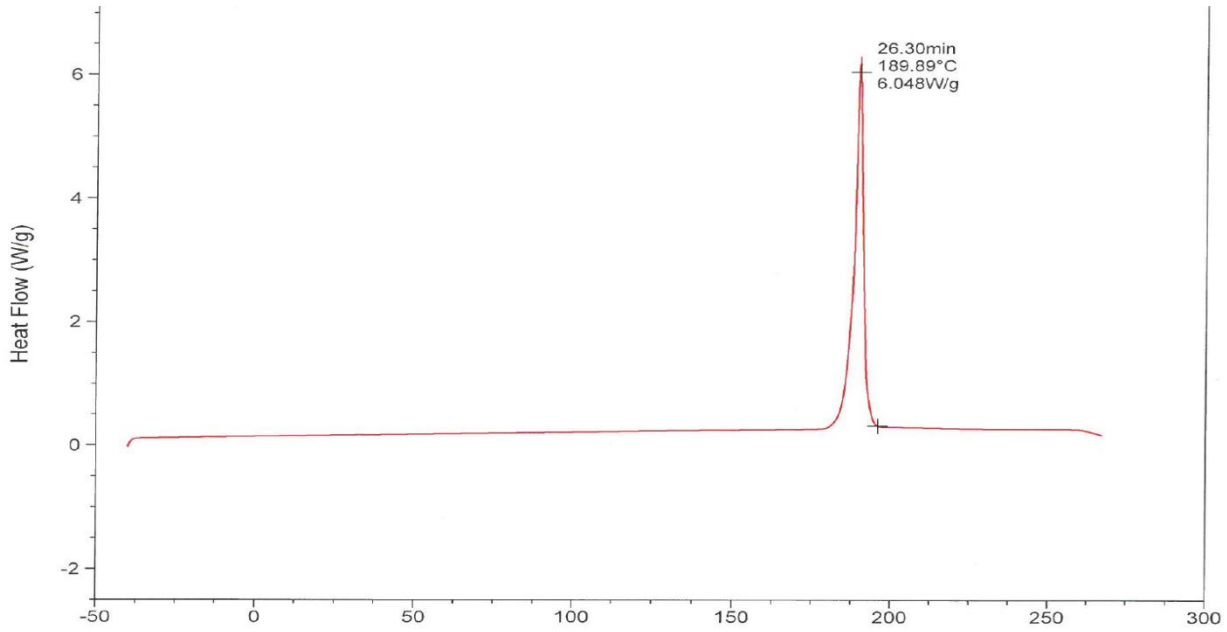


Figure 139 *DSC of HEI 2840 heated at constant flow. As it can be observed, at 189.89°C the temperature peak is defining the melting point.*

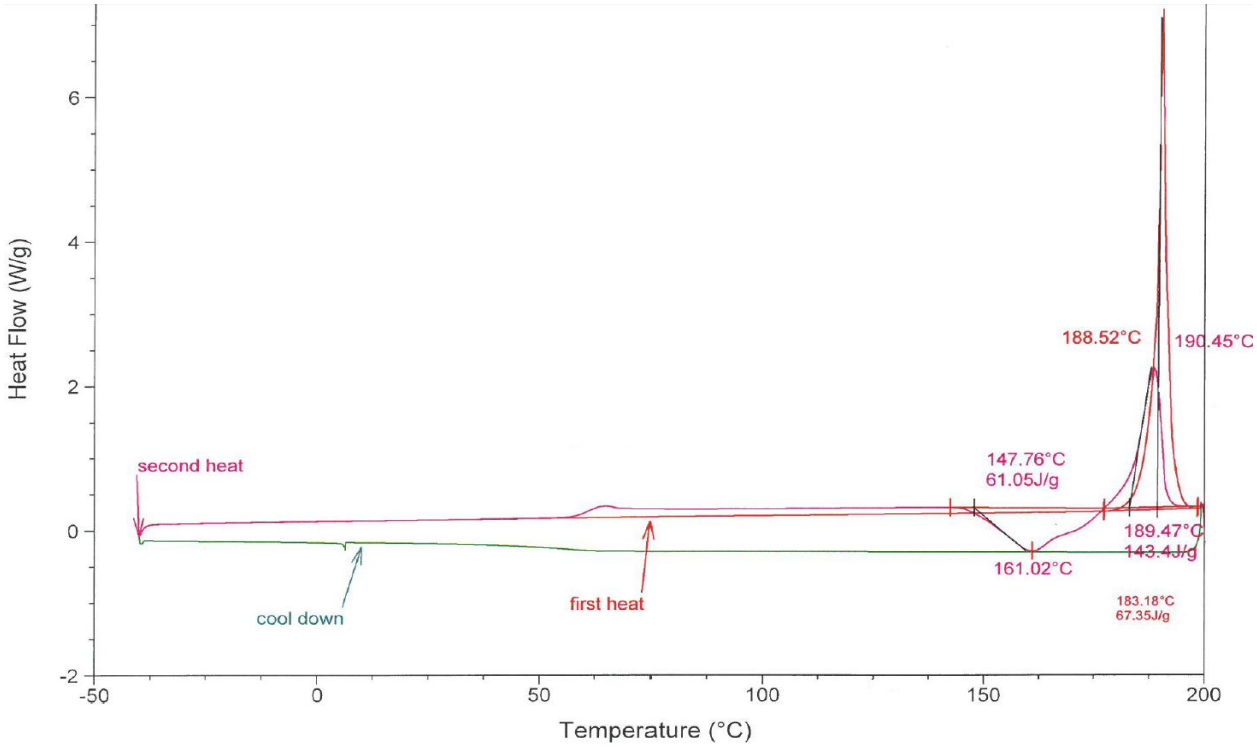


Figure 140 *DSC of HEI 2840 - two heating cycles. The first heating cycle occurs without events, as previously shown, and the cold one as well. However, the second heating cycle presents a glass transition at around 70°C, followed by a hot crystallization at 147.76°C and a shifted melting at 188.52°C because of crystallinity change. The DSC profile is resembling HEI2909.*

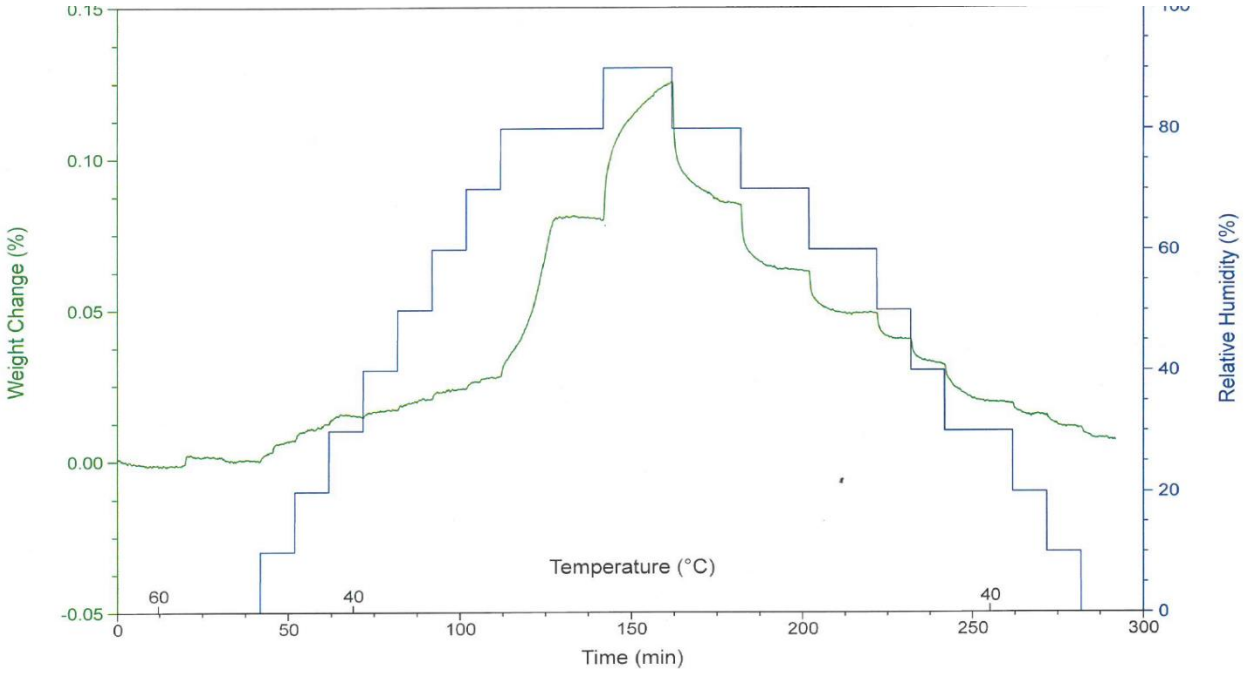


Figure 141 *DVS change in mass plot and water sorption limit measurement of HEI 2840. From the kinetic plot we can see that the adsorption mechanism is different from the desorption one, while the compound seems to bulk gain water; it eliminates it in steps.*

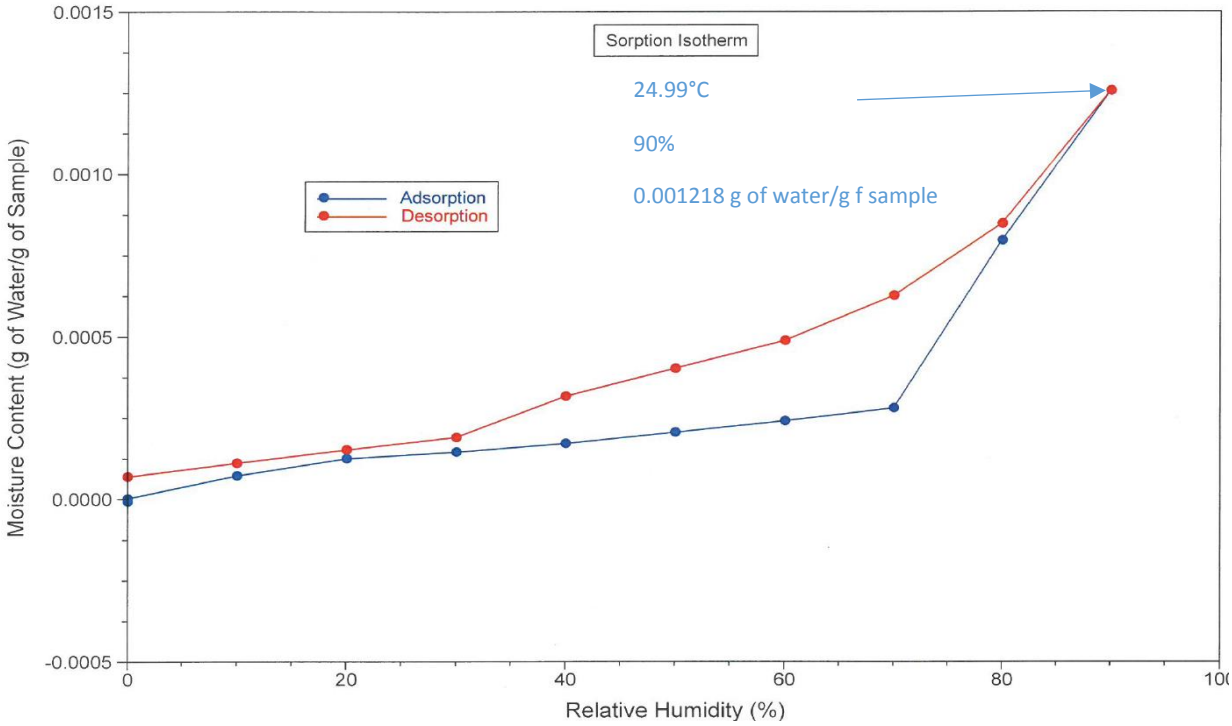


Figure 142 *DVS isotherm plot of HEI 2840. From the isotherm we can observe that the molecule is probably forming a hydrate but is little hygroscopic.*

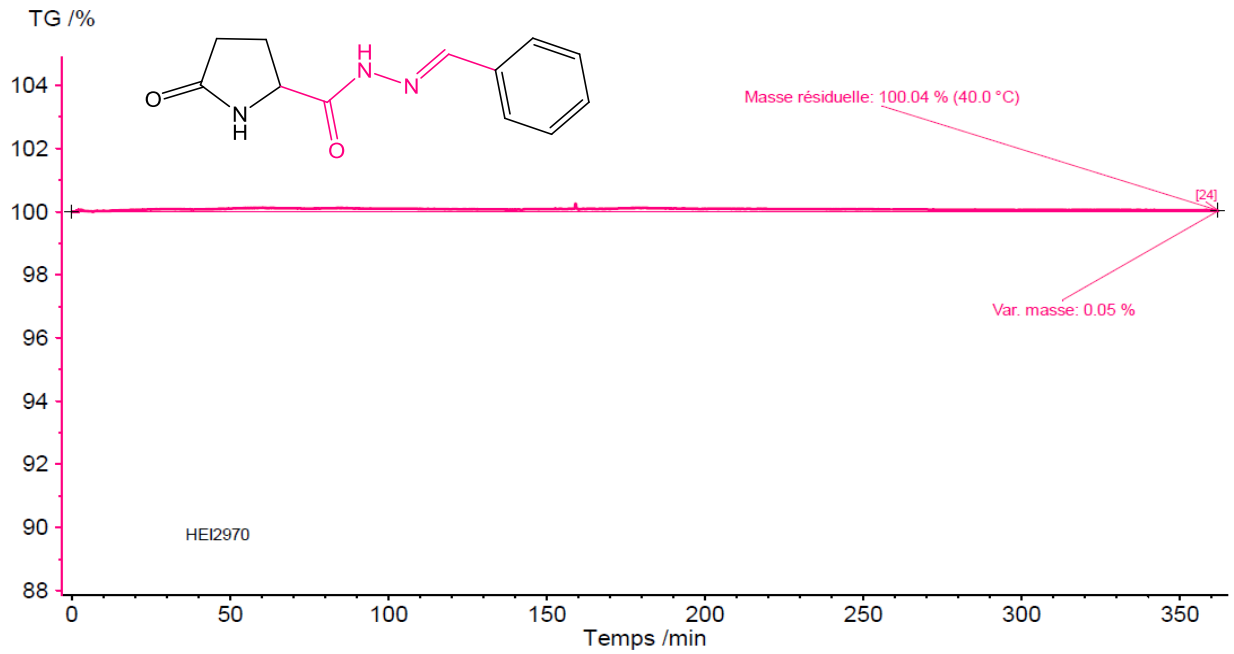


Figure 143 *TGA of HEI 261, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is very stable, having a little gain of 0.05% mass.*

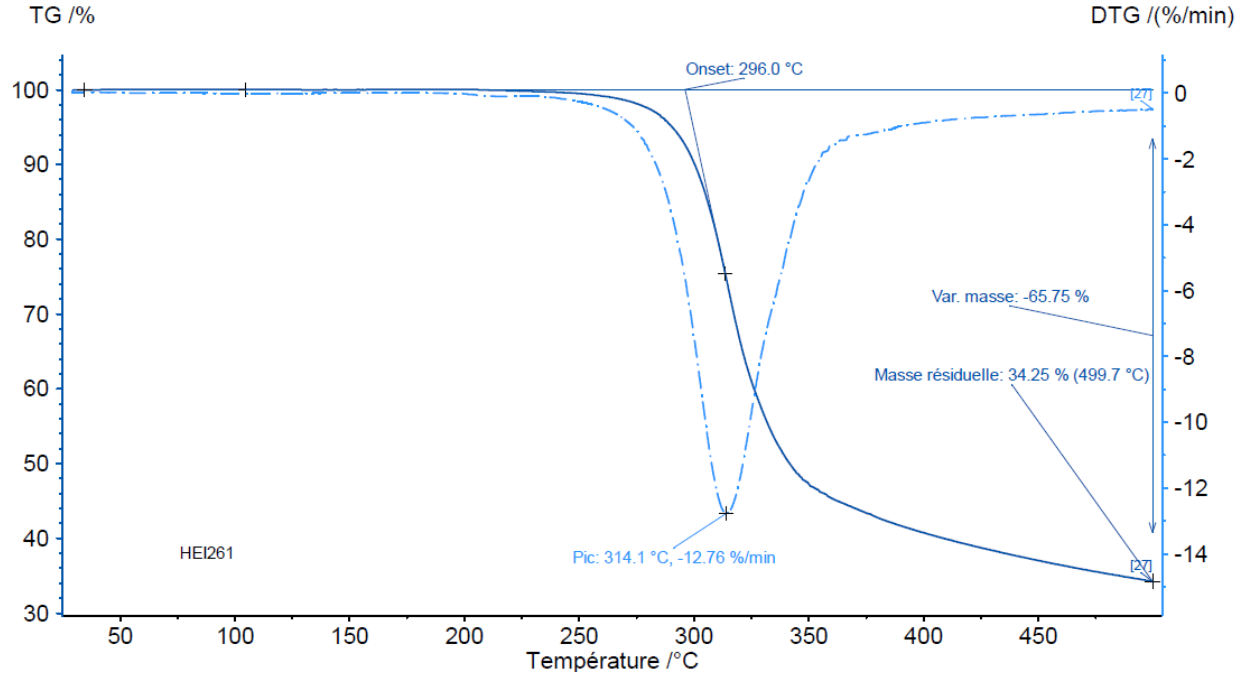


Figure 144 *TGA of the same compound HEI 261, up to 500°C. From the TGA profile we can observ the stability of the compound at high temperatures. For instance, at 314.1°C only 12.76% of the compounds gets degraded, and the residual mass at 500°C is also notable, of 34.25%, the profile being quite resembling with HEI2840.*

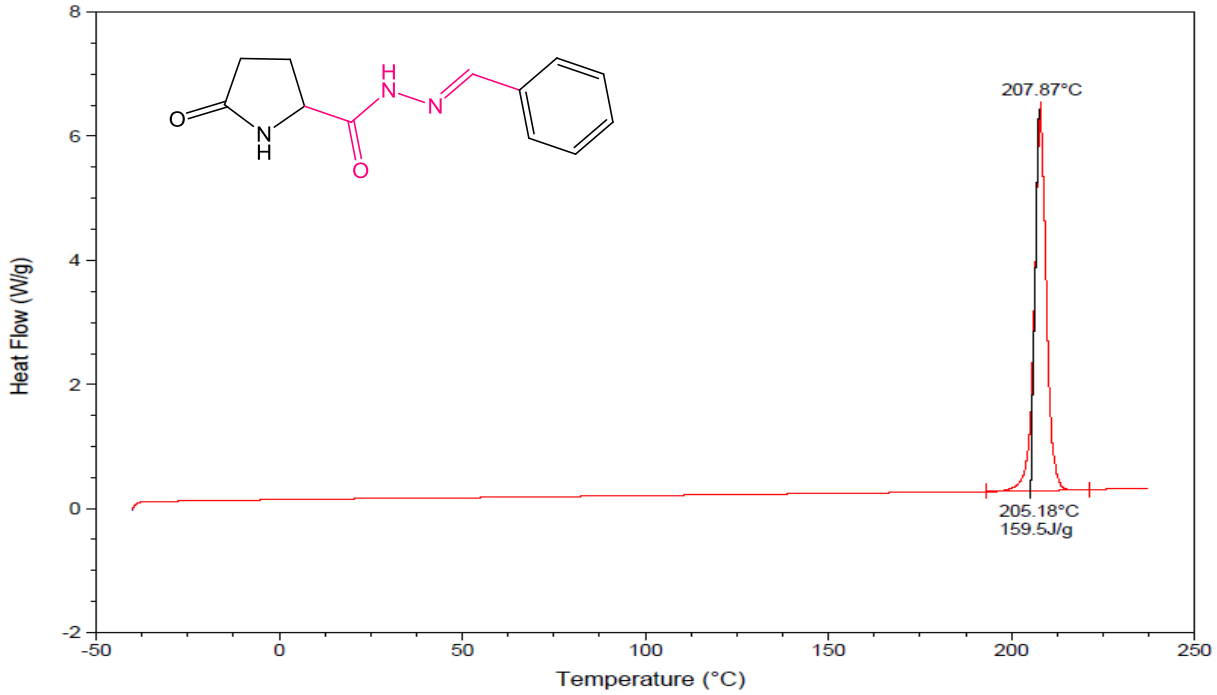


Figure 145 *DSC of HEI 261 heated at constant flow. As it can be observed, at 207.87°C the temperature peak is defining the melting point, the value being generally higher compared to previous seen.*

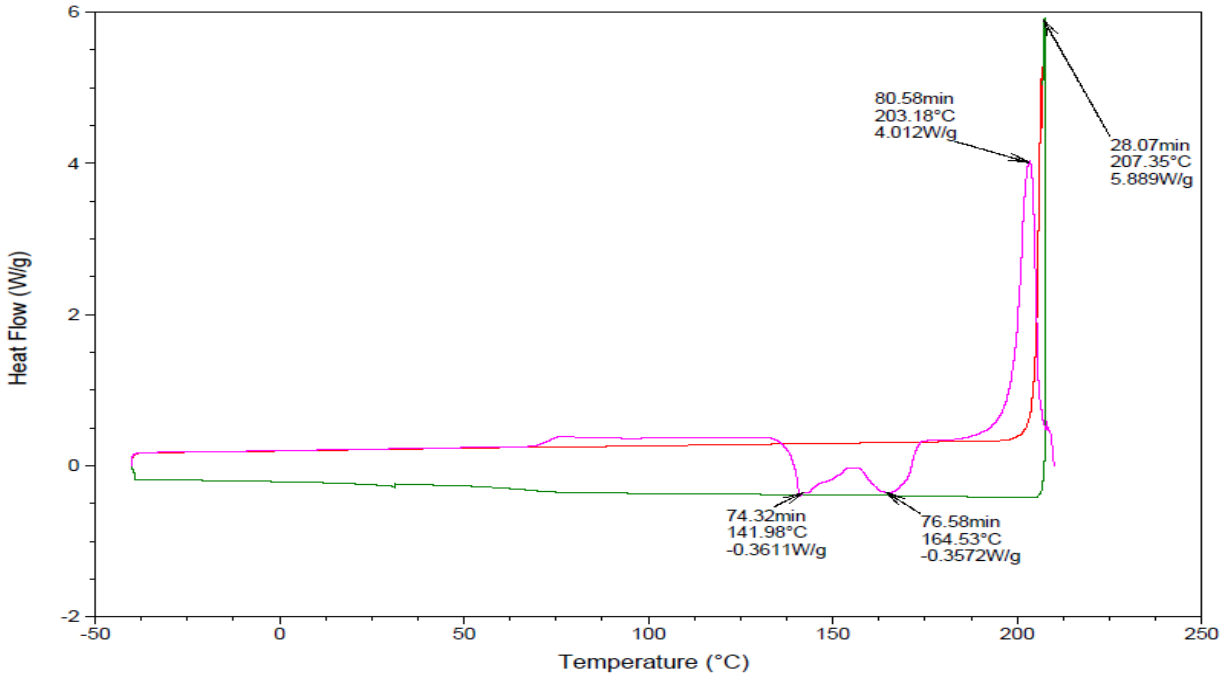


Figure 146 *DSC of HEI 261 - two heating cycles. The first heating cycle occurs without events, as previously shown, and the cold one as well. However, the second heating cycle presents a glass transition at around 70°C, followed by two crystalizations and a shifted melting due to previously change of crystallinity.*

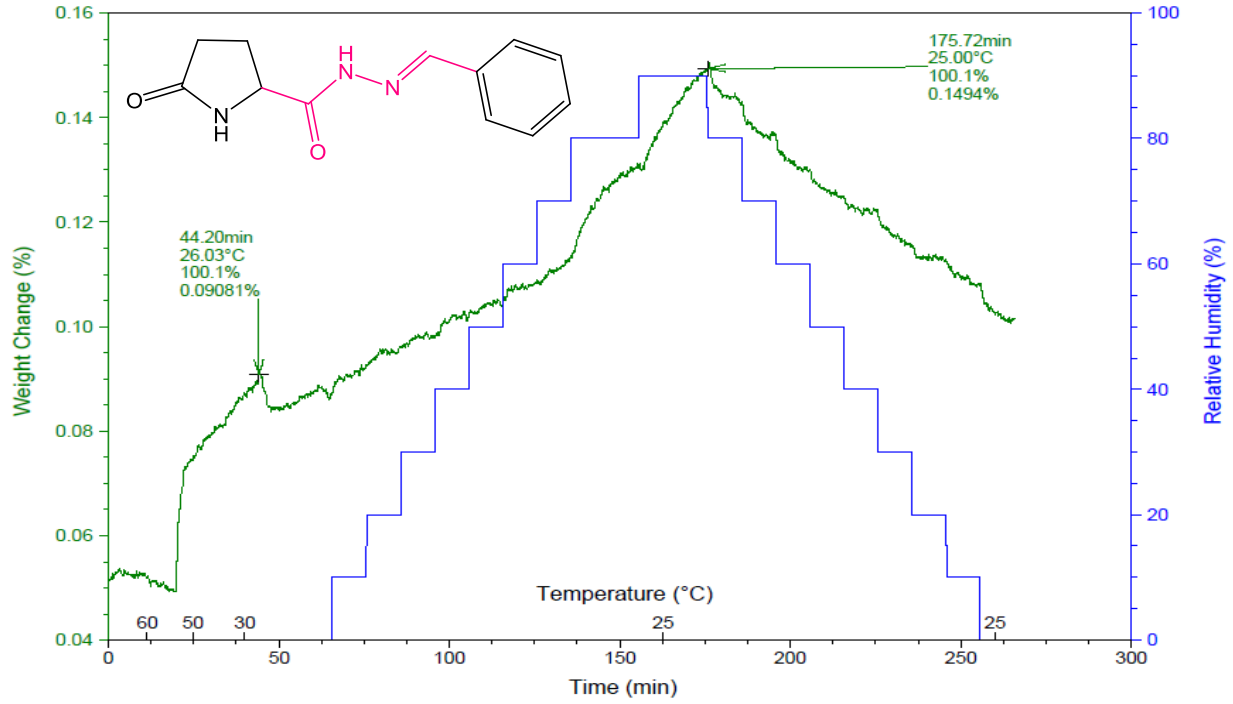


Figure 147 *DVS change in mass plot and water sorption limit measurement of HEI 261. As it can be seen, the plot is not really defined and the compound is not very hygroscopic, intaking only 0.149% of water.*

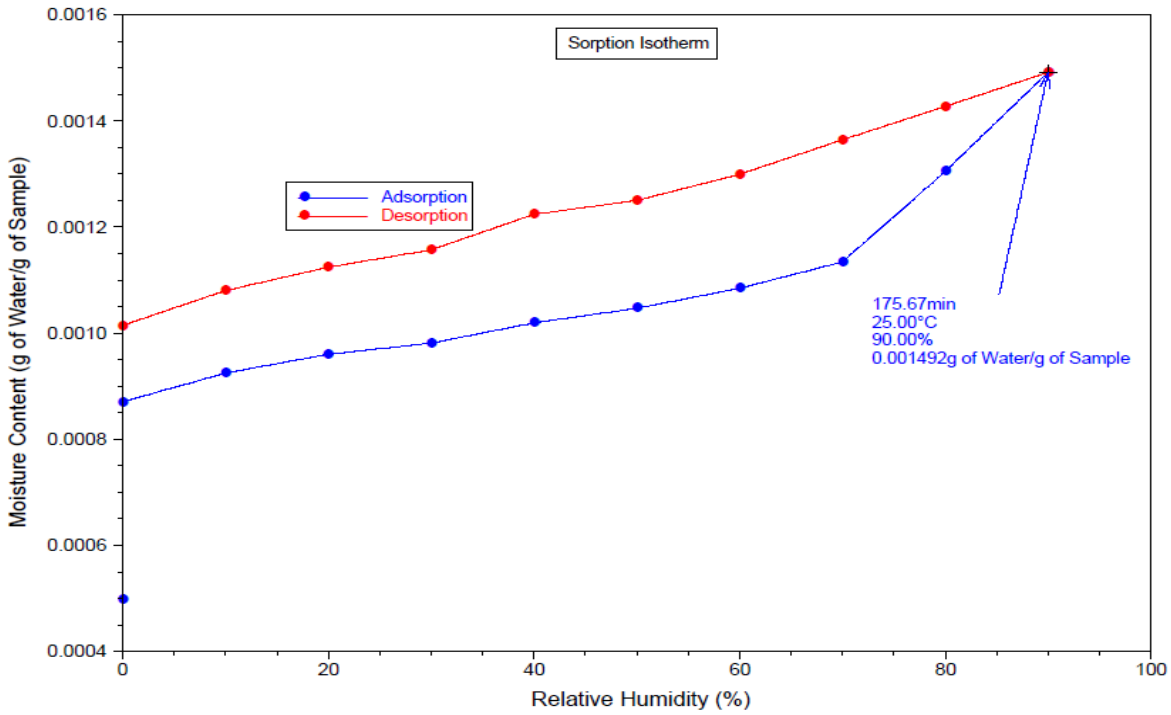


Figure 148 *DVS isotherm plot of HEI 261. From the isotherm as from the kinetic plot, a gradual intake of water can be seen, though modest.*

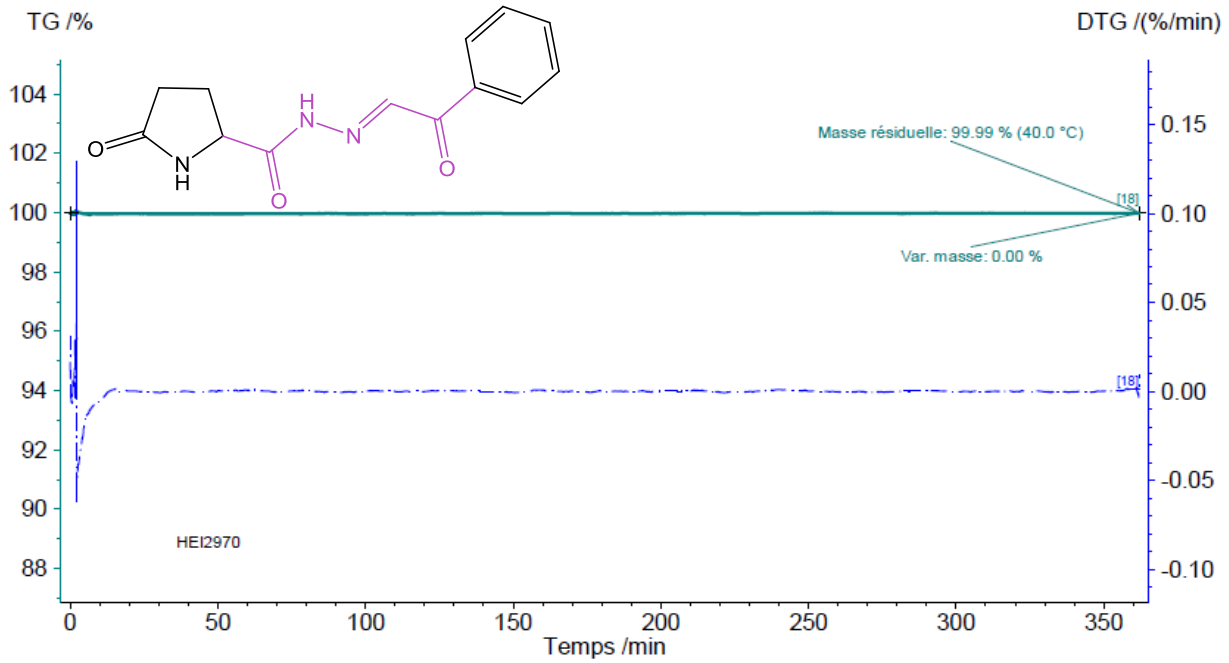


Figure 149 *TGA of HEI 3156, at 40°C for 8 hours. On the TGA profile, it can be observed that in air (20% O_2, 80% N_2), during an 8 hours analysis, the compound is very stable, having no mass variation at all.*

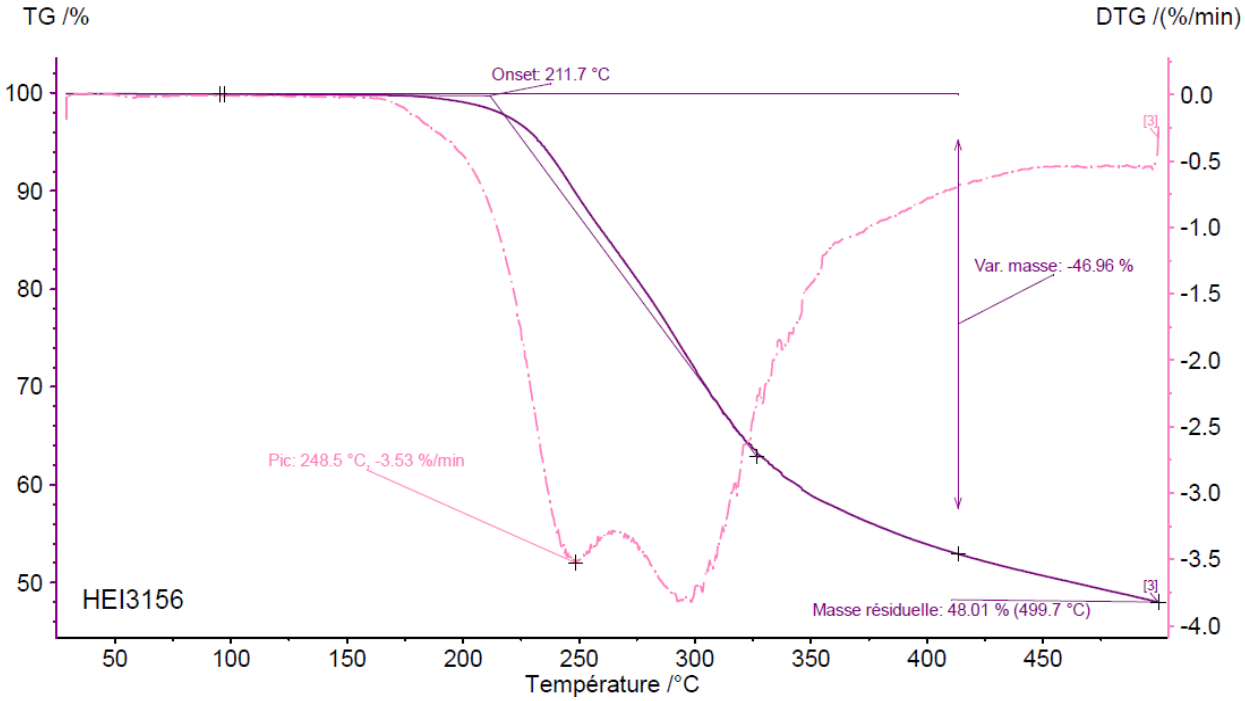


Figure 150 *TGA of the same compound HEI 3156, up to 500°C. From the TGA derivate we can observe two close inflexion points which might correspond to the two isomers cis and trans. Overall, the compound has a very stable profile and the residual mass at 500°C is the largest seen within the analyzed compounds, 48.01%.*

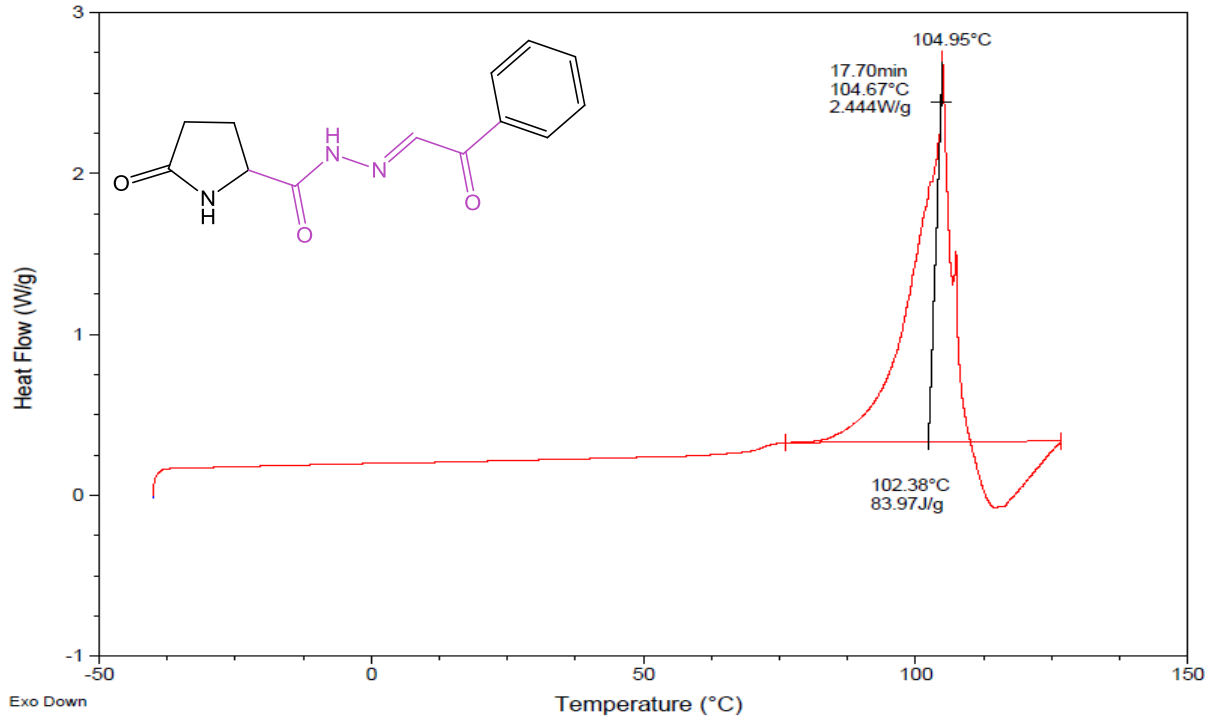


Figure 151 *DSC of HEI 3156 heated at constant flow. Surprisingly compared to the TGA profile, the melting observed is at 105°C followed by a hot crystallization.*

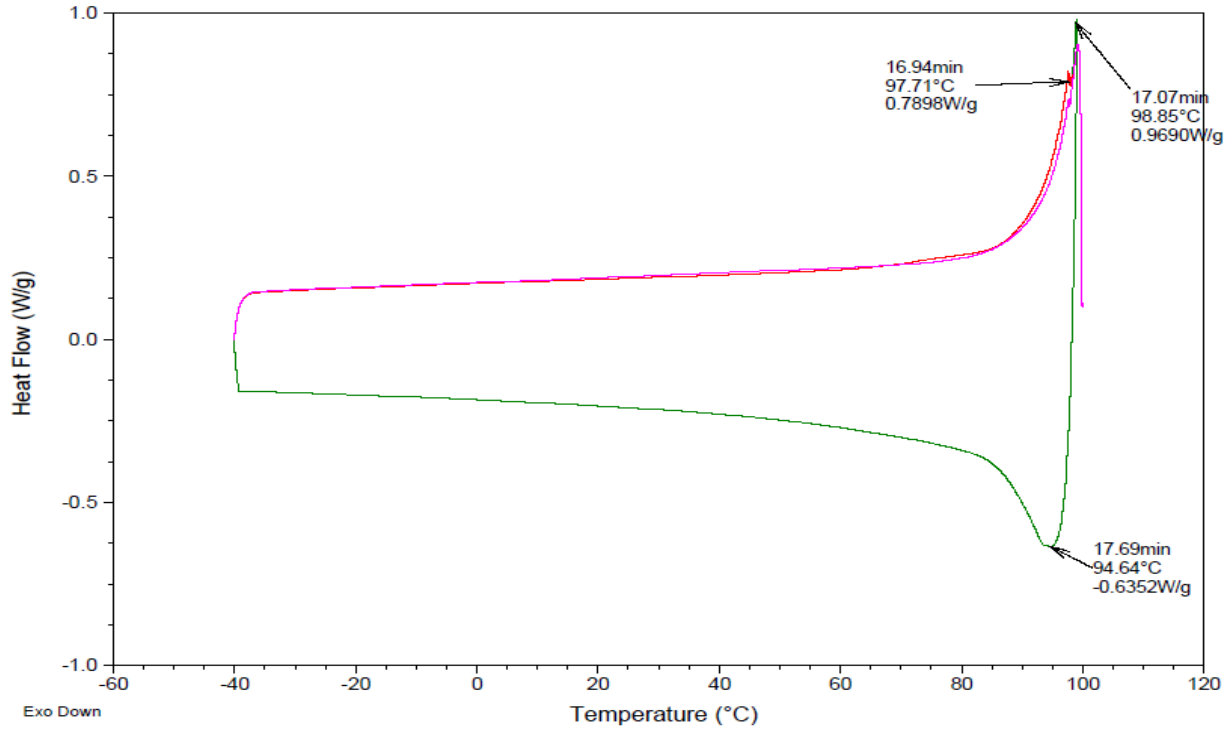


Figure 152 *DSC of HEI 3156 - two heating cycles. The first heating cycle occurs without events, as previously shown. During the cold cycle we can observe the same crystallization that could be seen in the first DSC. The second heating cycle has a little shift of the melting point.*

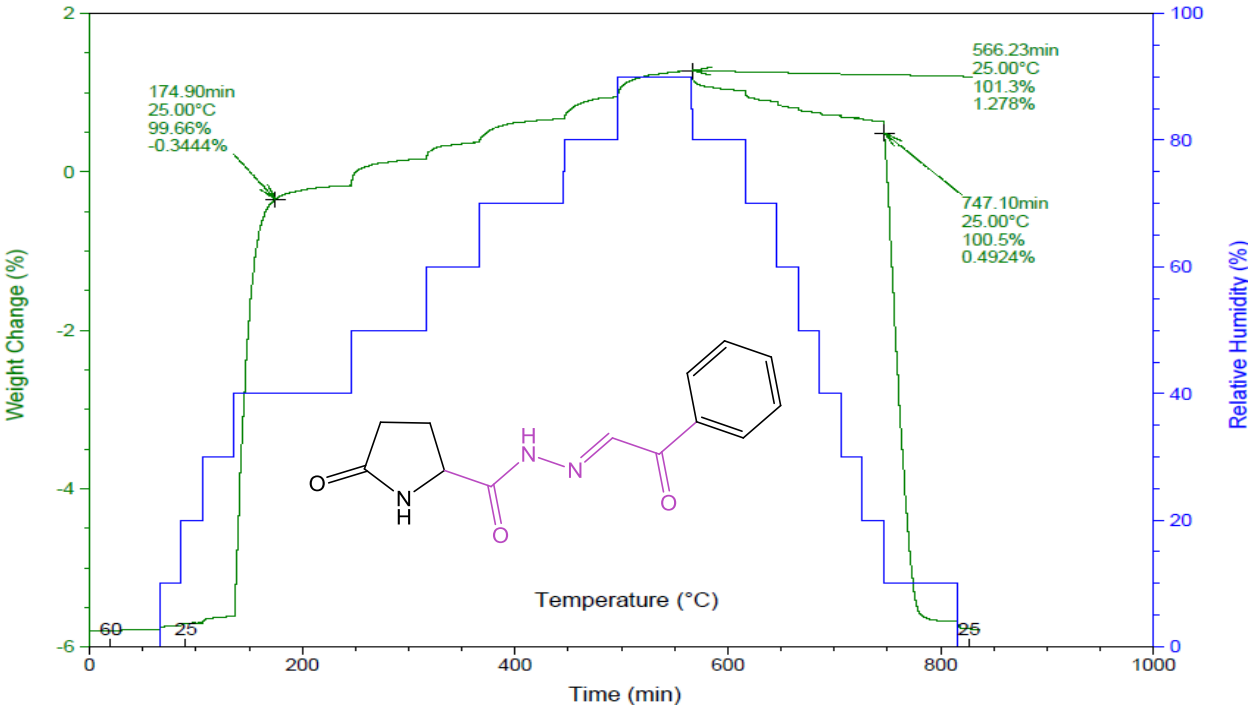


Figure 153 *DVS change in mass plot and water sorption limit measurement of HEI 3156. From the kinetic plot the adsorption seems to be normal in steps, and the compound seems hygroscopic. The desorption however is sudden.*

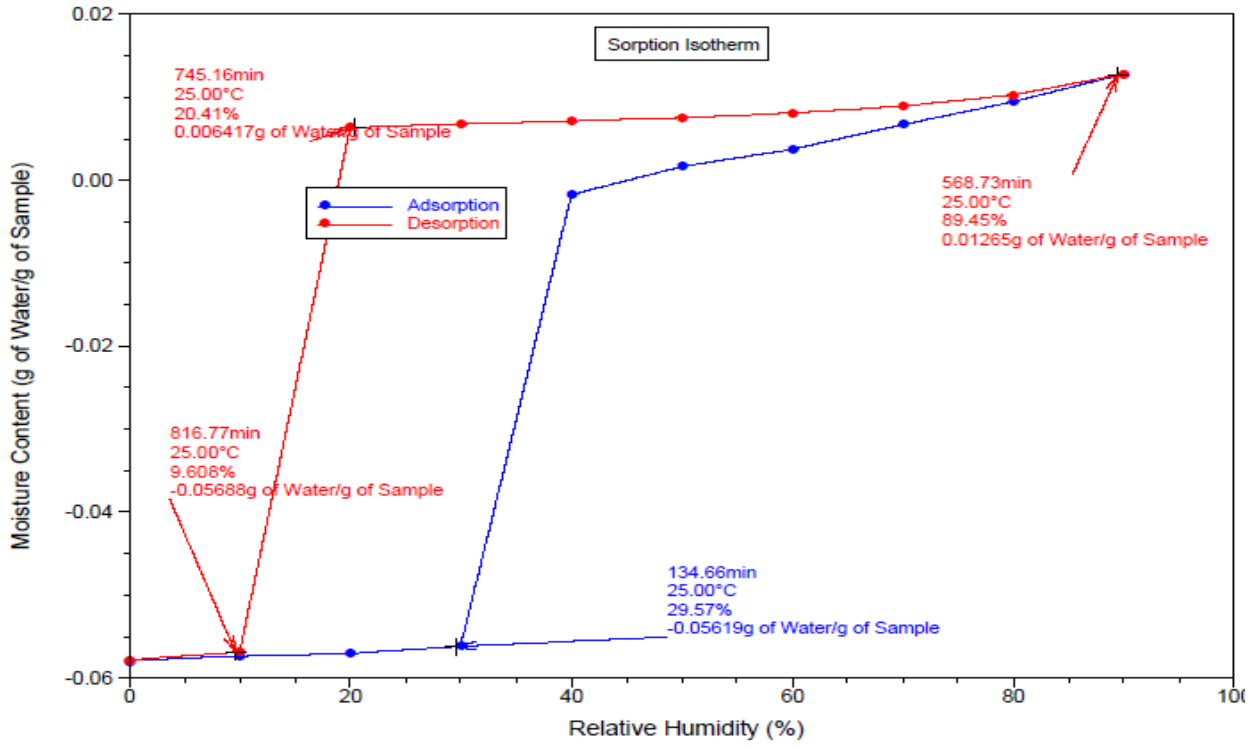


Figure 154 *DVS isotherm plot of HEI 3156. From the isotherm we can see clearly the formation of two hydrates, which is explaining the water intake.*

III.2.3 Conclusions considering thermal analysis results

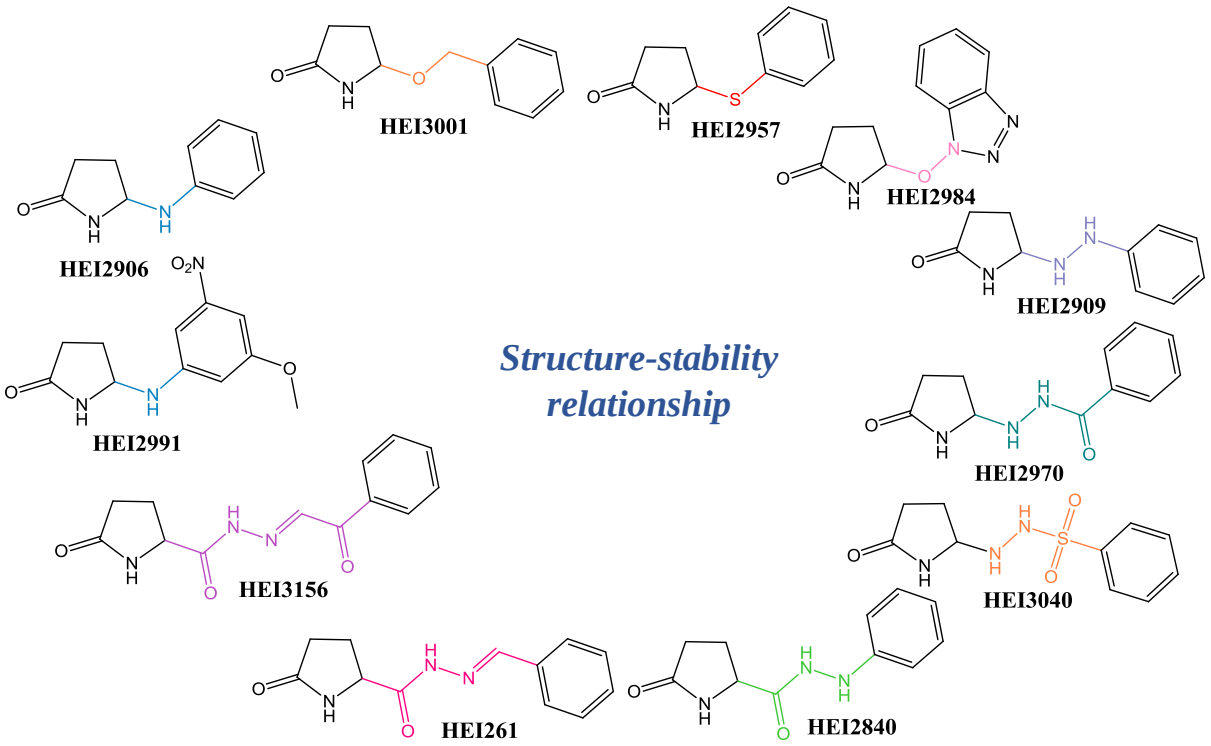


Figure 155 *Selected molecules for stability tests*

Thermal analysis instruments and its applications along with sorption analysis are very important in the characterization and profiling of molecules. Within this study we chose 11 molecules based on their linker type, for which we wanted to have a general overview on their stability in the frame of a possible formulation.

Starting with a single heteroatom link molecules, between the amino-, oxy-, and thio- molecules, respectively HEI 2906, 3001 and 2957, the most stable profile is the one of 5-(phenylamino)pyrrolidin-2-one. In air atmosphere we can notice that both amino- and oxy-derivative are perfectly stable while the thio- derivative seem to be air sensitive having 3% of mass variation during the 8 hours. Because of this quite important air sensitivity, HEI 3001 would not be a great candidate for a possible formulation. Going further with the amino- and oxy- derivatives, from the 500°C TGA we can observe that the alcohol derivative is more stable, with a higher degradation temperature while in the end the residual mass is smaller compared to the aniline´s derivative. However, from the DSC, observing the second cycle of both compounds we can sustain that HEI 2906 is more stable to temperature cycles, the oxy-derivatives suffering changes after the first heating cycle and besides this fact, another disadvantage of the alcohol derivative is the low m.p. of 91°C which would not be convenient for paints or materials needing a thermic treatment. In terms of hygroscopic profile, aniline

derivative is the most hygroscopic absorbing 1.85% water, oxy- derivative being a little less while the thio- derivative eliminates water. If we analyze the substituted compound HEI2991, we can observe that compared to HEI2906, the melting point is superior at 50°C of difference and is less soluble.

Going further with the *N*-oxy- derivative, the compound is more stable having a higher melting point, and does not degradate during heating cycles, as observed from DSC, while in terms of water intake, is a little less hygroscopic than the aniline derivate, retaining 1.6% water.

Comparing the hydrazide derivatives, we can notice that HEI 2840 is the most stable, the pterolactam derivatives being less stable than the pyroglutamic acid derivatives. Though the 3 molecules are stable in air, during the thermic treatment, HEI 2970 and 3040 are less stable, having lower melting points and DSC second cycles are revealing degradation from the first cycles.

Comparing other pyroglutamic acid derivatives, *N*-acylhydrazones HEI 261 and 3156, as expected, the first is more stable, most probably based on its intermolecular bonds. One notable difference between the two is the melting points which for the simple hydrazine is 207°C while for the oxo-derivative is 105°C. For the water affinity, HEI 3156 has a more hygroscopic profile, having a higher solubility.

Things considered, within the tested molecules, for a possible formulation, the greatest candidates based on their stabilities are aniline, carbazides and *N*-acyl hydrazine derivatives, though depending on the treatment, the compounds might present solubility problems, based on their DVS analysis.

CHAPTER V

HIGHLIGHTS AND PERPECTIVES

In the context of drug resistant bacteria and fungi, the research and study of new antibiotic and antifungal drugs will likely remain an active, complex, and evolving field. The present work encompasses the chemical synthesis and the *in vitro* test results of a large range of molecules on fungi and bacteria as well.

Through 5 chemical pathways, around the pyroglutamic acid and its derivatives, we were able to build and extend 5 chemical families as platforms for antibacterial and antifungal screening, as following:

1. 5-Pyrrolidin-2-ones
2. Hydrazides
3. *N*-Acylhydrazones
4. Hydrazones
5. Azines

The most important target this work envisioned, is *A. baumannii*, being the first description of this type of antibacterials. Two series, the series of hydrazides and hydrazones, were found to be selectively targeting *A. baumannii in vitro*, while their potency *in vivo* is to be tested after the evaluation and confirmation that these molecules are active on a larger panel of drug resistant *A. baumannii* strains. This work provided a lot of potentially important insights, creating new questions deserving further study. One of it being structure-activity relationship, which will be studied more deeply within the patents maturity project which were filed around the two *A. baumannii* families. In what perspectives are concerning, in the context of a possible scale-up, the efficiency of certain chosen chemical pathways has to be improved if the corresponding pathways are not efficient enough, in the frame of the same maturity project.

Moreover, considering the central hypothesis of the *A. baumannii* antibacterial families' mechanism of action linked to the antioxidant character of molecules, further tests are needed to understand it. DNA sequencing of the bacterium genome and the development of an optimized fluorescence-based assay to ascertain the mechanism will be further developed.

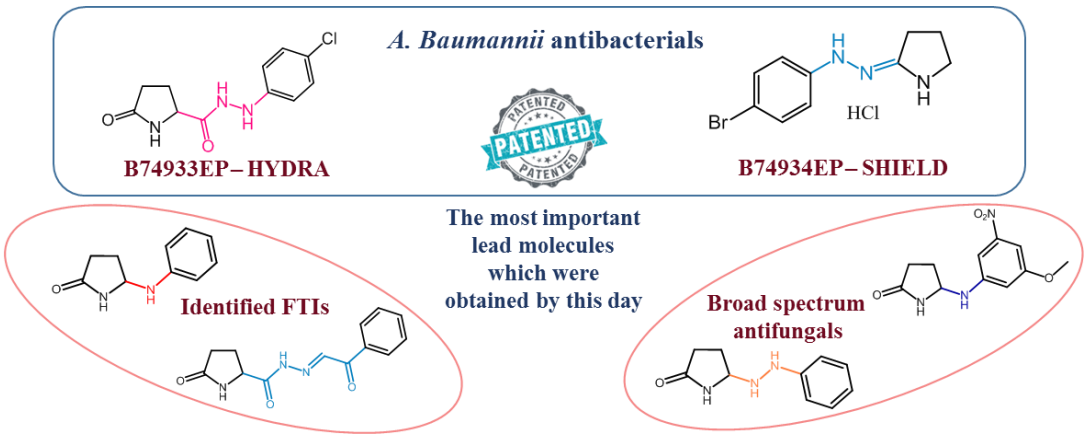


Figure 156 *The most important lead molecules within the project*

Secondly, new broad spectrum antifungal and anti-mold compounds were found, providing new possible paints antifungal additives. In the context of the developing paints with anti-fungal and anti-mildew properties, these molecules have been tested for their thermic stability and have shown long stability in air, and at high temperatures.Things considered, their broad activity, lack of cytotoxicity, and good thermal stability, are making these identified molecules, suitable for formulation.

Moreover, considering the other biological targets, some particular molecules designed in the context of the project proved to be important farnesyltansferase inhibitiors, becoming important HIT molecules, which could be developed in future projects.

The optimization of enantiomeric separation through different analytical techniques on some chiral molecules was done as well, serving as method material, enriching the knowledge around the pyro-scaffolded chiral molecules separations.

All the chemical methods and representative biological results are summarized in **Figure 165**.

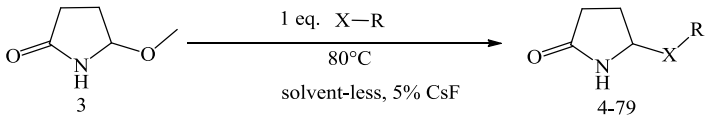
EXPERIMENTAL SECTION

1. Chemistry

Solvents were of analytical reagent grade and used without further purification, unless otherwise mentioned. Analytical thin-layer chromatographies (TLC) were performed on E. Merck 60 F254 silica gel plates. 1H NMR and 13C NMR spectra were obtained at 25 °C on a Varian 400-MR spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C).

Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to TMS as an internal standard. Coupling constants (*J*) are quoted in hertz. Column chromatographies were performed with a CombiFlash Rf Companion (Teledyne-Isco System) using RediSep packed columns. IR spectra were recorded on a Varian 640-IR FT-IR Spectrometer. Melting points were measured on a MPA 100 OptiMelt® apparatus. Elemental analyses (C, H, N, S) of new compounds were determined on a Thermo Electron apparatus by 'Pôle Chimie Moléculaire-Welience', Faculté de Sciences Mirande, Université de Bourgogne, Dijon, France. Yield refers to the isolated analytically pure material. The central molecule within this work, the pyroglutamic acid, was bought directly from Solabia group (France), a company specialized in active ingredients produced from plants. Other starting materials were purchased from Sigma Aldrich, Alfa Aesar and TCI, and were used without further purification.

1.1 Experimental procedures for the synthesis of 5-pyrolidone derivatives

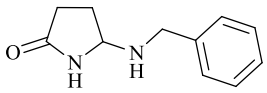


Scheme 52 General procedure for the synthesis of 5-pyrolidone derivatives

The nucleophilic reactant (17 mmol) was added to a mixture of 5-methoxypyrrolidin-2-one (1eq) and CsF (0.86 mmol, 5 mol%). The mixture was stirred under vacuum (20 mmHg), at a temperature of 80°C, until the NMR conversion showed no more progression or after caking of the reaction medium. Then, the crude product was precipitated by addition of Et_2O. Crude products were then recrystallized from EtOH or purified through flash liquid chromatography being eluted with a gradient of heptane / ethyl acetate, to afford the target derivatives 4-79.

5-(benzylamino)pyrrolidin-2-one[251] – 4

HEI 2907



Following the general procedure, 5-(benzylamino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with benzylamine (17.4 mmol, 1.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 5 as a white solid (2.6 g, 79% yield).

M.p. 106-107°C

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 7.34-7.27 (m, 5H, Ar*H*), 4.62 (d, *J* = 6.3 Hz, 1H, CH), 3.87 (d, *J* = 12.7 Hz, 1H, C*H*$_2$-N*H*), 3.82 (d, *J* = 12.7 Hz, 1H, C*H*$_2$-N*H*), 2. 38-2. 46 (m, 2H, C*H*$_2$C$\underline{H}_2$CH), 1.82 (m, 1H, CH$_2$C$\underline{H}_2$CH).

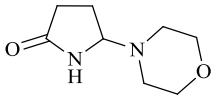
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.8 (C=O), 139.5 (C$_{IV}$), 128.6 (2 CHAr), 128.1 (2 CHAr), 121.3 (CHAr), 69.5 (CH), 50.0 (CH$_2$), 29.5 (CH$_2$), 28.7 (CH$_2$).

IR ν (cm^{-1}): 3264, 3029, 1677, 1487, 1262.

Anal. Calcd for C$_{11}$H$_{14}$N$_2$O: C, 69.45; H, 7.42; N, 14.73.

5-morpholinopyrrolidin-2-one[1] - 6

HEI 2477



Following the general procedure, 5-morpholinopyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with morpholine (17.4 mmol, 1.5 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white-off solid (2.0 g, 70% yield).

M.p. 141-142° C

^{1}H-NMR (400 MHz, CDCl$_3$): δ 7.29 (bs, 1H, N*H*), 4.48 (d, *J* = 7.4 Hz, 1H, C*H*), 3.73 (d, *J* = 5.2 Hz, 4H, C*H*$_2$), 2.38 (d, *J* = 5.2 Hz, 4H, C*H*$_2$), 2.30-2.26 (m, 3H, C*H*$_2$C*H*$_2$CH), 2.05 (m, 1H, CH$_2$C*H*$_2$CH).

[251] Kosugi Y., Hamaguchi H., Nagasaka T., Ozawa N., Ohki, S. Synthesis of 5-Amino-2-pyrrolidinone and Its Derivatives. *Heterocycles*, 1980, 14, p. 1245-1249.

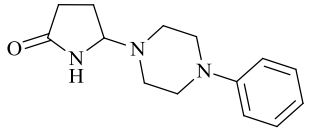
^{13}C- NMR (100 MHz, $CDCl_3$): δ 178.4 (C=O), 75.1 (CH), 66.8 (2 CH_2), 47.3 (2 CH_2), 29.4 (CH_2), 23.1 (CH_2).

IR ν (cm^{-1}): 3153, 2966, 2854, 1673, 1263, 1107, 1068.

Anal. Calcd for $C_8H_{14}N_2O_2$: C, 56.45; H, 8.29; N, 16.46.

5-(4-phenylpiperazin-1-yl)pyrrolidin-2-one[252] - 7

HEI 2948



Following the general procedure, 5-(4-phenylpiperazin-1-yl)pyrrolidin-2-one was obtained yield from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 1-phenylpiperazine (17.4 mmol, 2.8 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 8 as a white solid (4.0 g, 95% yield).

M.p. 174-175°C

^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.22 (d, *J* = 8.0 Hz, 2H, Ar*H*), 6.92 (d, *J* = 7.8 Hz, 2H, Ar*H*), 6.76 (t, *J* = 7.6 Hz,1H, Ar*H*), 4.41 (dd, *J* = 7.9 Hz, *J* = 2.25 Hz, 1H, C*H*), 3.11 (t, *J* = 5.0 Hz, 4H, NC*H*$_2$C*H*$_2$N), 2.66 (m, 4H, NC*H*$_2$C*H*$_2$N), 2.22-2.08 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.86 (m, 1H, CH$_2$C*H*$_2$CH).

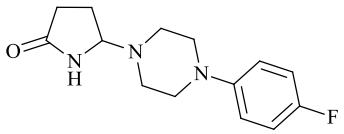
^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 177.2 (C=O), 151.4 (C_{IV}), 129.3 (2 CHAr), 119.2 (CHAr), 115.8 (2 CHAr), 74.4 (CH), 48.6 (2 CH_2), 46.8 (2 CH_2), 26.6 (CH_2), 23.7 (CH_2).

IR ν (cm^{-1}): 3214, 2825, 1686, 1651, 1241.

Anal. Calcd for $C_{14}H_{19}N_3O$: C, 68.54; H, 7.81; N, 17.13.

5-(4-(4-fluorophenyl)piperazin-1-yl)pyrrolidin-2-one – 8

HEI 2951



Following the general procedure, 5-(4-(4-fluorophenyl)piperazin-1-yl)pyrrolidin-2-one was

[252] Toja E., Gorini C., Zirotti C., Barzaghi F., Galliani G. Amnesia-reversal activity of a series of 5-alkoxy-1-arylsulfonyl-2-pyrrolidinones. *Eur. J. Med. Chem.* 1991, 26, p. 403-413.

obtained yield from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 1-(4-fluorophenyl)piperazine (17.4 mmol, 3.13 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (50 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (3.6 g, 78% yield).

M.p. 172-173°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.23 (bs, 1H, N*H*), 7.04 (t, *J* = 9.2 Hz, 2H, Ar*H*), 6.95 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz,1H, Ar*H*), 4.41 (d, *J* = 5.2 Hz, 1H, C*H*), 3.06 (bs, 4H, NC*H*$_2$C*H*$_2$-N), 2.65 (m, 2H, NC*H*$_2$C*H*$_2$N), 2.50 (m, 2H, NC*H*$_2$C*H*$_2$N), 2.20-2.08 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.89 (m, 1H, CH$_2$C*H*$_2$CH).

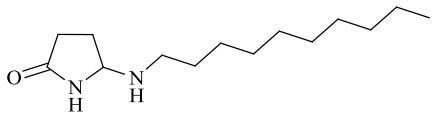
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.3 (C=O), 157.6 (C$_{IV}$, J_{C-F} = 233.9 Hz), 148.3 (C$_{IV}$, J_{C-F} = 1.5 Hz), 117.5 (2 CHAr, J_{C-F} = 7.6 Hz), 115.6 (2 CHAr, J_{C-F} = 21.3 Hz), 74.4 (CH), 49.4 (2 CH$_2$), 46.8 (2 CH$_2$), 29.7 (CH$_2$), 23.8 (CH$_2$).

IR ν (cm^{-1}): 3214, 2825, 1686, 1651, 1241.

Anal. Calcd for C$_{14}$H$_{18}$FN$_3$O: C, 63.86; H, 6.89; N, 15.96.

5-(decylamino)pyrrolidin-2-one – 9

HEI 2479



Following the general procedure, 5-(decylamino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (14.5 mmol, 1.67 g) with decan-1-amine (14.5 mmol, 2.27 g), in presence of 5 mol% CsF (0.69 mmol, 0.11 g). Dichloromethane (50 mL) and water (15 mL) were added and the aqueous phase was extracted twice with dichloromethane. The organic layers was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give the final product which was recrystallized in ether and ethanol affording 2.7 g of a cream solid, in 78% yield.

M.p. 70-71°C.

^{1}H- NMR (400 MHz, CDCl$_3$): δ 6.95 (bs, 1H, N*H*), 4.57 (t, *J* = 5.7 Hz, 1H, CH), 2.68 (m, 1H, C*H*$_2$CH$_2$CH), 2.57 (m, 1H, C*H*$_2$CH$_2$CH), 2.37 (m, 2H, CH$_2$C*H*$_2$CH), 2.29 (m, 1H, C*H*$_2$), 1.79 (m, 1H, N*H*), 1.49 (d, *J* = 7,1 Hz, 3H, C*H*$_2$), 1.26 (s, 14 H, C*H*$_2$), 0.89 (t, *J* = 7,1 Hz, 3H, C*H*$_3$).

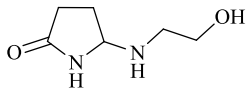
^{13}C- NMR (100 MHz, CDCl$_3$): δ, 178.0 (C=O), 69.9 (CH), 45.6 (CH$_2$), 31.9 (CH$_2$), 30.4 (CH$_2$), 29.6 (2 CH$_2$), 29.5 (CH$_2$), 29.4 (CH$_2$), 29.3 (CH$_2$), 27.2 (CH$_2$), 28.6 (CH$_2$), 22.6 (CH$_2$), 14.1 (CH$_3$).

IR ν (cm^{-1}): 3291, 2918, 2850, 1694, 1495, 1468, 1272.

Anal. Calcd for $C_{14}H_{28}N_2O$: C, 69.95; H, 11.74; N, 11.65; found: C, 69.72; H, 12.09; N, 11.58.

5-((2-hydroxyethyl)amino)pyrrolidin-2-one – 10

HEI 2908



Following the general procedure, 5-((2-hydroxyethyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 2-aminoethanol (17.4 mmol, 1.1 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound **10** as a white solid (1.75 g, 70% yield).

M.p. 136-137°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ ppm 8.09 (bs, 1H, N*H*), 4.51 (bs, 1H, CH), 4.34 (bs, 1H, N*H*), 3.41 (bs, 2H, CH_2), 2.67 (bs, 1H, OH), 2.15-2.01 (m, 5H, CH_2CH_2NH, CH_2CH_2CH), 1.65-1.60 (m, 1H, CH_2CH_2CH).

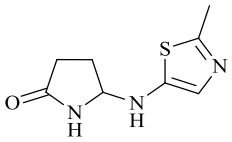
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 176.8 (C=O), 69.7 (CH), 61.3 (CH_2), 48.0 (CH_2), 47.5 (CH_2), 29.7 (CH_2), 28.3 (CH_2).

IR ν (cm^{-1}): 3223, 3079, 2850, 1668, 1455, 1261, 1060.

Anal. Calcd for $C_6H_{12}N_2O_2$: C, 49.98; H, 8.39; N, 19.43.

5-((5-methylthiazol-2-yl)amino)pyrrolidin-2-one – 11

HEI 2996



Following the general procedure, 5-((5-methylthiazol-2-yl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 2-methylthiazol-5-amine (17.4 mmol, 2.0 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The precipitated product was washed with ether several times in order to afford the wanted compound, as a white solid (1.85 g, 54% yield).

M.p. 166-167°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 7.82 (s, 1H, N*H*), 7.63 (s, 1H, N*H*), 6.69 (s, 1H, C*H*), 5.29 (s, 1H, C*H*), 2.50 (bs, 2H, CH_2CH_2CH), 2.30 (s, 3H, CH_3), 2.10 (bs, 1H, CH_2CH_2CH), 1.88 (bs,

1H, CH_2CH_2CH).

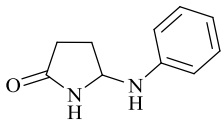
$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ 176.5 (C=O), 166.2 (C_{IV}), 136.0 (CH), 120.8 (C_{IV}), 65.4 (CH), 29.2 (CH_2), 28.1 (CH_2).

IR ν (cm^{-1}): 3280, 1670, 1450, 1280, 1109.

Anal. Calcd for $C_8H_{11}N_3OS$: C, 48.71; H, 5.62; N, 21.30; S, 16.26.

5-(phenylamino)pyrrolidin-2-one[253] – 12

HEI 2906



Following the general procedure, 5-(phenylamino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with aniline (17.4 mmol, 1.6 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 10 as a white solid (2.7 g, 88% yield).

M.p. 135-136 °C.

^1H NMR (DMSO-d_6, 400 MHz): δ 8.25 (bs, 1H, N*H*), 7.09 (t, *J* = 6 Hz, 2H, Ar*H*), 6.62 (d, *J* = 9.6 Hz, 2H, Ar*H*), 6.57 (d, *J* = 6.6 Hz, 1H, N*H*), 6.11 (d, *J* = 9.6 Hz, 1H, Ar*H*), 5.10 (bs, 1H, CH), 2. 46-2. 20 (m, 2H, CH_2CH_2CH), 2. 16-2. 05 (m, 1H, CH_2CH_2CH), 1. 95-1. 73 (m, 1H, CH_2CH_2CH).

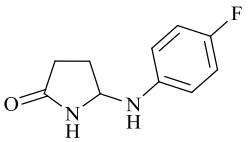
$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ 176.8 (C=O), 147.2 (C_{IV}), 129.4 (2 CHAr), 117.1 (CHAr), 114.4 (2 CHAr), 64.2 (CH), 29.5 (CH_2), 28.5 (CH_2).

IR ν (cm^{-1}): 3338, 3165, 1669, 1598, 1253.

Anal. Calcd for $C_{10}H_{12}N_2O$: C, 68.16; H, 6.86; N, 15.90.

5-((4-fluorophenyl)amino)pyrrolidin-2-one – 13

HEI 2910



[253] Priyadarshini S., Amal Joseph P.J., Lakshmi Kantam M. Copper catalyzed oxidative cross-coupling of aromatic amines with 2-pyrrolidinone: a facile synthesis of *N*-aryl-γ-amino-γ-lactams. *Tetrahedron*, 2014, 70, p. 6068-6074.

Following the general procedure, 5-((4-fluorophenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-fluoroaniline (17.4 mmol, 1.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 11 as a white solid (2.8 g, 82% yield).

M.p. 146-147°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.15 (bs, 1H, N*H*), 6.93 (t, *J* = 8.8 Hz, 2H, Ar*H*), 6.63 (dd, *J* = 8.8 Hz, *J* = 4.4 Hz, 2H, Ar*H*), 6.11 (d, *J* = 7.8 Hz, 1H, N*H*), 5.05 (bs, 1H, CH), 2.36-2.26 (m, 2H, C*H*$_2$CH$_2$CH), 2.14 -2.05 (m, 1H, CH$_2$C*H*$_2$CH), 1.83 (m, 1H, CH$_2$C*H*$_2$CH).

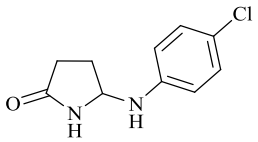
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.85 (C=O), 155.2 (C$_{IV}$, C-F, *J* = 232 Hz), 143.85 (C$_{IV}$), 115.7 (CHAr, *J* = 21.2 Hz), 114.3 (CHAr, *J* = 7.1 Hz), 64.7 (CH), 29.5 (CH$_2$), 28.4 (CH$_2$).

IR ν (cm^{-1}): 3284, 3177, 1683, 1505, 1245.

Anal. Calcd for C$_{10}$H$_{11}$FN$_2$O: C, 61.85; H, 5.71; N, 14.42; found: C, 61.55; H, 5.61; N, 14.22.

5-((4Chlorophenyl)amino)pyrrolidin-2-one – 14

HEI 2913



Following the general procedure, 5-((4Chlorophenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-chloroaniline (17.4 mmol, 2.2 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a grey solid (2.1 g, 57% yield).

M.p. 169-170°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 7.12 (d, 2H, ArH, *J* = 9.2 Hz) 6.63 (d, 2H, ArH, *J* = 9.2 Hz), 6.39 (bs, 1H, N*H*), 5.08 (s, 1H, CH), 2.25-2.31 (m, 2H, C*H*$_2$CH$_2$CH), 2.07-2.12 (m, 1H, CH$_2$C*H*$_2$CH), 1.84-1.79 (m, 1H, CH$_2$C*H*$_2$CH).

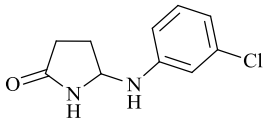
^{13}C-NMR (100 MHz, DMSO-d$_6$) δ ppm 176.8 (C$_{IV}$) 146.2 (C$_{IV}$), 129.0 (2 CHAr), 120.3 (C$_{IV}$), 114.8 (2 CHAr), 64.2 (CH), 29.5 (CH$_2$), 28.4 (CH$_2$).

IR ν (cm^{-1}): 3281, 3159, 3088, 1681, 1590, 1487, 1247, 1176.

Anal. Calcd for C$_{10}$H$_{11}$ClN$_2$O: C, 57.01; H, 5.26; N, 13.30; found: C, 61.55; H, 5.61; N, 14.22.

5-((3-chlorophenyl)amino)pyrrolidin-2-one – 15

HEI 2918



Following the general procedure, 5-((3-chlorophenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 3-chloroaniline (17.4 mmol, 2.2 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a white solid (2.8 g, 77% yield).

M.p. 139-140°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 7.10 (t, *J* = 7.9 Hz, 1H, Ar*H*), 6.66 (t, *J* = 2.2 Hz, 1H, Ar*H*), 6.57 (td, *J* = 7.9 Hz, *J* = 2.2 Hz, 2H, Ar*H*), 5.10 (t, *J* = 5.2 Hz, 1H, CH), 2.37-2.25 (m, 2H, C*H*$_2$CH$_2$CH), 2.12-2.06 (m, 1H, CH$_2$C*H*$_2$CH), 1.82-1.79 (m, 1H, CH$_2$C*H*$_2$CH).

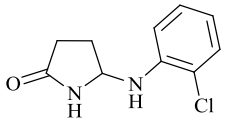
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.8 (C=O), 148.8 (C$_{IV}$), 134.1 (C$_{IV}$), 130.8 (CHAr), 116.4 (CHAr), 112.5 (CHAr), 112.0 (CHAr), 64.0 (CH), 29.5 (CH$_2$), 28.4 (CH$_2$).

IR ν (cm^{-1}): 3328, 3168, 1682, 1595, 1482, 1242, 1089.

Anal. Calcd for C$_{10}$H$_{11}$ClN$_2$O: C, 57.01; H, 5.26; N, 13.30.

5-((2-chlorophenyl)amino)pyrrolidin-2-one – 16

HEI 2966



Following the general procedure, 5-((2-chlorophenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 2-chloroaniline (17.4 mmol, 2.2 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a white solid (3.1 g, 85% yield).

M.p. 125-126°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.32 (s, 1H, N*H*), 7.28 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, Ar*H*), 7.14 (td, *J* = 8.8 Hz, *J* = 2.0 Hz, 1H, Ar*H*), 6.84 (d, *J* = 7.2 Hz, 1H, Ar*H*), 6.66 (td, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, Ar*H*), 5.61 (d, *J* = 8.8 Hz, 1H, N*H*), 5.22 (t, *J* = 4.0 Hz, 1H, C*H*), 2.40-2.25 (m, 2H, C*H*$_2$CH$_2$CH), 2.13-2.06 (m, 2H, CH$_2$C*H*$_2$CH).

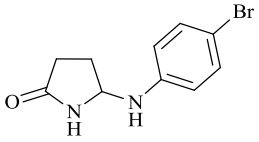
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): δ 176.9 (C=O), 142.7 (C_{IV}), 129.6 (C_{IV}), 128.4 (CHAr), 118.9 (C_{IV}), 118.3 (CH), 113.3 (CHAr), 64.2 (CH), 29.5 (CH_2), 28.1 (CH_2).

IR ν (cm^{-1}): 3398, 3169, 1692, 1597, 1497, 1265, 1181.

Anal. Calcd for $C_{10}H_{11}ClN_2O$: C, 57.01; H, 5.26; N, 13.30.

5-((4-bromophenyl)amino)pyrrolidin-2-one – 17

HEI 2917



Following the general procedure, x was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-bromoaniline (17.4 mmol, 3 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a white solid (3.6 g, 81% yield).

M.p. 161-162°C.

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 7.22 (d, 2H, ArH, *J* = 9.4 Hz), 6.60 (d, 2H, ArH, *J* = 9.4 Hz), 6.39 (bs, 1H, N*H*), 5.18 (bs, 1H, CH), 2.37-2.25 (m, 1H, CH_2CH_2CH), 2.15-2.04 (m, 1H, CH_2CH_2CH), 1.81-1.78 (m, 1H, CH_2CH_2CH).

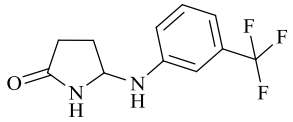
^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm 176.3 (C_{IV}), 146.0 (C_{IV}), 131.3 (2 CHAr), 114.8 (2 CHAr), 107.2 (C_{IV}), 63.8 (CH), 29.0 (CH_2), 27.8 (CH_2).

IR ν (cm^{-1}): 3283, 3161, 3082, 1680, 1583, 1485, 1245, 1176.

Anal. Calcd for $C_{10}H_{11}BrN_2O$: C, 47.08; H, 4.35; N, 10.98; found: C, 61.55; H, 5.61; N, 14.22

5-((3-(trifluoromethyl)phenyl)amino)pyrrolidin-2-one – 18

HEI 2911



Following the general procedure, 5-((3-(trifluoromethyl)phenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 3-(trifluoromethyl)aniline (17.4 mmol, 2.8 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a white solid (1.4 g, 33% yield).

M.p. 149-150°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.40 (s, 1H, N*H*), 7.32 (t, *J* = 8.2 Hz, 1H, Ar*H*), 6.91 (d, *J* = 8.2 Hz, 2H, Ar*H*), 6.87 (s, 1H, N*H*), 6.65 (d, *J* = 8.2 Hz, Ar*H*), 5.19 (td, *J* = 8.0 Hz, *J* = 3.5 Hz, 1H, CH), 2.41-2.20 (m, 2H, C*H*$_2$CH$_2$CH), 2.14 (m, 1H, CH$_2$C*H*$_2$CH), 1.84 (m, 1H, CH$_2$C*H*$_2$CH).

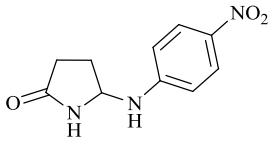
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.8 (C=O), 147.7 (C$_{IV}$), 130 (CHAr), 129.0 (q, J_{C-F} = 31.2 Hz, C$_{IV}$), 124.8 (q, J_{C-F} = 272.0 Hz, C$_{IV}$), 116.7 (CHAr), 113.0 (d, J_{C-F} = 3.8 Hz, CHAr), 109.3 (d, J_{C-F} = 3.8 Hz, CHAr), 63.9 (CH), 29.5 (CH$_2$), 28.4 (CH$_2$).

IR ν (cm^{-1}): 3314, 1695, 1612, 1451, 1286, 1107, 1068.

Anal. Calcd for C$_{11}$H$_{11}$F$_3$N$_2$O: C, 54.10; H, 4.54; N, 11.47.

5-((4-nitrophenyl)amino)pyrrolidin-2-one – 19

HEI 2912



Following the general procedure, 5-((4-nitrophenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-nitroaniline (17.4 mmol, 2.4 g) in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a yellow solid (2.3 g, 60% yield).

M.p. 200-201°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 8.03 (bs, 1H, N*H*), 8.02 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.74 (d, *J* = 8.8 Hz, 2H, Ar*H*), 5.28 (q, *J* = 3.4 Hz, 1H, C*H*), 2.41-2.23 (m, 2H, C*H*$_2$CH$_2$CH), 2.18-2.13 (m, 1H, CH$_2$C*H*$_2$CH), 1.89-1.85 (m, 1H, CH$_2$C*H*$_2$CH).

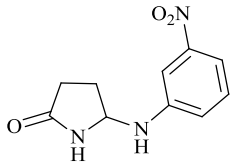
^{13}C-NMR (DMSO-d$_6$, 100 MHz): δ ppm 176.9 (C=O), 153.2 (C$_{IV}$), 137.0 (C$_{IV}$), 126.5 (3 CHAr), 112.1 (CHAr), 63.6 (CH), 29.2 (CH$_2$), 28.2 (CH$_2$).

IR ν (cm^{-1}): 3349, 3259, 1681, 1594, 1477, 1326, 1254, 1176, 1121.

Anal. Calcd for C$_{10}$H$_{11}$N$_3$O$_3$: C, 54.29; H, 5.01; N, 19.00.

5-((3-nitrophenyl)amino)pyrrolidin-2-one – 20

HEI 3222



Following the general procedure, 5-((3-nitrophenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 3-nitroaniline (17.4 mmol, 2.4 g) in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a yellow solid (1.9 g, 50% yield).

M.p. 170-171°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 8.39 (s, 1H, N*H*), 7.43 (d, *J* = 8.9 Hz, 2H, ArH), 7.38 (t, *J* = 8.9 Hz, 1H, ArH), 7.05 (d, *J* = 8.9 Hz, 1H, ArH), 6.88 (d, *J* = 8.4 Hz, 1H, N*H*), 5.23 (td, *J* = 7.7, 3.7 Hz, 1H, CH), 1.85-1.82 (m, 2H, C*H*$_2$CH$_2$CH), 2.17-2.10 (m, 1H, CH$_2$C*H*$_2$CH), 1.88 (m, 1H, CH$_2$C*H*$_2$CH).

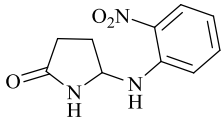
^{13}C-NMR (100 MHz, DMSO-d$_6$) δ ppm 176.2 (C=O), 148.7 (C$_{IV}$), 147.8 (C$_{IV}$), 130.0 (CHAr), 119.0 (CHAr), 110.8 (CHAr), 106.9 (CHAr), 63.4 (CH), 28.9 (CH$_2$), 27.8 (CH$_2$).

IR ν (cm^{-1}): 3349, 3259, 1681, 1594, 1477, 1326, 1254, 1176, 1121.

Anal. Calcd for C$_{10}$H$_{11}$N$_3$O$_3$: C, 54.29; H, 5.01; N, 19.00.

5-((2-nitrophenyl)amino)pyrrolidin-2-one – 21

HEI 3228



Following the general procedure, x was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 3-nitroaniline (17.4 mmol, 2.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a yellow solid (1.2 g, 30% yield).

M.p. 182-183° C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 8.52 (s, 1H, N*H*), 8.09 (dd, *J* = 9.2, 1.6 Hz, 1H, ArH), 7.97 (d, *J* = 7.5 Hz, 1H, N*H*), 7.57 (t, *J* = 9.2 Hz, 1H, ArH), 7.12 (d, *J* = 9.2 Hz, 1H, ArH), 6.80 (d, *J* = 7.5 Hz, 1H, N*H*), 5.43 (td, *J* = 7.5, 3.1 Hz, 1H, C*H*), 2.42-2.30 (m, 2H, C*H*$_2$CH$_2$CH), 2.20-1.25 (m, 1H, CH$_2$C*H*$_2$CH), 1.88 (m, 1H, CH$_2$C*H*$_2$CH).

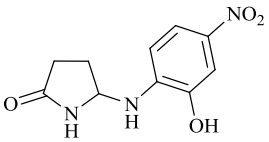
^{13}C-NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.0 (C=O), 143.4 (C$_{IV}$), 137.0 (CHAr), 132.3 (C$_{IV}$), 126.7 (CHAr), 117.0 (CHAr), 115.8 (CHAr), 64.0 (CH), 29.2 (CH$_2$), 28.5 (CH$_2$).

IR ν (cm^{-1}): 3345, 3156, 1686, 1342, 1228, 1178.

Anal. Calcd for $C_{10}H_{11}N_3O_3$: C, 54.29; H, 5.01; N, 19.00.

5-((2-hydroxy-4-nitrophenyl)amino)pyrrolidin-2-one – 22

HEI 3215



Following the general procedure, 5-((2-hydroxy-4-nitrophenyl)amino)pyrrolidin-2-on was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 2-amino-5-nitrophenol (17.4 mmol, 2.7 g) in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n-heptane*/ethyl acetate, the wanted compound being eluted in 100% ethyl acetate, as 4.0 g of a yellow solid, in 55% yield.

M.p. 169-170°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 10.42 (bs, 1H, O*H*), 8.34 (s, 1H, N*H*), 7.68 (d, *J* = 9.0 Hz, 1H, Ar*H*), 7.51 (d, *J* = 2.73 Hz, 1H, Ar*H*), 6.74 (d, *J* = 9.0 Hz, 1H, Ar*H*), 6.51 (d, *J* = 9.0 Hz, 1H, N*H*), 5.32 (td, *J* = 8.5, 3.8 Hz, 1H, C*H*), 2.35-2.39 (m, 2H, C*H*$_2$CH$_2$CH), 2.04-2.11 (m, 2H, CH$_2$C*H*$_2$CH).

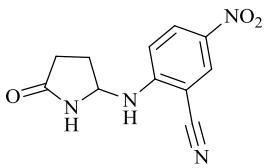
^{13}C-NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.0 (C=O), 143.7 (C$_{IV}$), 143.0 (C$_{IV}$), 136.9 (C$_{IV}$), 118.2 (CHAr), 109.0 (CHAr), 108.3 (CHAr), 63.6 (CH), 29.4 (CH$_2$), 27.9 (CH$_2$).

IR ν (cm^{-1}): 3404, 3332, 1658, 1589, 1475, 1255, 1221.

Anal. Calcd for $C_{10}H_{11}N_3O_4$: C, 50.63; H, 4.67; N, 17.71.

5-nitro-2-((5-oxopyrrolidin-2-yl)amino)benzonitrile – 23

HEI 3224



Following the general procedure, 5-nitro-2-((5-oxopyrrolidin-2-yl)amino)benzonitrile was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 2-cyano-4-nitroaniline (17.4 mmol, 2.8 g) in presence of 5 mol% CsF (0.86 mmol, 0.132 g).). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n-*

heptane/ethyl acetate, the wanted compound being eluted in 100% ethyl acetate, as 1.5 g of a yellow solid, in 35% yield.

M.p. 199-200°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 8.47 (d, J = 2.8 Hz, 1H, N*H*), 8.39 (d, *J* = 2.8 Hz, 1H, N*H*), 8.22 (dd, *J* = 10 Hz, *J* = 2.8 Hz, 1H, Ar*H*), 7.89 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.04 (d, *J* = 10 Hz, 1H, Ar*H*), 5.45 (td, *J* = 7.6, 2.5 Hz, 1H, C*H*), 2.43-2.40 (m, 2H, C*H*$_2$CH$_2$CH), 2.12-1.98 (m, 2H, CH$_2$C*H*$_2$CH).

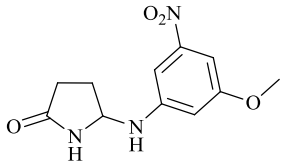
^{13}C-NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.2 (C=O), 153.1 (C_{IV}), 137.1 (C_{IV}), 131.4 (CHAr), 130.0 (CHAr), 116.4 (C_{IV}), 113.0 (CHAr), 95.3 (C_{IV}), 64.4 (CH), 29.2 (CH_2), 27.6 (CH_2).

IR ν (cm^{-1}): 3332, 3167, 3094, 2230, 1697, 1587, 1503, 1305, 1270, 1171.

Anal. Calcd for C$_{11}$H$_{10}$N$_4$O$_3$: C, 53.66; H, 4.09; N, 22.75.

5-((3-methoxy-5-nitrophenyl)amino)pyrrolidin-2-one – 24

HEI 2991



Following the general procedure, 5-((3-methoxy-5-nitrophenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 3-methoxy-5-nitroaniline (17.4 mmol, 2.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as an orange solid (2.4 g, 55% yield).

M.p. 194-195°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.53 (s, 1H, N*H*), 7.87 (d, *J* = 7.5 Hz, 1H, N*H*), 7.53 (s, 1H, Ar*H*), 7.31 (dd, *J* = 9.5 Hz, *J* = 2.5 Hz, 1H, Ar*H*), 7.12 (d, *J* = 7.5 Hz, 1H, Ar*H*), 5.42 (bs, 1H, CH), 3.76 (s, 3H, CH_3), 2.43-2.32 (m, 1H, C*H*$_2$CH$_2$CH), 2.22-2.11 (m, 1H, CH$_2$C<u>H$_2$</u>CH), 2.02-1.90 (m, 1H, CH$_2$C<u>H$_2$</u>CH) ppm.

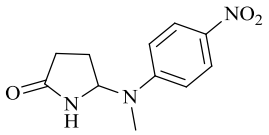
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.97 (C=O), 150.27 (C_{IV}), 138.99 (C_{IV}), 131.58 (C_{IV}), 127.16 (CHAr), 117.46 (CHAr), 107.25 (CHAr), 64.16 (CH), 56.08 (CH_3), 29.22 (CH_2), 28.63 (CH_2).

IR ν (cm^{-1}): 3359, 3169, 1695, 1511, 1345, 1232, 1178.

Anal. Calcd for C$_{11}$H$_{13}$N$_3$O$_4$: C, 52.59; H, 5.22; N, 16.73; found: C, 52.24; H, 4.89; N, 16.36.

5-(methyl(4-nitrophenyl)amino)pyrrolidin-2-one – 25

HEI 3220



Following the general procedure, 5-(methyl(4-nitrophenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with *N*-methyl-4-nitroaniline (17.4 mmol, 2.6 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 90% ethyl acetate, as 1.6 g of a yellow solid, in 40% yield.

M.p. 152-153°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 8.22 (s, 1H, N*H*), 8.08 (d, *J* = 9.5 Hz, 2H, Ar*H*), 6.98 (d, *J* = 9.5 Hz, 2H, Ar*H*), 5.83 (q, *J* = 3.4 Hz, 1H, C*H*), 2.82 (s, 3H, C*H*$_3$), 2.45-2.37 (m, 2H, C*H*$_2$CH$_2$CH), 2.17-2.23 (m, 1H, CH$_2$C*H*$_2$CH), 1.85 (m, 1H, CH$_2$C*H*$_2$CH).

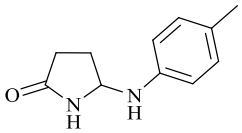
13C-NMR (DMSO-d$_6$, 100 MHz): δ ppm 176.8 (C=O), 154.0 (C$_{IV}$), 126.1 (2 ArH), 112.6 (2 ArH), 68.3 (CH), 30.5 (CH$_2$), 29.6 (CH$_2$), 25.1 (CH$_3$).

IR ν (cm^{-1}): 3151, 3066, 2892, 1693, 1583, 1462, 1311, 1238, 1081.

Anal. Calcd for C$_{11}$H$_{13}$N$_3$O$_3$: C, 56.16; H, 5.57; N, 17.86.

5-(p-tolylamino)pyrrolidin-2-one – 26

HEI 2915



Following the general procedure, 5-(p-tolylamino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with *p*-toluidine (17.4 mmol, 1.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (30 mL), filtered off and washed with ethanol (2x10 mL), affording compound as a white solid (2.8 g, 85% yield).

M.p. 139-140°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 6.90 (d, 2H, *J* = 8.2 Hz, Ar*H*) 6.54 (d, 2H, *J* = 8.2 Hz, Ar*H*), 5.93 (d, 1H, *J* = 8.2 Hz, N*H*), 5.05 (td, 1H, *J* = 8.2 Hz, *J* = 4.1 Hz, C*H*), 2.46-2.29

(m, 2H, C*H*$_2$CH$_2$CH), 2.15 (s, 3H, C*H*$_3$), 2.09-2.13 (m, 1H, CH$_2$C*H*$_2$CH), 1.85-1.78 (m, 1H, CH$_2$C*H*$_2$CH),

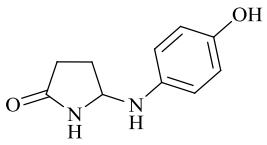
^{13}C-NMR (100 MHz, DMSO-d$_6$): δ ppm 176.8 (C$_{IV}$), 144.9 (C$_{IV}$), 129.8 (2 CHAr), 125.5 (C$_{IV}$), 113.6 (2 CHAr), 64.5 (CH), 29.5 (CH$_2$), 28.4 (CH$_2$), 20.5 (CH$_3$).

IR ν (cm^{-1}): 3283, 3161, 1680, 1583, 1485, 1246, 1176.

Anal. Calcd for C$_{11}$H$_{14}$N$_2$O: C, 69.45; H, 7.42; N, 14.73.

5-((4-hydroxyphenyl)amino)pyrrolidin-2-one – 27

HEI 2990



Following the general procedure, 5-((4-hydroxyphenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-aminophenol (17.4 mmol, 1.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 12 as a white solid (1.8 g, 50% yield).

M.p. 190-191°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.49 (s, 1H, N*H*), 8.16 (s,1H, N*H*), 6.48 (d, *J* = 9.1 Hz, *J* = 2.46 Hz, 2H, Ar*H*), 6.55 (d, *J* = 9.1 Hz, *J* = 2.46 Hz, 2H, Ar*H*), 5.47 (d, *J* = 9.5 Hz, 1H, CH), 4.98 (m, 1H, OH), 2.31-2.26 (m, 2H, C*H*$_2$CH$_2$CH), 2.12-2.04 (m, 1H, CH$_2$C<u>H$_2$</u>CH), 1.82-1.74 (m, 1H, CH$_2$C<u>H$_2$</u>CH).

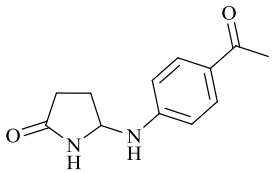
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.74 (C=O), 149.50 (C$_{IV}$), 139.76 (C$_{IV}$), 116.11 (2 CHAr), 115.12 (2 CHAr), 65.45 (CH), 29.56 (CH$_2$), 28.47 (CH$_2$), ppm.

IR ν (cm^{-1}): 3361, 3141, 1658, 1511, 1244, 1231.

Anal. Calcd for C$_{10}$H$_{12}$N$_2$O$_2$: C, 62.49; H, 6.29; N, 14.57; found: C, 62.49; H, 6.14; N, 14.20.

5-((4-acetylphenyl)amino)pyrrolidin-2-one – 28

HEI 2916



Following the general procedure, 5-((4-acetylphenyl)amino)pyrrolidin-2-one was obtained

from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 1-(4-aminophenyl)ethanone (17.4 mmol, 2.3 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 13 as a white solid (2.6 g, 70% yield).

M.p. 171-172°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 7.74 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.68 (d, *J* = 8.4 Hz, 2H, Ar*H*), 5.22 (td, *J* = 6.8 Hz, *J* = 3.2 Hz, 1H, C*H*), 2.36-2.29 (m, 2H, C*H*$_2$CH$_2$CH), 2.35 (s, 3H, C*H*$_3$), 2.16-2.11 (m, 1H, CH$_2$C*H*$_2$CH), 1.87-1.83 (m, 1H, CH$_2$C*H*$_2$CH).

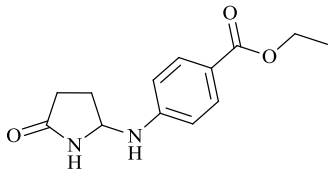
^{13}C-NMR (DMSO-d$_6$, 100 MHz): δ ppm 195.7 (C=O), 176.8 (C=O), 151.4 (C_{IV}), 130.8 (2CHAr), 126.2 (C_{IV}), 112.1 (2CHAr), 63.6 (CH), 29.4 (CH_2), 28.3 (CH_2), 26.4 (CH_3).

IR ν (cm^{-1}): 3280, 1686, 1576, 1424, 1240, 1162.

Anal. Calcd for C$_{12}$H$_{14}$N$_2$O$_2$: C, 66.04; H, 6.47; N, 12.84, found: C, 66.35; H, 6.31; N, 12.44.

ethyl 4-((5-oxopyrrolidin-2-yl)amino)benzoate –29

HEI 2914



Following the general procedure, ethyl 4-((5-oxopyrrolidin-2-yl)amino)benzoate was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with ethyl 4-aminobenzoate (17.4 mmol, 2.8 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (3.2 g, 74% yield).

M.p. 141-142°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 7.71 (d, *J* = 9.6 Hz, 2H, Ar*H*), 6.67 (d, *J* = 9.6 Hz, 2H, Ar*H*), 5.19 (q, *J* = 3.4 Hz, 1H, C*H*), 4.22 (q, *J* = 7.0 Hz, 1H, C*H*$_2$), 2.48-2.26 (m, 2H, C*H*$_2$CH$_2$CH), 2.14 (m, 1H, CH$_2$C*H*$_2$CH), 1.84-1.70 (m,1H, CH$_2$C*H*$_2$CH), 1.27 (t, *J* = 7.0 Hz, 1H, C*H*$_3$).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.9 (C=O), 166.2 (C=O), 151.4 (C_{IV}), 131.3 (2CHAr), 117.6 (C_{IV}), 112.2 (2N

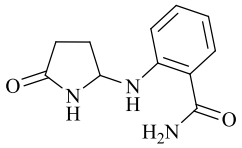
CHAr), 63.7 (CH), 60.1 (CH_2), 29.5 (CH_2), 28.4 (CH_2), 14.8 (CH_3).

IR ν (cm^{-1}): 3340, 3172, 3075, 1680, 1602, 1276, 1192.

Anal. Calcd for C$_{13}$H$_{16}$N$_2$O$_3$: C, 62.89; H, 6.50; N, 11.28.

2-((5-oxopyrrolidin-2-yl)amino)benzamide – 30

HEI 2987



Following the general procedure, 2-((5-oxopyrrolidin-2-yl)amino)benzamide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 2-aminobenzamide (17.4 mmol, 2.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 13 as a cream solid (3.2 g, 85% yield).

M.p. 195-196°C

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.47 (d, *J* = 6.6 Hz, 1H, N*H*), 8.43 (s, 1H, N*H*), 7.91 (s, 1H, N*H*), 7.62 (d, *J* = 6.6 Hz, 1H, N*H*), 7.26 (d, *J* = 6.6 Hz, 2H, Ar*H*), 6.75 (d, *J* = 6.6 Hz, 1H, Ar*H*), 6.62 (bs,1H, Ar*H*), 5.18 (bs, 1H, CH), 2.48-2.36 (bs, 1H, C*H*$_2$CH$_2$CH), 2.34-2.23 (bs, 1H, C*H*$_2$CH$_2$CH), 2.21-2.10 (bs,1H, CH$_2$C*H*$_2$CH), 1.84-1.70 (bs,1H, CH$_2$C*H*$_2$CH).

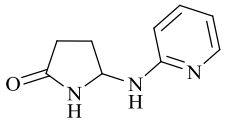
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.7 (C=O), 171.9 (C=O), 148.1 (C_{IV}), 133.0 (C_{IV}), 129.5 (CHAr), 115.7 (CHAr), 114.9 (CHAr), 112.7 (CHAr), 63.7 (CH), 29.4 (CH_2), 29.1 (CH_2).

IR ν (cm^{-1}): 3407, 3188, 1673, 1652, 1616, 1264.

Anal. Calcd for C$_{11}$H$_{13}$N$_3$O$_2$: C, 60.26; H, 5.98; N, 19.17; found: C, 59.85; H, 5.90; N, 19.07.

5-(pyridin-2-ylamino)pyrrolidin-2-one – 31

HEI 2622



Following the general procedure, 5-(pyridin-2-ylamino)pyrrolidin-2-one was obtained from the reaction of pyridin-2-amine (43 mmol, 5 g) with pyridin-2-amine (43 mmol, 4.9 g), in presence of 5 mol% CsF (2.07 mmol, 0.33 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound **31** as a white solid (4.6 g, 60% yield).

M.p. 158-159°C.

^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.10 (d, *J* = 5.8 Hz, 1H, CHAr), 7.45 (td, *J* = 8.1 Hz, *J* = 1.6 Hz, 1H, CHAr), 6.68 (t, *J* = 8.1 Hz, 1H, CHAr), 6.59 (bs, 1H, NH), 6.45 (d, *J* = 8.1 Hz, 1H, CHAr), 5.59 (td, *J* = 7.4 Hz, *J* = 4.9 Hz, 1H, CH), 4.74 (d, *J* = 7.0 Hz, 1H, NH), 2.62-2.41 (m,

2H, C*H*$_2$CH$_2$CH), 2.40-2.35 (m, 1H, CH$_2$C*H*$_2$CH), 1.90-1.61 (m, 1H, CH$_2$C*H*$_2$CH).

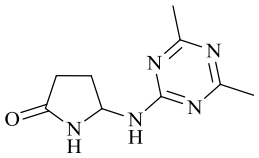
^{13}C-NMR ((100 MHz, CDCl$_3$): δ 176.2 (C=O), 157.1 (C$_{IV}$), 148.0 (CHAr), 137.4 (CHAr), 114.4 (CHAr), 109.0 (CHAr), 62.9 (CH), 29.0 (CH$_2$), 28.1 (CH$_2$).

IR ν (cm^{-1}): 3416, 3211, 1679, 1599, 1525, 1484, 1284, 1266.

Anal. Calcd for C$_9$H$_{11}$N$_3$O: C, 61.00; H, 6.26; N, 23.71; found: C, 60.86; H, 6.36; N, 23.43.

5-((4,6-dimethylpyrimidin-2-yl)amino)pyrrolidin-2-one – 32

HEI 3518



Following the general procedure, 5-((4,6-dimethylpyrimidin-2-yl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4,6-dimethyl-1,3,5-triazin-2-amine (17.4 mmol, 2.1 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 90% ethyl acetate, as 1.8 g of a white solid, in 51% yield.

M.p. 161-162°C.

^{1}H-NMR (400 MHz, CDCl$_3$): δ 6.90 (s, 1H, NH), 6.41 (s, 1H, CHAr), 5.64 (s, 1H, NH), 5.58 (m, 1H, CH), 2.52-2.57 (m, 1H, C*H*$_2$CH$_2$CH), 2.48-2.35 (m, 1H, CH$_2$C*H*$_2$CH), 2.27 (s, 6H, 2 CH$_3$), 1.93 (m, 1H, CH$_2$C*H*$_2$CH).

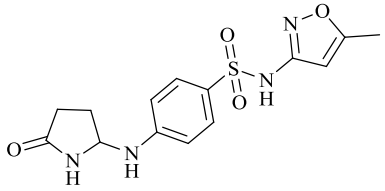
^{13}C-NMR ((100 MHz, CDCl$_3$): δ 176.8 (C=O), 167.8 (2 C$_{IV}$), 161.2 (C$_{IV}$), 111.2 (CHAr), 62.6 (CH), 29.1 (CH$_2$), 27.7 (CH$_2$), 23.8 (2 CH$_3$).

IR ν (cm^{-1}): 3266, 2973, 1672, 1566, 1540, 1314, 1215.

Anal. Calcd for C$_{10}$H$_{14}$N$_4$O: C, 58.24; H, 6.84; N, 27.17; found: C, 58.03; H, 6.64; N, 26.87.

5-((4-(((5-methylisoxazol-3-yl)methyl)sulfonyl)phenyl)amino)pyrrolidin-2-one – 33

HEI 3272



Following the general procedure, 5-((4-(((5-methylisoxazol-3-yl)methyl)sulfonyl) phenyl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one

(17.4 mmol, 2.0 g) with sulfamethoxazole (17.4 mmol, 4.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (30 mL), filtered off and washed with ethanol (3x10 mL), affording the compound as a white solid (3.2 g, 55% yield).

M.p. 231-232°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.00 (bs, 1H, N*H*), 8.36 (s, 1H, N*H*), 7.56 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.12 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.71 (d, *J* = 8.0 Hz, 2H, Ar*H*), 6.11 (s, 1H, N*H*), 5.18 (bs, 1H, C*H*), 2.50-2.34 (m, 2H, C*H*$_2$CH$_2$CH), 2.28 (s, 3H, C*H*$_3$), 2.1-2.12 (m, 2H, CH$_2$C*H*$_2$CH), 1.84-1.81 (m, 2H, CH$_2$C*H*$_2$CH),

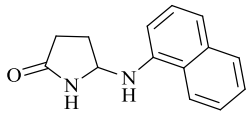
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.8 (C$_{IV}$), 170.4 (C$_{IV}$), 158.3 (C$_{IV}$), 151.1 (C$_{IV}$), 129.1 (2 CHAr), 126.1 (C$_{IV}$), 112.3 (2 CHAr), 95.7 (CHAr), 63.5 (CH), 29.4 (CH$_2$), 28.3 (CH$_2$), 12.5 (CH$_3$).

IR ν (cm^{-1}): 3387, 3220, 1681, 1596, 1325, 1157, 1095.

Anal. Calcd for C$_{14}$H$_{16}$N$_4$O$_4$S: C, 49.99; H, 4.79; N, 16.66; S, 9.53.

5-(naphthalen-1-ylamino)pyrrolidin-2-one – 34

HEI 2902



Following the general procedure, 5-(naphthalen-1-ylamino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 1-naphthylamine (17.4 mmol, 2.5 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a white solid (2.4 g, 60% yield).

M.p. 137-138°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 8.39 (bs, 1H, N*H*), 8.21 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.77 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.41 (m, 2H, Ar*H*), 7.27 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.19 (d, *J* = 7.6 Hz, 1H, Ar*H*), 6.65 (d, *J* = 7.6 Hz, 1H, Ar*H*), 6.46 (d, *J* = 8.4 Hz, 1H, N*H*), 5.31 (d, *J* = 3.4 Hz, 1H, C*H*), 2.45-2.32 (m, 2H, C*H*$_2$CH$_2$CH), 2.11-2.09 (m, 2H, CH$_2$C*H*$_2$CH).

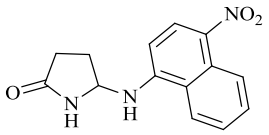
^{13}C-NMR (DMSO-d$_6$, 100 MHz): δ ppm 176.5 (C=O), 141.9 (C$_{IV}$), 133.9 (C$_{IV}$), 127.8 (CHAr), 126.5 (CHAr), 125.6 (CHAr), 124.0 (CHAr), 123.2 (CHAr), 121.9 (CHAr), 116.7 (C$_{IV}$), 105.0 (CHAr), 64.1 (CH), 29.1 (CH$_2$), 27.7 (CH$_2$).

IR ν (cm^{-1}): 3353, 3048, 1702, 1579, 1530, 1409, 1258.

Anal. Calcd for C$_{14}$H$_{14}$N$_2$O: C, 74.31; H, 6.24; N, 12.38.

5-((4-nitronaphthalen-1-yl)amino)pyrrolidin-2-one – 35

HEI 3223



Following the general procedure, 5-((4-nitronaphthalen-1-yl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-nitronaphthalen-1-amine (17.4 mmol, 3.3 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a white solid (2.5 g, 53% yield).

M.p. 204-205°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 8.83 (dd, *J* = 9.2 Hz, *J* = 1.0 Hz, 1H, N*H*), 8.48 (d, *J* = 8.0 Hz, 2H, Ar*H*), 8.40 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.90 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.77 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.61 (t, *J* = 8.0 Hz, 1H, Ar*H*), 6.77 (d, *J* = 9.2 Hz, 1H, N*H*), 5.52 (t, *J* = 5.1 Hz, 1H, C*H*), 2.45-2.35 (m, 2H, C*H*$_2$CH$_2$CH), 2.19-2.15 (m, 2H, CH$_2$C*H*$_2$CH).

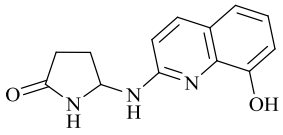
^{13}C-NMR (DMSO-d$_6$, 100 MHz): δ ppm 176.7 (C=O), 149.2 (C$_{IV}$), 133.5 (C$_{IV}$), 130.0 (CHAr), 129.5 (CHAr), 127.0 (C$_{IV}$), 125.4 (CHAr), 123.3 (CHAr), 123.0 (CHAr), 121.7 (C$_{IV}$), 102.5 (CHAr), 63.8 (CH), 28.8 (CH$_2$), 27.3 (CH$_2$).

IR ν (cm^{-1}): 3402, 3164, 3079, 1692, 1573, 1482, 1253, 1182.

Anal. Calcd for C$_{14}$H$_{13}$N$_3$O$_3$: C, 61.99; H, 4.83; N, 15.49.

5-((8-hydroxynaphthalen-2-yl)amino)pyrrolidin-2-one – 36

HEI 2997



Following the general procedure, 5-((8-hydroxynaphthalen-2-yl)amino)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 7-aminonaphthalen-1-ol (17.4 mmol, 2.8 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (1.9 g, 45% yield).

M.p. 122-123°C.

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 8.95 (bs, 1H, N*H*), 7.84 (d, *J* = 8.8 Hz, 1H, Ar*H*), 7.55 (bs, 1H, N*H*), 7.00 (m, 2H, Ar*H*), 6.84 (dd, *J* = 6.9 Hz, *J* = 1.9 Hz 1H, Ar*H*), 6.73 (d, *J* =

8.8 Hz, 1H, Ar*H*), 5.63 (bs, 1H, C*H*), 3.45 (bs, 1H, O*H*), 2.32-2.40 (m, 2H, C*H*$_2$CH$_2$CH), 2.12 (m, 1H, CH$_2$C*H*$_2$CH), 1.92 (m, 1H, CH$_2$C*H*$_2$CH).

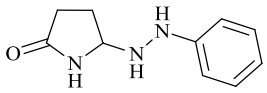
^{13}C-NMR (DMSO-d$_6$, 100 MHz): δ ppm 176.3 (C=O), 154.6 (C$_{IV}$), 137.7 (C$_{IV}$), 136.6 (C$_{IV}$), 136.6 (CHAr), 123.5 (CHAr), 122.2 (CHAr), 116.5 (C$_{IV}$), 112.8 (CHAr), 111.8 (CHAr), 61.8 (CH), 29.1 (CH$_2$), 27.4 (CH$_2$).

IR ν (cm^{-1}): 3364, 3161, 1694, 1612, 1529, 1266, 1241.

Anal. Calcd for C$_{14}$H$_{14}$N$_2$O$_2$: C, 69.41; H, 5.82; N, 11.56.

5-(2-phenylhydrazinyl)pyrrolidin-2-one – 37

HEI 2909



Following the general procedure, 5-(2-phenylhydrazinyl)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with phenylhydrazine (17.4 mmol, 1.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 16 as a white solid (2.8 g, 85% yield).

M.p. 102-103°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.04 (bd, 1H, N*H*), 7.07 (td, *J* = 7.9 Hz, *J* = 1.2 Hz, 2H, Ar*H*), 6.77 (dd, *J* = 9.11 Hz, *J* = 1.2 Hz, 2H, Ar*H*), 6.76 (bs, 1H, N*H*), 6.58 (td, *J* = 7.9 Hz, *J* = 1.2 Hz, 1H, Ar*H*), 4.78 (dd, *J* = 6.8 Hz, *J* = 1.3 Hz, 1H, N*H*), 4.48 (td, *J* = 6.8 Hz, *J* = 3.4 Hz, 1H, CH), 2.33-1.95 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.8-1.70 (m, 1H, CH$_2$C*H*$_2$CH).

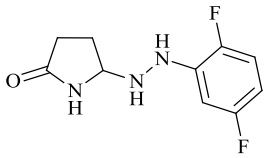
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.0 (C=O), 150.8 (C$_{IV}$), 129.0 (2 CHAr), 117.5 (CHAr), 112.4 (2 CHAr), 70.6 (CH), 29.7 (CH$_2$), 25.5 (CH$_2$).

IR ν (cm^{-1}): 3287, 3022, 1635, 1596, 1245.

Anal. Calcd for C$_{10}$H$_{13}$N$_3$O: C, 62.81; H, 6.85; N, 21.97; found: 62.64; H, 6.94; N, 21.91.

5-(2-(2,5-difluorophenyl)hydrazinyl)pyrrolidin-2-one – 38

HEI 2943



Following the general procedure, 5-(2-(2,5-difluorophenyl)hydrazinyl)pyrrolidin-2-one was

obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with (2,5-difluorophenyl)hydrazine (17.4 mmol, 2.5 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g) after 5 hours. The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (3x10 mL), affording the compound as a white solid (3.1 g, 79% yield).

M.p. 163-164°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.05 (s, 1H, N*H*), 7.02 (m, 2H, Ar*H*), 6.88 (m, 1H, Ar*H*), 6.34 (m, 1H, Ar*H*), 5.0 (d, *J* = 6.8 Hz, 1H, C*H*), 4.78 (q, *J* = 6.4 Hz, 1H, C*H*), = 2.25-2.19 (m, 2H, *CH*$_2$CH$_2$CH), 2.08 (m, 1H, CH$_2$*CH*$_2$CH), 1.81 (m, 1H, CH$_2$*CH*$_2$CH).

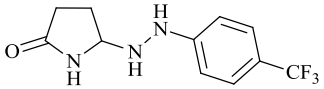
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.4 (C=O), 159.1 (dd, $J_{C\text{-}F}$ = 237.7 Hz, $J_{C\text{-}F}$ = 1.5 Hz C$_{IV}$), 139.8 (t, $J_{C\text{-}F}$ = 12.2 Hz, C$_{IV}$), 115.1 (dd, $J_{C\text{-}F}$ = 20.5 Hz, $J_{C\text{-}F}$ = 10.6 Hz, CHAr), 101.6 (dd, $J_{C\text{-}F}$ = 24.3 Hz, $J_{C\text{-}F}$ = 6.9 Hz, CHAr), 100.1 (dd, $J_{C\text{-}F}$ = 28.8 Hz, $J_{C\text{-}F}$ = 3.8 Hz, CHAr), 70.3 (CH), 29.1 (CH$_2$), 25.2 (CH$_2$).

IR ν (cm^{-1}): 3312, 3290, 1690, 1596, 1310, 1085.

Anal. Calcd for C$_{10}$H$_{13}$N$_3$O: C, 62.81; H, 6.85; N, 21.97.

5-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)pyrrolidin-2-one – 39

HEI 3192



Following the general procedure, 5-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (8.7 mmol, 1 g) with (4-(trifluoromethyl) phenyl)hydrazine (8.7 mmol, 1.5 g), in presence of 5 mol% CsF (0.43 mmol, 0.066 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 100% ethyl acetate, as 0.5 g of a yellow solid, in 20% yield.

M.p. 126-127°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 7.89 (s, 1H, N*H*), 7.46 (s, 1H, N*H*), 7.37 (d, *J* = 9.2 Hz, 2H, Ar*H*), 6.91 (d, *J* = 9.2 Hz, 2H, Ar*H*), 5.02 (dd, 1H, N*H*, *J* = 6.8 Hz), 4.49 (td, *J* = 6.8 Hz, *J* = 3.5 Hz, 1H, CH), 2.29-2.10 (m, 2H, C*H*$_2$CH$_2$CH), 2.09-1.97 (m, 1H, CH$_2$C*H*$_2$CH), 1.84-1.72 (m, 1H, CH$_2$C*H*$_2$CH).

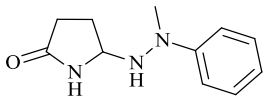
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.0 (C=O), 154.1 (C$_{IV}$), 126.5 (C$_{IV}$), 111.5 (4 CHAr), 70.5 (CH), 29.5 (CH$_2$), 25.4 (CH$_2$).

IR ν (cm^{-1}): 3303, 1698, 1613, 1321, 1095.

Anal. Calcd for C$_{11}$H$_{12}$F$_3$N$_3$O: C, 50.97; H, 4.67; N, 16.21; found: C, 51.16; H, 4.95; N, 15.86.

5-(2-methyl-2-phenylhydrazinyl)pyrrolidin-2-one – 40

HEI 3180



Following the general procedure, 5-(2-methyl-2-phenylhydrazinyl)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 1-methyl-1-phenylhydrazine (17.4 mmol, 2.1 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (2.1 g, 60% yield).

M.p. 136-137°C

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.01 (s, 1H, N*H*), 7.14 (dd, *J* = 7.1 Hz, *J* = 1.6 Hz, 2H, Ar*H*), 6.94 (d, *J* = 8.9 Hz, *J* = 1.2 Hz, 2H, Ar*H*), 6.65 (t, *J* = 7.0 Hz, 1H, Ar*H*), 4.70 (d, *J* = 3.8 Hz, 1H, N*H*), 4.65 (quin, *J* = 3.8 Hz, 1H, CH), 3.02 (s, 3H, CH_3), 2.34-2.21 (m, 1H, C*H*$_2$CH$_2$CH), 2.19-1.95 (m, 2H, C*H*$_2$C*H*$_2$CH), 1.79-1.67 (m, 1H, CH$_2$C*H*$_2$CH).

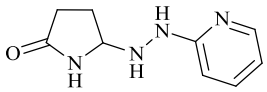
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.4 (C=O), 152.9 (C_{IV}), 128.9 (2 CHAr), 117.7 (CHAr), 113.4 (2 CHAr), 69.1 (CH), 39.3 (CH_3), 29.4 (CH_2), 26.0 (CH_2).

IR ν (cm^{-1}): 3214, 2928, 1681, 1596, 1503, 1256.

Anal. Calcd for C$_{11}$H$_{15}$N$_3$O: C, 64.37; H, 7.37; N, 20.47; found: C, 63.97; H, 7.10; N, 20.36.

5-(2-(pyridin-2-yl)hydrazinyl)pyrrolidin-2-one – 41

HEI 3179



Following the general procedure, 5-(2-(pyridin-2-yl)hydrazinyl)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 2-hydrazinylpyridine (17.4 mmol, 1.9 g), in presence of 5% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a cream solid (2.5 g, 75% yield).

M.p. 176-177°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 7.97 (s, 1H, N*H*), 7.96 (s, 1H, N*H*), 7.46 (d, *J* = 6.2 Hz, 1H, Ar*H*), 7.43 (s, 1H, Ar*H*), 6.80 (d, *J* = 8.3 Hz, 1H, Ar*H*), 6.57 (t, *J* = 6.2 Hz, 1H, Ar*H*), 5.04 (dd, *J* = 5.2 Hz, *J* = 1.3 Hz, 1H, N*H*), 4.50 (m, 1H, CH), 2.28-2.10 (m, 2H, C*H*$_2$CH$_2$CH), 2.09-1.96 (m, 1H, CH$_2$C*H*$_2$CH), 1.86-1.72 (m, 1H, CH$_2$C*H*$_2$CH).

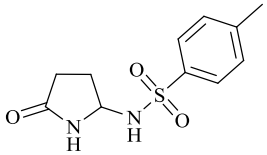
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): δ 177.0 (C=O), 161.5 (C_{IV}), 147.8 (CHAr), 137.7 (CHAr), 113.7 (CHAr), 106.8 (CHAr), 70.9 (CH), 29.6 (CH_2), 25.7 (CH_2).

IR ν (cm^{-1}): 3256, 2815, 1672, 1606, 1457, 1291.

Anal. Calcd for $C_9H_{12}N_4O$: C, 56.24; H, 6.29; N, 29.15; found: C, 56.04; H, 6.19; N, 28.86.

4-methyl-*N*-(5-oxopyrrolidin-2-yl)benzenesulfonamide – 42

HEI 3042



Following the general procedure, 4-methyl-*N*-(5-oxopyrrolidin-2-yl)benzenesulfonamide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-methylbenzenesulfonamide (17.4 mmol, 3.0 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 21 as a white solid (2.6 g, 60% yield).

M.p. 141-142°C

^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.43 (s, 1H, N*H*), 8.12 (s, 1H, N*H*), 7.82 (d, *J* = 8.1 Hz, *J* = 4.4 Hz, 2H, Ar*H*), 7.56-7.66 (m, 3H, Ar*H*), 4.99 (d, *J* = 7.4 Hz, 1H, CH), 2.26-2.15 (m, 2H, C*H*$_2$CH$_2$CH), 2.08 (s, 3H, CH_3), 2.00-1.88 (m, 1H, $CH_2C\underline{H_2}CH$), 1.57-1.47 (m, 1H, $CH_2C\underline{H_2}CH$).

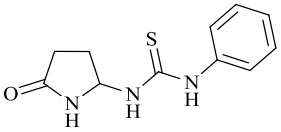
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): δ 176.6 (C=O), 142.4 (C_{IV}), 132.9 (C_{IV}), 129.8 (2 CHAr), 126.7 (2 CHAr), 65.2 (CH), 31.3 (CH_3), 28.8 (CH_2), 28.5 (CH_2).

IR ν (cm^{-1}): 3192, 3065, 1663, 1341, 1162.

Anal. Calcd for $C_{11}H_{14}N_2O_3S$: C, 51.95; H, 5.55; N, 11.02; S, 12.61; found: C, 59.63; H, 5.23; N, 11.48; S, 13.00.

1-(5-oxopyrrolidin-2-yl)-3-phenylthiourea – 45

HEI 2956



Following the general procedure, 1-(5-oxopyrrolidin-2-yl)-3-phenylthiourea was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 1-phenylthiourea (17.4 mmol, 2.6 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was

precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 24 as a yellow solid (2.5 g, 60% yield).

M.p. 169-170°C

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.50 (s, 1H, N*H*), 8.25 (d, *J* = 7.3 Hz, 1H, N*H*), 7.11 (t, 1H, Ar*H*), 8.13 (s, 1H, N*H*), 7.39 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.32 (t, *J* = 8.2 Hz, 2H, Ar*H*), 5.88 (t, *J* = 7.9 Hz, 1H, CH), 2.33-2.27 (m, 2H, C*H*$_2$CH$_2$CH), 2.11-2.05 (m, 1H, CH$_2$C*H*$_2$CH), 1.95-1.85 (m, 1H, CH$_2$C*H*$_2$CH).

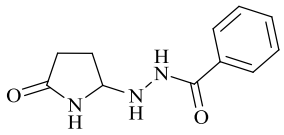
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 179.9 (C=S), 176.9 (C=O), 139.4 (C_{IV}), 129.0 (3 CHAr), 124.8 (CHAr), 123.7 (CHAr), 65.5 (CH), 29.2 (CH_2), 28.1 (CH_2).

IR ν (cm^{-1}): 3283, 3196, 1674, 1523, 1254.

Anal. Calcd for C$_{11}$H$_{13}$N$_3$OS: C, 56.15; H, 5.57; N, 17.86; S, 13.63; found: C, 56.18; H, 5.57; N, 17.74; S, 14.18.

N'-(5-oxopyrrolidin-2-yl)benzohydrazide – 46

HEI 2970



Following the general procedure, *N'*-(5-oxopyrrolidin-2-yl)benzohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with benzohydrazide (17.4 mmol, 2.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (3.62g, 95% yield).

M.p. 168-169°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.94 (d, *J* = 5.9 Hz, 1H, N*H*), 7.93 (s, 1H, N*H*), 7.82 (d, *J* = 7.6 Hz, 2H, Ar*H*), 7.53 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.47 (t, *J* = 7.6 Hz, 2H, Ar*H*), 5.48 (bs, 1H, C*H*), 4.60 (bs, 1H, N*H*), 2.38-1.83 (m, 4H, C*H*$_2$C*H*$_2$CH).

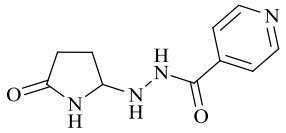
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.6 (C=O), 166.6 (C=O), 133.5 (C_{IV}), 131.8 (CHAr), 128.7 (2 CHAr), 127.8 (2 CHAr), 70.5 (CH), 29.3 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3249, 1646, 1460, 1290, 1241.

Anal. Calcd for C$_{11}$H$_{13}$N$_3$O$_2$: C, 60.26; H, 5.98; N, 19.17; found: C, 60.21; H, 5.91; N, 18.80.

_N'_-(5-oxopyrrolidin-2-yl)picolinohydrazide – 47

HEI 2947



Following the general procedure, _N'_-(5-oxopyrrolidin-2-yl)picolinohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with picolinohydrazide (17.4 mmol, 2.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The product was filtered and washed with ethanol (3x10 mL), affording the compound as a white solid (0.7 g, 35% yield).

M.p. 141-142°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.09 (t, _J_ = 6.8 Hz, 1H, N_H_), 8.93 (s, 1H, Ar_H_), 8.66 (dd, _J_ = 4.7 Hz, _J_ = 1.5 Hz, 1H, Ar_H_), 8.13 (d, _J_ = 8.2 Hz, 1H, Ar_H_), 7.95 (s, 1H, N_H_), 7.47 (dd, _J_ = 7.8 Hz, _J_ = 5.1 Hz, 1H, Ar_H_), 5.52 (t, _J_ = 5.8 Hz, 1H, CH), 4.58 (bs, 1H, N_H_), 2.45-2.22 (m, 2H, C_H_$_2$CH$_2$CH), 2.15-2.00 (m, 1H, CH$_2$C_H_$_2$CH), 1.82 (m, 1H, CH$_2$C_H_$_2$CH).

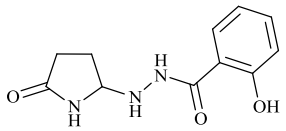
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.4 (C=O), 165.0 (C=O), 152.4 (C_{IV}), 148.8 (CHAr), 135.5 (CHAr), 129.2 (CHAr), 123.9 (CHAr), 70.2 (CH), 29.2 (CH_2), 25.5 (CH_2).

IR ν (cm^{-1}): 3360, 3288, 3064, 1674, 1645, 1274, 1104.

Anal. Calcd for C$_{10}$H$_{12}$N$_4$O$_2$: C, 54.54; H, 5.49; N, 25.44.

2-hydroxy-_N'_-(5-oxopyrrolidin-2-yl)benzohydrazide – 48

HEI 3238



Following the general procedure, 2-hydroxy-_N'_-(5-oxopyrrolidin-2-yl)benzohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (8.7 mmol, 1.0 g) with 2-hydroxybenzohydrazide (8.7 mmol, 1.3 g), in presence of 5 mol% CsF (0.43 mmol, 0.066 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (1.7 g, 80% yield).

M.p. 171-172°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 12.12 (s, 1H, O_H_), 10.11 (s, 1H, N_H_), 8.00 (s, 1H, N_H_), 7.82 (dd, _J_ = 7.8 Hz, _J_ = 1.6 Hz, 1H, Ar_H_), 7.40 (td, _J_ = 8.5 Hz, _J_ = 1.6 Hz, 1H, Ar_H_), 6.90 (m,

2H, Ar*H*), 5.62 (bs, 1H, C*H*), 4.64 (bs, 1H, N*H*), 2.25-2.21 (m, 2H, C*H*$_2$CH$_2$CH), 2.06-1.92 (m, 2H, CH$_2$C*H*$_2$CH).

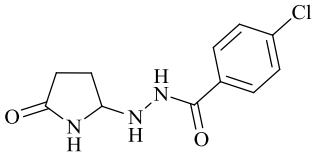
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 179.4 (C=O), 167.1 (C=O), 154.2 (C_{IV}), 131.9 (CHAr), 126.4 (CHAr), 117.9 (CHAr), 114.7 (CHAr), 68.9 (CH), 26.7 (CH_2), 22.0 (CH_2).

IR ν (cm^{-1}): 3259, 1644, 1469, 1284, 1210.

Anal. Calcd for C$_{11}$H$_{13}$N$_3$O$_3$: C, 56.16; H, 5.57; N, 17.86.

4-chloro-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide – 49

HEI 2939



Following the general procedure, 4-chloro-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-chlorobenzohydrazide (17.4 mmol, 2.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a white solid (2.2 g, 50% yield).

M.p. 197-198°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.99 (t, *J* = 3.8 Hz, 1H, N*H*), 7.96 (s, 1H, Ar*H*), 7.78 (d, *J* = 4.1 Hz, 2H, Ar*H*), 7.16 (t, *J* = 3.9 Hz, 1H, Ar*H*), 5.47 (bs, 1H, CH), 4.61 (t, *J* = 3.2 Hz, 1H, N*H*), 2.24 (m, 1H, C*H*$_2$CH$_2$CH), 2.10 (m, 1H, C*H*$_2$CH$_2$CH), 2.01 (m, 1H, CH$_2$C*H*$_2$CH), 1.91 (m, 1H, CH$_2$C*H*$_2$CH).

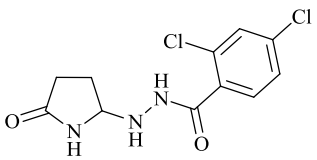
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.3 (C=O), 165.5 (C=O), 136.6 (C_{IV}), 132.3 (C_{IV}), 129.7 (2CHAr), 128.8 (2CHAr), 70.3 (CH), 29.2 (CH_2), 25.5 (CH_2).

IR ν (cm^{-1}): 3322, 3260, 1690, 1643, 1464, 1344, 1090.

Anal. Calcd for C$_{11}$H$_{12}$ClN$_3$O$_2$: C, 52.08; H, 4.77; N, 16.56.

2,4-dichloro-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide – 50

HEI 3008



Following the general procedure, 2,4-dichloro-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (0.84 mmol, 0.5 g) with 2,4-dichlorobenzohydrazide (0.84 mmol, 1.5 g), in presence of 5 mol% CsF (0.21 mmol, 0.033 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (0.5 g, 30% yield).

M.p. 238-239°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.88 (d, *J* = 6.5 Hz, 1H, N*H*), 7.86 (s, 1H, N*H*), 7.69 (d, *J* = 1.6 Hz, 1H, Ar*H*), 7.54 (d, *J* = 8.38 Hz, 1H, Ar*H*), 7.52 (d, *J* = 8.38 Hz, 1H, Ar*H*), 5.56 (t, *J* = 6.4 Hz, 1H, CH), 4.62 (t, *J* = 3.2 Hz, 1H, N*H*), 2.25 (m, 1H, C*H*$_2$CH$_2$CH), 2.12 (m, 1H, C*H*$_2$CH$_2$CH), 2.03 (m, 1H, CH$_2$C*H*$_2$CH), 1.90 (m, 1H, CH$_2$C*H*$_2$CH).

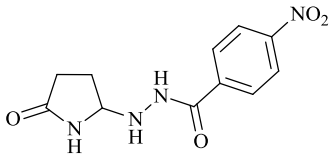
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.7 (C=O), 164.7 (C=O), 134.7 (C$_{IV}$), 134.1 (C$_{IV}$), 131.5 (C$_{IV}$), 130.6 (CHAr), 129.0 (CHAr), 127.1 (CHAr), 69.7 (CH), 28.7 (CH$_2$), 24.9 (CH$_2$).

IR ν (cm^{-1}): 3305, 3165, 1672, 1660, 1480, 1360, 1106.

Anal. Calcd for C$_{11}$H$_{11}$Cl$_2$N$_3$O$_2$: C, 45.85; H, 3.85; N, 14.58.

4-nitro-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide – 51

HEI 2940



Following the general procedure, 4-nitro-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-nitrobenzohydrazide (17.4 mmol, 3.1 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a yellow solid (2.5 g, 54% yield).

M.p. 209-210°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.27 (t, *J* = 5.6 Hz, 1H, N*H*), 8.33 (d, *J* = 9.1 Hz, 2H, Ar*H*), 8.07 (d, *J* = 9.1 Hz, 2H, Ar*H*), 8.03 (s, 1H, N*H*), 5.60 (t, *J* = 6.0 Hz, 1H, CH), 4.64 (bs, 1H, N*H*), 2.26-2.22 (m, 1H, C*H*$_2$CH$_2$CH), 2.13 (m, 1H, C*H*$_2$CH$_2$CH), 2.04 (m, 1H, CH$_2$C*H*$_2$CH), 1.92 (m, 1H, CH$_2$C*H*$_2$CH).

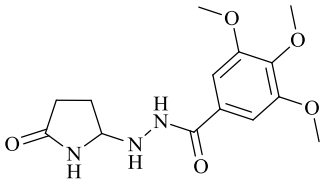
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.3 (C=O), 164.7 (C=O), 149.5 (C$_{IV}$), 139.3 (C$_{IV}$), 129.3 (2CHAr), 123.9 (2CHAr), 70.2 (CH), 29.3 (CH$_2$), 25.4 (CH$_2$).

IR ν (cm^{-1}): 3306, 3261, 1704, 1659, 1515, 1463, 1343, 1299.

Anal. Calcd for $C_{11}H_{12}N_4O_4$: C, 50.00; H, 4.58; N, 21.20.

3,4,5-trimethoxy-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide – 52

HEI 2941



Following the general procedure, 3,4,5-trimethoxy-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 3,4,5-trimethoxybenzohydrazide (17.4 mmol, 3.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g).The product was filtered and washed with ethanol (3x10 mL), affording the compound as a white solid (2.4g, 60% yield).

M.p. 170-171°C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.91 (d, *J* = 5.0 Hz, 1H, N*H*), 7.96 (s, 1H, N*H*), 7.18 (s, 2H, Ar*H*), 5.43 (t, *J* = 5.59 Hz, 1H, C*H*), 4.60 (s, 1H, N*H*), 3.82 (s, 6H, OCH_3), 3.70 (s, 3H, OCH_3), 2.23 (m, 1H, CH_2CH$_2$CH), 2.07 (m, 2H, CH_2CH_2CH), 1.92 (m, 1H, CH$_2$CH_2CH).

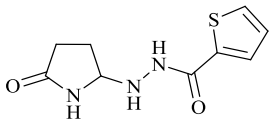
^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 177.4 (C=O), 166.1 (C=O), 153.0 (C_{IV}), 140.4 (C_{IV}), 128.6 (C_{IV}), 105.2 (2CHAr), 70.3 (CH), 60.5 (OCH_3), 56.4 ($2OCH_3$), 29.2 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3411, 3237, 1683, 1582, 1340, 1242, 1129.

Anal. Calcd for $C_{14}H_{19}N_3O_5$: C, 54.36; H, 6.19; N, 13.58.

N'-(5-oxopyrrolidin-2-yl)thiophene-2 carbohydrazide – 53

HEI 2944



Following the general procedure, *N'*-(5-oxopyrrolidin-2-yl)thiophene-2-carbohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with thiophene-2Carbohydrazide (17.4 mmol, 2.5 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a cream solid (2.15 g, 55% yield).

M.p. 179-178°C

^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.97 (d, *J* = 5.1 Hz, 1H, N*H*), 7.94 (s, 1H, N*H*), 7.77 (dd,

J = 7.0 Hz, *J* = 4.5 Hz, 2H, Ar*H*), 7.15 (t, *J* = 5.2 Hz, 1H, Ar*H*), 5.46 (t, *J* = 4.8 Hz, 1H, C*H*), 4.60 (bs, 1H, N*H*), 2.25 (m, 1H, C$H_2$$CH_2$CH), 2.09 (m, 1H, C$H_2$$CH_2$CH), 2.01 (m, 1H, CH_2CH_2CH), 1.92 (m, 1H, CH_2CH_2CH).

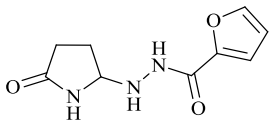
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.3 (C=O), 161.6 (C=O), 138.4 (C_{IV}), 131.3 (CHAr), 128.7 (CHAr), 128.4 (CHAr), 70.3 (CH), 29.2 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3212, 3097, 1660, 1634, 1309, 1243.

Anal. Calcd for $C_9H_{11}N_3O_2S$: C, 47.99; H, 4.92; N, 18.65; S, 14.23.

N'-(5-oxopyrrolidin-2-yl)furan-2-carbohydrazide – 54

HEI 3271



Following the general procedure, *N'*-(5-oxopyrrolidin-2-yl)furan-2-carbohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with furan-2-carbohydrazide (17.4 mmol, 2.2 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (0.3 g, 38% yield).

M.p. 184-185°C

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.47 (s, 1H, N*H*), 7.88 (s, 1H, N*H*), 7.76 (d, *J* = 1.0 Hz, 2H, Ar*H*), 7.36 (s, 1H, Ar*H*), 7.21 (s, 1H, Ar*H*), 6.82 (s, 1H, N*H*), 6.65 (m, 1H, C*H*), 2.46 (d, *J* = 11.2 Hz, 2H, C$H_2$$CH_2$CH), 2.31 (t, *J* = 7.2 Hz, 2H, CH_2CH_2CH).

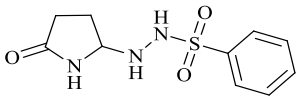
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 173.6 (C=O), 154.4 (C=O), 152.0 (C_{IV}), 147.1 (C_{IV}), 145.9 (CHAr), 114.9 (CHAr), 112.3 (CHAr), 79.6 (CH), 56.5 (CH), 32.0 (CH_2), 28.4 (CH_2).

IR ν (cm^{-1}): 3349, 3176, 3076, 1657, 1625, 1350, 1301, 1191.

Anal. Calcd for $C_9H_{11}N_3O_3$: C, 51.67; H, 5.30; N, 20.09.

N'-(5-oxopyrrolidin-2-yl)benzenesulfonohydrazide – 55

HEI 3040



Following the general procedure, *N'*-(5-oxopyrrolidin-2-yl)benzenesulfonohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with benzenesulfonohydrazide (17.4 mmol, 3.0 g), in presence of 5 mol% CsF (0.86 mmol, 0.132

g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (3.5 g, 80% yield).

M.p. 168-169°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.81 (d, *J* = 3.6 Hz, 1H, N*H*), 7.82 (d, *J* = 6.9 Hz, 1H, Ar*H*), 7.81 (s, 1H, N*H*), 7.62-7.56 (m, CHAr, 4H), 4.95 (q, *J* = 3.6 Hz, 1H, CH), 4.29 (td, *J* = 7.1 Hz, *J* = 1.8 Hz, 1H, N*H*), 2.20-1.78 (m, 4H, C*H*$_2$C<u>H$_2$</u>CH).

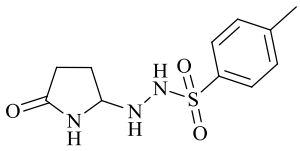
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.1 (C=O), 139.7 (C$_{IV}$), 133.0 (CHAr), 129.4 (2 CHAr),127.9 (2 CHAr), 70.8 (CH), 29.3 (CH$_2$), 25.1 (CH$_2$).

IR ν (cm^{-1}): 3302, 3208, 3090, 1695, 1314, 1155, 1089.

Anal. Calcd for C$_{10}$H$_{13}$N$_3$O$_3$S: C, 47.05; H, 5.13; N, 16.46; S, 12.56; found: C, 47.13; H, 5.49; N, 16.91; S, 12.84.

4-methyl-*N'*-(5-oxopyrrolidin-2-yl)benzenesulfonohydrazide –56

HEI 3035



Following the general procedure, 4-methyl-*N'*-(5-oxopyrrolidin-2-yl)benzenesulfonohydrazide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-methylbenzenesulfonohydrazide (17.4 mmol, 3.2 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (3x10 mL), affording the compound as a white solid (3.0 g, 65% yield).

M.p. 152-153°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.70 (d, *J* = 3.2 Hz, 1H, N*H*), 7.69 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.60 (s, 1H, N*H*), 7.38 (d, *J* = 8.0 Hz, 2H, Ar*H*), 4.91 (q, *J* = 3.6 Hz, 1H, C*H*), 4.27 (d, *J* = 1.6 Hz, 1H, N*H*), 2.37 (s, 3H, CH$_3$), 2.19-2.10 (m, 1H, C*H*$_2$CH$_2$CH), 2.09-2.04 (m, 2H, C*H*$_2$C*H*$_2$CH), 1.78 (m, 1H, CH$_2$C*H*$_2$CH).

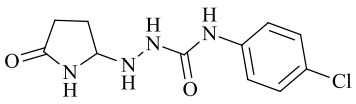
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.0 (C=O), 143.3 (C$_{IV}$), 136.8 (C$_{IV}$), 129.7 (2 CHAr), 127.9 (2 CHAr), 70.8 (CH), 29.3 (CH$_2$), 25.1 (CH$_2$), 21.4 (CH$_3$).

IR ν (cm^{-1}): 3281, 3073, 1686, 1338, 1164, 1091.

Anal. Calcd for C$_{11}$H$_{15}$N$_3$O$_3$S: C, 49.06; H, 5.61; N, 15.60; S, 11.91.

***N*-(4-chlorophenyl)-2-(5-oxopyrrolidin-2-yl)hydrazinecarboxamide – 57**

HEI 2946



Following the general procedure, *N*-(4-chlorophenyl)-2-(5-oxopyrrolidin-2-ylhydrazine carboxamide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with *N*-(4-chlorophenyl)hydrazinecarboxamide (17.4 mmol, 3.2 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a grey solid (1.4 g, 30% yield).

M.p. 180-181°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.69 (s, 1H, N*H*), 9.22 (s, 1H, N*H*), 7.91 (s, 1H, N*H*), 7.65 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.36 (t, *J* = 8.0 Hz, 2H, Ar*H*), 5.64 (s, 1H, C*H*), 4.50 (bs, 1H, N*H*), 2.31 (m, 1H, C*H*$_2$CH$_2$CH), 2.25 (m, 1H, C*H*$_2$CH$_2$CH), 2.17 (m, 1H, CH$_2$C*H*$_2$CH), 1.91 (m, 1H, CH$_2$C*H*$_2$CH).

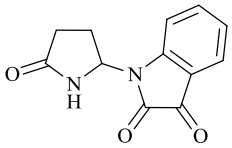
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 180.4 (C=O), 177.0 (C=O), 138.5 (C_{IV}), 128.9 (C_{IV}), 128.2 (2 CHAr), 126.5 (2 CHAr), 70.6 (CH), 29.3 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3286, 3241, 3190, 1672, 1549, 1251, 1211.

Anal. Calcd for C$_{11}$H$_{13}$ClN$_4$O$_2$: C, 49.17; H, 4.88; N, 20.85;

1-(5-oxopyrrolidin-2-yl)indoline-2,3-dione – 58

HEI 2972



Following the general procedure, 1-(5-oxopyrrolidin-2-yl)indoline-2,3-dione was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with indoline-2,3-dione (17.4 mmol, 2.6 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 27 as an orange solid (2.6 g, 65% yield).

M.p. 187-188°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.14 (s, 1H, N*H*), 7.68 (td, *J* = 7.64 Hz, *J* = 1.30 Hz, 1H, Ar*H*), 7.61 (dd, *J* = 7.64 Hz, *J* = 1.30 Hz, 1H, Ar*H*), 7.17 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.15 (d, *J* = 7.6 Hz, 1H, Ar*H*), 5.91 (dd, *J* = 9.0 Hz, *J* = 3.2 Hz, 1H, CH), 2.65-2.53 (m, 2H, C*H*$_2$CH$_2$CH), 2.34-2.20 (m, 2H, CH$_2$C<u>H$_2$</u>CH).

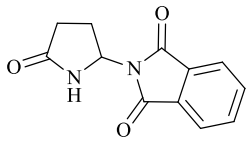
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): δ 183.5 (C=O), 177.2 (C=O), 158.23 (C=O), 149.59 (C_{IV}), 138.62 (CHAr), 125.27 (CHAr), 123.78 (CHAr), 118.23 (C_{IV}), 111.96 (CHAr), 62.6 (CH), 29.7 (CH_2), 24.1 (CH_2).

IR ν (cm^{-1}): 3216, 1742, 1679, 1606, 1468, 1254.

Anal. Calcd for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.38; N, 12.17; found: C, 62.26; H, 4.17; N, 11.82.

2-(5-oxopyrrolidin-2-yl)isoindoline-1,3-dione –59

HEI 2942



Following the general procedure, 2-(5-oxopyrrolidin-2-yl)isoindoline-1,3-dione was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with isoindoline-1,3-dione (17.4 mmol, 2.6 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (1.2 g, 30% yield).

M.p. 177-178°C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.33 (s, 1H, N*H*), 8.04 (s, 1H, N*H*), 7.91 (d, *J* = 9.2 Hz, 2H, Ar*H*), 7.85 (m, 2H, Ar*H*), 5.75 (d, *J* = 8.8 Hz, 1H, CH), 2.67-2.50 (m, 2H, C$H_2$$CH_2$CH), 2.34-2.14 (m, 2H, CH_2CH_2CH).

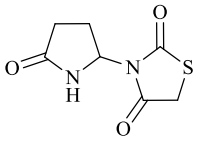
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): δ 177.9 (C=O), 169.7 (C=O), 167.6 (C=O), 135.1 (CHAr), 134.7 (CHAr), 133.0 (C_{IV}), 131.8 (C_{IV}), 123.6 (CHAr), 123.3 (CHAr), 60.2 (CH), 29.7 (CH_2), 25.3 (CH_2).

IR ν (cm^{-1}): 3186, 3058, 1713, 1688, 1307, 1051.

Anal. Calcd for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.38; N, 12.17.

3-(5-oxopyrrolidin-2-yl)thiazolidine-2,4-dione – 60

HEI 2995



Following the general procedure, 3-(5-oxopyrrolidin-2-yl)thiazolidine-2,4-dione was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with thiazolidine-2,4-dione (17.4 mmol, 2.0 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was

precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a white solid (2.3 g, 67% yield).

M.p. 186-187°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.05 (s, 1H, N*H*), 5.68 (d, *J* = 8.9 Hz, 1H, CH), 2.6-2.39 (m, 4H, C*H*$_2$CH$_2$CH, O=CC*H*$_2$C*H*$_2$C=O), 2.20-2.00 (m, 2H, CH$_2$C*H*$_2$CH).

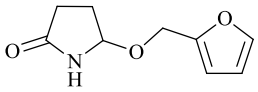
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.7 (C=O), 172.0 (C=O), 171.6 (C=O), 63.2 (CH), 33.5 (CH$_2$), 29.4 (CH$_2$), 24.1 (CH$_2$).

IR ν (cm^{-1}): 3037, 2943, 1747, 1662, 1277, 1149.

Anal. Calcd for C$_7$H$_8$N$_2$O$_3$S, 0.5H$_2$O: C, 40.18; H, 4.34; N, 13.39; S, 15.33; found: C, 40.57; H, 3.93; N, 13.52; S, 15.28.

5-(furan-2-ylmethoxy)pyrrolidin-2-one – 3

HEI 2976



Following the general procedure, 5-(benzyloxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with benzyl alcohol (17.4 mmol, 1.7 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a white solid (2.5 g, 80% yield).

M.p. 91-92°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.74 (s, 1H, N*H*), 7.63 (q, *J* = 0.8 Hz, 1H, CH), 6.43 (m, 2H, CH), 4.93 (dt, *J* = 6.3 Hz, *J* = 1.2 Hz, 1H, CH), 4.48 (d, *J* = 12.8 Hz, 1H, C*H*$_2$-O), 4.36 (d, *J* = 12.8 Hz, 1H, C*H*$_2$-O), 2.34-2.13 (m, 2H, C*H*$_2$CH$_2$CH), 2.14-2.0 (m, 1H, CH$_2$C*H*$_2$CH), 1.85 (m, 1H, CH$_2$C*H*$_2$CH).

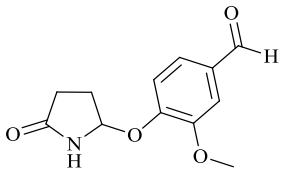
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.4 (C=O), 151.1 (C$_{IV}$), 143.0 (CH), 110.3 (CH), 109.4 (CH), 84.4 (CH), 60.0 (CH$_2$), 27.9 (CH$_2$), 27.4 (CH$_2$).

IR ν (cm^{-1}): 3182, 3099, 1680, 1278, 1059, 1037.

Anal. Calcd for C$_9$H$_1$1NO$_3$: C, 59.66; H, 6.12; N, 7.73; found: C, 59.16; H, 5.92; N, 7.43.

3-**methoxy-4-((5-oxopyrrolidin-2-yl)oxy)benzaldehyde – 63**

HEI 2999



Following the general procedure, 5-((3-hydroxy-4-methoxybenzyl)oxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-hydroxy-3-methoxybenzaldehyde (17.4 mmol, 2.6 g), in presence of 5% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 90% ethyl acetate, as a white solid (0.6 g, 15% yield).

M.p. 167-168°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.10 (s, 1H, CHO), 7.79 (s, 1H, N*H*), 7.56 (dd, *J* = 8.2 Hz, *J* = 1.6 Hz, 1H, Ar*H*), 7.47 (d, *J* = 1.6 Hz, 1H, Ar*H*), 6.88 (d, *J* = 8.2 Hz, 1H, Ar*H*), 5.19 (q, *J* = 4.3 Hz, 1H, CH), 3.83 (s, 3H, CH_3), 2.02-2.25 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.92-1.84 (m, 1H, CH_2C*H*$_2$CH).

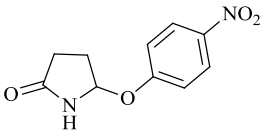
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 196.6 (HC=O), 177.0 (C=O), 152.1 (C_{IV}), 147.7 (C_{IV}), 125.6 (C_{IV}), 123.2 (CHAr), 115.3 (CHAr), 111.3 (CHAr), 56.8 (CH), 55.6 (OCH_3), 29.0 (CH_2), 25.3 (CH_2).

IR ν (cm^{-1}): 3204, 3158, 1650, 1233, 1154, 950.

Anal. Calcd for C$_{12}$H$_{13}$NO$_4$: C, 61.27; H, 5.57; N, 5.95; found: C, 61.08; H, 5.35; N, 6.02.

5-(4-nitrophenoxy)pyrrolidin-2-one – 64

HEI 3228



Following the general procedure, 5-(4-nitrophenoxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-nitrophenol (17.4 mmol, 2.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 90% ethyl acetate, as a yellow solid (0.4 g, 10% yield).

M.p. 186-187°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.36 (s, 1H, N*H*), 8.10 (s, 1H, Ar*H*), 8.08 (dd, *J* = 8.8 Hz, *J* = 2.8 Hz, 1H, Ar*H*), 8.03 (d, *J* = 2.8 Hz, 1H, Ar*H*), 7.00 (d, *J* = 8.8 Hz, 1H, Ar*H*),

4.88 (t, *J* = 6.6 Hz, 1H, CH), 2.55 (m, 1H, C*H*$_2$CH$_2$CH), 2.23 (m, 2H, C*H*$_2$C*H*$_2$CH), 1.72 (m, 1H, CH$_2$C*H*$_2$CH).

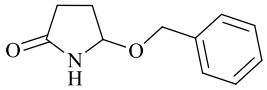
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.9 (C=O), 161.3 (C$_{IV}$), 139.9 (C$_{IV}$), 131.6 (CH), 125.1 (CH), 122.0 (CH), 115.8 (CH), 52.0 (CH$_2$), 29.9 (CH$_2$), 28.7 (CH$_2$).

IR ν (cm^{-1}): 3195, 3094, 1682, 1524, 1343, 1281.

Anal. Calcd for C$_{10}$H$_{10}$N$_2$O$_4$: C, 54.05; H, 4.54; N, 12.61.

5-(benzyloxy)pyrrolidin-2-one[2] –65

HEI 3001



Following the general procedure, 5-(benzyloxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with benzyl alcohol (17.4 mmol, 1.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (1.82 g, 55% yield).

M.p. 91-92°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.74 (s, 1H, N*H*), 7.30-7.36 (m, 5H, Ar*H*), 4.98 (d, *J* = 6.4 Hz, 1H, CH), 4.55 (d, *J* = 11.8 Hz, 1H, C*H*$_2$-O), 4.40 (d, *J* = 11.8 Hz, 1H, C*H*$_2$-O), 2.35-2.19 (m, 2H, C*H*$_2$CH$_2$CH), 2.05 (m, 1H, CH$_2$C*H*$_2$CH), 1.87 (m, 1H, CH$_2$C*H*$_2$CH).

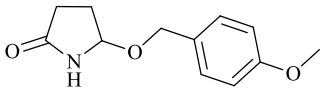
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 178.0 (C=O), 138.6 (C$_{IV}$), 128.7 (2 CHAr), 128.0 (2 CHAr), 127.8 (CHAr), 85.3 (CH), 68.4 (CH$_2$), 28.5 (CH$_2$), 28.0 (CH$_2$).

IR ν (cm^{-1}): 3182, 3099, 1680, 1278, 1059, 1037.

Anal. Calcd for C$_{11}$H$_{13}$NO$_2$: C, 69.09; H, 6.85; N, 7.32; found: C, 68.88; H, 6.96; N, 7.29.

5-((4-methoxybenzyl)oxy)pyrrolidin-2-one – 66

HEI 2992



Following the general procedure, 5-((4-methoxybenzyl)oxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with (4-methoxyphenyl)methanol (17.4 mmol, 2.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as

an off-white solid (1.2 g, 46% yield).

M.p. 71-72°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.78 (s, 1H, N*H*), 7.25 (d, *J* = 6.9 Hz, 2H, Ar*H*), 6.90 (d, *J* = 6.9 Hz, 2H, Ar*H*), 4.94 (d, *J* = 6.5 Hz, 1H), 4.45 (d, *J* = 11.6 Hz, 1H, CH_2), 4.30 (d, *J* = 11.6 Hz, 1H, CH_2), 3.73 (s, 3H, OCH_3), 2.32-2.14 (m, 2H, C*H*$_2$$CH_2CH$), 2.03 (m, 1H, CH_2C*H*$_2$CH), 1.88 (m, 1H, CH_2C*H*$_2$CH).

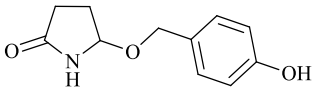
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 178.0 (C=O), 159.1 (C_{IV}), 130.4 (C_{IV}), 129.8 (2 CHAr), 114.1 (2 CHAr), 84.9 (CH), 68.0 (CH_2), 55.5 (CH_3), 28.5 (CH_2), 28.0 (CH_2).

IR ν (cm^{-1}): 3413, 3194, 2914, 1668, 1256, 1172, 1027.

Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33; found: C, 64.95; H, 6.91; N, 6.07.

5-((4-hydroxybenzyl)oxy)pyrrolidin-2-one – 67

HEI 2969



Following the general procedure, 5-((4-hydroxybenzyl)oxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 4-(hydroxymethyl)phenol (17.4 mmol, 2.1 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as an off-white solid (1.2 g, 33% yield).

M.p. 142-143°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.38 (s, 1H, O*H*), 8.76 (s, 1H, N*H*), 7.12 (d, *J* = 8.5 Hz, 2H, Ar*H*), 6.73 (d, *J* = 8.5 Hz, 2H, Ar*H*), 4.93 (d, *J* = 6.2 Hz, 1H, CH), 4.39 (d, *J* = 11.4 Hz, 1H, C*H*$_2$-O), 4.25 (d, *J* = 11.4 Hz, 1H, C*H*$_2$-O), 2.29-2.13 (m, 2H, C*H*$_2$$CH_2CH$), 2.06-1.98 (m, 1H, CH_2C*H*$_2$CH), 1.87 (m, 1H, CH_2C*H*$_2$CH).

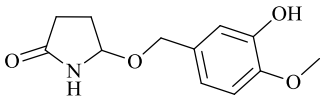
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 178.0 (C=O), 157.2 (C_{IV}), 130.0 (2 CHAr), 128.6 (C_{IV}), 115.4 (2 CHAr), 84.8 (CH), 68.3 (CH_2), 28.5 (CH_2), 28.0 (CH_2).

IR ν (cm^{-1}): 3203, 2935, 1668, 1516, 1454, 1227, 1056, 1037.

Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76; found: C, 63.36; H, 6.40; N, 6.91.

5-((3-hydroxy-4-methoxybenzyl)oxy)pyrrolidin-2-one – 68

HEI 2974



Following the general procedure, 5-((3-hydroxy-4-methoxybenzyl)oxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 5-(hydroxymethyl)-2-methoxyphenol (17.4 mmol, 2.7 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a white solid (1.2 g, 30% yield).

M.p. 121-122°C

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.93 (s, 1H, OH), 8.73 (bs, 1H, N*H*), 6.85 (d, *J* = 8.4 Hz, 1H, Ar*H*), 6.75 (d, *J* = 1.8 Hz, 1H, Ar*H*), 6.7 (dd, *J* = 8.4 Hz, *J* = 1.8 Hz, 1H, Ar*H*), 4.94 (d, *J* = 6.2 Hz, 1H, CH), 4.39 (d, *J* = 11.7 Hz, 1H, CH_2), 4.24 (d, *J* = 11.7 Hz, 1H, CH_2), 3.74 (s, 3H, CH_3), 2.33-2.13 (m, 2H, C*H*$_2$CH$_2$CH), 2.08-1.98 (m, 1H, CH$_2$C*H*$_2$CH), 1.92-1.83 (m, 1H, CH$_2$C*H*$_2$CH).

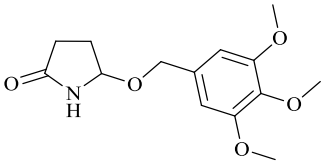
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 178.0 (C=O), 147.5 (C_{IV}), 146.8 (C_{IV}), 131.0 (C_{IV}), 119.1 (CHAr), 115.7 (CHAr), 112.3 (CHAr), 84.9 (CH), 68.3 (CH_2), 56.1 (CH_3), 28.5 (CH_2), 28.0 (CH_2).

IR ν (cm^{-1}): 3212, 3194, 1674, 1679, 1254, 1172, 1027.

Anal. Calcd for C$_{12}$H$_{15}$NO$_4$: C, 60.75; H, 6.37; N, 5.90; found: C, 60.36; H, 6.40; N, 6.12.

5-((3,4,5-trimethoxybenzyl)oxy)pyrrolidin-2-one – 69

HEI 2964



Following the general procedure, 5-((3,4,5-trimethoxybenzyl)oxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with (3,4,5-trimethoxyphenyl)methanol (17.4 mmol, 3.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, being obtained as a white solid (1.8 g, 40% yield).

M.p. 91-92°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.76 (s, 1H, N*H*), 6.63 (s, 2H, Ar*H*), 4.96 (d, *J* = 5.2 Hz,

1H, C*H*), 4.46 (d, *J* = 9.6 Hz, 1H, CH_2), 4.32 (d, *J* = 9.6 Hz, 1H, CH_2), 3.77 (s, 6H, OCH_3), 3.64 (s, 3H, OCH_3), 2.32 (m, 2H, CH_2CH$_2$CH), 2.07 (m, 1H, CH_2CH$_2$CH), 1.95 (m, 1H, CH$_2$CH_2CH).

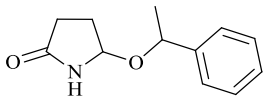
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 178.0 (C=O), 153.2 (2 C_{IV}), 134.1 (C_{IV}), 105.4 (2 CHAr), 85.0 (CH), 68.5 (CH_2), 60.4 (OCH_3), 56.2 (2 OCH_3), 28.5 (CH_2), 28.0 (CH_2).

IR ν (cm^{-1}): 3184, 3092, 1682, 1590, 1462, 1230, 1132.

Anal. Calcd for C$_{14}$H$_{19}$NO$_5$: C, 59.78; H, 6.81; N, 4.98.

5-(1-phenylethoxy)pyrrolidin-2-one – 70

HEI 2985



Following the general procedure, 5-((4-hydroxybenzyl)oxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 1-phenylethanol (17.4 mmol, 2.1 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 60% ethyl acetate, as an off-white solid (0.35 g, 10% yield).

M.p. 139-140°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.73 (s, 1H, N*H*), 7.38-7.21 (m, 5H, Ar*H*), 4.63 (m, 2H, 2C*H*), 2.27 (m, 1H, CH_2CH$_2$CH), 2.06-1.98 (m, 2H, CH_2CH_2CH), 1.80 (m, 1H, CH$_2$CH_2CH), 1.33 (d, *J* = 6.8 Hz, 3H, CH_3).

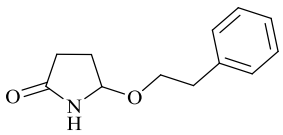
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 178.5 (C=O), 144.3 (C_{IV}), 129.4 (2 CHAr), 128.5 (CHAr), 127.3 (2 CHAr), 83.8 (CH), 74.3 (CH), 29.1 (CH_2), 29.1 (CH_2), 25.3 (CH_3).

IR ν (cm^{-1}): 3175, 3070, 1671, 1264, 1102.

Anal. Calcd for C$_{12}$H$_{15}$NO$_2$: C, 70.22; H, 7.37; N, 6.82.

5-phenethoxypyrrolidin-2-one – 71

HEI 2982



Following the general procedure, 5-phenethoxypyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 2-phenylethanol (17.4 mmol, 2.33 g), in

presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (0.25 g, 12% yield).

M.p. 144-145°C.

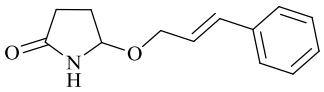
^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.66 (s, 1H, N*H*), 7.72 (m, 5H, Ar*H*), 4.89 (d, *J* = 6.5 Hz, 1H), 3.65 (q, *J* = 7.0 Hz, 1H, CH_2CH_2-O), 3.49 (q, *J* = 7.0 Hz, 1H, CH_2CH_2O), 2.79 (t, *J* = 7.9 Hz, 2H, CH_2CH_2O), 2.28-2.12 (m, 2H, CH_2CH_2CH), 2.06-1.96 (m, 1H, CH_2CH_2CH), 1.87-1.78 (m, 1H, CH_2CH_2CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.9 (C=O), 139.2 (C_{IV}), 129.3 (2 CHAr), 128.6 (2 CHAr), 126.5 (CHAr), 85.6 (CH), 67.7 (CH_2), 35.9 (CH_2), 28.5 (CH_2), 28.0 (CH_2).

IR ν (cm^{-1}): 3178, 3106, 2913, 1683, 1278, 1067, 1027.

Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82; found: C, 69.84; H, 7.51; N, 7.03.

5-(cinnamyloxy)pyrrolidin-2-one – 72



Following the general procedure, (E)-5-(cinnamyloxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with *trans*-cinnamyl alcohol (17.4 mmol, 2.3 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 100% ethyl acetate as an orange solid (2.0 g, 53% yield).

M.p. 66-67°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.76 (s, 1H, N*H*) , 7.45 (d, *J* = 7.6 Hz, 2H, Ar*H*), 7.33 (t, *J* = 7.6 Hz, 2H, Ar*H*), 7.25 (t, *J* = 7.6 Hz, 1H, Ar*H*), 6.62 (d, *J* = 16.3 Hz, 1H, CH), 6.36 (dt, *J* = 16.3 Hz, *J* = 6.1 Hz, 1H, CH), 4.98 (d, *J* = 5.6 Hz, 1H, CH), 4.15 (qd, *J* = 13.3 Hz, *J* = 1.6 Hz, 1H, CH_2), 4.04 (qd, *J* = 16.3 Hz, *J* = 6.1 Hz, 1H, CH_2), 2.34-2.17 (m, 2H, CH_2CH_2CH), 2.08-2.01 (m, 1H, CH_2CH_2CH), 1.92 (m, 1H, CH_2CH_2CH).

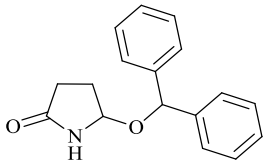
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 178.0 (C=O), 136.8 (C_{IV}), 131.7 (CH), 129.0 (2 CHAr), 128.0 (CH), 126.8 (2 CHAr), 126.7 (CHAr), 85.2 (CH), 67.2 (CH_2), 28.5 (CH_2), 28.1 (CH_2).

IR ν (cm^{-1}): 3187, 3099, 2905, 1682, 1274, 1057, 1027, 969.

Anal. Calcd for $C_{13}H_{15}NO_2$, 1/5 H_2O: C, 70.69; H, 7.03; N, 6.34; found: C, 70.82; H, 6.73; N, 6.24.

5-(benzhydryloxy)pyrrolidin-2-one – 73

HEI 2968



Following the general procedure, 5-(benzhydryloxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with diphenylmethanol (17.4 mmol, 3.2 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 90% ethyl acetate, as a white solid (1.1 g, 24% yield).

M.p. 98-99°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.79 (s, 1H, N*H*) 7.29-7.36 (m, 10H, Ar*H*), 5.63 (s, 1H, CH), 4.85 (d, *J* = 5.8 Hz 1H, CH), 2.35-2.25 (m, 1H, C*H*$_2$CH$_2$CH), 2.17 (m, 1H, CH$_2$C*H*$_2$CH), 2.10-1.85 (m, 2H, CH$_2$C*H*$_2$CH).

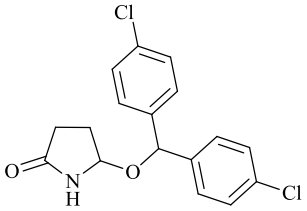
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 178.0 (C=O), 143.0 (C_{IV}), 142.3 (C_{IV}), 128.9 (2 CHAr), 128.7 (2 CHAr), 127.9 (CHAr), 127.6 (CHAr), 127.5 (2 CHAr), 126.8 (2 CHAr), 85.7 (CH), 78.7 (CH), 28.5 (CH_2), 28.2 (CH_2).

IR ν (cm^{-1}): 3183, 3104, 2935, 1697, 1283, 1059.

Anal. Calcd for C$_{17}$H$_{17}$NO$_2$: C, 76.38; H, 6.41; N, 5.24; found: C, 76.12; H, 6.26; N, 5.08.

5-(bis(4-chlorophenyl)methoxy)pyrrolidin-2-one – 74

HEI 2977



Following the general procedure, 5-(bis(4-chlorophenyl)methoxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with bis-(4-chlorophenyl)methanol (17.4 mmol, 4.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a white solid (0.6 g, 10% yield).

M.p. 172-173° C

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.79 (s, 1H, N*H*), 7.30-7.44 (m, 8H, Ar*H*), 5.65 (s, 1H, N*H*), 4.85 (d, 1H, *J* = 6.4 Hz, C*H*), 2.41-2.27 (m, 1H, C*H*$_2$CH$_2$CH), 2.21-2.18 (m, 1H, C*H*$_2$CH$_2$CH), 2.05-1.95 (m, 2H, CH$_2$C*H*$_2$CH).

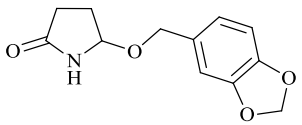
^{13}C-NMR (100 MHz, DMSO-d_6): δ 178.0 (C_{IV}), 141.6 (C_{IV}), 140.9 (C_{IV}), 132.7 (C_{IV}), 132.3 (C_{IV}), 129.4 (2 CHAr), 129.0 (2 CHAr), 128.8 (2 CHAr), 128.7 (2 CHAr), 83.9 (C_{IV}), 77.2 (CH), 28.4 (CH_2), 28.2 (CH_2).

IR ν (cm^{-1}): 3196, 3160, 2915, 1695, 1488, 1278, 1045.

Anal. Calcd for $C_{17}H_{15}Cl_2NO_2$: C, 60.73; H, 4.50; N, 4.17.

5-(benzo[d][1,3]dioxol-5-ylmethoxy)pyrrolidin-2-one – 75

HEI 2989



Following the general procedure, 5-(benzo[*d*][1,3]dioxol-5-ylmethoxy)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with benzo[*d*][1,3]dioxol-5-ylmethanol (17.4 mmol, 2.6 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was purified through flash liquid chromatography being eluted with a gradient of DCM/methanol, the wanted compound being eluted in 20% methanol, affording the compound as a white solid (0.4 g, 10% yield).

M.p. 134-135°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0 (s, 1H, N*H*), 6.83 (s, 1H, Ar*H*), 6.78 (s, 2H, Ar*H*), 5.95 (m, 2H, C*H*$_2$), 5.05 (d, *J* = 6.0 Hz, 1H, C*H*), 4.50 (d, *J* = 11.2 Hz, 1H, C*H*$_2$), 4.38 (d, *J* = 11.2 Hz, 1H, C*H*$_2$), 2.59 (m, 1H, C*H*$_2$CH$_2$CH), 2.30-2.22 (m, 2H, C*H*$_2$CH$_2$CH), 2.21 (m, 1H, CH$_2$C*H*$_2$CH), 2.15 (m, 1H, CH$_2$C*H*$_2$CH).

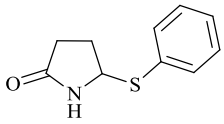
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 179.6 (C=O), 147.8 (C_{IV}), 147.3 (C_{IV}), 131.1 (C_{IV}), 121.5 (CHAr), 108.6 (CHAr), 108.2 (CHAr), 101.0 (CH_2), 85.2 (CH), 69.4 (CH_2), 28.4 (CH_2), 28.3 (CH_2).

IR ν (cm^{-1}): 3205, 3156, 1684, 1252, 1101, 1078.

Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95.

5-(phenylthio)pyrrolidin-2-one[254]-76

HEI 2957



Following the general procedure, 5-(phenylthio)pyrrolidin-2-one was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with thiophenol (17.4 mmol, 1.9 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the compound as a white solid (1.0 g, 30% yield).

M.p.75-76°C

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.48 (s, 1H, N*H*), 7.47 (dd, *J* = 7.9 Hz, *J* = 2.1 Hz, 2H, Ar*H*), 7.38 (m, 1H, Ar*H*), 7.35 (dd, *J* = 7.9 Hz, *J* = 2.1 Hz, 2H, Ar*H*), 5.16 (d, *J* = 7.4 Hz, 1H, CH), 2.43 (m, 1H, C*H*$_2$CH$_2$CH), 2.03-1.80 (m, 3H, C*H*$_2$C*H*$_2$CH).

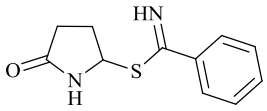
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 176.8 (C=O), 133.3 (2 CHAr), 132.9 (C$_{IV}$), 129.6 (2 CHAr), 128.2 (CHAr), 62.2 (CH), 29.2 (CH$_2$), 28.9 (CH$_2$).

IR ν (cm^{-1}): 3389, 3158, 1657, 1453, 1258.

Anal. Calcd for C$_{10}$H$_{11}$NOS: C, 62.15; H, 5.74; N, 7.25; S, 16.59.

5-oxopyrrolidin-2-yl benzimidothioate - 77

HEI 2956



Following the general procedure, 5-oxopyrrolidin-2-yl benzimidothioate was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with benzothioamide (17.4 mmol, 2.4 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product precipitates in diethyl ether. The solid was isolated by filtration under suction, after it was washed with ethanol, affording a yellow solid (1.5 g, 60% yield).

M.p. 153-154°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.61 (s, 1H, N*H*), 8.27 (s, 1H, N*H*), 7.67 (d, *J* = 7.2 Hz, 2H, Ar*H*), 7.42 (d, *J* = 7.2 Hz, 1H, Ar*H*), 7.34 (t, *J* = 7.2 Hz, 2H, Ar*H*), 6.04 (s, 1H, CH), 2.01

[254] Eguchi M., Zen Q., Korda A., Ojima I. Synthesis of pyrrolizidine alkaloids *via* rhodium-catalyzed silylformylation and amidocarbonylation. *Tetrahedron Lett.* 1993, 34, p. 915-918.

(m, 2H, C*H*$_2$CH$_2$CH), 2.01 (m, 1H, CH$_2$C*H*$_2$CH), 1.80 (m, 1H, CH$_2$C*H*$_2$CH).

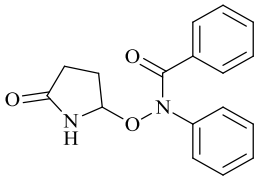
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.3 (C=O), 161.6 (C$_{IV}$), 138.4 (C$_{IV}$), 131.3 (CHAr), 128.7 (2 CHAr), 123.4 (2CHAr), 70.3 (CH), 29.2 (CH$_2$), 25.6 (CH$_2$).

IR ν (cm^{-1}): 3190, 3088, 1677, 1662, 1453, 1278, 1248, 1226.

Anal. Calcd for C$_{11}$H$_{12}$N$_2$OS: C, 59.97; H, 5.49; N, 12.72; S, 14.56; found: C, 58.40; H, 5.13; N, 12.35; S, 14.37.

_N_-((5-oxopyrrolidin-2-yl)oxy)-_N_-phenylbenzamide-78

HEI 2983



Following the general procedure, *N*-((5-oxopyrrolidin-2-yl)oxy)-*N*-phenylbenzamide was obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with *N*-hydroxy-*N*-phenylbenzamide (17.4 mmol, 3.7 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product precipitates in diethyl ether. The solid was isolated by filtration under suction, after it was washed with ethanol, affording 1.0 g of an orange solid, in 20% yield.

M.p. 144-145°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.04 (s, 1H, N*H*), 7.55 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.47 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.41-7.32 (m, 5H, Ar*H*), 7.27 (t, *J* = 8.4 Hz, 1H, Ar*H*), 5.34 (dd, *J* = 5.6 Hz, *J* = 1.2 Hz, 1H, CH), 2.22-2.10 (m, 1H, C*H*$_2$CH$_2$CH), 1.96-1.87 (m, 3H, CH$_2$C<u>H$_2$</u>CH).

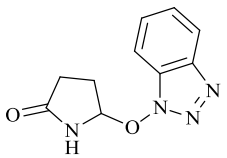
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 178.36 (C=O), 169.2 (C=O), 141.0 (C$_{IV}$), 135.4 (C$_{IV}$), 130.7 (2 CHAr), 129.5 (2 CHAr), 128.9 (2 CHAr), 128.3 (CHAr), 128.1 (CHAr), 127.9 (CHAr), 126.3 (CHAr), 89.0 (CH), 27.8 (CH$_2$), 26.3 (CH$_2$).

IR ν (cm^{-1}): 3202, 3057, 1709, 1674, 1491, 1300, 1291, 900, 700.

Anal. Calcd for C$_{17}$H$_{16}$N$_2$O$_3$: C, 68.91; H, 5.44; N, 9.45; found: C, 68.52; H, 5.34; N, 9.42.

5-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)oxy)pyrrolidin-2-one – 79

HEI 2984



Following the general procedure, 5-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)oxy)pyrrolidin-2-one was

obtained from the reaction of 5-methoxypyrrolidin-2-one (17.4 mmol, 2.0 g) with 1*H*-benzo[*d*][1,2,3]triazol-1-ol (17.4 mmol, 2.3 g), in presence of 5 mol% CsF (0.86 mmol, 0.132 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording the wanted compound as a grey solid (3.0 g, 81% yield).

M.p. 154-155°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.80 (s, 1H, N*H*), 7.96 (d, *J* = 7.1 Hz, 1H, Ar*H*), 7.90 (d, *J* = 7.1 Hz, 1H, Ar*H*), 7.73 (d, *J* = 7.1 Hz, 1H, Ar*H*), 7.47 (t, *J* = 7.1 Hz, 1H, Ar*H*), 6.54 (d, *J* = 7.6 Hz, 1H, CH), 2.75-2.54 (m, 2H, C*H*$_2$CH$_2$CH), 2.44-2.27 (m, 2H, CH$_2$C*H*$_2$CH).

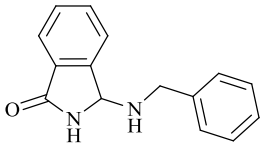
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.6 (C=O), 133.7 (C_{IV}), 131.0 (CHAr), 130.1 (C_{IV}), 125.0 (CHAr), 115.3 (CHAr), 112.3 (CHAr), 68.6 (CH), 28.9 (CH_2), 27.4 (CH_2).

IR ν (cm^{-1}): 3204, 3099, 1708, 1489, 1394, 1346, 1233, 1195, 1098.

Anal. Calcd for C$_{10}$H$_{10}$N$_4$O$_2$: C, 55.04; H, 4.62; N, 25.68; found: C, 54.68; H, 4.51; N, 25.33.

3-(benzylamino)isoindolin-1-one – 83

HEI 3161



Following the general procedure, 3-(benzylamino)isoindolin-1-one was obtained from the reaction of 3-methoxyisoindolin-1-one (3 mmol, 0.5 g) with phenylmethanamine (3 mmol, 0.3 g), in presence of 5 mol% CsF (0.15 mmol, 0.02 g The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 45 as a white solid (0.44 g, 61% yield).

M.p. 154-155°C.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.88 (s, 1H, N*H*), 7.63 (d, J = 7.4Hz, 1H, Ar*H*), 7.60 (m, 2H, Ar*H*), 7.49 (m, 1H, Ar*H*), 7.34 (d, J = 7.4Hz, 2H, Ar*H*), 7.31 (t, J = 7.4Hz, 2H, Ar*H*), 7.20 (t, J = 7.4 Hz, 1H, Ar*H*), 5.38 (d, J = 9.5 Hz, 1H, C*H*), 3.74 (q, J = 6.8 Hz, 1H, C*H*$_2$NH), 3.64 (q, J = 6.8 Hz, 1H, C*H*$_2$NH), 3.10 (q, 1H, N*H*).

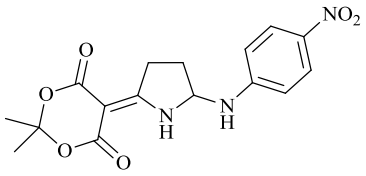
^{13}C{1H}NMR (100 MHz, DMSO-d$_6$): δ 169.3 (C=O), 146.6 (C_{IV}), 141.0 (C_{IV}), 133.4 (C_{IV}), 132.1 (CHAr), 129.2 (CHAr), 128.6 (2 CHAr), 128.4 (2 CHAr), 127.1 (CHAr), 124.3 (CHAr), 122.9 (CHAr), 70.0 (CH), 48.2 (CH_2).

IR ν (cm-1): 3285, 3067, 2976, 1697, 1360, 1135.

Anal. Calcd for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76; found: C, 75.27; H, 5.80; N, 11.65.

2,2-dimethyl-5-(5-((4-nitrophenyl)amino)pyrrolidin-2-ylidene)-1,3-dioxane-4,6-dione – 84

HEI 3214



Following the general procedure, 2,2-dimethyl-5-(5-((4-nitrophenyl)amino)pyrrolidin-2-ylidene)-1,3-dioxane-4,6-dione was obtained from the reaction of 5-(5-methoxypyrrolidin-2-ylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (8.3 mmol, 2.0 g) with 4-nitroaniline (8.3 mmol, 1.1 g), in presence of 5 mol% CsF (0.41 mmol, 0.06 g). The crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2x10 mL), affording compound 46 as a dark green solid (1.93 g, 65% yield).

M.p. 216-217°C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.45 (s, 1H, N*H*), 8.06 (d, J = 9.0 Hz, 1H, Ar*H*), 7.82 (d, J = 9.0 Hz, 1H, N*H*), 6.88 (d, J = 9.0 Hz, 2H, Ar*H*), 5.77 (dt, J = 8.2 Hz, J = 3.7 Hz, 1H, C*H*), 3.38-3.28 (m, 2H, C*H*$_2$CH$_2$CH), 2.49 (m, 1H, CH$_2$C*H*$_2$CH), 1.95 (m, 1H, CH$_2$C*H*$_2$CH), 1.59 (s, 6H, 2 C*H*$_3$).

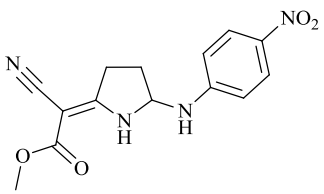
^{13}C{1H}NMR (100 MHz, DMSO-d_6): δ 175.6 (C_{IV}), 165.1 (C=O), 162.54 (C=O), 152.6 (C_{IV}), 137.8 (C_{IV}), 126.6 (3 CHAr), 112.4 (CHAr), 102.9 (C_{IV}), 81.9 (C_{IV}), 70.0 (CH), 34.0 (CH_2), 28.1 (CH_2), 26.7 (CH_3), 26.5 (CH_3).

IR ν (cm^{-1}): 3309, 1654, 1549, 1448, 1330, 1306, 1261.

Anal. Calcd for $C_{16}H_{17}N_3O_6$: C, 55.33; H, 4.93; N, 12.10; found: C, 55.09; H, 4.53; N, 12.05.

(Z)-methyl 2-cyano-2-(5-((4-nitrophenyl)amino)pyrrolidin-2-ylidene)acetate - 85

HEI 3221



Following the general procedure, (Z)-methyl 2-cyano-2-(5-((4-nitrophenyl)amino)pyrrolidin-2-ylidene)acetate was obtained from the reaction of (Z)-methyl 2-cyano-2-(5-

methoxypyrrolidin-2-ylidene)acetate (2.54 mmol, 0.5 g) with 4-nitroaniline (2.54 mmol, 0.35 g), in presence of 5% CsF (0.127 mmol, 0.019 g). The crude product precipitates in diethyl ether. The crude product was purified through flash liquid chromatography being eluted with a gradient of n-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as 0.4 g of a

purple solid, in 51% yield.

M.p. 180-181°C.

^{1}H NMR (400 MHz, DMSO-d$_6$): δ 9.72 (s, 1H, N*H*), 8.04 (d, J = 9.3 Hz, 2H, Ar*H*), 7.78 (d, J = 9.3 Hz, 1H, N*H*), 6.86 (d, J = 9.3 Hz, 2H, Ar*H*), 5.72 (dt, J = 8.2 Hz, J = 3.3 Hz, 1H, C*H*), 3.64 (s, 3H, C*H*$_3$), 3.06 (m, 1H, C*H*$_2$CH$_2$CH), 2.90 (m, 1H, C*H*$_2$CH$_2$CH), 2.46 (m, 1H, CH$_2$C*H*$_2$CH), 1.91 (m, 1H, CH$_2$C*H*$_2$CH).

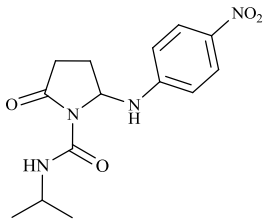
^{13}C{1H}NMR (100 MHz, DMSO-d$_6$): δ 172.9 (C$_{IV}$), 166.88 (C=O), 152.6 (C$_{IV}$), 137.6 (C$_{IV}$), 126.5 (3 CHAr), 118.8 (CHAr), 112.4 (C$_{IV}$), 66.8 (C$_{IV}$), 70.8 (CH), 51.6 (CH$_3$), 32.3 (CH$_2$), 28.2 (CH$_2$).

IR ν (cm-1): 3263, 2208, 1687, 1592, 1335, 1303, 1253.

Anal. Calcd for C$_{14}$H$_{14}$N$_4$O$_4$: C, 55.63; H, 4.67; N, 18.53; found: C, 55.27; H, 4.55; N, 18.13.

_N_-isopropyl-2-((4-nitrophenyl)amino)-5-oxopyrrolidine-1-carboxamide– 87

HEI 3018



N-isopropyl-2-((4-nitrophenyl)amino)-5-oxopyrrolidine-1-carboxamide was obtained from the reaction of 5-((4-nitrophenyl)amino)pyrrolidin-2-one (4.5 mmol, 1 g) with isopropyl isocyanate (6.5 mmol, 0.65 mL), in 30 mL toluene, under nitrogen atmosphere, at 80°C, for 12 hs. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 50% ethyl acetate, as a yellow solid (1.1 g, 42% yield).

M.p. 207-208°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.21 (d, , *J* = 7.5 Hz, 1H, N*H*), 8.12 (d, *J* = 9.7 Hz, 2H, C*H*), 7.75 (d, *J* = 8.7 Hz, 1H, N*H*), 6.78 (d, *J* = 9.7 Hz, 2H, Ar*H*), 5.97 (t, *J* = 7.5 Hz, 1H, Ar*H*), 3.82 (sext, *J* = 6.3 Hz, 1H, C*H*), 2.86 (m, 2H, C*H*$_2$CH$_2$CH), 2.54 -2.38 (m, 2H, C*H*$_2$C*H*$_2$CH),

1.80 (m, 1H, CH_2CH_2CH), 1.12 (m, 6H, CH_3).

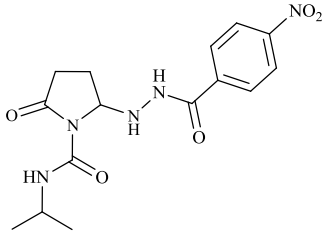
$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ 176.8 (C=O), 152.2 (C_{IV}), 150.4 (C_{IV}), 136.4 (C_{IV}), 125.8 (2CHAr), 111.6 (2CHAr), 66.1 (CH), 41.3 (CH), 30.8 (CH_2), 25.3 (CH_2), 22.4 (CH_3), 22.3 (CH_3).

IR ν (cm^{-1}): 3289, 2976, 1716, 1669, 1595, 1538, 1505, 1477, 1322, 1223.

Anal. Calcd for $C_{14}H_{18}N_4O_4$: C, 54.89; H, 5.92; N, 18.29.

N-isopropyl-2-(2-(4-nitrobenzoyl)hydrazinyl)-5-oxopyrrolidine-1-carboxamide – 88

HEI 3013



N-isopropyl-2-(2-(4-nitrobenzoyl)hydrazinyl)-5-oxopyrrolidine-1-carboxamide was obtained from the reaction of 4-nitro-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide (3.7 mmol, 1 g) with isopropyl isocyanate (5.5 mmol, 0.55 mL), in 30 mL toluene, under nitrogen atmosphere, at 80°C, for 12 hs. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a yellow solid (0.4 g, 30% yield).

M.p. 135-136°C.

^1H NMR (DMSO-d_6, 400 MHz): δ 10.49 (2s, 1H, N*H*), 8.33 (d, *J* = 8.5 Hz, 2H, Ar*H*), 8.10 (d, *J* = 8.5 Hz, 2H, Ar*H*), 7.69 (d, *J* = 113.6 Hz, 1H, N*H*), 6.61 (m, 1H, N*H*), 5.97 (s, 1H, Ar*H*), 3.82 (sext, *J* = 6.5 Hz, 1H, C*H*), 2.33-2.11 (m, 4H, CH_2CH_2CH), 1.05 (d, *J* = 6.6 Hz, 6H, CH_3).

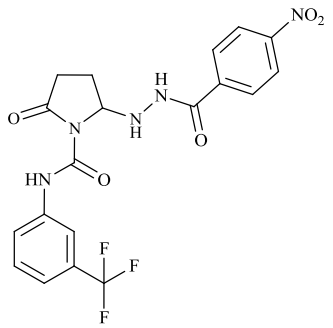
$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ 176.9 (C_{IV}), 176.7 (C_{IV}), 166.0 (C_{IV}), 165.3 (C_{IV}), 155.8 (C_{IV}), 149.7 (C_{IV}), 138.9 (C_{IV}), 130.0 (CH), 129.8 (CH), 123.4 (2CH), 66.7 (CH), 66.9 (CH), 42.1 (CH), 29.3 (CH_2), 29.0 (CH_2), 25.8 (CH_2), 24.7 (CH_2), 23.2 ($2CH_3$).

IR ν (cm^{-1}): 3243, 2974, 1677, 1523, 1348, 1274, 1245.

Anal. Calcd for $C_{15}H_{19}N_5O_5$: C, 51.57; H, 5.48; N, 20.05.

2-(2-(4-nitrobenzoyl)hydrazinyl)-5-oxo-*N*-(3-(trifluoromethyl)phenyl)pyrrolidine-1-carboxamide – 89

HEI 3016



N-isopropyl-2-(2-(4-nitrobenzoyl)hydrazinyl)-5-oxopyrrolidine-1-carboxamide was obtained from the reaction of 4-nitro-*N'*-(5-oxopyrrolidin-2-yl)benzohydrazide (3.7 mmol, 1 g) with isopropyl isocyanate (5.5 mmol, 0.55 mL), in 30 mL toluene, under nitrogen atmosphere, at 80°C, for 12 hs. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a yellow solid (0.4 g, 30% yield).

M.p. 227-228°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.81 (2s, 1H, N*H*), 9.16 (s, 1H, NH), 8.37 (d, *J* = 8.5 Hz, 2H, Ar*H*), 8.13 (t, *J* = 8.5 Hz, 2H, Ar*H*), 7.90 (2s, 1H, NH), 7.88 (s, 1H, Ar*H*), 7.79 (s, 1H, Ar*H*), 7.49 (t, *J* = 8.5 Hz, 2H, Ar*H*), 7.31 (d, *J* = 8.5 Hz, 1H, Ar*H*), 6.61 (2d, *J* = 8.0 Hz, 1H, N*H*), 2.45-2.20 (m, 2H, C*H*$_2$C*H*$_2$CH), 2.18-1.96 (m, 2H, C*H*$_2$C*H*$_2$CH).

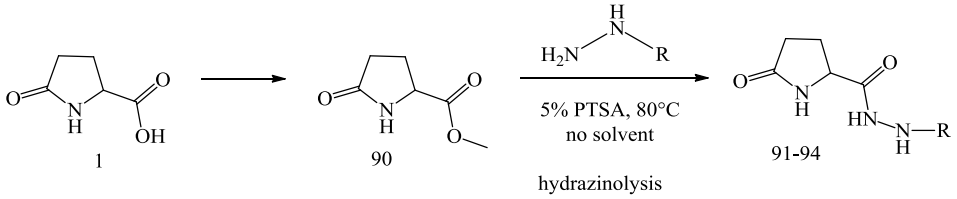
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.1 (C=O), 177.0 (C=O), 166.3 (C_{IV}), 165.5 (C_{IV}), 154.7 (C_{IV}), 149.9 (C_{IV}), 140.7 (2CH), 138.5 (2CH), 130.1 (CH), 130.0 (2CH), 129.9 (CH), 124.8 (q, J = 274 Hz, CF_3), 129.5 (q, J = 30 Hz, C_{IV}), 124.3 (2CH), 124.0 (CH), 123.9 (CH), 119.3 (q, *J* = 4.8 Hz, CH), 116.8 (q, *J* = 4.8 Hz, CH), 67.4 (CH), 67.1 (CH), 29.3 (CH_2), 28.9 (CH_2), 25.8 (CH_2), 24.6 (CH_2).

IR ν (cm^{-1}): 3284, 3177, 1683, 1523, 1505, 1245.

Anal. Calcd for C$_{15}$H$_{19}$N$_5$O$_5$: C, 51.57; H, 5.48; N, 20.05; O, 22.90.

1.2 Experimental procedures for the synthesis of pyrrolidine-based carbohydrazides derivatives

1.2.1 The synthesis of pyroglutamic acid carbohydrazides *via* hydrazinolysis

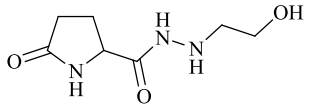


Scheme 53 General synthetic route of carbohydrazides via hydrazinolysis

This first pathway is a two-steps process based on modification of carboxylic acid **1**. The carboxylic acid **1** is methylated by action of $ZrCl_4$ with CH_3SO_3H in MeOH, in presence of molecular sieves 3Å at reflux to give methyl ester **90** in quantitative yield after 24 hours.[4] The obtained methyl pyroglutamate **90** is submitted to hydrazinolysis with free hydrazine derivatives in presence of PTSA as an acid catalyst, to afford carbohydrazides **91**-**94**. This pathway offers the possibility of a simple purification such as EtOH washing of the compound and recrystallization if needed. No side products were observed for this type of reaction.

_N'_-(2-hydroxyethyl)-5-oxopyrrolidine-2-carbohydrazide-91

HEI 3232



Ratio conformer 1/conformer 2: 92/08

Following the general procedure, *N'*-(2-hydroxyethyl)-5-oxopyrrolidine-2-carbohydrazide was obtained through the hydrazinolysis reaction of methyl-pyroglutamate (2 g, 13.9 mmol) and 2-hydrazinylethanol (1.04 g, 13.9 mmol), in the presence of a catalytic amount of PTSA, 5% (0.24 g). The mixture was stirred at 80° for 24 h. The precipitate was then washed with ethanol affording the compound as a white solid (1.1 g, 42 % yield).

M.p. 157-158°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.47 (s, 1H, N*H*, conformer 1), 8.44 (s, 1H, N*H*, conformer 2), 7.78 (s, 1 H, N*H*, conformer 1), 7.54 (s, 1H, N*H*, conformer 2), 4.96 (bs, 1H, O*H*, conformers 1+2), 4.49 (t, *J* = 5.8 Hz, 1H, C*H*, conformers 1+2), 3.95 (q, *J* = 4.4 Hz, 1H, C*H*, conformers 1+2), 3.44 (d, *J* = 5.83 Hz, 2H, C*H*$_2$, conformers 1+2), 2.74 (d, *J* = 5.83 Hz, 2H, C*H*$_2$, conformers 1+2), 2.20-2.07 (m, 3H, C*H*$_2$, conformers 1+2), 1.95 (m, 1H, C*H*$_2$, conformers 1+2).

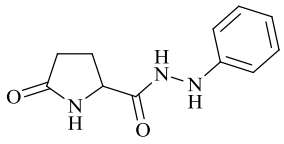
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.2 (C=O, conformer 1), 177.1 (C=O, conformer 2), 170.8 (C=O, conformers 1+2), 58.6 (CH, conformer 1), 58.2 (CH, conformer 2), 54.3 (CH_2, conformer 1), 53.7 (CH_2, conformer 2), 53.3 (CH_2, conformer 1), 52.1 (CH_2, conformer 2), 29.1 (CH_2, conformers 1+2), 25.0 (CH_2, conformers 1+2).

IR ν (cm^{-1}): 3260, 2924, 1685, 1657, 1272, 1065.

Anal. Calcd for $C_7H_{13}N_3O_3$: C, 44.91; H, 7.00; N, 22.45.

5-oxo-_N'_-phenylpyrrolidine-2-carbohydrazide-92

HEI 2840



Ratio conformer 1/conformer 2: 91/09

Following the general procedure, 5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide was obtained through the hydrazinolysis reaction of methyl-pyroglutamate (2 g, 13.9 mmol) and phenylhydrazine (1.5 g, 13.9 mmol), in the presence of a catalytic amount of PTSA, 5% (0.24 g). The mixture was stirred at 80° for 24 h. The precipitate was then washed with ethanol affording the compound as a white solid (2.0 g, 66 % yield).

M.p. 191-192°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.83 (d, *J* = 2.4 Hz, 1H, N*H*, conformer 1), 9.16 (s, 1H, N*H*, conformer 2), 7.91 (s, 1H, N*H*, conformer 1 + 2), 7.73 (d, *J* = 2.4 Hz, 1H, N*H*, conformer 1), 7.63 (s, 1H, N*H*, conformer 2), 7.21 (t, *J* = 8.3 Hz, 2H, Ar*H*, conformer 2), 7.13 (t, *J* = 8.3 Hz, 2H, Ar*H*, conformer 1), 6.75 (d, *J* = 8.3 Hz, 3H, Ar*H*, conformer 2), 6.75 (d, *J* = 8.3 Hz, 3H, Ar*H*, conformer 1), 4.36 (q, *J* = 4.3 Hz, 1 H, C*H*, conformer 2), 4.11 (q, *J* = 4.3 Hz, 1 H, C*H*, conformer 1), 2.32 (m, 1H, CH_2, conformers 1+2), 2.19-2.03 (m, 2H, CH_2, conformers 1+2), 1.95 (m, 1H, CH_2, conformers 1+2).

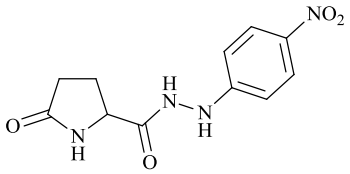
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ 177.7 (C=O), 172.6 (C=O), 149.5 (C_{IV}), 129.1 (2CHAr), 119.0 (CHAr), 112.6 (2CHAr), 54.8 (CH), 29.7 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3249, 1646, 1460, 1290, 1241.

Anal. Calcd for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98; N, 19.17.

***N'*-(4-nitrophenyl)-5-oxopyrrolidine-2-carbohydrazide – 93**

HEI 3037



Ratio conformer 1/conformer 2: 95/05

Following the general procedure, *N'*-(4-nitrophenyl)-5-oxopyrrolidine-2-carbohydrazide was obtained through the hydrazinolysis reaction of methyl-pyroglutamate (2 g, 13.9 mmol) and (4-nitrophenyl)hydrazine (2.4 g, 13.9 mmol), in the presence of a catalytic amount of PTSA, 5%

(0.24 g). The mixture was stirred at 80° for 24 h. The precipitate was then washed with ethanol, the compound as a white solid (2.1 g, 57 % yield).

M.p. 261-262°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.2 (s, 1H, N*H*, conformer 1), 9.48 (s, 1H, N*H*, conformer 2), 9.13 (s, 1 H, N*H*, conformer 2), 9.10-9.08 (s, 1H, N*H*, conformers 1+2), 8.10 (d, *J* = 9.4 Hz, 2H, Ar*H*, conformer 2), 8.06 (d, *J* = 9.4 Hz, 2H, Ar*H*, conformer 1), 7.95 (s, 1H, N*H*, conformer 1), 6.80 (d, *J* = 9.4 Hz, 2H, Ar*H*, conformer 2), 6.79 (d, *J* = 9.4 Hz, 2H, Ar*H*, conformer 1), 4.14 (q, *J* = 5.3 Hz, 1H, C*H*, conformers 1+2), 2.29 (m, 1H, CH_2, conformers 1+2), 2.22-2.15 (m, 2H, CH_2, conformers 1+2), 1.91 (m, 1H, CH_2, conformers 1+2).

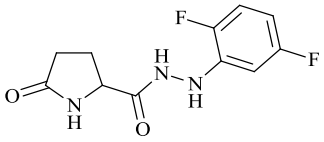
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.2 (C=O), 172.2 (C=O), 154.6 (C_{IV}), 138.0 (C_{IV}), 125.7 (2CHAr), 110.5 (2CHAr), 54.3 (CH), 29.1 (CH_2), 25.0 (CH_2).

IR ν (cm^{-1}): 3320, 3195, 2970, 1692, 1656, 1593, 1480, 1328, 1228, 832.

Anal. Calcd for $C_{11}H_{12}N_4O_4$: C, 50.00; H, 4.58; N, 21.20.

N'-(2,5-difluorophenyl)-5-oxopyrrolidine-2-carbohydrazide – 94

HEI 3036



Ratio conformer 1/conformer 2: 95/05

Following the general procedure, *N'*-(2,5-difluorophenyl)-5-oxopyrrolidine-2-carbohydrazide was obtained through the hydrazinolysis reaction of methyl-pyroglutamate (2 g, 13.9 mmol) and (2,5-difluorophenyl)hydrazine (2.0 g, 13.9 mmol), in the presence of a catalytic amount of PTSA, 5% (0.24 g). The mixture was stirred at 80° for 24 h. The precipitate was then washed with ethanol affording the compound as a white solid (1.4 g, 40 % yield).

M.p. 214-215°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.96 (d, *J* = 3.1 Hz, 1H, N*H*, conformer 1), 9.25 (s, 1H, N*H*, conformer 2), 8.31 (s, 1H, N*H*, conformer 2), 8.01 (s, 1H, N*H*, conformer 1), 7.96 (s, 1H, N*H*, conformer 1), 7.62 (s, 1H, N*H*, conformer 2), 7.09 (m, 1H, Ar*H*, conformers 1+2), 6.59 (m, 1H, Ar*H*, conformers 1+2), 6.49 (m, 1H, Ar*H*, conformers 1+2), 4.36 (q, *J* = 4.4 Hz, 1H, C*H*, conformer 2), 4.10 (q, *J* = 4.4 Hz, 1H, C*H*, conformer 1), 2.32 (m, 1H, CH_2, conformers 1+2), 2.18-2.13 (m, 2H, CH_2, conformers 1+2), 1.97 (m, 1H, CH_2, conformers 1+2).

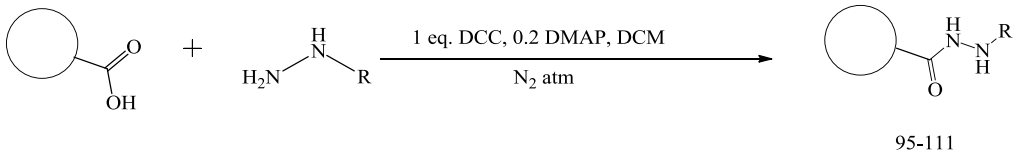
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.7 (C=O), 172.7 (C=O), 159.4 (d, C_{IV}, *J* = 239 Hz), 146.6 (dd, C_{IV} , *J* = 239 Hz, *J* = 2.3 Hz), 138.7 (dd, C_{IV}, *J* = 13 Hz, *J* = 10.3 Hz), 116.1 (dd,

CHAr, *J* =20.5 Hz, *J* = 10.7 Hz), 104.2 (dd, CHAr, *J* = 25 Hz, *J* = 8.5 Hz), 100.5 (dd, CHAr, *J* = 29 Hz, *J* = 3.8 Hz), 54.8 (CH), 29.7 (CH_2), 25.4 (CH_2).

IR ν (cm^{-1}): 3310, 3268, 3019, 1677, 1656, 1632, 1510, 1266, 1184, 846.

Anal. Calcd for $C_{11}H_{11}F_2N_3O_2$: C, 51.77; H, 4.34; N, 16.46.

1.2.2 The synthesis of carbohydrazides *via* a peptidic coupling protocol

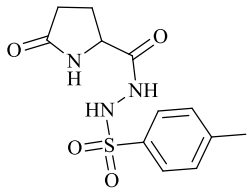


Scheme 54 General synthetic route of carbohydrazides via peptidic coupling

An alternative pathway to obtain carbohydrazides derivatives is based on the coupling of carboxylic acid derivatives with hydrazine derivatives. For realizing this coupling, 1equivalent of *N,N'*-dicyclohexylcarbodiimide (DCC) was employed in presence of 0.2 equivalents of base, 4-dimethylaminopyridine (DMAP), in an inert organic solvent, such as methylene chloride (DCM). The reaction was carried out at room temperature, under nitrogen. The reaction mixture was first washed with ethanol to remove the excess of 1,3-dicyclohexyl urea (DCU), and after purified through flash liquid chromatography being eluted with a gradient of *n*-heptane / ethyl acetate, to afford the target derivatives.

4-methyl-*N'*-(5-oxopyrrolidine-2-carbonyl) benzene sulfonohydrazide – 95

HEI 3038



Ratio conformer 1/conformer 2: 94/06

Following the general procedure, 4-methyl-*N'*-(5-oxopyrrolidine-2-carbonyl)benzenesulfono-hydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol) and 4-methylbenzenesulfonohydrazide (2.88 g, 15.5 mmol), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was

filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (2.0 g, 43% yield).

M.p. 190-191°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.2 (d, *J* = 3.6 Hz, 1H, N*H*, conformer 1), 9.89 (s, 1H, N*H*, conformer 2), 9.84 (d, *J* = 3.6 Hz, 1H, N*H*, conformer 1), 9.39 (s, 1H, N*H*, conformer 2), 7.72 (s, 1H, N*H*, conformers 1+2), 7.71 (d, *J* = 8.1 Hz, 2H, Ar*H*, conformers 1+2), 7.45 (d, *J* = 8.1 Hz, 2H, Ar*H*, conformer 2), 7.37 (d, *J* = 8.1 Hz, 2H, Ar*H*, conformer 1), 4.40 (q, *J* = 4.3 Hz, 1H, C*H*, conformer 2), 3.93 (q, *J* = 4.3 Hz, 1H, C*H*, conformer 1), 2.38 (s, 3H, CH_3, conformer 2), 2.40 (s, 3H, CH_3, conformer 1), 2.30 (m, 1H, CH_2, conformers 1+2), 2.01-1.91 (m, 2H, CH_2, conformers 1+2), 1.78 (m, 1H, CH_2, conformers 1+2).

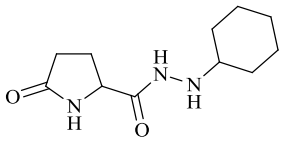
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.7 (C=O), 171.6 (C=O), 143.8 (C_{IV}), 136.2 (C_{IV}), 129.7 (2CHAr), 128.2 (2CHAr), 54.2 (CH), 29.3 (CH_2), 25.7 (CH_2), 21.5 (CH_3).

IR ν (cm^{-1}): 3370, 3046, 2858. 1712, 1657, 1349, 1162, 1080, 696.

Anal. Calcd for $C_{12}H_{15}N_3O_4S$: C, 48.47; H, 5.08; N, 14.13; S, 10.78

***N'*-cyclohexyl-5-oxopyrrolidine-2-carbohydrazide 96**

HEI 3231



Ratio conformer 1/conformer 2: 93/07

Following the general procedure, *N'*-cyclohexyl-5-oxopyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol) and cyclohexylhydrazine hydrochloride (2.33 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (0.53 g, 15% yield).

M.p. 141-142°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.41 (s, 1H, N*H*, conformer 1), 8.43 (s, 1H, N*H*, conformer 2), 7.78 (s, 1 H, N*H*, conformer 1), 7.53 (s, 1H, N*H*, conformer 2), 4.65 (bs, 1H, C*H*, conformers 1+2), 3.95 (t, *J* = 3.6 Hz, 1H, C*H*, conformers 1+2), 2.55 (m, 1H, CH_2, conformers 1+2), 2.20-2.00 (m, 3H, CH_2, conformers 1+2), 1.90-1.70 (m, 4H, CH_2, conformers 1+2), 1.15-1.05 (m, 6H, CH_2, conformers 1+2).

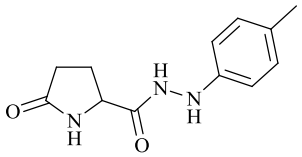
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.7 (C=O), 171.5 (C=O), 58.2 (CH), 54.8 (CH), 31.3 (CH_2), 31.2 (CH_2), 29.7 (CH_2), 26.1 (CH_2), 25.6 (CH_2), 24.4 (2CH_2).

IR ν (cm^{-1}): 3261, 3232, 2929, 2859, 1684, 1666, 1265.

Anal. Calcd for $C_{11}H_{19}N_3O_2$: C, 58.64; H, 8.50; N, 18.65.

N'-(4-toluyl)-5-oxopyrrolidine-2-carbohydrazide – 97

HEI 3136



Ratio conformer 1/conformer 2: 90/10

Following the general procedure, 5-oxo-*N'*-(p-tolyl)pyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol) and *p*-tolylhydrazine hydrochloride (2.45 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (0.9 g, 25% yield).

M.p. 228-229°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.80 (d, *J* = 4.3 Hz, 1H, N*H*, conformer 1), 9.11 (s, 1H, N*H*, conformer 2), 7.90 (s, 1 H, N*H*, conformer 1), 7.71 (s, 1H, N*H*, conformer 2), 7.61 (s, 1H, N*H*, conformer 2), 7.56 (d, *J* = 3.1 Hz, 1H, N*H*, conformer 1), 7.02 (d, *J* = 8.1 Hz, 2H, Ar*H*, conformer 2), 6.95 (d, *J* = 8.1 Hz, 2H, Ar*H*, conformer 1), 6.64 (d, *J* = 8.1 Hz, 2H, Ar*H*, conformers 1+2), 4.37 (q, *J* = 4.4 Hz, 1H, C*H*, conformer 2), 4.08 (q, *J* = 4.4 Hz, 1H, C*H*,

conformer 1), 2.30 (m, 1H, C*H*$_2$, conformers 1+2), 2.18-2.13 (m, 2H, C*H*$_2$, conformers 1+2), 2.16 (s, 3H, C*H*$_3$, conformers 1+2), 1.88 (m, 1H, C*H*$_2$, conformers 1+2).

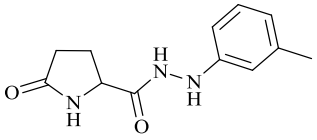
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.7 (C=O, conformer 1), 176.9 (C=O, conformer 2), 172.5 (C=O, conformers 1+2), 147.2 (C$_{IV}$, conformer 1), 146.5 (C$_{IV}$, conformer 2), 129.8 (2 Ar*H*, conformer 2), 129.5 (2 Ar*H*, conformer 1), 128.4 (C$_{IV}$, conformer 2), 127.6 (C$_{IV}$, conformer 1), 112.9 (2 Ar*H*, conformers 1+2), 54.8 (CH, conformer 1), 52.7 (CH, conformer 2), 29.7 (CH$_2$, conformers 1+2), 25.3 (CH$_2$, conformers 1+2), 20.60 (CH$_3$, conformers 1+2).

IR ν (cm^{-1}): 3283, 2986, 1659, 1511, 1285, 1232, 813.

Anal. Calcd for C$_{12}$H$_{15}$N$_3$O$_2$: C, 61.79; H, 6.48; N, 18.01.

N'-(3-toluyl)-5-oxopyrrolidine-2-carbohydrazide – 98

HEI 3227



Ratio conformer 1/conformer 2: 90/10

Following the general procedure, 5-oxo-*N'*-(*m*-tolyl)pyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol), *m*-tolylhydrazine hydrochloride (2.45 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (1.1 g, 30% yield).

M.p. 168-169°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.80 (d, *J* = 4.7 Hz, 1H, N*H*, conformer 1), 9.12 (s, 1H, N*H*, conformer 2), 7.92 (s, 1 H, N*H*, conformer 1), 7.84 (s, 1H, N*H*, conformer 2), 7.65 (d, *J* = 2.7 Hz, 1H, N*H*, conformer 1), 7.62 (s, 1H, N*H*, conformer 2), 7.07 (t, *J* = 7.8 Hz, 1H, Ar*H*, conformer 2), 7.03 (t, *J* = 7.8 Hz, 1H, Ar*H*, conformer 1), 6.52 (d, *J* = 7.8 Hz, 1H, Ar*H*, conformer 2), 6.51 (m, 3 H, Ar*H*, conformers 1+2), 4.37 (q, *J* = 4.2 Hz, 1H, C*H*, conformer 2), 4.09 (qd, *J* = 4.4, 0.8 Hz, 1H, C*H*, conformer 1), 2.29 (m, 1H, C*H*$_2$, conformers 1+2), 2.22-2.12

(m, 2H, CH_2, conformers 1+2), 2.20 (s, 3H, CH_3, conformers 1+2), 1.88 (m, 1H, CH_2, conformers 1+2).

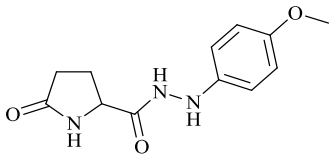
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.2 (C=O, conformer 1), 176.3 (C=O, conformer 2), 172.0 (C=O, conformers 1+2), 149.0 (C_{IV}, conformer 1), 148.3 (C_{IV}, conformer 2), 138.1 (C_{IV}, conformer 2), 137.7 (C_{IV}, conformer 1), 128.8 (CHAr, conformer 2), 128.5 (CHAr, conformer 1), 120.1 (CHAr, conformer 2), 119.3 (CHAr, conformer 1), 112.7 (CHAr, conformers 1+2), 109.4 (CHAr, conformers 1+2), 54.2 (CH, conformer 1), 52.2 (CH, conformer 2), 29.1 (CH_2, conformer 1), 29.0 (CH_2, conformer 2), 25.1 (CH_2, conformer 1), 24.7 (CH_2, conformer 2), 21.2 (CH_3, conformers 1+2).

IR ν (cm^{-1}): 3298, 3206, 1697, 1664, 1597, 1259, 1087.

Anal. Calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.01.

N'-(4-methoxyphenyl)-5-oxopyrrolidine-2-carbohydrazide – 99

HEI 3128



Ratio conformer 1/conformer 2: 90/10

Following the general procedure, *N'*-(4-methoxyphenyl)-5-oxopyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol), (4-methoxyphenyl)hydrazine hydrochloride (2.7 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (1.35 g, 35% yield).

M.p. 141-142°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.80 (d, *J* = 3.3 Hz, 1H, N*H*, conformer 1), 9.05 (s, 1H, N*H*, conformer 2), 7.89 (s, 1 H, N*H*, conformer 1), 7.56 (s, 1H, N*H*, conformer 2), 7.41 (d, *J* = 3.3 Hz, 1H, N*H*, conformers 1+2), 6.78 (d, *J* = 9.1 Hz, 2H, Ar*H*, conformer 2), 6.77 (d, *J* = 9.1 Hz, 2H, Ar*H*, conformer 1), 6.67 (d, *J* = 9.1 Hz, 2H, Ar*H*, conformers 1+2), 4.39 (q, *J* = 4.3

Hz, 1H, C*H*, conformer 2), 4.08 (q, *J* = 4.3 Hz, 1H, C*H*, conformer 1), 3.70 (s, 3H, OCH_3, conformer 2), 3.65 (s, 3H, OCH_3, conformer 2), 2.30 (m, 1H, CH_2, conformers 1+2), 2.18-2.13 (m, 2H, CH_2, conformers 1+2), 1.89 (m, 1H, CH_2, conformers 1+2).

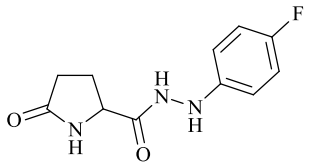
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.2 (C=O, conformer 1), 176.3 (C=O, conformer 2), 171.9 (C=O, conformers 1+2), 153.0 (C_{IV}, conformer 2), 152.6 (C_{IV}, conformer 1), 142.8 (C_{IV}, conformer 1), 142.1 (C_{IV}, conformer 2), 114.3 (2CHAr, conformer 2), 114.1 (2CHAr, conformer 1), 113.6 (2CHAr, conformer 1), 113.5 (2CHAr, conformer 2), 55.2 (CH_2, conformers 1+2), 54.2 (CH_3, conformer 1), 52.2 (CH_3, conformer 2), 29.1 (CH_2, conformer 1), 29.0 (CH_2, conformer 2), 25.0 (CH_2, conformer 1), 24.8 (CH_2, conformer 2).

IR ν (cm^{-1}): 3200, 3085, 2832, 1696, 1659, 1508, 1248, 1216, 1031, 833.

Anal. Calcd for $C_{12}H_{15}N_3O_3$: C, 57.82; H, 6.07; N, 16.86.

N'-(4-fluorophenyl)-5-oxopyrrolidine-2-carbohydrazide – 100

HEI 3213



Ratio conformer 1/conformer 2: 91/09

Following the general procedure, *N'*-(4-fluorophenyl)-5-oxopyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol), (4-fluorophenyl)hydrazine hydrochloride (2.5 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (0.85 g, 23% yield).

M.p. 221-222°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.86 (d, *J* = 2.9 Hz, 1H, N*H*, conformer 1), 9.19 (s, 1H, N*H*, conformer 2), 7.90 (s, 1 H, N*H*, conformers 1+2), 7.72 (d, *J* = 2.9 Hz, 1H, N*H*, conformer 1), 7.55 (s, 1H, N*H*, conformer 2), 7.08 (t, *J* = 9.0 Hz, 2H, Ar*H*, conformer 2), 6.98 (t, *J* = 9.0 Hz, 2H, Ar*H*, conformer 1), 6.73 (q, *J* = 4.8 Hz, 2H, Ar*H*, conformers 1+2), 4.30 (q, *J* = 4.3

Hz, 1H, C*H*, conformer 2), 4.09 (q, *J* = 4.3 Hz, 1H, C*H*, conformer 1), 2.33 (m, 1H, CH_2, conformers 1+2), 2.19-2.11 (m, 2H, CH_2, conformers 1+2), 1.91 (m, 1H, CH_2, conformers 1+2).

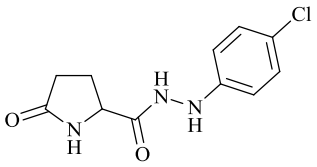
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.7 (C=O), 172.6 (C=O), 156.3 (d, C_{IV}, *J* = 232 Hz, C-F), 146.1 (C_{IV}, *J* = 2.2 Hz), 115.5 (d, 2CHAr, *J* = 24.0 Hz), 113.8 (d, 2CHAr, *J* = 6.9 Hz), 54.8 (CH), 29.7 (CH_2), 25.5 (CH_2).

IR ν (cm^{-1}): 3331, 3273, 1687, 1654, 1648, 1506, 1269, 1207, 826.

Anal. Calcd for $C_{11}H_{12}FN_3O_2$: C, 55.69; H, 5.10; N, 17.71.

N'-(4-chlorophenyl)-5-oxopyrrolidine-2-carbohydrazide – 101

HEI 3216



Ratio conformer 1/conformer 2: 92/08

Following the general procedure, *N'*-(4-chlorophenyl)-5-oxopyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol), (4-chlorophenyl)hydrazine hydrochloride (2.75 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (0.78 g, 20% yield).

M.p. 234-235°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.89 (d, *J* = 2.7 Hz, 1H, N*H*, conformer 1), 9.21 (s, 1H, N*H*, conformer 2), 8.11 (s, 1 H, N*H*, conformer 2), 7.94 (s, 1H, N*H*, conformer 1), 7.92 (d, *J* = 2.7 Hz, 1H, N*H*, conformer 1), 7.62 (s, 1H, N*H*, conformer 2), 7.24 (d, *J* = 8.5 Hz, 2H, Ar*H*, conformer 2), 7.16 (d, *J* = 8.5 Hz, 2H, Ar*H*, conformer 1), 6.71 (d, *J* = 8.5 Hz, 2H, Ar*H*, conformers 1+2), 4.36 (q, *J* = 4.1 Hz, 1H, C*H*, conformer 2), 4.08 (q, *J* = 4.1 Hz, 1H, C*H*, conformer 1), 2.30 (m, 1H, CH_2, conformers 1+2), 2.18-2.11 (m, 2H, CH_2, conformers 1+2), 1.92 (m, 1H, CH_2, conformers 1+2).

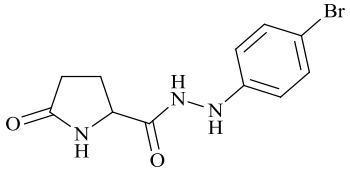
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 177.2 (C=O), 172.1 (C=O), 148.0 (C_{IV}), 128.4 (2CHAr), 121.8 (C_{IV}), 113.6 (2CHAr), 54.2 (CH), 29.1 (CH_2), 25.0 (CH_2).

IR ν (cm^{-1}): 3276, 3098, 3032, 1664, 1645, 1490, 1267, 1224, 1087.

Anal. Calcd for $C_{11}H_{12}ClN_3O_2$: C, 52.08; H, 4.77; N, 16.56.

N'-(4-bromophenyl)-5-oxopyrrolidine-2-carbohydrazide – 102

HEI 3230



Ratio conformer 1/conformer 2: 93/07

Following the general procedure, *N'*-(4-bromophenyl)-5-oxopyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol), (4-bromophenyl)hydrazine hydrochloride (3.5 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (0.82 g, 18% yield).

M.p. 212-213°C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.89 (d, *J* = 2.9 Hz, 1H, N*H*, conformer 1), 9.18 (s, 1H, N*H*, conformer 2), 8.67 (s, 1 H, N*H*, conformer 2), 7.95 (d, *J* = 2.9 Hz, 1H, N*H*, conformer 1), 7.91 (s, 1H, N*H*, conformer 1), 7.59 (s, 1H, N*H*, conformer 2), 7.33 (d, *J* = 8.7 Hz, 2H, Ar*H*, conformer 2), 7.29 (d, *J* = 8.7 Hz, 2H, Ar*H*, conformer 1), 6.67 (d, *J* = 8.7 Hz, 2H, Ar*H*, conformers 1+2), 4.30 (q, *J* = 3.8 Hz, 1H, C*H*, conformer 2), 4.05 (q, *J* = 3.8 Hz, 1H, C*H*, conformer 1), 2.33 (m, 1H, CH_2, conformers 1+2), 2.19-2.13 (m, 2H, CH_2, conformers 1+2), 1.90 (m, 1H, CH_2, conformers 1+2).

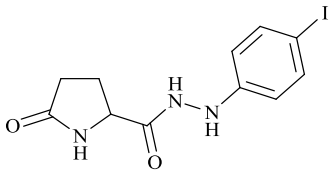
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 177.7 (C=O), 172.6 (C=O), 148.9 (C_{IV}), 131.7 (2CHAr), 114.6 (2CHAr), 109.8 (C_{IV}), 54.8 (CH), 29.7 (CH_2), 25.5 (CH_2).

IR ν (cm^{-1}): 3278, 3097, 2901, 1672, 1666, 1487, 1266, 1225, 1071.

Anal. Calcd for $C_{11}H_{12}BrN_3O_2$: C, 44.31; H, 4.06; N, 14.09.

_N'_-(4-iodophenyl)-5-oxopyrrolidine-2-carbohydrazide – 103

HEI 3240



Ratio conformer 1/conformer 2: 90/10

Following the general procedure, _N'_-(4-iodophenyl)-5-oxopyrrolidine-2-carbohydrazide was obtained through the hydrazinolysis reaction of methyl-pyroglutamate (0.62 g, 4.2 mmol) and (4-iodophenyl)hydrazine (1 g, 4.2 mmol), in the presence of a catalytic amount of PTSA, 5% (0.08 g). The mixture was stirred at 80°C for 24 h. The precipitate was then washed with ethanol affording the compound as a white solid (0.32 g, 20% yield).

M.p. 187-188°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.88 (d, *J* = 2.7 Hz, 1H, N*H*, conformer 1), 9.21 (s, 1H, N*H*, conformer 2), 8.10 (s, 1 H, N*H*, conformer 2), 7.96 (d, *J* = 3.4 Hz, 1H, N*H*, conformer 1), 7.90 (s, 1H, N*H*, conformer 1), 7.16 (s, 1H, N*H*, conformer 2), 7.51 (d, *J* = 9.1 Hz, 2H, Ar*H*, conformer 2), 7.43 (d, *J* = 9.1 Hz, 2H, Ar*H*, conformer 1), 7.10 (d, *J* = 9.1 Hz, 2H, Ar*H*, conformer 2), 6.57 (d, *J* = 9.1 Hz, 2H, Ar*H*, conformer 1), 4.18 (m, 1H, C*H*, conformer 2), 4.08 (q, *J* = 4.5 Hz, 1H, C*H*, conformer 1), 2.32 (m, 1H, CH_2, conformers 1+2), 2.19-2.08 (m, 2H, CH_2, conformers 1+2), 1.90 (m, 1H, CH_2, conformers 1+2).

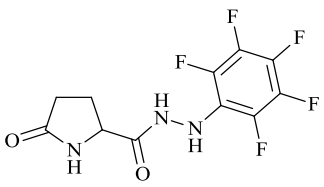
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.7 (C=O), 172.6 (C=O), 149.4 (C_{IV}), 137.5 (2CHAr), 115.2 (2CHAr), 80.3 (C_{IV}), 54.8 (CH), 29.7 (CH_2), 25.5 (CH_2).

IR ν (cm^{-1}): 3205, 2960, 1667, 1654, 1483, 1260, 1060.

Anal. Calcd for C$_{11}$H$_{12}$IN$_3$O$_2$: C, 38.28; H, 3.50; N, 12.17.

_N'_-(perfluorophenyl)-5-oxopyrrolidine-2-carbohydrazide – 105

HEI 3122



Ratio conformer 1/conformer 2: 93/07

Following the general procedure, 5-oxo-*N'*-(perfluorophenyl)pyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol), (perfluorophenyl)hydrazine hydrochloride (3.6 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (1.2 g, 25% yield).

M.p. 218-219°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.25 (s, 1H, N*H*, conformer 1), 9.42 (s, 1H, N*H*, conformer 2), 8.25 (s, 1H, N*H*, conformer 2), 8.02 (s, 1H, N*H*, conformer 1), 7.85 (s, 1H, N*H*, conformer 1), 7.77 (s, 1H, N*H*, conformer 2), 4.37 (bs, 1 H, C*H*, conformer 2), 4.04 (q, *J* = 3.6 Hz, 1 H, C*H*, conformer 1), 2.23 (m, 1H, CH_2, conformers 1+2), 2.15-2.08 (m, 2H, CH_2, conformers 1+2), 1.85 (m, 1H, CH_2, conformers 1+2).

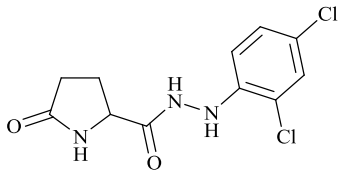
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.8 (C=O), 172.8 (C=O), 139.1 (m, 2C$_{IV}$), 136.7 (m, 2C$_{IV}$,), 135.5 (tt, C$_{IV}$, *J* = 13.6 Hz, *J* = 3.7 Hz), 133.1 (tt, C$_{IV}$, *J* = 13.7 Hz, *J* = 4.4 Hz), 124.9 (td, C$_{IV}$, *J* = 11.3 Hz, *J* = 3.0 Hz), 54.4 (CH), 29.5 (CH$_2$), 25.5 (CH$_2$).

IR ν (cm^{-1}): 3277, 3210, 3023, 1684, 1661, 1523, 1275, 1010, 963.

Anal. Calcd for C$_{11}$H$_8$F$_5$N$_3$O$_2$: C, 42.73; H, 2.61; N, 13.59.

N'-(2,4-dichlorophenyl)-5-oxopyrrolidine-2-carbohydrazide – 105

HEI 3133



Ratio conformer 1/conformer 2: 95/05

Following the general procedure, *N'*-(2,4-dichlorophenyl)-5-oxopyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol), (2,4-dichlorophenyl)hydrazine hydrochloride (3.3 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and

dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (1.12 g, 27% yield).

M.p. 221-222°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.02 (s, 1H, N*H*, conformer 1), 9.31 (s, 1H, N*H*, conformer 2), 8.16 (s, 1H, N*H*, conformer 2), 7.92 (s, 1H, N*H*, conformer 1), 7.66 (s, 1H, N*H*, conformers 1+2), 7.42 (s, 1H, Ar*H*, conformer 2), 7.41 (s, 1H, Ar*H*, conformer 1), 7.24 (bs, 1H, Ar*H*, conformer 2), 7.22 (d, *J* = 9.2 Hz, 1H, Ar*H*, conformer 1), 6.82 (bs, 1H, Ar*H*, conformer 2), 6.79 (d, *J* = 9.2 Hz, 1H, Ar*H*, conformer 1), 4.29 (bs, 1 H, C*H*, conformer 2), 4.11 (bs, 1H, C*H*, conformer 1), 2.32 (m, 1H, CH_2, conformers 1+2), 2.20-1.92 (m, 2H, CH_2, conformers 1+2), 1.98 (m, 1H, CH_2, conformers 1+2).

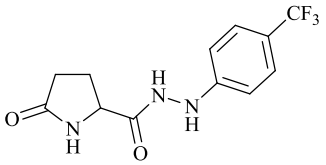
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.7 (C=O), 172.7 (C=O), 144.1 (C_{IV}), 128.8 (CHAr), 128.1 (CHAr), 122.4 (C_{IV}), 118.0 (C_{IV}), 114.4 (CHAr), 54.8 (CH), 29.6 (CH_2), 25.4 (CH_2).

IR ν (cm^{-1}): 3313, 3230, 2970, 1679, 1654, 1492, 1271, 1095, 802.

Anal. Calcd for $C_{11}H_{11}Cl_2N_3O_2$: C, 45.85; H, 3.85; N, 14.58.

N'-(4-trifluoro methylphenyl)-5-oxopyrrolidine-2-carbohydrazide – 106

HEI 3126



Ratio conformer 1/conformer 2: 94/06

Following the general procedure, 5-oxo-*N'*-(4-(trifluoromethyl)phenyl)pyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (0.73 g, 5.6 mmol), (4-(trifluoromethyl)phenyl)hydrazine (0.99 g, 5.6 mmol), in presence of dicyclohexyl carbodiimide (1.1 g, 5.5 mmol) and dimethylaminopyridine (0.06 g, 1.55 mmol) which were dissolved in 50 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (0.32 g, 20% yield).

M.p. 101-102°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.0 (s, 1H, N*H*, conformer 1), 9.34 (s, 1H, N*H*, conformer 2), 8.54 (s, 1 H, N*H*, conformer 2), 8.40 (s, 1H, N*H*, conformer 1), 7.93 (s, 1H, N*H*, conformer 1), 7.66 (s, 1H, N*H*, conformer 2), 7.53 (d, *J* = 8.3 Hz, 2H, Ar*H*, conformer 2), 7.46 (d, *J* = 8.3 Hz, 2H, Ar*H*, conformer 1), 6.82 (d, *J* = 8.3 Hz, 2H, Ar*H*, conformers 1+2), 4.33 (bs, 1H, C*H*, conformer 2), 4.12 (q, *J* = 4.5 Hz, 1H, C*H*, conformer 1), 2.31 (m, 1H, CH_2, conformers 1+2), 2.23-2.13 (m, 2H, CH_2, conformers 1+2), 1.88 (m, 1H, CH_2, conformers 1+2).

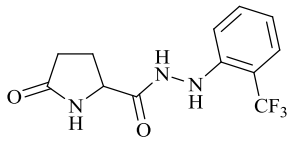
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.7 (C=O), 172.7 (C=O), 152.7 (C_{IV}), 126.6 (q, 2CHAr, *J* = 3.9 Hz), 124.4 (q, C_{IV}, *J* = 270 Hz), 119.2 (2CHAr), 118.7 (q, C_{IV}, *J* = 32 Hz), 54.8 (CH), 29.7 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3354, 3279, 3033, 2946, 1652, 1617, 1332, 1099, 1067.

Anal. Calcd for $C_{12}H_{12}F_3N_3O_2$: C, 50.18; H, 4.21; N, 14.63.

N'-(2-trifluoro methylphenyl)-5-oxopyrrolidine-2-carbohydrazide – 107

HEI 3212



Ratio conformer 1/conformer 2: 92/08

Following the general procedure, 5-oxo-*N'*-(2-(trifluoromethyl)phenyl)pyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (0.73 g, 5.6 mmol), (2-(trifluoromethyl)phenyl)hydrazine (0.99 g, 5.6 mmol), in presence of dicyclohexyl carbodiimide (1.1 g, 5.5 mmol) and dimethylaminopyridine (0.06 g, 1.55 mmol) which were dissolved in 50 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (0.40 g, 25% yield).

M.p. 150-151°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.1 (s, 1H, N*H*, conformer 1), 9.36 (s, 1H, N*H*, conformer 2), 7.980 (s, 1 H, N*H*, conformer 2), 7.93 (s, 1 H, N*H*, conformer 1), 7.65 (s, 1 H, N*H*, conformer 2), 7.48 (m, 3H, Ar*H*+N*H*, conformers 1+2), 7.02 (d, *J* = 8.2 Hz, 1H, Ar*H*, conformer 2), 6.98 (d, *J* = 8.2 Hz, 1H, Ar*H*, conformer 1), 6.88 (t, *J* = 8.2 Hz, 1H, Ar*H*, conformers 1+2), 4.30 (q, *J* = 4.3 Hz, 1H, C*H*, conformer 2), 4.13 (q, *J* = 4.3 Hz, 1H, C*H*, conformer 1), 2.33 (m, 1H,

CH_2, conformers 1+2), 2.19-2.11 (m, 2H, CH_2, conformers 1+2), 1.91 (m, 1H, CH_2, conformers 1+2).

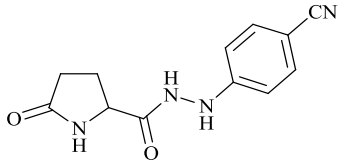
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.8 (C=O), 172.6 (C=O), 146.4 (q, C_{IV}, J = 1.7 Hz), 133.8 (s, CHAr), 126.5 (q, C_{IV}, *J* =5.3 Hz), 125.0 (q, C_{IV}, *J* = 271 Hz), 118.7 (s, CHAr), 113.5 (s, CHAr), 54.8 (CH), 29.6 (CH_2), 25.3 (CH_2).

IR ν (cm^{-1}): 3288, 3058, 2951, 1655, 1632, 1312, 1064.

Anal. Calcd for $C_{12}H_{12}F_3N_3O_2$: C, 50.18; H, 4.21; N, 14.63.

N'-(4-cyanophenyl)-5-oxopyrrolidine-2-carbohydrazide – 108

HEI 3219



Ratio conformer 1/conformer 2: 95/05

Following the general procedure, *N'*-(4-cyanophenyl)-5-oxopyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol), 4-hydrazinylbenzonitrile hydrochloride (2.62 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (1.13 g, 30% yield).

M.p. 250-251°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.05 (s, 1H, N*H*, conformer 1), 9.4 (s, 1H, N*H*, conformer 2), 8.76 (s, 1H, N*H*, conformer 2), 8.64 (s, 1H, N*H*, conformer 1), 7.93 (s, 1H, N*H*, conformers 1+2), 7.64 (d, *J* = 8.6 Hz, 2H, Ar*H*, conformer 2), 7.56 (d, *J* = 8.6 Hz, 2H, Ar*H*, conformer 1), 6.79 (d, *J* = 8.6 Hz, 2H, Ar*H*, conformer 2), 6.78 (d, *J* = 8.6 Hz, 2H, Ar*H*, conformer 1), 4.30 (bs, 1 H, C*H*, conformer 2), 4.12 (q, *J* = 3.7 Hz, 1 H, C*H*, conformer 1), 2.34 (m, 1H, CH_2, conformers 1+2), 2.20-1.90 (m, 2H, CH_2, conformers 1+2), 1.97 (m, 1H, CH_2, conformers 1+2).

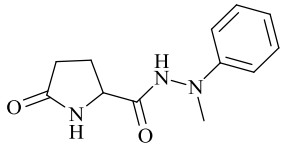
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 177.2 (C=O), 172.2 (C=O), 152.5 (C_{IV}), 133.3 (2CHAr), 119.9 (C_{IV}), 111.6 (2CHAr), 98.9 (C_{IV}), 54.3 (CH), 29.1(CH_2), 25.0 (CH_2).

IR ν (cm^{-1}): 3333, 3295, 3216, 2970, 2212, 1695, 1657, 1607, 1503, 1229, 822.

Anal. Calcd for $C_{12}H_{12}N_4O_2$: C, 59.01; H, 4.95; N, 22.94.

N'-methyl-5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide – 109

HEI 3187



Ratio conformer 1/conformer 2: 92/08

Following the general procedure, *N'*-methyl-5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2.0 g, 15.5 mmol), 1-methyl-1-phenylhydrazine (1.9 g, 15.5 mmol), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (2.17 g, 60% yield).

M.p. 219-220°C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.13 (s, 1H, N*H*, conformer 1), 9.35 (s, 1H, N*H*, conformer 2), 7.90 (s, 1H, N*H*, conformer 1), 7.62 (s, 1H, N*H*, conformer 2), 7.23 (t, *J* = 8.3 Hz, 2H, Ar*H*, conformer 2), 7.21 (t, *J* = 8.3 Hz, 2H, Ar*H*, conformer 1), 6.79 (d, *J* = 8.3 Hz, 3H, Ar*H*, conformer 2), 6.77 (t, *J* = 8.3 Hz, 3H, Ar*H*, conformer 1), 4.22 (bs, 1 H, C*H*, conformer 2), 4.06 (q, *J* = 3.5 Hz, 1 H, C*H*, conformer 1), 3.09 (s, 3H, CH_3, conformer 2), 3.07 (s, 3H, CH_3, conformer 1), 2.23 (m, 1H, CH_2, conformers 1+2), 2.18-2.12 (m, 2H, CH_2, conformers 1+2), 1.90 (m, 1H, CH_2, conformers 1+2),

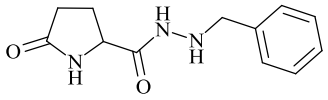
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 177.7 (C=O), 171.7 (C=O), 150.1 (C_{IV}), 129.2 (2CHAr), 118.8 (CHAr), 112.8 (2CHAr), 54.8 (CH), 29.7 (CH_2), 25.3 (CH_2), 25.3.

IR ν (cm^{-1}): 3335, 3202, 3078, 2970, 1669, 1451, 1245.

Anal. Calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.01.

_N'_-benzyl-5-oxopyrrolidine-2-carbohydrazide – 110

HEI 3269



Ratio conformer 1/conformer 2: 91/09

Following the general procedure, 5-oxo-_N'_-(p-tolyl)pyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol) and benzylhydrazine hydrochloride (2.45 g, 15.5 mmol) which at first was released in situ in a presence of sodium methanoate (3.5 mL, 30% aqueous methanolic solution), in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (1.4 g, 39% yield).

M.p. 134-135°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.43 (d, _J_ = 6.0 Hz, 1H, N_H_, conformer 1), 8.57 (s, 1H, N_H_, conformer 2), 7.75 (s, 1 H, N_H_, conformer 1), 7.47 (s, 1H, N_H_, conformer 2), 7.33-7.25 (m, 5H, Ar_H_, conformers 1+2), 5.26 (d, _J_ = 5.6 Hz, 1H, N_H_, conformer 1), 4.53 (s, 1H, N_H_, conformer 2), 3.90 (q, _J_ = 3.2 Hz, 1H, C_H_, conformers 1+2), 3.85 (t, _J_ = 4.4 Hz, 2H, CH_2, conformers 1+2), 2.16-2.05 (m, 3H, CH_2, conformers 1+2), 1.75 (m, 1H, CH_2, conformers 1+2).

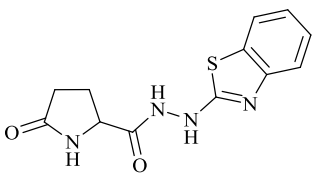
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.8 (C=O), 171.8 (C=O, conformer 2), 139.0 (C_{IV}), 129.0 (2CHAr, conformer 2), 128.6 (2CHAr, conformer 1), 127.4 (CHAr), 54.9 (CH_2), 54.7 (CH), 29.6 (CH_2), 25.7 (CH_2).

IR ν (cm^{-1}): 3247, 1701, 1658, 1537, 1256, 1078.

Anal. Calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.01.

_N'_-(benzo[d]thiazol-2-yl)-5-oxopyrrolidine-2-carbohydrazide – 111

HEI 3270



Ratio conformer 1/conformer 2: 94/06

Following the general procedure, *N'*-(benzo[d]thiazol-2-yl)-5-oxopyrrolidine-2-carbohydrazide was obtained from the coupling reaction of 5-oxopyrrolidine-2-carboxylic acid (2 g, 15.5 mmol), 2-hydrazinylbenzo[d]thiazole (2.55 g, 15.5 mmol) in presence of dicyclohexyl carbodiimide (3.2 g, 15.5 mmol) and dimethylaminopyridine (0.19 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (2.1 g, 50% yield).

M.p. 251-252°C

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.52 (s, 1H, N*H*, conformer 1), 9.89 (s, 1H, N*H*, conformer 1), 7.96 (s, 1H, N*H*, conformer 1), 7.77 (d, *J* = 7.6 Hz, 1H, Ar*H*, conformers 1+2), 7.46 (d, *J* = 8.0 Hz, 1H, Ar*H*, conformers 1+2), 7.28 (t, *J* = 7.6 Hz, 1H, Ar*H*, conformers 1+2), 7.10 (d, *J* = 7.6 Hz, 1H, Ar*H*, conformers 1+2), 4.41 (bs, 1 H, C*H*, conformer 2), 4.14 (q, *J* = 4.0 Hz, 1 H, C*H*, conformer 1), 2.20 (m, 1H, CH_2, conformers 1+2), 2.18 (m, 2H, CH_2, conformers 1+2), 1.97 (m, 1H, CH_2, conformers 1+2).

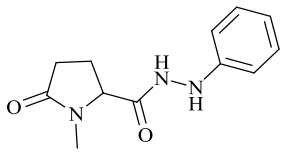
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.8 (C=O), 172.9 (C=O), 170.4 (C_{IV}), 152.6 (C_{IV}), 130.6 (C_{IV}), 126.3 (CHAr), 122.1 (CHAr), 121.8 (CHAr), 119.3 (CHAr), 54.7 (CH), 29.5 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3191, 2876, 1674, 1607, 1531, 1444, 1262.

Anal. Calcd for $C_{12}H_{12}N_4O_2S$: C, 52.16; H, 4.38; N, 20.28; S, 11.60.

1-methyl-5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide – 112

HEI 3132



Ratio conformer 1/conformer 2: 91/09

1-methyl-5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide was obtained from the reaction of hydrazinolysis of methyl 1-methyl-5-oxopyrrolidine-2-carboxylate (2 g, 12.7 mmol) and phenylhydrazine (1.37 g, 12.7 mmol), in the presence of a catalytic amount of $ZrCl_4$, (10%, 0.3 g). The mixture was stirred at 80°C for 24 h. The crude product was purified *via* flash column

chromatography with a gradual amount of acetate/heptane (0:100 v/v) affording the compound as a white solid (0.9 g, 30% yield).

M.p. 187-188°C

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.0 (d, *J* = 2.0 Hz, 1H, N*H*, conformer 1), 9.29 (s, 1H, N*H*, conformer 2), 8.01 (s, 1H, N*H*, conformer 2), 7.84 (d, *J* = 2.0 Hz, 1H, N*H*, conformer 1), 7.23 (t, *J* = 7.7 Hz, 2H, Ar*H*, conformer 2), 7.16 (t, *J* = 7.7 Hz, 2H, Ar*H*, conformer 1), 6.78 (d, *J* = 7.7 Hz, 3H, Ar*H*, conformer 2), 6.71 (d, *J* = 7.7, 1.3 Hz, 3H, Ar*H*, conformer 1), 4.44 (q, *J* = 3.6 Hz, 1 H, C*H*, conformer 2), 4.13 (q, *J* = 3.6 Hz, 1 H, C*H*, conformer 1), 2.66 (s, 3H, CH_3, conformer 1), 2.62 (s, 3H, CH_3, conformer 2), 2.28-2.18 (m, 3H, CH_2, conformers 1+2), 1.87 (m, 1H, CH_2, conformers 1+2).

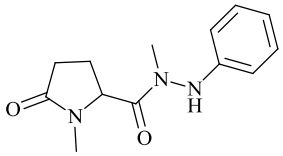
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 174.9 (C=O), 171.4 (C=O), 149.5 (C_{IV}), 129.2 (2CHAr), 119.1 (CHAr), 112.5 (2CHAr), 60.7 (CH), 29.6 (CH_2), 28.4 (CH_2), 22.9 (CH_3).

IR ν (cm^{-1}): 3341, 3246, 3029, 2961, 1671, 1495, 1222.

Anal. Calcd for C$_{12}$H$_{15}$N$_3$O$_2$: C, 61.79; H, 6.48; N, 18.01.

N,1-dimethyl-5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide – 113

HEI 3205



Ratio conformer 1/conformer 2: 44/56

N,1-dimethyl-5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide was obtained through the alkylation reaction of 5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide (2 g, 9.1 mmol), and iodomethane (1.5 g, 10.9 mmol), in presence of sodium hydride (60% dispersion in mineral oil, 0.04 g, 0.91 mmol), in 50 mL THF. The mixture was stirred at room temperature for 12 h. The crude product which was purified via flash column chromatography with a gradual amount of acetate/heptane (0:100 v/v) affording the compound as a white solid (0.8 g, 35% yield).

M.p. 187-188°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 7.60 (s, 1H, N*H*, conformer 1), 7.58 (s, 1H, N*H*, conformer 2), 7.33 (d, *J* = 7.3 Hz, 2H, Ar*H*, conformer 1), 7.30 (d, *J* = 7.3 Hz, 2H, Ar*H*, conformer 2), 6.97 (m, 1H, Ar*H*, conformers 1+2), 6.77 (dt, *J* = 8.8, 1.3 Hz, 2H, Ar*H*, conformers 1+2), 4.54 (q, *J* = 5.1 Hz, 1 H, C*H*, conformer 2), 4.33 (q, *J* = 5.1 Hz, 1 H, C*H*, conformer 1), 3.08 (s, 3H, CH_3, conformer 2), 3.07 (s, 3H, CH_3, conformer 1), 2.82 (s, 3H, CH_3, conformer 2), 2.80

(s, 3H, C*H*$_3$, conformer 1), 2.30 (m, 1H, C*H*$_2$, conformers 1+2), 2.09-1.91 (m, 2H, C*H*$_2$, conformers 1+2), 1.87 (m, 1H, C*H*$_2$, conformers 1+2).

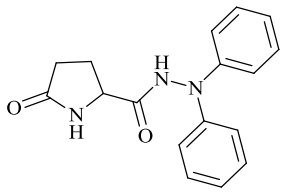
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.0 (C=O, conformer 1), 174.5 (C=O, conformer 2), 174.4 (C=O, conformers 1+2), 147.2 (C_{IV}, conformer 1), 146.9 (C_{IV}, conformer 2), 129.4 (2CHAr, conformer 2), 129.3 (2CHAr, conformer 1), 120.3 (CHAr, conformer 2), 119.9 (CHAr, conformer 1), 113.5 (2CHAr, conformer 2), 113.4 (2CHAr, conformer 1), 52.9 (CH, conformer 2), 52.5 (CH, conformer 1), 29.2 (CH_2, conformer 1), 29.0 (CH_2, conformer 2), 27.8 (CH_2, conformer 1), 27.4 (CH_2, conformer 2), 24.8 (CH_3, conformers 1+2), 24.5 (CH_3, conformers 1+2).

IR ν (cm^{-1}): 3230, 2980, 2896, 1689, 1671, 1249.

Anal. Calcd for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99.

5-oxo-*N'*,*N'*-diphenylpyrrolidine-2-carbohydrazide – 114

HEI 3173



Ratio conformer 1/conformer 2: 96/04

5-oxo-*N'*,*N'*-diphenylpyrrolidine-2-carbohydrazide was obtained through the alkylation reaction of 5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide (2 g, 9.1 mmol), and iodobenzene (1.85 g, 9.1 mmol), in presence of CuI (0.08 g, 0.45 mmol), Cs_2CO_3 (5.9 g, 18.1 mmol), and dimethylethylenediamine (0.8 g, 9.1 mmol) in 50 mL THF. The mixture was stirred at room temperature for 12 h. The crude product which was purified via flash column chromatography with a gradual amount of acetate/heptane (0:100 v/v) affording the compound as a white solid (0.94 g, 35% yield).

M.p. 213-214°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.74 (s, 1H, N*H*, conformer 1), 10.17 (s, 1H, N*H*, conformer 2), 7.96 (s, 1H, N*H*, conformer 1), 7.65 (s, 1H, N*H*, conformer 2), 7.33 (t, *J* = 7.6 Hz, 4H, Ar*H*, conformer 2), 7.30 (t, *J* = 7.6 Hz, 4H, Ar*H*, conformer 1), 7.12 (d, *J* = 7.6 Hz, 4H, Ar*H*, conformer 2), 7.10 (d, *J* = 7.6 Hz, 4H, Ar*H*, conformer 1), 6.99 (t, *J* = 7.6 Hz, 2H, Ar*H*, conformers 1+2), 4.38 (m, 1H, C*H*, conformer 2), 4.14 (q, *J* = 4.1 Hz, 1 H, C*H*, conformer 1), 2.32 (m, 1H, C*H*$_2$, conformers 1+2), 2.20-2.13 (m, 2H, C*H*$_2$, conformers 1+2), 1.86 (m, 1H, C*H*$_2$, conformers 1+2).

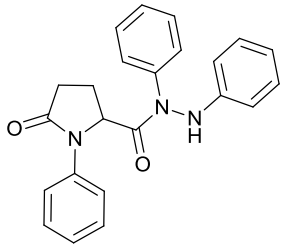
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 177.7 (C=O), 172.2 (C=O), 146.0 ($2C_{IV}$), 129.5 (4CHAr), 122.7 (2CH), 119.2 (4CHAr), 54.8 (CH), 29.6 (CH_2), 25.3 (CH_2).

IR ν (cm^{-1}): 3289, 2970, 1688, 1649, 1492, 1263, 1198, 749.

Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23.

5-oxo-*N,N'*,1-triphenylpyrrolidine-2-carbohydrazide – 115

HEI 3181



Ratio conformer 1/conformer 2: 93/07

5-oxo-*N,N'*,1-triphenylpyrrolidine-2-carbohydrazide was obtained through the alkylation reaction of 5-oxo-*N'*-phenylpyrrolidine-2-carbohydrazide (2 g, 9.1 mmol), and iodobenzene (1.85 g, 9.1 mmol), in presence of CuI (0.08 g, 0.45 mmol), Cs_2CO_3 (5.9 g, 18.1 mmol), and dimethylethylenediamine (0.8 g, 9.1 mmol) in 50 mL THF. The mixture was stirred at room temperature for 12 h. The crude product which was purified via flash column chromatography with a gradual amount of acetate/heptane (0:100 v/v) affording the compound as a white solid (0.67 g, 20% yield).

M.p. 188-189°C

^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.01 (s, 1H, N*H*, conformer 1), 10.43 (s, 1H, N*H*, conformer 2), 7.52 (t, *J* = 7.4 Hz, 2H, Ar*H*, conformers 1+2), 7.41 (t, *J* = 7.4 Hz, 2H, Ar*H*, conformers 1+2), 7.21 (t, *J* = 7.4 Hz, 6H, Ar*H*, conformers 1+2), 6.96 (t, *J* = 7.4 Hz, 1H, Ar*H*, conformers 1+2), 6.88 (d, *J* = 7.4 Hz, 4H, Ar*H*, conformers 1+2), 5.39 (q, *J* = 4.4 Hz, 1H, C*H*, conformer 2), 4.91 (q, *J* = 4.4 Hz, 1H, C*H*, conformer 1), 2.61 (m, 2 H, CH_2, conformers 1+2), 2.02 (m, 1H, CH_2, conformers 1+2).

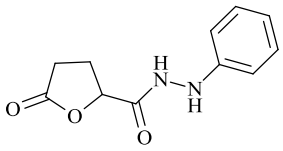
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 174.4 (C=O), 170.9 (C=O), 145.7 ($2C_{IV}$), 138.7 (C_{IV}), 129.9 (4CHAr), 129.1 (3CHAr), 125.4 (CH), 122.7 (CHAr), 122.0 (3CHAr), 118.9 (3CHAr), 60.7 (CH), 31.2 (CH_2), 22.7 (CH_2).

IR ν (cm^{-1}): 3202, 3035, 1706, 1671, 1493, 1205, 746.

Anal. Calcd for $C_{23}H_{21}N_3O_2$: C, 74.37; H, 5.70; N, 11.31.

5-oxo-*N'*-phenyltetrahydrofuran-2-carbohydrazide – 116

HEI 3194



Ratio conformer 1/conformer 2: 89/11

Following the general procedure, 5-oxo-*N'*-phenyltetrahydrofuran-2-carbohydrazide was obtained from the coupling reaction of 5-oxotetrahydrofuran-2-carboxylic acid (2.02 g, 15.5 mmol), phenylhydrazine (1.7 g, 15.5 mmol), in presence of dicyclohexyl carbodiimide (3.1 g, 15.4 mmol) and dimethylaminopyridine (0.18 g, 1.55 mmol) which were dissolved in 100 mL of methylene chloride. The mixture was stirred at room temperature for 24 h, under nitrogen atmosphere. The precipitated dicyclohexylurea was filtered. After washing with methylene chloride, the solution was concentrated to a crude product which was purified via flash column chromatography with a gradual amount of acetate/heptane (0:100 v/v) affording the compound as a white solid (0.85 g, 25% yield).

M.p. 113-114°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.10 (s, 1H, N*H*, conformer 1), 9.42 (s, 1H, N*H*, conformer 2), 8.01 (s, 1H, N*H*, conformer 2), 7.82 (s, 1H, N*H*, conformer 1), 7.22 (t, *J* = 8.4 Hz, 2H, Ar*H*, conformer 2), 7.23 (t, *J* = 8.4 Hz, 2H, Ar*H*, conformer 1), 6.76 (d, *J* = 8.4 Hz, 3H, Ar*H*, conformers 1+2), 5.21 (t, *J* = 7.5 Hz, 1 H, C*H*, conformer 2), 4.99 (t, *J* = 7.5 Hz, 1 H, C*H*, conformer 1), 2.56 (m, 3H, CH_2, conformers 1+2), 2.19-2.14 (m, 1H, CH_2, conformers 1+2).

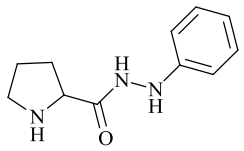
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 177.7 (C=O, conformer 2), 177.3 (C=O, conformer 1), 174.0 (C=O, conformer 2), 169.6 (C=O, conformer 1), 149.2 (C_{IV}, conformer 1), 148.6 (C_{IV}, conformer 2), 129.5 (2CHAr, conformer 2), 129.2 (2CHAr, conformer 1), 120.2 (CHAr, conformer 2), 119.2 (CHAr, conformer 1), 112.8 (2CHAr, conformer 2), 112.6 2Ar*H*, conformer 1), 76.3 (CH, conformer 1), 76.1 (CH, conformer 2), 27.5 (CH_2, conformer 1), 27.1 (CH_2, conformer 2), 25.9 (CH_2, conformers 1+2).

IR ν (cm^{-1}): 3291, 3228, 1765, 1651, 1600, 1484, 1161, 1058, 758.

Anal. Calcd for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72.

N'-phenylpyrrolidine-2-carbohydrazide – 117

HEI 3193



Ratio conformer 1/conformer 2: 94/06

Following the general procedure, *N'*-phenylpyrrolidine-2-carbohydrazide was obtained through the hydrazinolysis reaction methyl pyrrolidine-2-carboxylate (2 g, 15.5 mmol), phenylhydrazine (1.7 g, 15.5 mmol), in the presence of a catalytic amount of PTSA, 5% (0.24 g). The mixture was stirred at 80°C for 12 h. The precipitate was then washed with ethanol, affording the compound as a white solid (1.3 g, 40 % yield).

M.p. 148-149°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.56 (s, 1H, N*H*, conformer 1), 9.16 (s, 1H, N*H*, conformer 2), 7.89 (s, 1H, N*H*, conformer 2), 7.63 (s, 1H, N*H*, conformer 1), 7.12 (t, *J* = 9.5 Hz, 2H, Ar*H*, conformer 2), 7.11 (t, *J* = 9.5 Hz, 2H, Ar*H*, conformer 1), 6.67 (d, *J* = 9.5 Hz, 3H, Ar*H*, conformers 1+2), 3.63 (q, *J* = 5.9 Hz, 1 H, C*H*, conformer 2), 3.60 (q, *J* = 5.9 Hz, 1 H, C*H*, conformer 1), 2.89-2.80 (m, 3 H, CH_2, conformers 1+2), 1.91 (m, 1H, CH_2, conformers 1+2), 1.72-1.63 (m, 3H, CH_2, conformers 1+2).

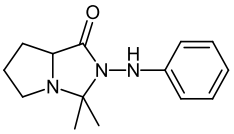
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 174.4 (C=O), 149.8 (C_{IV}), 129.1 (2CHAr), 118.8 (CHAr), 112.5 (2CHAr), 59.8 (CH), 47.30 (CH_2), 30.9 (CH_2), 26.2 (CH_2).

IR ν (cm^{-1}): 3216, 2871, 1676, 1603, 1493, 1087, 752.

Anal. Calcd for $C_{11}H_{15}N_3O$: C, 64.37; H, 7.37; N, 20.47.

3,3-dimethyl-2-(phenylamino)tetrahydro-1H-pyrrolo[1,2-c]imidazole-1,5(6H)-dione – 118

HEI 3188



The product is obtained as a major conformer (>97%).

Following the general procedure, *N'*-phenylpyrrolidine-2-carbohydrazide was obtained from the reaction methyl pyrrolidine-2-carboxylate (2 g, 15.5 mmol), phenylhydrazine (1.7 g, 15.5 mmol), in the presence of a catalytic amount of PTSA, 5% (0.24 g). The mixture was stirred at 80° for 12 h. The precipitate was then washed with acetone affording the compound as a white solid (0.3 g, 8 % yield).

M.p. 165-166°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 7.94 (s, 1H, N*H*), 7.13 (t, *J* = 8.0 Hz, 2H, Ar*H*), 7.34 (s, 1H, N*H*, conformer 2), 7.12 (t, *J* = 9.5 Hz, 2H, Ar*H*, conformer 2), 7.11 (t, *J* = 9.5 Hz, 2H, Ar*H*, conformer 1), 6.67 (d, *J* = 9.5 Hz, 3H, Ar*H*, conformers 1+2), 3.63 (q, *J* = 5.9 Hz, 1 H, C*H*, conformer 2), 3.60 (q, *J* = 5.9 Hz, 1 H, C*H*, conformer 1), 2.89-2.80 (m, 2 H, CH_2, conformers 1+2), 1.91 (m, 1H, CH_2, conformers 1+2), 1.72-1.63 (m, 3H, CH_2, conformers 1+2), 1.32 (s, 6H, 2CH_3, conformers 1+2).

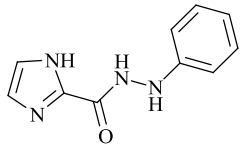
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 175.1 (C=O), 149.1 (C$_{IV}$), 126.6 (2CHAr), 118.6 (CHAr), 112.0 (2CHAr), 78.7 (C$_{IV}$), 60.4 (CH), 48.45 (CH_2), 25.7 (CH_2), 24.9 (2CH_3), 21.0 (CH_2).

IR ν (cm^{-1}): 3182, 3023, 2969, 2889, 2848, 1705, 1601, 1387, 1151, 759.

Anal. Calcd for C$_{14}$H$_{19}$N$_3$O: C, 68.54; H, 7.81; N, 17.13.

N'-phenyl-1H-imidazole-2-carbohydrazide – 119

HEI 3211



The product is obtained as a sole isomer.

Following the general procedure, *N'*-phenyl-1H-imidazole-2-carbohydrazide was obtained from the coupling reaction of 1H-imidazole-2-carboxylic acid (1 g, 8.9 mmol), phenylhydrazine (0.96 g, 8.9 mmol), in presence of dicyclohexyl carbodiimide (1.83 g, 8.9 mmol) and dimethylaminopyridine (0.22 g, 8.9 mmol) were dissolved in 50 mL of methylene chloride. The mixture was stirred at room temperature for 24 h. After filtering and washing (methylene chloride) of the precipitated dicyclohexylurea, the solution was concentrated to a crude product which was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (0.27 g, 14% yield).

M.p. 243-244°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 13.09 (s, 1H, N*H*), 10.26 (d, *J* = 2.9 Hz, 1H, N*H*), 7.85 (d, *J* = 2.9 Hz, 1H, N*H*), 7.32 (d, *J* = 2.9 Hz, 1H, C*H*), 7.15 (m, 3H, Ar*H*), 6.71 (d, *J* = 7.7 Hz, 2H, Ar*H*), 6.67 (s, 1H, C*H*).

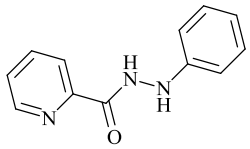
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 158.4 (C=O), 149.1 (C$_{IV}$), 139.7 (C$_{IV}$), 128.9 (CH), 128.5 (2CHAr), 119.9 (CH), 118.3 (CHAr), 112.0 (2CHAr).

IR ν (cm^{-1}): 3316, 3119, 3026, 2920, 1640, 1530, 1439, 1124, 880.

Anal. Calcd for $C_{10}H_{10}N_4O$: C, 59.40; H, 4.98; N, 27.71.

N'-phenylpicolinohydrazide – 120

HEI 3177



The product was obtained as a sole conformer.

Following the general procedure, *N'*-phenylpicolinohydrazide was obtained through the hydrazinolysis reaction of methyl picolinate (2 g, 15.4 mmol) with phenylhydrazine (1.6 g, 14 mmol), in the presence of a catalytic amount of PTSA, 5% (0.24 g). The mixture was stirred at 80° for 12 h. The crude product was purified via flash column chromatography (methanol/chloroform 2:10 v/v) affording the compound as a white solid (1.8 g, 60% yield).

M.p. 186-187°C.

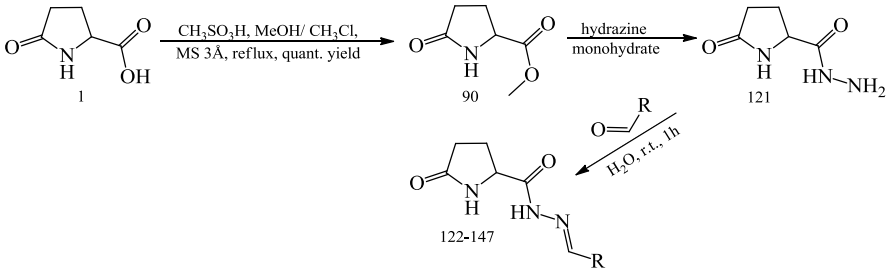
^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.55 (s, 1H, N*H*), 8.70 (d, *J* = 5.7 Hz, 1H, Ar*H*), 8.04 (m, 2H, Ar*H*), 7.91 (s, 1H, N*H*), 7.64 (t, *J* = 7.4 Hz, 1H, Ar*H*), 7.15 (t, *J* = 7.4 Hz, 2H, Ar*H*), 6.78 (d, *J* = 7.4 Hz, 2H, Ar*H*), 6.69 (t, *J* = 7.4 Hz, 1H, Ar*H*).

^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): 164.4 (C=O), 150.2 (C_{IV}), 149.7 (C_{IV}), 149.1 (CHAr), 146.4 (CHAr), 138.2 (CHAr), 129.1 (2CHAr), 127.2 (CHAr), 122.7 (CHAr), 119.0 (CHAr), 112.8 (CHAr).

IR ν (cm^{-1}): 3219, 3013, 1656, 1493, 1465, 1242, 743.

Anal. Calcd for $C_{12}H_{11}N_3O$: C, 67.59; H, 5.20; N, 19.71.

1.3 Experimental procedures for the synthesis of pyroglutamic acid *N*-acyl hydrazones



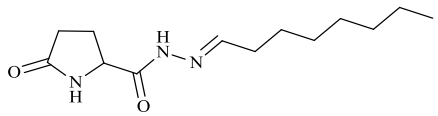
Scheme 55 General procedure for the synthesis of pyroglutamic acid N-acylhydrazones

Following the general scheme, the *N*-acylhydrazones were obtained from the condensation reaction of 5-oxopyrrolidine-2-carbohydrazide **121,** which was preliminary

obtained in a large quantity through the hydrazinolysis of methyl-pyroglutamate, with the corresponding aldehyde. The reaction was conducted at room temperature for one hour, in green solvents such as water or ethanol to afford the wanted *N*-acylhydrazones. No side products were observed for this type of reaction.

_N'_-octylidene-5-oxopyrrolidine-2-carbohydrazide – 122

HEI 3140



Following the general procedure, *N'*-octylidene-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with octanal (13.9 mmol, 1.8 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (0.5 g, 13 % yield).

M.p. 88-89°C.

Isomer 1 (48%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.00 (s, 1H, N*H*), 7.67 (s, 1H, C*H*), 7.29 (t, *J* = 5.0 Hz, 1H, C*H*), 4.70 (q, *J* = 4.4 Hz, 1H, C*H*), 2.40-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 2.08 (m, 1H, C*H*$_2$C*H*$_2$CH), 1.87 (bs, 2H, C*H*$_2$), 1.26 (bs, 10H, C*H*$_2$), 0.87 (t, *J* = 5.0 Hz, 3H, C*H*$_3$).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.7 (C=O), 173.3 (C=O), 148.5 (C_{IV}), 53.3 (CH), 32.0 (CH_2), 31.6 ($3CH_2$), 29.5 (CH_2), 26.1 (CH_2), 25.2 (CH_2), 22.5 (CH_2), 14.3 (CH_3).

Isomer 2 (52%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.06 (s, 1H, N*H*), 7.79 (s, 1H, C*H*), 7.52 (t, *J* = 5.0 Hz, 1H, C*H*), 3.96 (q, *J* = 4.4 Hz, 1H, C*H*), 2.40-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 2.08 (m, 1H, C*H*$_2$C*H*$_2$CH), 1.87 (bs, 2H, C*H*$_2$), 1.26 (bs, 10H, C*H*$_2$), 0.87 (t, *J* = 5.0 Hz, 3H, C*H*$_3$).

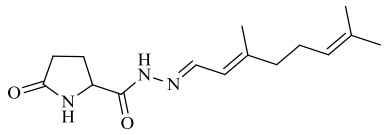
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 168.8 (C=O), 152.4 (C_{IV}), 55.2 (CH), 32.3 (CH_2), 29.6 (CH_2), 28.9 ($3CH_2$), 26.4 (CH_2), 25.5 (CH_2), 22.5 (CH_2), 14.3 (CH_3).

IR ν (cm^{-1}): 3207, 3071, 2920, 1670, 1560, 1233, 1153.

Anal. Calcd for $C_{13}H_{23}N_3O_2$: C, 61.63; H, 9.15; N, 16.59; found: C, 61.32; H, 9.46; N, 16.61.

_N'_-((E)-3,7-dimethylocta-2,6-dien-1-ylidene)-5-oxopyrrolidine-2-carbohydrazide – 123

HEI 3163



Following the general procedure, *N'*-((E)-3,7-dimethylocta-2,6-dien-1-ylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with cytral (13.9 mmol, 2.1 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (0.5 g, 13 % yield).

M.p. 123-124°C.

Isomer 1 (53%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.03 (s, 1H, N*H*), 7.95 (d, *J* = 10.6 Hz, 1H, C*H*), 7.67 (s, 1H, C*H*), 5.89 (d, *J* = 9.3 Hz, 1H, C*H*), 5.07 (bs, 1H, N*H*), 4.70 (q, *J* = 3.3 Hz, 1H, C*H*), 2.40-1.90 (m, 4H, C*H$_2$*C*H$_2$*CH), 2.13 (s, 4H, C*H$_2$*), 1.95 (s, 3H, C*H$_3$*), 1.58 (s, 6H, C*H$_3$*).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.7 (C=O), 173.4 (C=O), 148.1 (C_{IV}), 144.2 (CH), 131.8 (C_{IV}), 123.9 (CH), 121.9 (CH), 53.1 (CH), 40.7 (CH), 29.6 (CH_2), 26.2 (CH_2), 25.9 (CH_2), 25.6 (CH_2), 18.0 (CH_3), 17.2 ($2CH_3$).

Isomer 2 (47%)

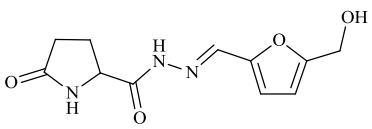
^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.13 (s, 1H, N*H*), 8.17 (d, *J* = 10.6 Hz, 1H, C*H*), 7.82 (s, 1H, C*H*), 5.93 (d, *J* = 9.3 Hz, 1H, C*H*), 5.07 (bs, 1H, N*H*), 4.01 (q, *J* = 3.3 Hz, 1H, C*H*), 2.40-1.90 (m, 4H, C*H$_2$*C*H$_2$*CH), 2.12 (s, 4H, C*H$_2$*), 1.92 (s, 3H, C*H$_3$*), 1.64 (s, 6H, C*H$_3$*).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 168.9 (C=O), 148.7 (C_{IV}), 147.2 (CH), 131.8 (C_{IV}), 123.9 (CH), 121.8 (CH), 55.5 (CH), 40.7 (CH), 29.6 (CH_2), 26.3 (CH_2), 25.8 (CH_2), 25.2 (CH_2), 18.0 (CH_3), 17.3 ($2CH_3$).

IR ν (cm^{-1}): 3303, 3195, 2966, 2913, 1665, 1246, 1227, 1090.

Anal. Calcd for $C_{15}H_{23}N_3O_2$, 3/4 H_2O: C, 59.78; H, 8.58; N, 13.94; found: C, 59.49; H, 8.42; N, 13.72.

N'-((5-(hydroxymethyl)furan-2-yl)methylene)-5-oxopyrrolidine-2-carbohydrazide – 124 HEI 3164



Following the general procedure, *N'*-((5-(hydroxymethyl)furan-2-yl)methylene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-

carbohydrazide (3.9 mmol, 0.6 g) with 5-(hydroxymethyl)furan-2-carbaldehyde (3.9 mmol, 0.5 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as an off-white solid (2.4 g, 60 % yield).

M.p. 63-64°C.

Isomer 1 (43%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.20 (s, 1H, N*H*), 7.86 (s, 1H, C*H*), 7.72 (s, 1H, N*H*), 6.84 (d, *J* = 2.4 Hz, 1H, C*H*), 6.43 (d, *J* = 2.4 Hz, 1H, C*H*), 5.35 (t, *J* = 5.0 Hz, 1H, O*H*), 4.83 (q, *J* = 4.0 Hz, 1H, C*H*), 4.44 (d, *J* = 5.0 Hz, 2H, CH_2), 2.40-2.10 (m, 3H, CH_2CH_2CH), 1.95 (m, 1H, CH$_2$CH_2CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.9 (C=O), 173.8 (C=O), 158.1 (C$_{IV}$), 148.8 (C$_{IV}$), 134.5 (CH), 114.8 (CH), 109.6 (CH), 56.5 (CH$_2$), 53.1 (CH), 29.6 (CH$_2$), 25.2 (CH$_2$).

Isomer 2 (57%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.45 (s, 1H, N*H*), 8.08 (s, 1H, C*H*), 7.85 (s, 1H, N*H*), 6.84 (d, *J* = 2.4 Hz, 1H, C*H*), 6.43 (d, *J* = 2.4 Hz, 1H, C*H*), 5.42 (t, *J* = 5.0 Hz, 1H, O*H*), 4.44 (d, *J* = 5.0 Hz, 2H, CH_2), 4.09 (q, *J* = 4.0 Hz, 1H, C*H*), 2.40-2.10 (m, 3H, CH_2CH_2CH), 1.95 (m, 1H, CH$_2$CH_2CH).

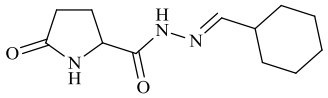
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.9 (C=O), 169.3 (C=O), 158.3 (C$_{IV}$), 148.8 (C$_{IV}$), 137.6 (CH), 115.4 (CH), 109.6 (CH), 56.5 (CH$_2$), 55.4 (CH), 29.6 (CH$_2$), 25.6 (CH$_2$).

IR ν (cm^{-1}): 3285, 3214, 3038, 1675, 1649, 1622, 1573, 1222.

Anal. Calcd for C$_{11}$H$_{13}$N$_3$O$_4$, H$_2$O: C, 49.07; H, 5.62; N, 15.61; found: C, 48.90; H, 5.56; N, 15.40.

N'-(cyclohexylmethylene)-5-oxopyrrolidine-2-carbohydrazide – 125

HEI 3138



Following the general procedure, *N'*-(cyclohexylmethylene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with cyclohexanecarbaldehyde (13.9 mmol, 1.6 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (2.1 g, 60 % yield).

M.p. 205-206°C.

Isomer 1 (49%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 10.98 (s, 1H, N*H*), 7.66 (s, 1H, N*H*), 7.22 (d, *J* = 5.0 Hz, 1H, C*H*), 4.69 (q, *J* = 4.7 Hz, 1H, C*H*), 2.4-2.10 (m, 1H, C*H*), 2.4-2.10 (m, 3H, CH_2CH_2CH), 2.01 (m, 1H, CH$_2$CH_2CH), 1.3-1.18 (m, 10H, CH_2).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.7 (C=O), 173.5 (C=O), 155.8 (CH), 53.4 (CH), 30.1 (2CH$_2$), 29.7 (CH), 29.5 (CH$_2$), 26.0 (CH$_2$), 25.5 (2CH$_2$), 25.4 (CH$_2$).

Isomer 2 (51%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.0 (s, 1H, N*H*), 7.78 (s, 1H, N*H*), 7.44 (d, *J* = 5.0 Hz, 1H, C*H*), 3.96 (q, *J* = 4.7 Hz, 1H, C*H*), 2.4-2.10 (m, 1H, C*H*), 2.4-2.10 (m, 3H, CH_2CH_2CH), 2.01 (m, 1H, CH$_2$CH_2CH), 1.76-1.60 (m, 10H, CH_2).

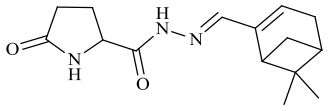
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 168.8 (C=O), 151.8 (CH), 55.3 (CH), 30.0 (CH), 29.9 (CH$_2$), 29.5 (CH$_2$), 25.9 (2CH$_2$), 25.6 (2CH$_2$), 25.1 (CH$_2$).

IR ν (cm^{-1}): 3434, 3216, 2929, 2853, 1670, 1550, 1241;

Anal. Calcd for C$_{12}$H$_{19}$N$_3$O$_2$, 1H$_2$O: C, 56.45; H, 8.29; N, 16.46; found: C, 56.61; H, 8.47; N, 16.68.

N'-((6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)methylene)-5-oxopyrrolidine-2-carbohydrazide – 126

HEI 3165



Following the general procedure, *N'*-((6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)methylene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with myrtenal (13.9 mmol, 2.1 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording compound as a white solid (2.4 g, 60 % yield).

M.p. 138-139°C.

Isomer 1 (44%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.14 (s, 1H, N*H*), 7.80 (s, 1H, C*H*), 7.65 (s, 1H, C*H*), 5.99 (s, 1H, N*H*), 4.70 (q, *J* = 4.0 Hz, 1H, C*H*), 2.78 (t, *J* = 2.8, 1H, C*H*), 2.50-1.90 (m, 8H, CH_2), 0.75 (s, 6H, CH_3).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.6 (C=O), 173.7 (C=O), 148.8 (CH), 145.2 (CH), 131.7 (C$_{IV}$), 53.5 (CH), 37.6 (CH), 32.9 (CH$_2$), 31.0 (CH$_2$), 29.5 (CH$_2$), 26.4 (C$_{IV}$), 24.9 (CH$_2$), 21.2 (CH$_3$).

Isomer 2 (56%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.18 (s, 1H, N*H*), 7.81 (s, 1H, C*H*), 7.60 (s, 1H, C*H*), 6.08 (s, 1H, N*H*), 4.04 (q, *J* = 4.0 Hz, 1H, C*H*), 2.80 (t, *J* = 2.8, 1H, C*H*), 2.50-1.90 (m, 8H, C*H*$_2$), 1.32 (s, 6H, C*H*$_3$).

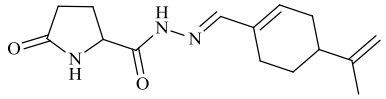
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 169.0 (C=O), 149.0 (CH), 145.5 (CH), 132.8 (C_{IV}), 55.4 (CH), 37.5 (CH), 32.4 (CH_2), 30.0 (CH_2), 29.6 (CH_2), 26.4 (C_{IV}), 25.5 (CH_2), 21.3 (CH_3).

IR ν (cm^{-1}): 3298, 3188, 3038, 2922, 2871, 1684, 1553, 1212, 1087;

Anal. Calcd for $C_{15}H_{21}N_3O_2$; 2/3 H_2O: C, 62.70; H, 7.83; N, 14.62; found: C, 62.96; H, 7.47; N, 16.32.

5-**oxo-*N'*-((4-(prop-1-en-2-yl)cyclohex-1-en-1 yl)methylene)pyrrolidine-2-carbohydrazide – 127**

HEI 3166



Following the general procedure, 5-oxo-*N'*-((4-(prop-1-en-2-yl)cyclohex-1-en-1-yl) methylene) pyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with perillaldehyde (13.9 mmol, 2.1 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (1.5 g, 40 % yield).

M.p. 177-178°C.

Isomer 1 (55%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.12 (s, 1H, N*H*), 7.82 (s, 1H, C*H*), 7.59 (s, 1H, C*H*), 6.12 (s, 1H, N*H*), 4.73 (bs, 2H, C*H*$_2$), 4.70 (bs, 1H, C*H*), 2.50-1.70 (m, 9H, C*H*$_2$), 1.72 (s, 3H, C*H*$_3$), 1.42 (m, 1H, C*H*$_2$).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.6 (C=O), 173.7 (C=O), 151.1 (C_{IV}), 149.2 (CH), 135.2 (C_{IV}), 134.9 (CH), 109.6 (CH_2), 53.5 (CH), 40.6 (CH), 31.2 (CH_2), 29.6 (CH_2), 26.8 (CH_2), 25.0 (CH_2), 23.5 (CH_2), 21.0 (CH_3).

Isomer 2 (45%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.16 (s, 1H, N*H*), 7.81 (s, 1H, C*H*), 7.68 (s, 1H, C*H*), 6.18 (s, 1H, N*H*), 4.73 (bs, 2H, C*H*$_2$), 4.04 (q, *J* = 4 Hz, 1H, C*H*), 2.50-1.70 (m, 9H, C*H*$_2$), 1.72 (s, 3H, C*H*$_3$), 1.42 (m, 1H, C*H*$_2$).

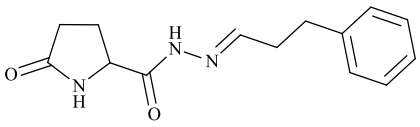
$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ ppm 177.8 (C=O), 169.0 (C=O), 149.1 (C_{IV}), 147.2 (CH), 136.7 (C_{IV}), 135.8 (CH), 109.6 (CH_2), 55.4 (CH), 40.7 (CH), 31.3 (CH_2), 29.7 (CH_2), 26.8 (CH_2), 25.6 (CH_2), 23.8 (CH_2), 21.0 (CH_3).

IR ν (cm^{-1}): 3289, 3201, 3047, 2939, 1680, 1638, 1554, 1222, 1089;

Anal. Calcd for $C_{15}H_{21}N_3O_2$: C, 65.43; H, 7.69; N, 15.26; found: C, 65.38; H, 7.77; N, 15.57.

5-oxo-*N'*-(3-phenylpropylidene)pyrrolidine-2-carbohydrazide – 128

HEI 3141



Following the general procedure, 5-oxo-*N'*-(3-phenylpropylidene)pyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 3-phenylpropanal (13.9 mmol, 1.9 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white crystals (1.5 g, 42 % yield).

M.p. 209-210°C.

Isomer 1 (48%):

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.04 (s, 1H, N*H*), 7.66 (s, 1H, N*H*), 7.35 (t, J = 5.6 Hz, 1H, C*H*), 7.36-7.16 (m, 5H, Ar*H*), 4.67 (q, *J* = 3.6 Hz, 1H, C*H*), 2.82 (m, 4H, CH_2), 2.5-2.15 (m, 3H, CH_2CH_2CH), 1.94 (m, 1H, CH_2CH_2CH).

$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ ppm 177.8 (C=O), 173.4 (C=O), 147.7 (CH), 141.3 (C_{IV}), 128.8 (2CHAr), 129.7 (2CHAr), 126.3 (CHAr), 53.3 (CH), 34.0 (CH_2), 32.0 (CH_2), 29.5 (CH_2), 25.1 (CH_2).

Isomer 2 (52%):

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.12 (s, 1H, N*H*), 7.80 (s, 1H, N*H*), 7.55 (t, J = 5.6 Hz, 1H, C*H*), 7.36-7.16 (m, 5H, Ar*H*), 3.97 (q, *J* = 3.6 Hz, 1H, C*H*), 2.52 (m, 4H, CH_2), 2.5-2.15 (m, 3H, CH_2CH_2CH), 1.98 (m, 1H, CH_2CH_2CH).

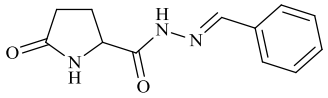
$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ ppm 177.8 (C=O), 168.8 (C=O), 151.5 (CH), 141.3 (C_{IV}), 128.8 (2CHAr), 129.7 (2CHAr), 126.4 (CHAr), 55.2 (CH), 33.6 (CH_2), 32.3 (CH_2), 29.5 (CH_2), 25.5 (CH_2).

IR ν (cm^{-1}): 3293, 3215, 3051, 1666, 1579, 1242, 1241;

Anal. Calcd for $C_{14}H_{17}N_3O_2$, H_2O: C, 60.63; H, 6.91; N, 15.15; found: C, 60.29; H, 6.65; N, 15.12.

_N'_-benzylidene-5-oxopyrrolidine-2-carbohydrazide – 129

HEI 261



Following the general procedure, _N'_-benzylidene-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with benzaldehyde (13.9 mmol, 1.5 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (2.5 g, 78 % yield).

M.p. 209-210°C.

Isomer 1 (54%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.48 (s, 1H, N*H*), 8.01 (s, 1H, C*H*), 7.77 (s, 1H, N*H*), 7.70 (m, 2H, Ar*H*), 7.42 (m, 3H, Ar*H*), 4.92 (q, *J* = 4.7 Hz, 1H, C*H*), 2.5-2.15 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.98 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 174.0 (C=O), 144.0 (CH), 134.5 (C$_{IV}$), 130.3 (CHAr), 129.2 (2CHAr), 127.3 (2CHAr), 53.5 (CH), 29.6 (CH$_2$), 25.3 (CH$_2$).

Isomer 2 (46%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.53 (s, 1H, N*H*), 8.27 (s, 1H, C*H*), 7.89 (s, 1H, N*H*), 7.71 (m, 2H, Ar*H*), 7.45 (m, 3H, Ar*H*), 4.11 (q, *J* = 4.7 Hz, 1H, C*H*), 2.5-2.15 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.98 (m, 1H, CH$_2$C*H*$_2$CH).

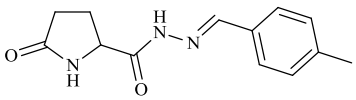
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.9 (C=O), 169.4 (C=O), 147.9 (CH), 134.5 (C$_{IV}$), 130.6 (CHAr), 129.2 (2CHAr), 127.5 (2CHAr), 55.4 (CH), 29.7 (CH$_2$), 25.6 (CH$_2$).

IR ν (cm^{-1}): 3293, 3215, 3051, 1666, 1579, 1242, 1241.

Anal. Calcd for C$_{12}$H$_{13}$N$_3$O$_2$: C, 62.33; H, 5.67; N, 18.17; found: C, 62.15; H, 5.84; N, 18.48.

_N'_-(4-methylbenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 130

HEI 3146



Following the general procedure, _N'_-(4-methylbenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 4-methylbenzaldehyde (13.9 mmol, 1.7 g), in 20 mL of water. The crude

product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (3.2 g, 93 % yield).

M.p. 229-230°C.

Isomer 1 (52%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.40 (s, 1H, N*H*), 7.97 (s, 1H, C*H*), 7.75 (s, 1H, N*H*), 7.56 (d, *J* = 8.6 Hz, 2H, Ar*H*), 7.25 (d, *J* = 8.6 Hz, 2H, Ar*H*), 4.88 (q, *J* = 4.6 Hz, 1H, C*H*), 2.21-2.12 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.96 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.9 (C=O), 173.9 (C=O), 144.1 (CH), 140.1 (C_{IV}), 131.8 (C_{IV}), 129.8 (2CHAr), 127.2 (2CHAr), 53.5 (CH), 29.6 (CH_2), 25.3 (CH_2), 21.4 (CH_3).

Isomer 2 (48%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.46 (s, 1H, N*H*), 8.21 (s, 1H, C*H*), 7.85 (s, 1H, N*H*), 7.60 (d, *J* = 8.6 Hz, 2H, Ar*H*), 7.26 (d, *J* = 8.6 Hz, 2H, Ar*H*), 4.12 (q, *J* = 4.6 Hz, 1H, C*H*), 2.20-2.08 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.96 (m, 1H, CH$_2$C*H*$_2$CH).

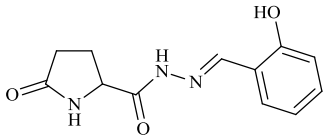
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 169.2 (C=O), 147.9 (CH), 140.4 (C_{IV}), 131.8 (C_{IV}), 129.8 (2CHAr), 127.5 (2CHAr), 55.4 (CH), 29.7 (CH_2), 25.6 (CH_2), 21.4 (CH_3).

IR ν (cm^{-1}): 3259, 2820, 1678, 1607, 1397, 1280.

Anal. Calcd for C$_{13}$H$_{15}$N$_3$O$_2$: C, 63.66; H, 6.16; N, 17.13; found: C, 63.70; H, 5.99; N, 17.17.

N'-(2-hydroxybenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 131

HEI 3162



Following the general procedure, *N'*-(2-hydroxybenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 2-hydroxybenzaldehyde (13.9 mmol, 1.7 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (3.4 g, 97 % yield).

M.p. 240-241°C.

Isomer 1 (33%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.40 (s, 1H, N*H*), 10.10 (s, 1H, N*H*), 8.23 (s, 1H, C*H*), 7.74 (s, 1H, O*H*), 7.69 (dd, *J* = 7.3 Hz, *J* = 1.0 Hz, 1H, Ar*H*), 7.23 (td, *J* = 7.9 Hz, *J* = 1.0

Hz, 1H, Ar*H*), 6.89 (dd, *J* = 7.3 Hz, *J* = 1.0 Hz, 1H, Ar*H*), 4.92 (q, *J* = 4.3 Hz, 1H, C*H*), 2.20-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.92 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.2 (C=O), 173.1 (C=O), 156.2 (C$_{IV}$), 141.0 (CH), (CHAr), 131.0 (CHAr), 126.1 (C$_{IV}$), 120.1 (CHAr), 118.5 (CHAr), 116.0 (CHAr), 53.0 (CH), 29.0 (CH$_2$), 24.6 (CH$_2$).

Isomer 2 (67%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.75 (s, 1H, N*H*), 11.02 (s, 1H, N*H*), 8.47 (s, 1H, C*H*), 7.87 (s, 1H, O*H*), 7.55 (dd, *J* = 7.3 Hz, *J* = 1.0 Hz, 1H, Ar*H*), 7.30 (td, *J* = 7.9 Hz, *J* = 1.0 Hz, 1H, Ar*H*), 6.91 (dd, *J* = 7.3 Hz, *J* = 1.0 Hz, 1H, Ar*H*), 4.11 (q, *J* = 4.3 Hz, 1H, C*H*), 2.20-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.92 (m, 1H, CH$_2$C*H*$_2$CH).

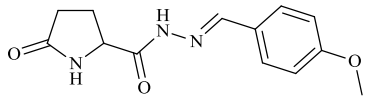
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.3 (C=O), 168.7 (C=O), 157.2 (C$_{IV}$), 147.6 (CH), (CHAr), 131.4 (CHAr), 129.1 (C$_{IV}$), 119.1 (CHAr), 118.5 (CHAr), 116.2 (CHAr), 54.1 (CH), 29.1 (CH$_2$), 25.0 (CH$_2$).

IR ν (cm^{-1}): 3294, 3180, 2986, 2906, 1661, 1610, 1572, 1274; 1222.

Anal. Calcd for C$_{12}$H$_{13}$N$_3$O$_3$: C, 58.29; H, 5.30; N, 16.99; found: C, 58.31; H, 5.42; N, 17.14.

N'-(4-methoxybenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 132

HEI 3160



Following the general procedure, *N'*-(4-methoxybenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 4-methoxybenzaldehyde (13.9 mmol, 2 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (2.6 g, 70 % yield).

M.p. 229-230°C.

Isomer 1 (52%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.35 (s, 1H, N*H*), 7.95 (s, 1H, C*H*), 7.76 (s, 1H, N*H*), 7.62 (td, *J* = 8.5 Hz, *J* = 2.0 Hz, 2H, Ar*H*), 7.00 (d, *J* = 8.5 Hz, 2H, Ar*H*), 4.90 (q, *J* = 4.2 Hz, 1H, C*H*), 3.80 (s, 3H, OC*H*$_3$), 2.21-2.12 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.96 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 173.8 (C=O), 161.1 (C$_{IV}$), 143.9 (CH), 128.8 (2CHAr), 127.1 (C$_{IV}$), 114.7 (2CHAr), 55.7 (OCH$_3$), 53.5 (CH), 29.6 (CH$_2$), 25.6 (CH$_2$).

Isomer 2 (48%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.40 (s, 1H, N*H*), 8.20 (s, 1H, C*H*), 7.88 (s, 1H, N*H*), 7.64 (td, *J* = 8.5 Hz, *J* = 2.0 Hz, 2H, Ar*H*), 7.00 (d, *J* = 8.5 Hz, 2H, Ar*H*), 4.11 (q, *J* = 4.2 Hz, 1H, C*H*), 3.80 (s, 3H, OC*H*$_3$), 2.21-2.12 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.96 (m, 1H, CH$_2$C*H*$_2$CH).

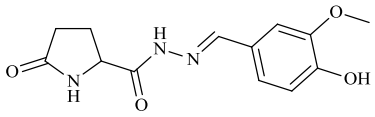
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.9 (C=O), 169.1 (C=O), 161.3 (C$_{IV}$), 147.8 (CH), 129.1 (2CHAr), 127.1 (C$_{IV}$), 114.7 (2CHAr), 55.7 (OCH$_3$), 55.4 (CH), 29.7 (CH$_2$), 25.3 (CH$_2$).

IR ν (cm^{-1}): 3294, 3199, 3027, 2912, 1662, 1607, 1244, 1174.

Anal. Calcd for C$_{13}$H$_{15}$N$_3$O$_3$: C, 59.76; H, 5.79; N, 16.08; found: C, 59.46; H, 5.70; N, 16.04.

N'-(4-hydroxy-3-methoxybenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 133

HEI 3147



Following the general procedure, *N'*-(4-hydroxy-3-methoxybenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 4-hydroxy-3-methoxybenzaldehyde (13.9 mmol, 2.1 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (3.1 g, 80 % yield).

M.p. 136-137°C.

Isomer 1 (47%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.29 (s, 1H, N*H*), 9.48 (s, 1H, C*H*), 7.87 (s, 1H, N*H*), 7.38 (s, 2H, Ar*H*), 7.25 (dd, *J* = 7.8 Hz, *J* = 2.6 Hz, 2H, Ar*H*), 6.80 (d, *J* = 6.0 Hz, 1 H, O*H*), 4.91 (q, *J* = 4.2 Hz, 1H, C*H*), 3.81 (s, 3H, C*H*$_3$), 2.3-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 2.08 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 173.8 (C=O), 149.2 (CH), 148.4 (C$_{IV}$), 144.4 (C$_{IV}$), 125.9 (C$_{IV}$), 121.6 (CHAr), 115.9 (CHAr), 110.0 (CHAr), 56.0 (OCH$_3$), 53.5 (CH), 29.7 (CH$_2$), 25.2 (CH$_2$).

Isomer 2 (53%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.32 (s, 1H, N*H*), 9.53 (s, 1H, C*H*), 8.11 (s, 1H, N*H*), 7.85 (s, 2H, Ar*H*), 7.26 (dd, *J* = 8.0 Hz, *J* = 2.6 Hz, 2H, Ar*H*), 6.83 (d, *J* = 6.0 Hz, 1 H, O*H*), 4.10 (q, *J* = 4.3 Hz, 1H, C*H*), 3.33 (s, 3H, C*H*$_3$), 2.3-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 2.08 (m, 1H, CH$_2$C*H*$_2$CH).

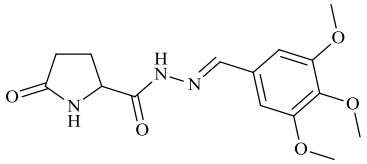
$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ ppm 177.9 (C=O), 168.9 (C=O), 149.5 (CH), 148.4 (C_{IV}), 144.4 (C_{IV}), 125.9 (C_{IV}), 122.5 (CHAr), 115.8 (CHAr), 109.5 (CHAr), 56.0 (OCH_3), 55.4 (CH), 29.6 (CH_2), 25.7 (CH_2).

IR ν (cm^{-1}): 3489, 3205, 3069, 2942, 1667, 1513, 1243.

Anal. Calcd for $C_{13}H_{15}N_3O_4$, H_2O: C, 52.88; H, 5.80; N, 14.23; found: C, 52.91; H, 5.74; N, 14.38.

5-oxo-*N'*-(3,4,5-trimethoxybenzylidene)pyrrolidine-2-carbohydrazide – 134

HEI 3145



Following the general procedure, 5-oxo-*N'*-(3,4,5-trimethoxybenzylidene)pyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 3,4,5-trimethoxybenzaldehyde (13.9 mmol, 2.7 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (3.1 g, 70 % yield).

M.p. 255-256°C.

Isomer 1 (50%)

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.50 (s, 1H, N*H*), 7.91 (s, 1H, C*H*), 7.78 (s, 1H, N*H*), 7.00 (s, 2H, Ar*H*), 4.93 (q, *J* = 3.8 Hz, 1H, C*H*), 3.83 (s, 6H, OCH_3), 3.70 (s, 3H, OCH_3), 2.21-2.12 (m, 3H, CH_2CH_2CH), 1.98 (m, 1H, CH_2CH_2CH).

$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ ppm 177.9 (C=O), 174.1 (C=O), 153.6 (2C_{IV}), 147.9 (CH), 139.5 (C_{IV}), 130.0 (C_{IV}), 104.5 (2CHAr), 60.5 (OCH_3), 56.4 (2OCH_3), 53.6 (CH), 29.5 (CH_2), 25.2 (CH_2).

Isomer 2 (40%)

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.50 (s, 1H, N*H*), 8.18 (s, 1H, C*H*), 7.88 (s, 1H, N*H*), 7.00 (s, 2H, Ar*H*), 4.12 (q, *J* = 3.8 Hz, 1H, C*H*), 3.83 (s, 6H, OCH_3), 3.70 (s, 3H, OCH_3), 2.21-2.12 (m, 3H, CH_2CH_2CH), 1.98 (m, 1H, CH_2CH_2CH).

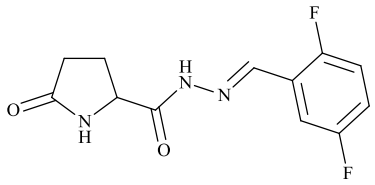
$^{13}C\{^1H\}$NMR (DMSO-d_6, 100 MHz): δ ppm 177.9 (C=O), 169.3 (C=O), 153.6 (2C_{IV}), 143.8 (CH), 139.5 (C_{IV}), 130.0 (C_{IV}), 104.8 (2CHAr), 60.5 (OCH_3), 56.4 (2OCH_3), 55.4 (CH), 29.7 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3439, 3193, 3062, 1681, 1553, 1238.

Anal. Calcd for $C_{15}H_{19}N_3O_5$, H_2O: C, 53.09; H, 6.24; N, 12.38; found: C, 52.97; H, 6.34; N, 12.59.

N'-(2,5-difluorobenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 135

HEI 3159



Following the general procedure, *N'*-(2,5-difluorobenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 2,5-difluorobenzaldehyde (13.9 mmol, 2 g), in 20 mL of ethanol. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (3.5 g, 94 % yield).

M.p. 241-242°C.

Isomer 1 (60%)

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.68 (s, 1H, N*H*), 8.17 (s, 1H, C*H*), 7.83 (s, 1H, N*H*), 7.68 (m, 1H, Ar*H*), 7.35 (m, 2H, Ar*H*), 4.97 (q, *J* = 3.9 Hz, 1H, C*H*), 2.23-2.12 (m, 3H, CH_2CH_2CH), 1.94 (m, 1H, CH$_2$CH_2CH).

^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ ppm 177.9 (C=O), 174.3 (C=O), 160.0 (C_{IV}), 157.6 (C_{IV}), 135.8 (CH), 123.6 (C_{IV}), 118.4 (2CHAr), 112.5 (CHAr), 53.5 (CH), 29.6 (CH_2), 25.1 (CH_2).

Isomer 2 (40%)

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.79 (s, 1H, N*H*), 8.48 (s, 1H, C*H*), 7.92 (s, 1H, N*H*), 7.58 (m, 1H, Ar*H*), 7.34 (m, 2H, Ar*H*), 4.16 (q, *J* = 3.9 Hz, 1H, C*H*), 2.23-2.12 (m, 3H, CH_2CH_2CH), 1.94 (m, 1H, CH$_2$CH_2CH).

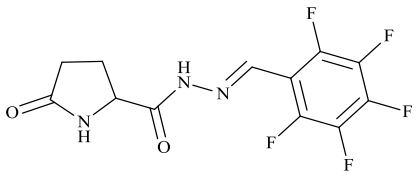
^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ ppm 177.8 (C=O), 169.5 (C=O), 158.5 (C_{IV}), 156.1 (C_{IV}), 139.7 (CH), 123.4 (C_{IV}), 118.1 (2CHAr), 112.1 (CHAr), 55.8 (CH), 29.6 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3191, 3073, 2827, 1678, 1276, 1240.

Anal. Calcd for $C_{12}H_{11}F_2N_3O_2$: C, 53.93; H, 4.15; N, 15.72; found: C, 53.82; H, 4.54; N, 16.10.

5-oxo-*N'*-((perfluorophenyl)methylene)pyrrolidine-2-carbohydrazide – 136

HEI 3158



Following the general procedure 5-oxo-*N'*-((perfluorophenyl)methylene)pyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 2,3,4,5,6-pentafluorobenzaldehyde (13.9 mmol, 2.7 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (4 g, 90 % yield).

M.p. 212-213°C.

Isomer 1 (62%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.78 (s, 1H, N*H*), 8.06 (s, 1H, C*H*), 7.39 (s, 1H, N*H*), 4.77 (q, *J* = 4.2 Hz, 1H, C*H*), 2.35 (m, 1H, CH_2CH$_2$CH), 2.18 (m, 2H, CH_2CH_2CH), 1.94 (m, 1H, CH$_2$CH_2CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.1 (C=O), 173.7 (C=O), 143.2 (2C$_{IV}$), 138.5 (C$_{IV}$), 136.0 (2C$_{IV}$), 131.6 (CH), 109.4 (C$_{IV}$), 53.0 (CH), 28.9 (CH$_2$), 24.5 (CH$_2$).

Isomer 2 (38%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.84 (s, 1H, N*H*), 8.38 (s, 1H, C*H*), 7.88 (s, 1H, N*H*), 4.10 (q, *J* = 4.2 Hz, 1H, C*H*), 2.35 (m, 1H, CH_2CH$_2$CH), 2.18 (m, 2H, CH_2CH_2CH), 1.94 (m, 1H, CH$_2$CH_2CH).

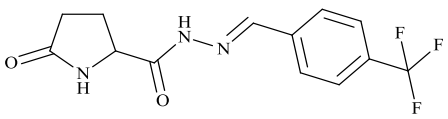
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.3 (C=O), 169.1 (C=O), 145.6 (2C$_{IV}$), 139.5 (C$_{IV}$), 136.0 (2C$_{IV}$), 135.8 (CH), 109.4 (C$_{IV}$), 55.1 (CH), 29.0 (CH$_2$), 25.0 (CH$_2$).

IR ν (cm^{-1}): 3439, 3201, 3066, 1687, 1523, 1496, 1236.

Anal. Calcd for C$_{12}$H$_8$F$_5$N$_3$O$_2$, H$_2$O: C, 42.49; H, 2.97; N, 12.39; found: C, 42.55; H, 2.70; N, 12.46.

5-oxo-*N'*-(4-(trifluoromethyl)benzylidene)pyrrolidine-2-carbohydrazide – 137

HEI 3157



Following the general procedure 5-oxo-*N'*-(4-(trifluoromethyl)benzylidene)pyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 4-(trifluoromethyl)benzaldehyde (13.9 mmol, 2.4 g), in 20 mL of water. The

crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (2.0 g, 50 % yield).

M.p. 187-188°C.

Isomer 1 (54%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.67 (s, 1H, N*H*), 8.08 (s, 1H, C*H*), 7.78 (m, 1H, N*H*), 7.78 (m, 4H, Ar*H*), 4.95 (q, *J* = 4.7 Hz, 1H, C*H*), 2.51-2.12 (m, 3H, CH_2CH_2CH), 1.98 (m, 1H, CH$_2$CH_2CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.7 (C=O), 174.3 (C=O), 142.4 (CH), 138.5 (C_{IV}), 130.0 (C_{IV}), 127.9 (2CHAr), 126.1 (2CHAr), 123.2 (CH), 53.5 (CH), 29.6 (CH_2), 25.2 (CH_2).

Isomer 2 (46%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.73 (s, 1H, N*H*), 8.34 (s, 1H, C*H*), 7.94 (m, 1H, N*H*), 7.94 (m, 4H, Ar*H*), 4.14 (q, *J* = 4.7 Hz, 1H, C*H*), 2.51-2.12 (m, 3H, CH_2CH_2CH), 1.98 (m, 1H, CH$_2$CH_2CH).

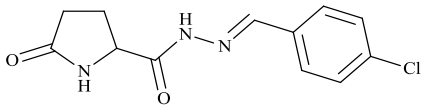
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.9 (C=O), 169.7 (C=O), 146.2 (CH), 138.5 (C_{IV}), 130.0 (C_{IV}), 128.1 (2CHAr), 126.1 (2CHAr), 123.2 (CH), 55.4 (CH), 29.6 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3411, 3219, 3083, 2940, 1667, 1552, 1325, 1125, 1066.

Anal. Calcd for C$_{13}$H$_{12}$F$_3$N$_3$O$_2$, H$_2$O: C, 49.21; H, 4.45; N, 13.24; found: C, 49.41; H, 4.62; N, 13.29.

N'-(4-chlorobenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 138

HEI 3142



Following the general procedure, *N'*-(4-chlorobenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 4-chlorobenzaldehyde (13.9 mmol, 2 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (1.5 g, 40 % yield).

M.p. 251-252°C.

Isomer 1 (54%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.53 (s, 1H, N*H*), 7.98 (s, 1H, C*H*), 7.76 (s, 1H, N*H*), 7.72 (d, *J* = 8.5 Hz, 2H, Ar*H*), 7.52 (d, *J* = 8.5 Hz, 2H, Ar*H*), 4.90 (q, *J* = 4.0 Hz, 1H, C*H*), 2.21-2.12 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.94 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 174.1 (C=O), 142.8 (CH), 134.75 (C$_{IV}$), 133.5 (C$_{IV}$), 129.4 (2CHAr), 129.3 (2CHAr), 53.5 (CH), 29.6 (CH$_2$), 25.2 (CH$_2$).

Isomer 2 (46%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.59 (s, 1H, N*H*), 8.24 (s, 1H, C*H*), 7.87 (s, 1H, N*H*), 7.75 (d, *J* = 8.5 Hz, 2H, Ar*H*), 7.55 (d, *J* = 8.5 Hz, 2H, Ar*H*), 4.10 (q, *J* = 4.0 Hz, 1H, C*H*), 2.21-2.12 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.94 (m, 1H, CH$_2$C*H*$_2$CH).

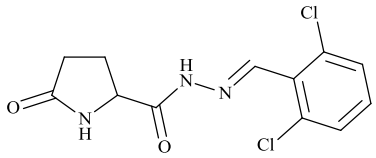
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.7 (C=O), 169.5 (C=O), 146.6 (CH), 135.01 (C$_{IV}$), 133.5 (C$_{IV}$), 129.2 (2CHAr), 128.9 (2CHAr), 55.4 (CH), 29.6 (CH$_2$), 25.6 (CH$_2$).

IR ν (cm^{-1}): 3279, 3206, 3006, 1665, 1575, 1248, 1090.

Anal. Calcd for C$_{12}$H$_{12}$ClN$_3$O$_2$: C, 54.25; H, 4.55; N, 15.82; found: C, 54.03; H, 4.55; N, 15.94.

N'-(2,6-dichlorobenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 139

HEI 3144



Following the general procedure, *N'*-(2,6-dichlorobenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 2,6-dichlorobenzaldehyde (13.9 mmol, 2.4 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (2.5 g, 62 % yield).

M.p. 224-225°C.

Isomer 1 (67%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.72 (s, 1H, N*H*), 8.26 (s, 1H, C*H*), 7.71 (s, 1H, N*H*), 7.54 (d, *J* = 7.7 Hz, 2H, Ar*H*), 7.42 (t, *J* = 7.7 Hz, 1H, Ar*H*), 4.77 (q, *J* = 4.3 Hz, 1H, C*H*), 2.41 (m, 2H, C*H*$_2$CH$_2$CH), 2.20 (m, 1H, CH$_2$C*H*$_2$CH), 2.10 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 174.3 (C=O), 139.4 (CH), 134.3 (2C$_{IV}$), 131.5 (C$_{IV}$), 130.8 (CHAr), 130.0 (2 CHAr), 53.5 (CH), 29.5 (CH$_2$), 25.5 (CH$_2$).

Isomer 2 (33%)

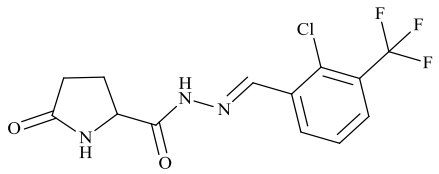
^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.81 (s, 1H, N*H*), 8.46 (s, 1H, C*H*), 7.87 (s, 1H, N*H*), 7.57 (d, *J* = 7.7 Hz, 2H, Ar*H*), 7.45 (t, *J* = 7.7 Hz, 1H, Ar*H*), 4.11 (q, *J* = 4.3 Hz, 1H, C*H*), 2.40–2.10 (m, 4H, C*H*$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 169.6 (C=O), 143.5 (CH), 131.7 (2C$_{IV}$), 131.6 (C$_{IV}$), 130.9 (CHAr), 129.5 (2 CHAr), 55.5 (CH), 29.5 (CH$_2$), 25.5 (CH$_2$).

IR ν (cm^{-1}): 3439, 3209, 3017, 1669, 1549, 1232, 1145.

Anal. Calcd for C$_{12}$H$_{11}$Cl$_2$N$_3$O$_2$, H$_2$O: C, 45.30; H, 4.12; N, 13.21; found: C, 45.49; H, 3.97; N, 13.39.

N'-(2-chloro-3-(trifluoromethyl)benzylidene)-5-oxopyrrolidine-2-carbohydrazide – 140 HEI 3207



Following the general procedure *N'*-(2-chloro-3-(trifluoromethyl)benzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (9.2 mmol, 1.3 g) with 2-chloro-3-(trifluoromethyl)benzaldehyde (9.2 mmol, 2.0 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (1.3 g, 43 % yield).

M.p. 143-144°C.

Isomer 1 (55%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.78 (s, 1H, N*H*), 8.48 (s, 1H, C*H*), 8.27 (d, *J* = 7.2 Hz, 1H, C*H*), 7.90 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.80 (s, 1H, N*H*), 7.61 (d, *J* = 8.0 Hz, 2H, Ar*H*), 4.93 (q, *J* = 4.4 Hz, 1H, C*H*), 2.35-2.20 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.98 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.3 (C=O), 173.8 (C=O), 138.5 (CH), 133.8 (C$_{IV}$), 130.9 (CHAr), 130.6 (C$_{IV}$), 128.7 (C$_{IV}$), 127.9 (2CHAr), 124.0 (C$_{IV}$), 55.1 (CH), 29.0 (CH$_2$), 24.6 (CH$_2$).

Isomer 2 (45%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.90 (s, 1H, N*H*), 8.76 (s, 1H, C*H*), 8.22 (d, *J* = 7.2 Hz, 1H, C*H*), 7.90 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.88 (s, 1H, N*H*), 7.65 (d, *J* = 7.6 Hz, 2H, Ar*H*), 4.12 (q, *J* = 4.4 Hz, 1H, C*H*), 2.35-2.20 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.98 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.2 (C=O), 169.2 (C=O), 142.3 (CH), 133.9

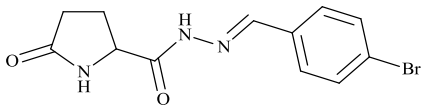
(C_{IV}), 130.9 (CHAr), 130.4 (C_{IV}), 128.5 (C_{IV}), 127.9 (2CHAr), 121.3 (C_{IV}), 53.0 (CH), 29.1 (CH_2), 25.0 (CH_2).

IR ν (cm^{-1}): 3214, 2922, 2853, 1677, 1563, 1239.

Anal. Calcd for $C_{13}H_{11}ClF_3N_3O_2$: C, 46.79; H, 3.32; N, 12.59; found: C, 46.34; H, 2.95; N, 12.40.

N'-(4-bromobenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 141

HEI 3143



Following the general procedure, *N'*-(4-bromobenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 4-bromobenzaldehyde (13.9 mmol, 2.6 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (2.5 g, 60 % yield).

M.p. 255-256°C.

Isomer 1 (53%)

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.53 (s, 1H, N*H*), 7.97 (s, 1H, C*H*), 7.76 (s, 1H, N*H*), 7.65 (m, 4H, Ar*H*), 4.90 (q, *J* = 4.3 Hz, 1H, C*H*), 2.21-2.12 (m, 3H, CH_2CH_2CH), 1.94 (m, 1H, CH_2CH_2CH).

^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ ppm 177.7 (C=O), 174.1 (C=O), 142.9 (CH), 133.8 (C_{IV}), 132.2 (2CHAr), 129.2 (2CHAr), 123.5 (C_{IV}), 53.5 (CH), 29.6 (CH_2), 25.2 (CH_2).

Isomer 2 (47%)

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.59 (s, 1H, N*H*), 8.23 (s, 1H, C*H*), 7.86 (s, 1H, N*H*), 7.65 (m, 4H, Ar*H*), 4.10 (q, *J* = 4.3 Hz, 1H, C*H*), 2.21-2.12 (m, 3H, CH_2CH_2CH), 1.94 (m, 1H, CH_2CH_2CH).

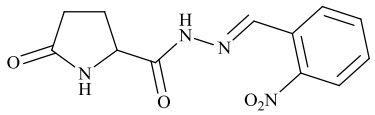
^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ ppm 177.8 (C=O), 169.5 (C=O), 146.7 (CH), 133.9 (C_{IV}), 132.3 (2CHAr), 129.4 (2CHAr), 123.8 (C_{IV}), 55.4 (CH), 29.6 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3299, 3210, 3012, 2928, 1665, 1249, 1092, 1067.

Anal. Calcd for $C_{12}H_{12}BrN_3O_2$: C, 46.47; H, 3.90; N, 13.55; found: C, 46.50; H, 3.95; N, 13.88.

N'-(2-nitrobenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 142

HEI 3155



Following the general procedure, *N'*-(2-nitrobenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 2-nitrobenzaldehyde (13.9 mmol, 2.1 g), in 20 mL of ethanol. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a yellow solid (3.2 g, 83 % yield).

M.p. 207-208°C.

Isomer 1 (57%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.74 (s, 1H, N*H*), 8.36 (s, 1H, C*H*), 8.07 (bs, 2H, Ar*H*),7.88 (s, 1H, N*H*), 7.78 (s, 1H, Ar*H*), 7.65 (s, 1H, Ar*H*), 4.86 (q, *J* = 4.2 Hz, 1H, C*H*), 2.2-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.98 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 174.3 (C=O), 148.5 (C$_{IV}$), 139.6 (CH), 133.9 (CHAr), 130.9 (CHAr), 129.05 (C$_{IV}$), 128.5 (CHAr), 124.9 (CHAr), 53.5 (CH), 29.5 (CH$_2$), 25.2 (CH$_2$).

Isomer 2 (43%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.87 (s, 1H, N*H*), 8.66 (s, 1H, C*H*), 8.07 (bs, 1H, N*H*), 7.78 (s, 3H, Ar*H*), 7.68 (s, 1H, Ar*H*), 4.12 (bs, 1H, C*H*), 2.2-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.98 (m, 1H, CH$_2$C*H*$_2$CH).

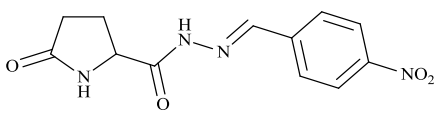
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 169.8 (C=O), 148.6 (C$_{IV}$), 143.5 (CH), 134.2 (CHAr), 131.8 (CHAr), 129.05 (C$_{IV}$), 128.8 (CHAr), 125.1 (CHAr), 55.5 (CH), 29.6 (CH$_2$), 25.5 (CH$_2$).

IR ν (cm^{-1}): 3371, 3195, 3059, 1706, 1679, 1558, 1520, 1339, 1233.

Anal. Calcd for C$_{12}$H$_{12}$N$_4$O$_4$: C, 52.17; H, 4.38; N, 20.28; found: C, 52.17; H, 4.11; N, 19.89.

***N'*-(4-nitrobenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 143**

HEI 3154



Following the general procedure, *N'*-(4-nitrobenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 4-nitrobenzaldehyde (13.9 mmol, 2.1 g), in 20 mL of ethanol. The crude product was filtered

and washed with ethanol (2x10 mL), affording the compound as a yellow solid (4 g, 88 % yield).

M.p. 239-240°C.

Isomer 1 (53%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.84 (s, 1H, N*H*), 8.28 (d, *J* = 8.9 Hz, 2H, Ar*H*), 8.1 (s, 1H, C*H*), 7.96 (d, *J* = 8.9 Hz, 2H, Ar*H*), 7.78 (s, 1H, N*H*), 4.92 (q, *J* = 4.3 Hz, 1H, C*H*), 2.2-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.95 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.3 (C=O), 173.9 (C=O), 147.6 (C_{IV}), 141.3 (CH), 140.3 (C_{IV}), 127.7 (2CHAr), 123.9 (2CHAr), 53.0 (CH), 29.0 (CH_2), 24.6 (CH_2).

Isomer 2 (47%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.75 (s, 1H, N*H*), 8.36 (s, 1H, C*H*), 8.29 (d, *J* = 8.9 Hz, 2H, Ar*H*), 7.97 (d, *J* = 8.9 Hz, 2H, Ar*H*), 7.90 (s, 1H, N*H*), 4.38 (q, *J* = 4.3 Hz, 1H, C*H*), 2.2-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.95 (m, 1H, CH$_2$C*H*$_2$CH).

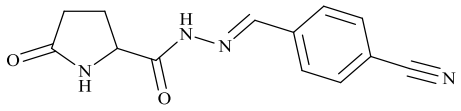
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.3 (C=O), 169.3 (C=O), 147.6 (C_{IV}), 145.0 (CH), 140.3 (C_{IV}), 127.9 (2CHAr), 124.0 (2CHAr), 54.9 (CH), 29.1 (CH_2), 25.1 (CH_2).

IR ν (cm^{-1}): 3333, 3115, 2962, 2839, 1684, 1342; 1252, 1145.

Anal. Calcd for C$_{12}$H$_{12}$N$_4$O$_4$: C, 52.17; H, 4.38; N, 20.28; found: C, 52.04; H, 4.19; N, 19.96.

N'-(4-cyanobenzylidene)-5-oxopyrrolidine-2-carbohydrazide – 144

HEI 3139



Following the general procedure, *N'*-(4-cyanobenzylidene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 4-formylbenzonitrile (13.9 mmol, 1.8 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (1.9 g, 53 % yield).

M.p. 241-242°C.

Isomer 1 (57%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.72 (s, 1H, N*H*), 7.05 (s, 1H, C*H*), 7.89 (m, 4H, Ar*H*), 7.81 (s, 1H, N*H*), 4.95 (q, *J* = 3.9 Hz, 1H, C*H*), 2.4-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 2.01 (m, 1H, CH$_2$C*H*$_2$CH).

$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): δ ppm 177.7 (C=O), 174.3 (C=O), 142.2 (CH), 139.9 (C_{IV}), 133.1 (2CHAr), 127.9 (2CHAr), 119.1 (C_{IV}), 112.2 (C_{IV}), 53.5 (CH), 29.6 (CH_2), 25.2 (CH_2).

Isomer 2 (43%)

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.73 (s, 1H, N*H*), 8.32 (s, 1H, C*H*), 7.92 (s, 1H, N*H*), 7.9 (m, 4H, Ar*H*), 4.16 (q, *J* = 3.9 Hz, 1H, C*H*), 2.4-2.10 (m, 3H, CH_2CH_2CH), 2.01 (m, 1H, CH_2CH_2CH)

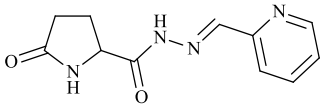
.**$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz):** δ ppm 177.9 (C=O), 169.8 (C=O), 146.0 (CH), 139.0 (C_{IV}), 133.1 (2CHAr), 128.1 (2CHAr), 119.1 (C_{IV}), 112.4 (C_{IV}), 55.5 (CH), 29.6 (CH_2), 25.6 (CH_2).

IR ν (cm^{-1}): 3223, 3082, 2223, 1685, 1556, 1236, 1205.

Anal. Calcd for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.86; found: C, 60.89; H, 4.66; N, 22.16.

5-oxo-*N'*-(pyridin-2-ylmethylene)pyrrolidine-2-carbohydrazide – 145

HEI 3149



Following the general procedure, 5-oxo-*N'*-(pyridin-2-ylmethylene)pyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with picolinaldehyde (13.9 mmol, 1.5 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (1.5 g, 46 % yield).

M.p. 223-224°C.

Isomer 1 (57%)

^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 11.67 (s, 1H, N*H*), 8.60 (s, 1H, N*H*), 8.04 (s, 1H, C*H*), 6.79 (m, 2H, Ar*H*), 7.42 (m, 1H, Ar*H*), 4.91 (q, *J* = 4.3 Hz, 1H, C*H*), 2.20-2.10 (m, 3H, CH_2CH_2CH), 1.93 (m, 1H, CH_2CH_2CH).

$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): δ ppm 177.7 (C=O), 174.2 (C=O), 153.5 (C_{IV}), 149.9 (CHAr), 144.6 (CH), 137.3 (CHAr), 124.7 (CHAr), 120.1 (CHAr), 53.5 (CH), 29.6 (CH_2), 25.2 (CH_2).

Isomer 2 (43%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.75 (s, 1H, N*H*), 8.60 (s, 1H, N*H*), 8.27 (s, 1H, C*H*), 6.79 (m, 2H, Ar*H*), 7.42 (m, 1H, Ar*H*), 4.11 (q, *J* = 4.2 Hz, 1H, C*H*), 2.20-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.93 (m, 1H, CH$_2$C*H*$_2$CH).

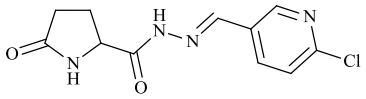
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 169.7 (C=O), 153.5 (C$_{IV}$), 147.0 (CHAr), 148.2 (CH), 137.3 (CHAr), 124.9 (CHAr), 120.4 (CHAr), 55.5 (CH), 29.6 (CH$_2$), 25.6 (CH$_2$).

IR ν (cm^{-1}): 3212, 3082, 2923, 1681, 1559, 1240, 1207.

Anal. Calcd for C$_{11}$H$_{12}$N$_4$O$_2$: C, 56.89; H, 5.21; N, 24.12; found: C, 56.78; H, 4.99; N, 23.95.

N'-((6-chloropyridin-3-yl)methylene)-5-oxopyrrolidine-2-carbohydrazide – 146

HEI 3206



Following the general procedure, *N'*-((6-chloropyridin-3-yl)methylene)-5-oxopyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 6-chloronicotinaldehyde (13.9 mmol, 2 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white solid (3.3 g, 90 % yield).

M.p. 254-255°C.

Isomer 1 (56%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.70 (s, 1H, N*H*), 8.69 (s, 1H, Ar*H*), 8.18 (td, *J* = 7.8 Hz, *J* = 1.8 Hz, 1H, Ar*H*), 8.03 (s, 1H, C*H*), 7.80 (s, 1H, N*H*), 7.58 (t, *J* = 7.8 Hz, 1H, Ar*H*), 4.91 (q, *J* = 4.1 Hz, 1H, C*H*), 2.20-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.94 (m, 1H, CH$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.8 (C=O), 174.2 (C=O), 151.2 (C$_{IV}$), 149.2 (CHAr), 140.0 (CH), 137.1 (CHAr), 130.1 (C$_{IV}$), 125.0 (CHAr), 53.5 (CH), 29.6 (CH$_2$), 25.1 (CH$_2$).

Isomer 2 (44%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 11.76 (s, 1H, N*H*), 8.69 (s, 1H, Ar*H*), 8.32 (s, 1H, C*H*), 8.18 (td, *J* = 7.8 Hz, *J* = 1.8 Hz, 1H, Ar*H*), 7.92 (s, 1H, N*H*), 7.59 (t, *J* = 7.8 Hz, 1H, Ar*H*), 4.15 (q, *J* = 4.1 Hz, 1H, C*H*), 2.20-2.10 (m, 3H, C*H*$_2$C*H*$_2$CH), 1.95 (m, 1H, CH$_2$C*H*$_2$CH).

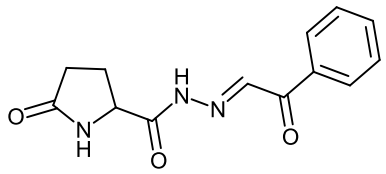
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 177.7 (C=O), 169.7 (C=O), 151.5 (C$_{IV}$), 149.4 (CHAr), 143.9 (CH), 137.2 (CHAr), 130.1 (C$_{IV}$), 125.1 (CHAr), 55.4 (CH), 29.6 (CH$_2$), 25.6 (CH$_2$).

IR ν (cm^{-1}): 3295, 2994, 2912, 1665, 1590, 1466, 1245.

Anal. Calcd for $C_{11}H_{11}ClN_4O_2$: C, 49.54; H, 4.16; N, 21.01; found: C, 49.90; H, 4.11; N, 21.23.

5-oxo-*N'*-(2-oxo-2-phenylethylidene)pyrrolidine-2-carbohydrazide – 147

HEI 3156



Following the general procedure, 5-oxo-*N'*-(2-oxo-2-phenylethylidene)pyrrolidine-2-carbohydrazide was obtained from the reaction of 5-oxopyrrolidine-2-carbohydrazide (13.9 mmol, 2.0 g) with 2-oxo-2-phenylacetaldehyde (13.9 mmol, 1.9 g), in 20 mL of water. The crude product was filtered and washed with ethanol (2x10 mL), affording the compound as a white crystals (1.4 g, 40 % yield).

M.p. 146-147°C.

Isomer 1 (63%)

^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 12.04 (s, 1H, N*H*), 8.05 (s, 1H, N*H*), 7.99 (d, *J* = 7.6 Hz, 1H, C*H*), 7.78 (d, *J* = 10.4 Hz, 2H, Ar*H*), 7.55 (t, J = 7.6 Hz, 3H, Ar*H*), 4.71 (q, *J* = 3.6 Hz, 1H, C*H*), 2.32-1.90 (m, 4H, C*H*$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 190.0 (C=O), 177.7 (C=O), 175.0 (C=O), 141.3 (CH), 136.1 (C_{IV}), 133.53 (CHAr), 130.3 (2CHAr), 128.8 (2CHAr), 53.5 (CH), 29.5 (CH_2), 24.9 (CH_2).

Isomer 2 (37%)

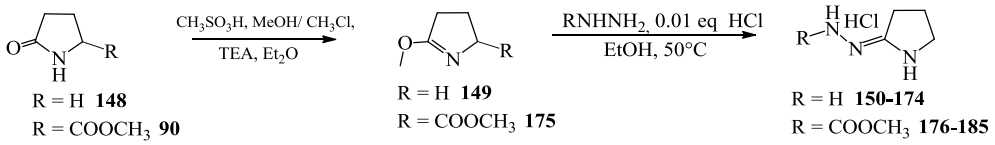
^{1}H-NMR (DMSO-d$_6$, 400 MHz): δ ppm 12.04 (s, 1H, N*H*), 8.04 (s, 1H, N*H*), 7.99 (d, *J* = 7.6 Hz, 1H, C*H*), 7.78 (d, *J* = 10.4 Hz, 2H, Ar*H*), 7.67 (t, J = 7.6 Hz, 3H, Ar*H*), 4.17 (bs, 1H, C*H*), 2.32-1.90 (m, 4H, C*H*$_2$C*H*$_2$CH).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): δ ppm 189.9 (C=O), 175.0 (C=O), 170.8 (C=O), 144.6 (CH), 136.1 (C_{IV}), 133.53 (CHAr), 130.2 (2CHAr), 128.9 (2CHAr), 55.6 (CH), 29.3 (CH_2), 25.5 (CH_2).

IR ν (cm^{-1}): 3444, 3192, 3060, 1686, 1555, 1347, 1218.

Anal. Calcd for $C_{13}H_{13}N_3O_3$, H_2O: C, 56.31; H, 5.45; N, 15.15; found: C, 56.15; H, 5.17; N, 14.97.

1.4 Experimental procedures for the synthesis of pyrrolidine hydrazones

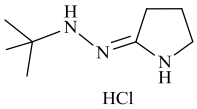


Scheme 56 General procedure for the synthesis of pyrrolidine hydrazones

In a first step, the lactam derivative is transformed to its iminoether. The iminoether was firstly obtained in a larger quantity, by treatment of lactam with dimethylsulfate under heating, for at least 12 hours. The resulting iminoether salt was slowly dropped in a solution of TEA in ether and the mixture was stirred with a polytron-type apparatus, after which, the organic layer containg iminoether was separated. The hydrazine derivatives are then added to a solution of iminoether in presence of a catalytic amount of acid. In a typical experiment, intermediate is dissolved in ethanol in presence of 1% mol equivalent of HCl and phenylhydrazine derivatives are added dropwise. The mixture is then heated to 60° until completion of the reaction.

2-(2-(tert-butyl) hydrazono)pyrrolidine hydrochloride – 150

HEI 3347



Following the general procedure, 2-(2-(tert-butyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and tert-butylhydrazine hydrochloride (2.6 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 6 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (2.7 g, 70 % yield).

M.p. 244-245°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.75 (s, 1H, N*H*), 9.50 (s, 1H, N*H*), 5.50 (s, 1H, N*H*), 3.51 (t, *J* = 7.2 Hz, 2H, CH_2), 2.85 (t, *J* = 8.0 Hz, 2H, CH_2), 2.10 (t, *J* = 7.6 Hz, 2H, CH_2), 1.06 (s, 9H, CH_3).

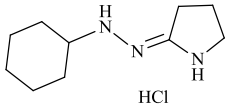
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.5 (C_{IV}), 54.7 (C_{IV}), 46.7 (CH_2), 28.8 (CH_2), 27.2 ($3CH_3$), 21.1 (CH_2).

IR ν (cm^{-1}): 3154, 2947, 1680, 1356, 1306, 1220, 1052.

Anal. Calcd for $C_8H_{18}ClN_3$: C, 50.12; H, 9.46; N, 21.92.

2-(2-cyclohexyl hydrazono)pyrrolidine hydrochloride – 151

HEI 3349



Following the general procedure, 2-(2-cyclohexylhydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and cyclohexylhydrazine hydrochloride (3.0 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the coumpound as a white solid (2.4 g, 55 % yield).

M.p. 170-171°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.05 (s, 1H, N*H*), 9.47 (s, 1H, N*H*), 5.71 (s, 1H, N*H*), 3.49 (bs, 2H, CH_2), 2.80 (bs, 2H, CH_2), 2.79 (bs, 1H, C*H*), 1.81 (bs, 2H, CH_2), 1.81-1.68 (m, 6H, CH_2) 1.20-1.08 (m, 5H, CH_2).

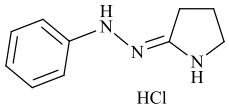
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 169.2 (C_{IV}), 58.08 (CH), 46.8 (CH_2), 30.7 ($2CH_2$), 28.7 (CH_2), 26.0 (CH_2), 24.4 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3171, 2923, 2852, 1688, 1454, 1305, 1057.

Anal. Calcd for $C_{10}H_{20}ClN_3$: C, 55.16; H, 9.26; N, 19.30.

2-(2-phenylhydrazono) pyrrolidine hydrochloride – 152

HEI 3337



Following the general procedure, 2-(2-phenylhydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and phenylhydrazine (2.2 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in

MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (1.3 g, 30 % yield).

M.p. 230-231°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.63 (s, 1H, N*H*), 10.43 (s, 1H, N*H*), 8.75 (s, 1H, N*H*), 7.27 (q, *J* = 7.2 Hz, 2H, Ar*H*), 6.88 (m, 3H, Ar*H*), 3.56 (t, *J* = 7.2 Hz, 2H, CH_2), 2.9 (t, *J* = 8.0 Hz, 2H, CH_2), 2.18 (t, 1H, C*H*), 1.81 (bs, *J* = 7.2 Hz, 2H, CH_2).

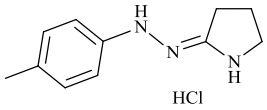
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.9 (C_{IV}), 146.8 (C_{IV}), 129.4 (2 CHAr), 121.1 (CHAr), 113.8 (2 CHAr), 47.3 (CH_2), 29.0 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3097, 2994, 1696, 1494, 1313, 1247, 1064.

Anal. Calcd for $C_{10}H_{14}ClN_3$: C, 56.74; H, 6.67; N, 19.85.

2-(2-(*p*-tolyl)hydrazono) pyrrolidine hydrochloride – 153

HEI 3343



Following the general procedure, 2-(2-(p-tolyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and p-tolylhydrazine hydrochloride (3.2 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the coumpound as a white solid (2.5 g, 55 % yield).

M.p. 249-250°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.58 (s, 1H, N*H*), 9.99 (s, 1H, N*H*), 8.59 (s, 1H, N*H*), 7.05 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.77 (d, *J* = 8.4 Hz, 2H, Ar*H*), 3.56 (t, *J* = 7.2 Hz, 2H, CH_2), 2.96 (t, *J* = 8.0 Hz, 2H, CH_2), 2.21 (s, 3H, CH_3), 2.14 (m, 2H, CH_2).

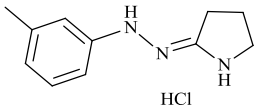
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.7 (C_{IV}), 144.4 (C_{IV}), 129.9 (C_{IV}), 129.8 (2CHAr), 114.1 (2CHAr), 49.5 (CH_2), 28.9 (CH_2), 21.1 (CH_2), 20.6 (CH_3).

IR ν (cm^{-1}): 3149, 2968, 1704, 1513, 1311, 1253, 1066.

Anal. Calcd for $C_{11}H_{16}ClN_3$: C, 58.53; H, 7.14; N, 18.62.

2-(2-(*m*-tolyl) hydrazono) pyrrolidine hydrochloride - 154

HEI 3344



Following the general procedure, 2-(2-(m-tolyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and m-tolylhydrazine hydrochloride (3.2 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (1.8 g, 40 % yield).

M.p. 234-235°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.60 (s, 1H, N*H*), 10.00 (s, 1H, N*H*), 8.69 (s, 1H, N*H*), 7.12 (t, *J* = 7.2 Hz, 1H, Ar*H*), 6.80 (d, *J* = 9.2 Hz 3H, Ar*H*), 3.54 (t, *J* = 6.8 Hz, 2H, CH_2), 2.98 (t, *J* = 8.0 Hz, 2H, CH_2), 2.25 (s, 3H, CH_3), 2.08 (m, 2H, CH_2).

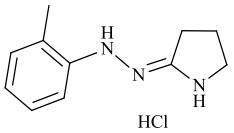
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.8 (C_{IV}), 146.8 (C_{IV}), 138.7 (C_{IV}), 129.3 (CHAr), 121.9 (CHAr), 114.3 (CHAr), 111.1 (CHAr), 47.3 (CH_2), 28.9 (CH_2), 21.6 (CH_3) 21.1 (CH_2).

IR ν (cm^{-1}): 3099, 2876, 1704, 1592, 1488, 1310, 1170, 1063.

Anal. Calcd for C$_{11}$H$_{16}$ClN$_3$: C, 58.53; H, 7.14; N, 18.62.

2-(2-(*o*-tolyl)hydrazono) pyrrolidine hydrochloride - 155

HEI 3345



Following the general procedure, 2-(2-(o-tolyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and o-tolylhydrazine hydrochloride (3.2 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (2.3 g, 50 % yield).

M.p. 256-257°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.70 (s, 1H, N*H*), 9.95 (s, 1H, N*H*), 8.13 (s, 1H, N*H*), 7.08 (d, *J* = 7.2 Hz, 2H, Ar*H*), 6.80 (m, 2H, Ar*H*), 3.56 (t, *J* = 7.2 Hz, 2H, CH_2), 3.01 (t, *J* = 8.0 Hz, 2H, CH_2), 2.24 (s, 3H, CH_3), 2.18 (m, 2H, CH_2).

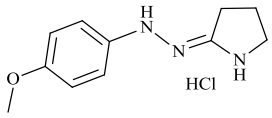
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.5 (C_{IV}), 144.3 (C_{IV}), 130.8 (CHAr), 126.9 (CHAr), 123.9 (CHAr), 120.9 (CHAr), 112.05 (C_{IV}), 47.2 (CH_2), 29.0 (CH_2), 21.1 (CH_2), 17.7 (CH_3).

IR ν (cm^{-1}): 3173, 1687, 1591, 1311, 1252, 1049.

Anal. Calcd for C$_{11}$H$_{16}$ClN$_3$: C, 58.53; H, 7.14; N, 18.62.

2-(2-(4-methoxyphenyl) hydrazono)pyrrolidine hydrochloride – 156

HEI 3341



Following the general procedure, 2-(2-(4-methoxyphenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and (4-methoxyphenyl)hydrazine hydrochloride (3.5 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (2.2 g, 45 % yield).

M.p. 237-238°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.53 (s, 1H, N*H*), 10.00 (s, 1H, N*H*), 8.44 (s, 1H, N*H*), 6.84 (s, 4H, Ar*H*), 3.69 (s, 3H, CH_3), 3.56 (t, *J* = 7.2 Hz, 2H, CH_2), 2.95 (t, *J* = 7.6 Hz, 2H, CH_2), 2.21 (s, 3H, OCH_3), 2.14 (t, *J* = 7.2 Hz, 2H, CH_2).

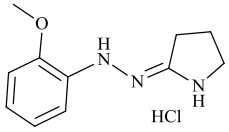
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.6 (C_{IV}), 154.5 (C_{IV}), 140.3 (C_{IV}), 115.6 (2CHAr), 114.8 (2CHAr), 55.8 (OCH_3), 47.2 (CH_2), 28.9 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3149, 3092, 2983, 1704, 1506, 1235, 1172, 1031.

Anal. Calcd for C$_{11}$H$_{16}$ClN$_3$O: C, 54.66; H, 6.67; N, 17.38.

(2-(2-methoxyphenyl)hydrazono)pyrrolidine hydrochloride - 157

HEI 3421



Following the general procedure, (2-(2-methoxyphenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (0.5 g, 5 mmol) and *o*-tolylhydrazine hydrochloride (0.9 g, 5 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was purified by flash LC, using a gradient of solvent heptane/ethyl acetate, the wanted product being eluted at 80% ethyl acetate affording the compound as a grey solid (0.8 g, 66 % yield).

M.p. 262-263°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.34 (bs, 1H, N*H*), 9.97 (s, 1H, N*H*), 8.06(s, 1H, N*H*), 6.97 (d, *J* = 8.7 Hz, 1H, Ar*H*), 6.89 (m, 2H, Ar*H*), 6.86 (dd, *J* = 7.0, *J* = 1.9 Hz, 1H, Ar*H*), 3.81 (s, 3H, CH_3), 3.55 (t, *J* = 7.0 Hz, 2H, CH_2), 2.98 (t, *J* = 7.0 Hz, 2H, CH_2), 2.18 (q, *J* = 7.0 Hz, 2H, CH_2).

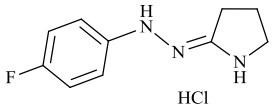
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.2 (C_{IV}), 146.8 (C_{IV}), 134.8 (C_{IV}), 121.0 (CHAr), 120.6 (CHAr), 112.6 (CHAr), 110.8 (CH), 55.4 (CH_3), 46.8 (CH), 28.5 (CH_2), 20.5 (CH_2).

IR ν (cm^{-1}): 3185, 1698, 1596, 1506, 1456, 1306, 1253, 1229, 1033.

Anal. Calcd for $C_{11}H_{16}ClN_3O$: C, 54.66; H, 6.67; N, 17.38.

2-(2-(4-fluorophenyl) hydrazono)pyrrolidine hydrochloride - 158

HEI 3340



Following the general procedure, 2-(2-(4-fluorophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and (4-fluorophenyl)hydrazine hydrochloride (3.3 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (4.0 g, 87 % yield).

M.p. 251-252°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.71 (s, 1H, N*H*), 10.05 (s, 1H, N*H*), 8.81 (s, 1H, N*H*), 7.10 (t, *J* = 9.2 Hz, 2H, Ar*H*), 6.91 (dd, *J* = 9.2 Hz, *J* = 2.4 Hz, 2H, Ar*H*), 3.56 (t, *J* = 7.2 Hz, 2H, CH_2), 2.96 (t, *J* = 8.0 Hz, 2H, CH_2), 2.16 (t, *J* = 7.2 Hz, 2H, CH_2).

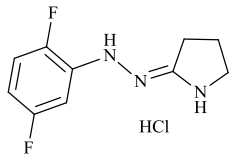
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 170.8 (C_{IV}), 157.4 (C_{IV}, J_{C-F} = 233.3 Hz), 143.3 (d, C_{IV}, J = 2.2 Hz), 115.9 (2CHAr, J_{C-F} = 22.8 Hz), 115.9 (2CHAr, J_{C-F} = 7.6 Hz), 47.3 (CH_2), 29.0 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3162, 3089, 2971, 1694, 1505, 1313, 1212, 1155.

Anal. Calcd for $C_{10}H_{13}ClFN_3$: C, 52.29; H, 5.70; N, 18.29.

2-(2-(2,5-difluorophenyl) hydrazono)pyrrolidine hydrochloride - 159

HEI 3346



Following the general procedure, 2-(2-(2,5-difluorophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and (2,5-difluorophenyl)hydrazine (2.9 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (1.0 g, 20 % yield).

M.p. 247-248°C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.75 (s, 1H, N*H*), 10.14 (s, 1H, N*H*), 8.92 (s, 1H, N*H*), 7.20 (m, 1H, Ar*H*), 6.91 (dd, *J* = 7.2 Hz, *J* = 3.2 Hz, 1H, Ar*H*), 6.70 (d, *J* = 5.2 Hz, 1H, Ar*H*), 3.58 (t, *J* = 6.8 Hz, 2H, CH_2), 3.01 (t, *J* = 7.6 Hz, 2H, CH_2), 2.19 (t, *J* = 7.6 Hz, 2H, CH_2).

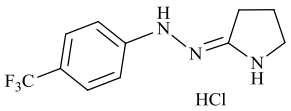
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 171.2 (C_{IV}), 159.3 (dd, C_{IV}, *J* = 237 Hz, *J* = 1.5 Hz), 147.0 (dd, C_{IV} , *J* = 237 Hz), 136.1 (dd, C_{IV}, *J* = 13 Hz, *J* = 10.7 Hz), 116.1 (dd, CHAr, *J* =20.0 Hz, *J* = 10.0 Hz), 106.7 (dd, CHAr, *J* = 33 Hz, *J* = 7.6 Hz), 102.2 (dd, CHAr, *J* = 28.5 Hz, *J* = 3.0 Hz), 47.5 (CH_2), 29.2 (CH_2), 21.0 (CH_2).

IR ν (cm^{-1}): 3144, 2957, 1696, 1504, 1434, 1312, 1157, 1052.

Anal. Calcd for $C_{10}H_{12}ClF_2N_3$: C, 48.49; H, 4.88; N, 16.97.

2-(2-(4-trifluoromethylphenyl) hydrazono)pyrrolidine hydrochloride – 160

HEI 3356



Following the general procedure, 2-(2-(4-(trifluoromethyl)phenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (0.55 g, 20.1 mmol) and (4-(trifluoromethyl) phenyl)hydrazine (1 g, 5.6 mmol), in the presence of 37% HCl (0.05 mmol), in 15 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (1.5 g, 95 % yield).

M.p. 243-244°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.81 (s, 1H, N*H*), 10.13 (s, 1H, N*H*), 9.35 (s, 1H, N*H*), 7.59 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.00 (d, *J* = 8.4 Hz, 2H, Ar*H*), 3.57 (td, *J* = 6.8 Hz, *J* = 4.0 Hz 2H, C*H*$_2$), 3.08 (t, *J* = 7.6 Hz, 2H, C*H*$_2$), 2.18 (t, *J* = 7.2 Hz, 2H, C*H*$_2$).

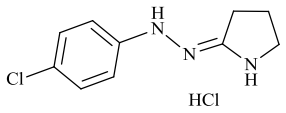
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.0 (C$_{IV}$), 150.2 (C$_{IV}$), 143.3 (C$_{IV}$), 126.8 (C$_{IV}$, $J_{C\text{-}F}$ = 3.2 Hz), 123.8 (C$_{IV}$, $J_{C\text{-}F}$ = 269.6 Hz), 113.4 (4CHAr), 47.5 (CH$_2$), 29.1 (CH$_2$), 21.1 (CH$_2$).

IR ν (cm^{-1}): 3088, 2980, 1698, 1615, 1315, 1100.

Anal. Calcd for C$_{11}$H$_{13}$ClF$_3$N$_3$: C, 47.24; H, 4.68; N, 15.02.

2-(2-(4-chlorophenyl) hydrazono)pyrrolidine hydrochloride – 161

HEI 3338



Following the general procedure, 2-(2-(4-chlorophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and (4-chlorophenyl)hydrazine hydrochloride (3.5 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (3.2 g, 65 % yield).

M.p. 244-245°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.68 (bs, 1H, N*H*), 10.08 (s, 1H, N*H*), 8.94 (bs, 1H, N*H*), 7.30 (t, *J* = 8.8 Hz, 2H, Ar*H*), 6.90 (dd, *J* = 8.4 Hz, *J* = 1.9 Hz, 2H, Ar*H*), 3.56 (t, *J* = 6.8 Hz, 2H, C*H*$_2$), 2.98 (t, *J* = 6.8 Hz, 2H, C*H*$_2$), 2.16 (t, *J* = 6.8 Hz, 2H, C*H*$_2$).

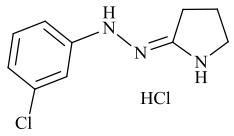
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.9 (C$_{IV}$), 145.9 (C$_{IV}$), 129.1 (2CHAr), 124.5 (C$_{IV}$), 115.5 (2CHAr), 47.4 (CH$_2$), 29.0 (CH$_2$), 21.1 (CH$_2$).

IR ν (cm^{-1}): 3120, 3079, 2968, 1698, 1488, 1314, 1253.

Anal. Calcd for $C_{10}H_{13}Cl_2N_3$: C, 48.80; H, 5.32; N, 17.07.

2-(2-(3-chlorophenyl)hydrazono)pyrrolidine hydrochloride – 162

HEI 3413



Following the general procedure, 2-(2-(3-chlorophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (1.0 g, 10 mmol) and (3-chlorophenyl)hydrazine hydrochloride (1.7 g, 10.1 mmol), in 20 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording the wanted compound as a white solid (0.73 g, 30 % yield).

M.p. 242-243°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.79 (bs, 1H, N*H*), 10.09 (s, 1H, N*H*), 9.13 (bs, 1H, N*H*), 7.25 (t, *J* = 8.0 Hz, 1H, Ar*H*), 6.92 (m, 2H, Ar*H*), 6.85 (d, *J* = 8.0 Hz, 1H, Ar*H*), 3.55 (bs, 2H, CH_2), 2.98 (t, *J* = 6.8 Hz, 2H, CH_2), 2.16 (bs, 2H, CH_2).

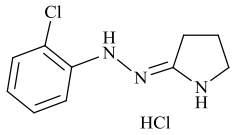
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.0 (C_{IV}), 148.6 (C_{IV}), 134.1 (C_{IV}), 131.1 (CHAr), 120.6 (CHAr), 113.2 (CHAr), 112.6 (CHAr), 47.4 (CH_2), 29.1 (CH_2), 21.0 (CH_2).

IR ν (cm^{-1}): 3166, 3084, 2969, 1690, 1587, 1471, 1307, 1248.

Anal. Calcd for $C_{10}H_{13}Cl_2N_3$: C, 48.80; H, 5.32; N, 17.07.

2-(2-(2-chlorophenyl)hydrazono)pyrrolidine hydrochloride – 163

HEI 3415



Following the general procedure, 2-(2-(2-chlorophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (1.0 g, 10 mmol) and (2-chlorophenyl)hydrazine hydrochloride (1.7 g, 10.1 mmol), in 20 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording the wanted compound as a white solid (1.4 g, 56% yield).

M.p. 246-247°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.77 (s, 1H, N*H*), 10.06 (s, 1H, N*H*), 8.45 (bs, 1H, N*H*), 7.38 (dd, *J* = 8.0 Hz, *J* = 1.2 Hz, 1H, Ar*H*), 7.26 (td, *J* = 8.0 Hz, *J* = 1.2 Hz, 1H, Ar*H*), 6.99 (dd, *J* = 8.0 Hz, *J* = 1.2 Hz, 1H, Ar*H*), 6.90 (td, *J* = 8.0 Hz, *J* = 1.2 Hz, 1H, Ar*H*), 3.56 (t, *J* = 7.0 Hz, 2H, CH_2), 3.02 (t, *J* = 8.2 Hz, 2H, CH_2), 2.20 (t, *J* = 7.0 Hz, 2H, CH_2).

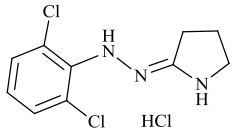
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.9 (C_{IV}), 142.6 (C_{IV}), 130.1 (CHAr), 128.4 (CHAr), 122.0 (CHAr), 118.7 (C_{IV}), 114.4 (CHAr), 47.5 (CH_2), 29.2 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3169, 2963, 1690, 1595, 1493, 1302, 1159.

Anal. Calcd for $C_{10}H_{13}Cl_2N_3$: C, 48.80; H, 5.32; N, 17.07.

2-(2-(2,6-dichlorophenyl) hydrazono)pyrrolidine hydrochloride – 164

HEI 3339



Following the general procedure, 2-(2-(2,6-dichlorophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and (2,6-dichlorophenyl)hydrazine hydrochloride (4.6 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording **164** as a white solid (3.0 g, 55 % yield).

M.p. 249-250°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.9 (s, 1H, N*H*), 10.25 (s, 1H, N*H*), 8.00 (s, 1H, N*H*), 7.48 (t, *J* = 7.6 Hz, 2H, Ar*H*), 7.12 (t, *J* = 8.0 Hz, 2H, Ar*H*), 3.65 (t, *J* = 7.2 Hz, 2H, CH_2), 2.86 (t, *J* = 7.6 Hz, 2H, CH_2), 2.17 (quin, *J* = 7.6 Hz, 2H, CH_2).

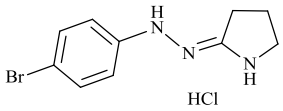
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 169.5 (C_{IV}), 138.7 (C_{IV}), 129.9 (2CHAr), 126.3 (2CHAr), 125.8 (C_{IV}), 47.5 (CH_2), 29.1 (CH_2), 21.3 (CH_2).

IR ν (cm^{-1}): 3214, 2978, 2814, 1698, 1446, 1303, 1265.

Anal. Calcd for $C_{10}H_{12}Cl_3N_3$: C, 42.81; H, 4.31; N, 14.98.

2-(2-(4-chlorophenyl) hydrazono)pyrrolidine hydrochloride – 165

HEI 3411



Following the general procedure, 2-(2-(4-bromophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (0.5 g, 5 mmol) and (4-bromophenyl)hydrazine hydrochloride (1.1 g, 5 mmol) in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording the wanted as a white solid (1.0 g, 68 % yield).

M.p. 246-247°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.54 (s, 1H, N*H*), 10.09 (s, 1H, N*H*), 8.64 (s, 1H, N*H*), 7.42 (d, *J* = 8.9 Hz, 2H, Ar*H*), 6.84 (d, *J* = 8.9 Hz, 2H, Ar*H*), 3.57 (t, *J* = 6.9 Hz, 2H, CH_2), 2.99 (t, *J* = 8.0 Hz, 2H, CH_2), 2.17 (quin, *J* = 6.9 Hz, 2H, CH_2).

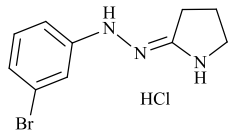
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.9 (C_{IV}), 146.3 (C_{IV}), 132.0 (2CHAr), 115.9 (2CHAr), 112.2 (C_{IV}), 47.4 (CH_2), 29.0 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3124, 2966, 1703, 1588, 1487, 1314, 1282, 1254, 1071.

Anal. Calcd for $C_{10}H_{13}BrClN_3$: C, 41.33; H, 4.51; N, 14.46.

2-(2-(3-chlorophenyl) hydrazono)pyrrolidine hydrochloride – 166

HEI 3412



Following the general procedure, 2-(2-(3-bromophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (0.5 g, 5 mmol) and (3-bromophenyl)hydrazine hydrochloride (1.1 g, 5 mmol) in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording the wanted as a white solid (0.8 g, 54 % yield).

M.p. 247-248°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.67 (bs, 1H, N*H*), 10.03 (bs, 1H, N*H*), 9.04 (s, 1H, N*H*), 7.21 (dd, *J* = 8.4 Hz, *J* = 3.2 Hz, 1H, Ar*H*), 7.04 (d, *J* = 7.4 Hz, 2H, Ar*H*), 6.87 (d, *J* = 7.4 Hz, 1H, Ar*H*), 3.55 (s, 2H, CH_2), 2.99 (t, *J* = 7.0 Hz, 2H, CH_2), 2.16 (t, *J* = 7.0 Hz, 2H, CH_2).

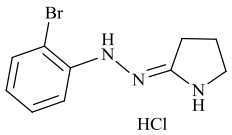
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.8 (C_{IV}), 148.7 (C_{IV}), 131.4 (CHAr), 123.4 (CHAr), 122.7 (C_{IV}), 116.0 (CHAr), 112.9 (CHAr), 47.4 (CH_2), 29.1 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3145, 2971, 1693, 1596, 1478, 1312, 1242.

Anal. Calcd for $C_{10}H_{13}BrClN_3$: C, 41.33; H, 4.51; N, 14.46.

2-(2-(2-chlorophenyl) hydrazono)pyrrolidine hydrochloride – 167

HEI 3422



Following the general procedure, 2-(2-(2-bromophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (0.5 g, 5 mmol) and (2-bromophenyl)hydrazine hydrochloride (1.1 g, 5 mmol) in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording the wanted as a white solid (0.75 g, 60 % yield).

M.p. 227-228°C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.75 (s, 1H, N*H*), 10.03 (s, 1H, N*H*), 8.23 (s, 1H, N*H*), 7.55 (dd, *J* = 7.9 Hz, *J* = 1.2 Hz, 1H, Ar*H*), 7.30 (t, *J* = 7.9 Hz, 1H, Ar*H*), 6.97 (dd, *J* = 7.9 Hz, *J* = 1.2 Hz, 1H, Ar*H*), 6.86 (t, *J* = 7.9 Hz, 1H, Ar*H*), 3.57 (t, *J* = 7.9 Hz, 2H, CH_2), 2.99 (t, *J* = 7.9 Hz, 2H, CH_2), 2.18 (quin, *J* = 7.9 Hz, 2H, CH_2).

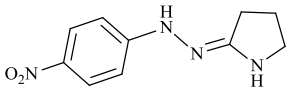
^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): 170.7 (C_{IV}), 143.6 (C_{IV}), 133.3 (CHAr), 129.0 (CHAr), 122.6 (C_{IV}), 114.6 (CHAr), 108.3 (CHAr), 47.5 (CH_2), 29.2 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3168, 2962, 1690, 1593, 1489, 1301, 1020.

Anal. Calcd for $C_{10}H_{13}BrClN_3$: C, 41.33; H, 4.51; N, 14.46.

2-(2-(4-nitrophenyl) hydrazono)pyrrolidine – 168

HEI 3342



Following the general procedure, 2-(2-(4-nitrophenyl)hydrazono)pyrrolidine was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and (4-nitrophenyl)hydrazine (3.0 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording the compound as a red solid (1.5 g, 30 % yield).

M.p. 237-238°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 9.17 (s, 1H, N*H*), 7.98 (dd, *J* = 8.0 Hz, *J* = 1.2 Hz, 2H, Ar*H*), 6.78 (d, *J* = 9.2 Hz, 2H, Ar*H*), 6.72 (s, 1H, N*H*), 3.37 (m, 2H, CH_2), 2.49 (m, 2H, CH_2), 2.06 (m, 2H, CH_2).

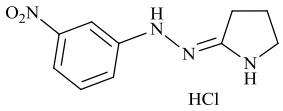
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 156.4 (C_{IV}), 152.3 (C_{IV}), 135.8 (C_{IV}), 126.7 (3CHAr), 110.1 (CHAr), 45.3 (CH_2), 29.4 (CH_2), 22.4 (CH_2).

IR ν (cm^{-1}): 3250, 1633, 1583, 1461, 1283, 1252, 1110.

Anal. Calcd for $C_{10}H_{12}N_4O_2$: C, 54.54; H, 5.49; N, 25.44.

2-(2-(4-nitrophenyl) hydrazono)pyrrolidine hydrochloride – 169

HEI 3414



Following the general procedure, 2-(2-(3-nitrophenyl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (0.5 g, 5 mmol) and (3-nitrophenyl)hydrazine (1 g, 5 mmol). The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording the wanted compound as a yellow solid (0.8 g, 62 % yield).

M.p. 253-254°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.75 (s, 1H, N*H*), 10.20 9 (s, 1H, N*H*), 9.36 (s, 1H, N*H*), 7.73 (dd, *J* = 8.2 Hz, *J* = 2.0 Hz, 1H, Ar*H*), 7.66 (t, *J* = 2.0 Hz, 1H, Ar*H*), 7.55 (t, *J* = 8.2 Hz, 1H, Ar*H*), 7.33 (dd, *J* = 8.2 Hz, *J* = 2.0 Hz, 1H, Ar*H*), 3.57 (t, *J* = 6.8 Hz, 2H, CH_2), 3.01 (t, *J* = 7.4 Hz, 2H, CH_2), 2.19 (quin, *J* = 7.4 Hz, 2H, CH_2).

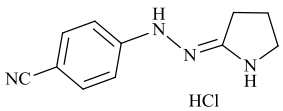
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.2 (C_{IV}), 149.0 (C_{IV}), 148.2 (CHAr), 130.9 (CHAr), 120.0 (C_{IV}), 115.4 (CHAr), 107.6 (CHAr), 47.5 (CH_2), 29.2 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3153, 3078, 2988, 1692, 1523, 1349, 1311.

Anal. Calcd for $C_{10}H_{13}ClN_4O_2$: C, 46.79; H, 5.10; N, 21.83.

2-(2-(4-cyanophenyl) hydrazono)pyrrolidine hydrochloride – 170

HEI 3352



Following the general procedure, 4-(2-(pyrrolidin-2-ylidene)hydrazinyl)benzonitrile hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (1.0 g,

10.0 mmol) and 4-hydrazinylbenzonitrile hydrochloride (1.7 g, 10.0 mmol), in the presence of 37% HCl (0.1 mmol), in 15 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording 6 as a white solid (0.7 g, 30 % yield).

M.p. 231-232°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.72 (s, 1H, N*H*), 10.18 (s, 1H, N*H*), 9.51 (s, 1H, N*H*), 7.68 (d, *J* = 6.8 Hz, 2H, Ar*H*), 6.97 (d, *J* = 6.8 Hz, 2H, Ar*H*), 3.58 (t, *J* = 6.8 Hz, 2H, CH_2), 3.00 (t, *J* = 8 Hz, 2H, CH_2), 2.19 (t, *J* = 8.4 Hz, 2H, CH_2).

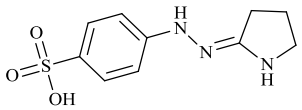
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.1 (C_{IV}), 150.7 (C_{IV}), 134.0 (2CHAr), 120.0 (C_{IV}), 113.6 (2CHAr), 102.0 (C_{IV}), 47.6 (CH_2), 29.2 (CH_2), 21.0 (CH_2).

IR ν (cm^{-1}): 3048, 2964, 2854, 2212, 1694, 1605, 1518, 1312, 1264, 1169.

Anal. Calcd for $C_{11}H_{13}ClN_4$: C, 55.82; H, 5.54; N, 23.67.

4-(2-(pyrrolidin-2-ylidene)hydrazinyl)benzenesulfonic – 171

HEI 3416



Following the general procedure, 4-(2-(pyrrolidin-2-ylidene)hydrazinyl)benzenesulfonic acid was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (1.0 g, 10 mmol) and 4-hydrazinylbenzenesulfonic acid (1.8 g, 10 mmol) in 20 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording the wanted compound as a cream solid (2.0 g, 78 % yield).

M.p. 345-346°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.16 (s, 1H, O*H*), 10.11 (s, 1H, N*H*), 8.55 (s, 1H, N*H*), 7.48 (d, *J* = 9.1 Hz, 2H, Ar*H*), 6.78 (d, *J* = 9.1 Hz, 2H, Ar*H*), 3.58 (t, *J* = 7.8 Hz, 2H, CH_2), 2.98 (t, *J* = 7.8 Hz, 2H, CH_2), 2.18 (t, *J* = 7.8 Hz, 2H, CH_2).

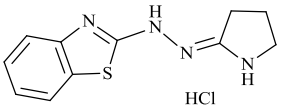
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.5 (C_{IV}), 146.0 (C_{IV}), 141.5 (C_{IV}), 126.5 (2CHAr), 112.0 (2CHAr), 46.9 (CH_2), 28.4 (CH_2), 20.5 (CH_2).

IR ν (cm^{-1}): 3187, 2908, 1706, 1606, 1347, 1170, 1125.

Anal. Calcd for $C_{10}H_{13}N_3O_3S$: C, 47.05; H, 5.13; N, 16.46; S, 12.56.

2-(2-(pyrrolidin-2-ylidene)hydrazinyl)benzo[d]thiazole hydrochloride – 172

HEI 3353



Following the general procedure, 2-(2-(pyrrolidin-2-ylidene)hydrazinyl)benzo[d]thiazole hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2.0 g, 20.1 mmol) and 2-hydrazinylbenzo[*d*]thiazole (3.3 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acetyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a yellowish solid (1.33 g, 25 % yield).

M.p. 237-238°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 12.2 (s, 1H, N*H*), 9.86 (s, 1H, N*H*), 7.70 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.30 (d, *J* = 4.8 Hz, 2H, Ar*H*), 7.12 (m, 2H, Ar*H*), 3.59 (t, *J* = 6.8 Hz, 2H, CH_2), 2.95 (t, *J* = 8.0 Hz, 2H, CH_2), 2.20 (t, *J* = 7.6 Hz, 2H, CH_2).

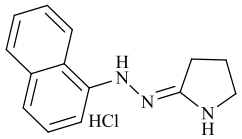
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 165.9 (C_{IV}), 164.9 (C_{IV}), 142.3 (C_{IV}), 126.6 (2CHAr), 122.1 (2CHAr), 114.5 (C_{IV}), 47.0 (CH_2), 28.6 (CH_2), 20.6 (CH_2).

IR ν (cm^{-1}): 2931, 1679, 1607, 1463, 1393, 1305, 1248.

Anal. Calcd for C$_{11}$H$_{13}$ClN$_4$S: C, 49.16; H, 4.88; N, 20.85; S, 11.93.

2-(2-(naphthalen-1-yl)hydrazono)pyrrolidine hydrochloride – 173

HEI 3420



Following the general procedure, 2-(2-(naphthalen-1-yl)hydrazono)pyrrolidine hydrochloride was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (0.5 g, 5 mmol) and naphthalen-1-ylhydrazine hydrochloride (1.0 g, 5 mmol) in 20 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording a white solid (1.0 g, 76 % yield).

M.p. 256-257°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.74 (s, 1H, N*H*), 10.04 (s, 1H, N*H*), 9.16 (s, 1H, N*H*), 8.17 (d, *J* = 5.3 Hz, 1H, Ar*H*), 7.89 (m, 1H, Ar*H*), 7.54 (t, *J* = 5.4 Hz, 2H, Ar*H*), 7.47 (d, *J* =

7.8 Hz, 1H, Ar*H*), 7.39 (t, *J* = 7.8 Hz, 1H, Ar*H*), 7.39 (d, *J* = 7.2 Hz, 1H, Ar*H*), 3.59 (t, *J* = 7.0 Hz, 2H, CH_2), 3.09 (t, *J* = 8.0 Hz, 2H, CH_2), 2.21 (t, *J* = 8.0 Hz, 2H, CH_2).

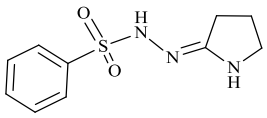
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 170.6 (C_{IV}), 141.7 (C_{IV}), 134.2 (C_{IV}), 128.5 (CHAr), 126.6 (CHAr), 126.5 (CHAr), 125.4 (CHAr), 122.9 (C_{IV}), 122.5 (CHAr), 120.9 (CHAr), 106.6 (CHAr), 47.4 (CH_2), 29.2 (CH_2), 21.1 (CH_2).

IR ν (cm^{-1}): 3234, 2956, 1686, 1583, 1526, 1403, 1310.

Anal. Calcd for $C_{14}H_{16}ClN_3$: C, 64.24; H, 6.16; N, 16.05.

N'-(pyrrolidin-2-ylidene) benzenesulfonohydrazide– 174

HEI 3348



Following the general procedure, *N'*-(pyrrolidin-2-ylidene)benzenesulfonohydrazide was obtained through the reaction of 5-methoxy-3,4-dihydro-2*H*-pyrrole (2 g, 20.1 mmol) and benzenesulfonohydrazide (3.4 g, 20.1 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was washed with ethanol, affording the compound as a white solid (2.9 g, 60 % yield).

M.p. 191-192°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 8.81 (bs, 1H, N*H*), 7.80 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.54 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.81 (bs, 1H, N*H*), 3.25 (t, *J* = 6.4 Hz, 2H, CH_2), 2.17 (bs, 2H, CH_2), 1.83 (t, *J* = 8.4 Hz, 2H, CH_2).

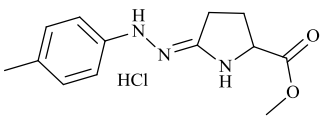
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 163.4 (C_{IV}), 140.2 (C_{IV}), 132.4 (CHAr), 129.0 (2CHAr), 127.8 (2CHAr), 45.3 (CH_2), 29.0 (CH_2), 22.1 (CH_2).

IR ν (cm^{-1}): 3247, 1688, 1549, 1262, 1132, 1108, 1067.

Anal. Calcd for $C_{10}H_{13}N_3O_2S$: C, 50.19; H, 5.48; N, 17.56; S, 13.40.

Methyl 5-(2-(p-tolyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride – 176

HEI 3407



Following the general procedure methyl 5-(2-(p-tolyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-2-carboxylate (1 g, 6.4 mmol) and p-tolylhydrazine hydrochloride (1 g, 6.4 mmol), in 30 mL

EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent. The obtained mixture was washed with ethanol, affording an off-white solid (1.54 g, 85 % yield).

M.p. 204-205°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.91 (bs, 1H, N*H*), 10.36 (s, 1H, N*H*), 8.69 (s, 1H, N*H*), 7.03 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 6.79 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 4.61 (q, *J* = 4.4 Hz, 1H, C*H*), 3.73 (s, 3H, CH_3), 3.03 (t, *J* = 7.0 Hz, 2H, CH_2), 2.66-2.49 (m, 1H, CH_2), 2.32-2.25 (m, 1H, CH_2), 2.25 (s, 3H, CH_3).

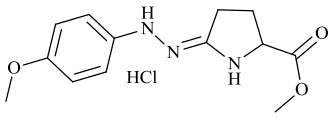
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.0 (C_{IV}), 170.9 (C_{IV}), 143.6 (C_{IV}), 129.6 (C_{IV}), 129.3 (2CHAr), 113.7 (2CHAr), 59.4 (CH), 52.5 (CH_3), 27.5 (CH_2), 25.2 (CH_2), 20.1 (CH_3).

IR ν (cm^{-1}): 3168, 2947, 1745, 1692, 1513, 1276, 1211.

Anal. Calcd for C$_{13}$H$_{18}$ClN$_3$O$_2$: C, 55.03; H, 6.39; N, 14.81.

Methyl 5-(2-(4-methoxyphenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride – 177

HEI 3403



Following the general procedure methyl 5-(2-(4-methoxyphenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-2-carboxylate (0.5 g, 3.2 mmol) and (4-methoxyphenyl)hydrazine hydrochloride (0.55 g, 3.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent. The obtained mixture was washed with ethanol, affording an off-white solid (0.74 g, 78 % yield).

M.p. 197-198°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.91 (bs, 1H, N*H*), 10.35 (s, 1H, N*H*), 8.53 (s, 1H, N*H*), 6.85 (d, *J* = 1.6 Hz, 4H, Ar*H*), 4.61 (q, *J* = 4.4 Hz, 1H, C*H*), 3.71 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 3.06 (t, *J* = 8.2 Hz, 2H, CH_2), 2.66-2.49 (m, 1H, CH_2), 2.32-2.18 (m, 1H, CH_2).

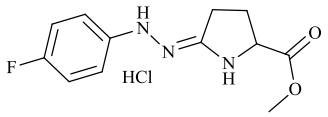
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.0 (C=O), 170.9 (C_{IV}), 154.1 (C_{IV}), 139.5 (C_{IV}), 115.3 (2CHAr), 114.4 (2CHAr), 59.4 (CH), 55.2 (CH_3), 52.5 (CH_3), 27.5 (CH_2), 25.2 (CH_2).

IR ν (cm^{-1}): 3173, 2950, 2861, 1749, 1689, 1510, 1238, 1177, 1031.

Anal. Calcd for C$_{13}$H$_{18}$ClN$_3$O$_3$: C, 52.09; H, 6.05; N, 14.02.

Methyl 5-(2-(4-fluorophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride – 178

HEI 3404



Following the general procedure methyl 5-(2-(4-fluorophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-2-carboxylate (1.0 g, 6.4 mmol) and (4-fluorophenyl)hydrazine hydrochloride (1.0 g, 6.4 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent. The obtained mixture was washed with ethanol, affording an off-white solid (1.5 g, 80 % yield).

M.p. 217-218°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 12.12 (s, 1H, N*H*), 10.45 (s, 1H, N*H*), 8.90 (s, 1H, N*H*), 7.12 (t, *J* = 8.4 Hz, 2H, Ar*H*), 6.92 (d, *J* = 2.4 Hz, 2H, Ar*H*), 4.63 (t, *J* = 2.4 Hz, 1H, C*H*), 3.71 (s, 3H, CH_3), 3.05 (d, *J* = 6.4 Hz, 2H, CH_2), 2.64 (m, 1H, CH_2), 2.24 (d, *J* = 6.8 Hz, 1H, CH_2).

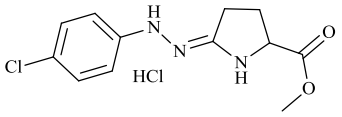
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.8 (C_{IV}), 170.7 (C_{IV}), 157.6 (d, $C_{IV,}$ J_{C-F} = 235.4 Hz), 143.0 (C_{IV}), 116.0 (d, CHAr, J_{C-F} = 22.0 Hz), 115.6 (d, CHAr, J_{C-F} = 8.3 Hz), 60.0 (CH), 53.0 (CH_3), 28.1 (CH_2), 25.7 (CH_2).

IR ν (cm^{-1}): 3178, 2957, 2864, 1737, 1687, 1507, 1278, 1214.

Anal. Calcd for C$_{12}$H$_{15}$ClFN$_3$O$_2$: C, 50.09; H, 5.25; N, 14.60.

Methyl 5-(2-(4-chlorophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride – 179

HEI 3402



Following the general procedure methyl 5-(2-(3-chlorophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-2-carboxylate (1 g, 6.4 mmol) and (4-chlorophenyl)hydrazine hydrochloride (1.2 g, 6.4 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent. The obtained mixture was washed with ethanol, affording an off-white solid (1.36 g, 70 % yield).

M.p. 227-228°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.95 (bs, 1H, N*H*), 10.51 (s, 1H, N*H*), 8.97 (s, 1H, N*H*), 7.32 (dd, *J* = 8.65 Hz, *J* = 1.92 Hz, 1H, Ar*H*), 6.91 (dd, *J* = 8.65 Hz, *J* = 1.92 Hz, 1H, Ar*H*), 4.64 (q, *J* = 4.3 Hz, 1H, C*H*), 3.74 (s, 3H, C*H*$_3$), 3.07 (t, *J* = 8.74 Hz, 2H, C*H*$_2$), 2.61-2.51 (m, 1H, C*H*$_2$), 2.32-2.25 (m, 1H, C*H*$_2$).

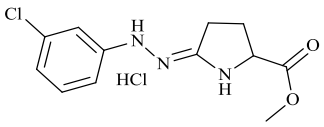
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.4 (C_{IV}), 170.8 (C_{IV}), 145.0 (C_{IV}), 128.7 (2 CHAr), 124.4 (C_{IV}), 115.1 (CHAr), 59.6 (CH), 52.5 (CH_3), 27.6 (CH_2), 25.1 (CH_2).

IR ν (cm^{-1}): 3159, 2953, 1755, 1693, 1489, 1270, 1253, 1093.

Anal. Calcd for C$_{12}$H$_{15}$Cl$_2$N$_3$O$_2$: C, 47.38; H, 4.97; N, 13.81.

Methyl 5-(2-(3-chlorophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride – 180

HEI 3410



Following the general procedure methyl 5-(2-(3-chlorophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-2-carboxylate (1.0 g, 6.4 mmol) and (3-chlorophenyl)hydrazine hydrochloride (1.2 g, 6.4 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent. The obtained mixture was washed with ethanol, affording an off-white solid (1.5 g, 77 % yield).

M.p. 217-218°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 12.14 (bs, 1H, N*H*), 10.51 (s, 1H, N*H*), 9.18 (s, 1H, N*H*), 7.30 (t, *J* = 8.1 Hz, 1 H, Ar*H*), 6.94 (d, *J* = 3.1 Hz, 2 H, Ar*H*), 6.83 (d, *J* = 8.1 Hz, *J* = 1.92 Hz, 1 H, Ar*H*), 4.65 (q, *J* = 3.9 Hz, 1H, C*H*), 3.71 (s, 3H, C*H*$_3$), 3.07 (t, *J* = 8.4 Hz, 2H, C*H*$_2$), 2.61-2.51 (m, 1H, C*H*$_2$), 2.32-2.25 (m, 1H, C*H*$_2$).

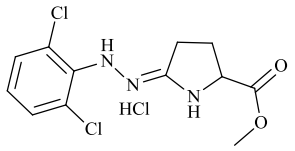
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.5 (C_{IV}), 170.8 (C_{IV}), 147.7 (C_{IV}), 133.6 (C_{IV}), 130.6 (CHAr), 120.3 (CHAr), 112.8 (CHAr), 112.1 (CHAr), 59.6 (CH), 52.5 (CH_3), 27.6 (CH_2), 25.1 (CH_2).

IR ν (cm^{-1}): 3163, 2952, 1750, 1692, 1597, 1477, 1272, 1214.

Anal. Calcd for C$_{12}$H$_{15}$Cl$_2$N$_3$O$_2$: C, 47.38; H, 4.97; N, 13.81.

Methyl 5-(2-(2,6-dichlorophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride – 181

HEI 934



Following the general procedure methyl 5-(2-(2,6-dichlorophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-2-carboxylate (1.0 g, 6.4 mmol) and (2,6-dichlorophenyl)hydrazine hydrochloride (1.35 g, 6.4 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent. The obtained mixture was washed with ethanol, affording an off-white solid (1.3 g, 60 % yield).

M.p. 189-190°C.

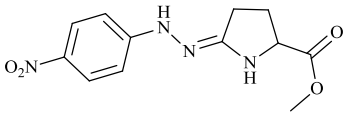
^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 12.12 (bs, 1H, N*H*), 10.60 (s, 1H, N*H*), 8.13 (s, 1H, N*H*), 7.49 (m, 2H, Ar*H*), 7.17 (m, 1H, Ar*H*), 4.72 (bs, 1H, C*H*), 3.73 (s, 3H, CH_3), 2.94 (t, *J* = 6.75 Hz, 2H, CH_2), 2.57 (m, 1H, CH_2), 2.25 (bs, 1H, CH_2).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.3 (C_{IV}), 170.4 (C_{IV}), 138.6 (C_{IV}), 129.9 (2CHAr), 126.8 (CHAr), 126.2 (2C_{IV}), 60.3 (CH), 53.0 (CH_3), 28.2 (CH_2), 25.8 (CH_2).

IR ν (cm^{-1}): 3202, 2950, 1748, 1695, 1567, 1449, 1204, 1096.

Anal. Calcd for $C_{12}H_{14}Cl_3N_3O_2$: C, 42.56; H, 4.17; N, 12.41.

Methyl 5-(2-(4-nitrophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride – 182 HEI 3406



Following the general procedure, methyl 5-(2-(4-nitrophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-2-carboxylate (0.5 g, 3.2 mmol) and (4-nitrophenyl)hydrazine (0.49 g, 3.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h and the precipitate was filtered off. The obtained solid was washed with ethanol, affording the wanted compound as a red solid (0.5 g, 57 % yield).

M.p. 204-205°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 10.64 (bs, 1H, N*H*), 9.59 (s, 1H, Ar*H*), 8.64 (dd, J = 4.8 Hz, *J* = 1.2 HZ, 1H, Ar*H*), 8.17 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.46 (dd, *J* = 8.0 Hz, *J* = 4.4 Hz, 1H, Ar*H*), 4.34 (m, 1H, C*H*), 3.68 (s, 3H, CH_3), 2.56-2.50 (m, 2H, CH_2), 2.35-2.29 (m, 1H, CH_2), 2.07-2.00 (m, 1H, CH_2).

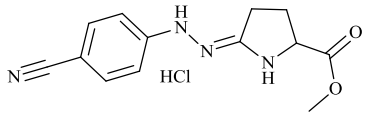
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 173.3 (C_{IV}), 171.8 (C_{IV}), 161.5 (C_{IV}), 159.6 (C_{IV}), 151.4 (CHAr), 149.0 (CHAr), 135.4 (CHAr), 123.6 (CHAr), 58.6 (CH), 52.5 (CH_3), 28.3 (CH_2), 26.1 (CH_2).

IR ν (cm^{-1}): 3507, 3253, 1740, 1667, 1591, 1460, 1310, 1228.

Anal. Calcd for $C_{12}H_{14}N_4O_4$: C, 51.80; H, 5.07; N, 20.13.

Methyl 5-(2-(4-nitrophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride – 183

HEI 3405



Following the general procedure methyl 5-(2-(4-nitrophenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-2-carboxylate (0.5 g, 3.2 mmol) and 4-hydrazinylbenzonitrile hydrochloride (0.5 g, 3.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent. The obtained mixture was washed with ethanol, affording an off-white solid (0.56 g, 60 % yield).

M.p. 221-222°C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.64 (bs, 1H, N*H*), 9.59 (s, 1H, N*H*), 9.07 (s, 1H, N*H*), 7.72 (dd, *J* = 8.9 Hz, *J* = 2.2 Hz, 2H, Ar*H*), 6.99 (d, *J* = 8.9 Hz, 2H, Ar*H*), 4.66 (q, *J* = 4.3 Hz, 1H, C*H*), 3.73 (s, 3H, CH_3), 3.08 (t, *J* = 7.9 Hz, 2H, CH_2), 2.66-2.49 (m, 1H, CH_2), 2.32-2.21 (m, 1H, CH_2).

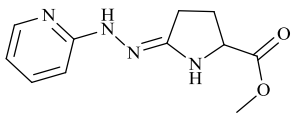
$^{13}C\{^{1}H\}$NMR (DMSO-d_6, 100 MHz): 171.6 (C=O), 170.7 (C_{IV}), 149.9 (C_{IV}), 133.5 (CHAr), 133.2 (CHAr), 119.4 (C_{IV}), 113.5 (CHAr), 113.1 (CHAr), 101.7 (C_{IV}), 59.8 (CH), 52.5 (CH_3), 27.7 (CH_2), 25.0 (CH_2).

IR ν (cm^{-1}): 2996, 2963, 2218, 1751, 1681, 1609, 1519, 1217, 1175.

Anal. Calcd for $C_{13}H_{15}ClN_4O_2$: C, 52.98; H, 5.13; N, 19.01.

Methyl 5-(2-(pyridin-2-yl)hydrazono)pyrrolidine-2-carboxylate– 184

HEI 3409



Following the general procedure methyl 5-(2-(pyridin-2-yl)hydrazono)pyrrolidine-2-carboxylate was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-

2-carboxylate (1 g, 6.4 mmol) and 2-hydrazinylpyridine (0.92 g, 6.4 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent. The obtained mixture was washed with ethanol, affording an off-white solid (0.64 g, 37 % yield).

M.p. 185-186°C.

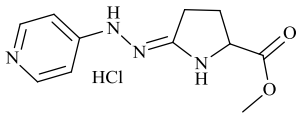
^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 12.06 (bs, 1H, N*H*), 10.41 (s, 1H, N*H*), 9.10 (s, 1H, N*H*), 7.23 (t, *J* = 6.75 Hz, 1H, Ar*H*), 7.07 (m, 2H, Ar*H*), 6.89 (d, *J* = 6.75 Hz, 1H, Ar*H*), 4.64 (d, *J* = 5.05 Hz, 1H, C*H*), 3.71 (s, 3H, CH_3), 3.05 (t, *J* = 7.0 Hz, 2H, CH_2), 2.62-2.49 (m, 1H, CH_2), 2.24 (m, 1H, CH_2).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.4 (C_{IV}), 148.4 (C_{IV}), 131.4 (CHAr), 123.6 (C_{IV}), 122.7 (CHAr), 116.0 (CHAr), 113.0 (CHAr), 60.1 (CH), 53.0 (CH_3), 28.2 (CH_2), 25.7 (CH_2).

IR ν (cm^{-1}): 3162, 2951, 2858, 1749, 1689, 1594, 1475, 1271, 1212.

Anal. Calcd for C$_{11}$H$_{15}$ClN$_4$O$_2$: C, 48.80; H, 5.58; N, 20.70.

Methyl 5-(2-(pyridin-4-yl)hydrazono)pyrrolidine-2-carboxylate hydrochloride – 185 HEI 3408



Following the general procedure methyl 5-(2-(4-methoxyphenyl)hydrazono)pyrrolidine-2-carboxylate hydrochloride was obtained through the reaction of methyl 5-methoxy-3,4-dihydro-2*H*-pyrrole-2-carboxylate (1.0 g, 6.4 mmol) and 4-hydrazinylpyridine hydrochloride (0.92 g, 6.4 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent. The obtained mixture was washed with ethanol, affording an off-white solid (1.05 g, 60 % yield).

M.p. 185-186°C.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 12.06 (bs, 1H, N*H*), 10.41 (s, 1H, N*H*), 9.10 (s, 1H, N*H*), 7.23 (t, *J* = 6.75 Hz, 1H, Ar*H*), 7.07 (s,2H, Ar*H*), 6.89 (d, *J* = 6.75 Hz, 1H, Ar*H*), 4.64 (d, *J* = 5.05 Hz, 1H, C*H*), 3.71 (s, 3H, CH_3), 3.05 (t, *J* = 7.0 Hz, 2H, CH_2), 2.62-2.49 (m, 1H, CH_2), 2.24 (m, 1H, CH_2).

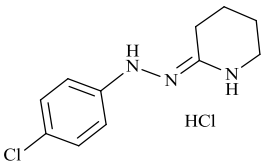
^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 171.4 (C_{IV}), 148.4 (C_{IV}), 131.4 (CHAr), 123.6 (C_{IV}), 122.7 (CHAr), 116.0 (CHAr), 113.0 (CHAr), 60.1 (CH), 53.0 (CH_3), 28.2 (CH_2), 25.7 (CH_2).

IR ν (cm^{-1}): 3162, 2951, 2858, 1749, 1689, 1594, 1475, 1271, 1212.

Anal. Calcd for C$_{11}$H$_{15}$ClN$_4$O$_2$: C, 48.80; H, 5.58; N, 20.70.

2-(2-(4-chlorophenyl)hydrazono)piperidine hydrochloride – 188

HEI 3368



(2-(4-Chlorophenyl)hydrazono)piperidine hydrochloride was obtained through the reaction of 6-methoxy-2,3,4,5-tetrahydropyridine (2.0 g, 17.6 mmol) and (4-chlorophenyl)hydrazine hydrochloride (3.1 g, 17.6 mmol), in the presence of 37% HCl (0.2 mmol), in 30 mL EtOH. The mixture was stirred at 50° for 3 h. The crude product was obtained by the evaporation of left solvent and after transformed into the corresponding salt, employing acethyl chloride in MeOH. The obtained salt was washed with ethanol, affording the compound as a white solid (4.5 g, 100 % yield).

M.p. 267-268°C.

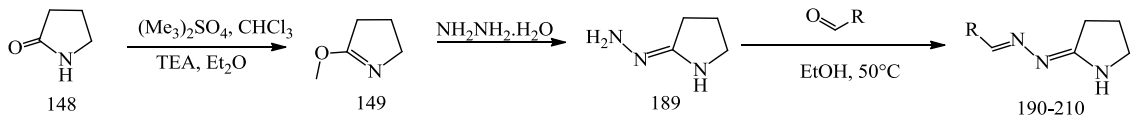
^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 11.35 (s, 1H, N*H*), 9.68 (s, 1H, N*H*), 8.72 (s, 1H, N*H*), 7.28 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.89 (d, *J* = 8.4 Hz, 2H, Ar*H*), 3.26 (m, 2H, CH_2), 2.71 (m, 2H, CH_2), 1.77 (m, 4H, CH_2).

^{13}C{^{1}H}NMR (DMSO-d$_6$, 100 MHz): 165.3 (C_{IV}), 145.9 (C_{IV}), 129.1 (2CHAr), 124.5 (C_{IV}), 115.6 (2CHAr), 41.3 (CH_2), 24.0 (CH_2), 21.1 (CH_2), 17.7 (CH_2).

IR ν (cm^{-1}): 3081, 2976, 1658, 1593, 1488, 1412, 1250.

Anal. Calcd for $C_{11}H_{15}Cl_2N_3$: C, 50.78; H, 5.81; N, 16.15.

1.5 Experimental procedures for the synthesis of pyrrolidino-azines starting from the 2-hydrazonopyrrolidine

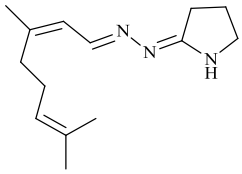


Scheme 57 The synthesis of pyrrolidino-azines

The same iminoether which was previously used to form hydrazonopyrrolidine derivativatives, was employed in the reaction with hydrazine hydrate, to afford derivative **189** in quantitative yield. Without any purification, **189** was used directly in reactions with different aldehydes, giving access to a wide variety of azine linker derivatives **190**-**211**.

((3,7-dimethylocta-2,6-dien-1-ylidene)hydrazono)pyrrolidine – 190

HEI 3445



Ratio isomer 1/isomer 2: 92/8

Following the general procedure, 3,7-dimethylocta-2,6-dien-1-ylidene)hydrazono) pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine **189** (5 mmol, 0.5 g) with citral (0.76 g, 5 mmol), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 60% ethyl acetate, as a yellow solid (0.74 g, 64% yield).

M.p. 66-67°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (d, *J* = 9.7 Hz, C*H*, 1H, isomer 1+2), 6.05 (d, *J* = 9.2 Hz, 2H, C*H*, isomer 1+2), 5.10 (bs, 1H, N*H*, isomer 1+2), 3.47 (t, *J* = 6.7 Hz, 2H, CH_2, isomer 1), 3.40 (t, *J* = 6.7 Hz, 2H, CH_2, isomer 2), 2.85 (m, 1H, CH_2, isomer 2), 2.59 (t, *J* = 8.2 Hz, 2H, CH_2, isomer 1), 2.30 (m, 3H, CH_2, isomer 2), 2.15 (bs, 4H, CH_2, isomer 1+2), 2.08 (quin, *J* = 7.7 Hz, 2H, CH_2, isomer 1+2), 1.87 (s, 3H, CH_3, isomer 1+2), 1.68 (s, 3H, CH_3, isomer 1), 1.66 (s, 3H, CH_3, isomer 2), 1.60 (s, 3H, CH_3, isomer 1), 1.59 (s, 3H, CH_3, isomer 2).

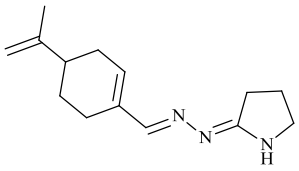
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 166.5 (C_{IV}, isomer 1+2), 152.8 (CH, isomer 1), 152.4 (CH, isomer 2), 148.3 (C_{IV}, isomer 2), 147.9 (C_{IV}, isomer 1), 132.3 (C_{IV}, isomer 2), 132.1 (C_{IV}, isomer 1), 123.5 (CH, isomer 1), 123.4 (CH, isomer 2), 122.5 (CH, isomer 1), 116.7 (CH, isomer 2), 44.8 (CH_2, isomer 1), 42.0 (CH_2, isomer 2), 40.2 (CH_2, isomer 1), 29.7 (CH_2, isomer 2), 28.8 (CH_2, isomer 1), 27.0 (CH_2, isomer 2), 26.2 (CH_2, isomer 1), 25.6 (CH_2, isomer 1+2), 24.4 (CH_3, isomer 2), 21.8 (CH_3, isomer 1), 20.9 (CH_3, isomer 2), 17.7 (CH_3, isomer 1+2), 17.2 (CH_3, isomer 1).

IR ν (cm^{-1}): 3142, 2966, 1625, 1553, 1380, 1289, 1106.

Anal. Calcd for $C_{14}H_{23}N_3$: C, 72.06; H, 9.93; N, 18.01.

4-**(prop-1-en-2-yl)cyclohex-1-en-1-yl)methylene)hydrazono)pyrrolidine– 191**

HEI 3444


Ratio isomer 1/isomer 2: 98/2

Following the general procedure, ((4-(prop-1-en-2-yl)cyclohex-1-en-1-yl)methylene) hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with perillaldehyde (5 mmol, 0.75 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a white solid (0.35 g, 30% yield).

M.p. 125-126°C.

Description of the majoritary isomer.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (s, C*H*), 6.10 (d, *J* = 4.7 Hz, 2H, C*H*), 6.07 (s, N*H*), 4.73 (dd, *J* = 4.7 Hz, *J* = 0.8 Hz, 2H, C*H*), 3.48 (t, *J* = 6.7 Hz, 2H, CH_2), 2.58 (t, *J* = 8.5 Hz, 2H, CH_2), 2.36-2.11 (m, 4H, CH_2), 2.07 (quin, *J* = 7.0 Hz, 2H, CH_2), 1.91-1.86 (m, 1H, C*H*), 1.75 (m, 2H, CH_3), 1.59-1.45 (m, 1H, C*H*).

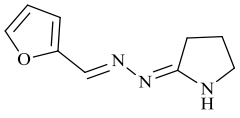
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 166.9 (C_{IV}), 156.6 (C_{IV}), 149.5 (CH), 136.0 (C_{IV}), 135.6 (CH), 108.9 (CH_2), 44.8 (CH_2), 41.2 (CH), 31.5 (CH_2), 28.8 (CH_2), 27.1 (CH_2), 24.2 (CH_2), 21.9 (CH_2), 20.8 (CH_2).

IR ν (cm^{-1}): 3134, 2927, 1642, 1635, 1569, 1399, 1287, 1030.

Anal. Calcd for $C_{14}H_{21}N_3$: C, 72.69; H, 9.15; N, 18.16.

2-((furan-2-ylmethylene)hydrazono)pyrrolidine – 192

HEI 3432


Ratio isomer 1/isomer 2: 92/8

Following the general procedure, 2-((furan-2-ylmethylene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with furfural (5 mmol, 0.5 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a white solid (0. 5 g, 56% yield).

M.p. 165-166°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (s, C*H*, isomer 1), 8.06 (s, 1H, C*H*, isomer 2), 7.48 (s, 1H, Ar*H*, isomer 1+2), 6.65 (d, *J* = 2.0 Hz, 1H, Ar*H*, isomer 1), 6.60 (bs, 1H, Ar*H*, isomer 2),

6.46 (d, *J* = 2.0 Hz, 2H, Ar*H*, isomer 1+2), 6.27 (bs, 1H, N*H*, isomer 1+2), 3.48 (t, *J* = 7.4 Hz, 2H, CH_2, isomer 1), 3.44 (t, *J* = 7.4 Hz, 2H, CH_2, isomer 1), 2.94 (t, *J* = 7.4 Hz, 2H, CH_2, isomer 2), 2.63 (t, *J* = 7.4 Hz, 2H, CH_2, isomer 1), 2.10 (quin, *J* = 7.4 Hz, 2H, CH_2, isomer 1), 1.87 (m, 2H, CH_2, isomer 2).

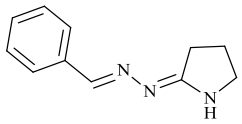
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 169.3 (C_{IV}, isomer 2), 167.6 (C_{IV}, isomer 1), 151.3 (C_{IV}, isomer 2), 151.0 (C_{IV}, isomer 1), 143.9 (CH, isomer 1), 143.6 (CH, isomer 2), 142.3 (CH, isomer 1), 141.1 (CH, isomer 2), 112.4 (CHAr, isomer 1+2), 111.7 (CH, isomer 1), 111.5 (CH, isomer 2), 44.9 (CH_2, isomer 1), 44.2 (CH_2, isomer 2), 28.9 (CH_2, isomer 1), 28.9 (CH_2, isomer 1+2), 26.5 (CH_2, isomer 2), 21.8 (CH_2, isomer 1).

IR ν (cm^{-1}): 3334, 3095, 2874, 1624, 1582, 1480, 1294, 1007.

Anal. Calcd for $C_9H_{11}N_3O$: C, 61.00; H, 6.26; N, 23.71.

2-((benzylidenehydrazono)pyrrolidine – 193

HEI 3427



Ratio isomer 1/isomer 2: 93/7

Following the general procedure, 2-((benzylidenehydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (10.1 mmol, 1.0 g) with benzaldehyde (10.1 mmol, 1.1 g), in 50 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a white solid (0.85 g, 45% yield).

M.p. 109-110°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, C*H*, isomer 1), 8.20 (s, 1H, C*H*, isomer 2), 7.71 (m, 2H, Ar*H*, isomer 1+2), 7.36 (m, 3H, Ar*H*, isomer 1+2), 6.28 (s, 1H, N*H*, isomer 1+2), 3.50 (t, *J* = 7.2 Hz, 2H, CH_2, isomer 1), 3.41 (t, *J* = 7.2 Hz, 2H, CH_2, isomer 2), 2.96 (m, 2H, CH_2, isomer 2), 2.65 (t, *J* = 7.2 Hz, 2H, CH_2, isomer 1), 2.30 (t, *J* = 7.2 Hz, 2H, CH_2, isomer 2), 2.13 (quin, *J* = 7.2 Hz, 2H, CH_2, isomer 1).

Only the majoritary isomer is visible in ^{13}C NMR.

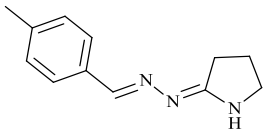
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 167.7 (C_{IV}), 153.2 (CH), 135.5 (C_{IV}), 129.5 (CHAr), 128.5 (2 CHAr), 127.5 (2 CHAr), 44.9 (CH_2), 28.9 (CH_2), 21.8 (CH_2).

IR ν (cm^{-1}): 2934, 2866, 1681, 1633, 1585, 1446, 1288.

Anal. Calcd $C_{11}H_{13}N_3$: C, 70.56; H, 7.00; N, 22.44.

2-((4-methylbenzylidene)hydrazono)pyrrolidine – 194

HEI 3425



Ratio isomer 1/isomer 2: 93/7

Following the general procedure, 2-((4-methylbenzylidene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 4-methylbenzaldehyde (5 mmol, 0.6 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as an off-white solid (0.6 g, 60% yield).

M.p. 165-166°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, C*H*, isomer 1), 8.19 (s, 1H, C*H*, isomer 2), 7.60 (d, *J* = 7.2 Hz, 2H, Ar*H*, isomer 1+2), 7.17 (d, *J* = 7.2 Hz, 2H, Ar*H*, isomer 1+2), 6.25 (s, 1H, N*H*, isomer 1+2), 3.50 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 1), 3.44 (m, 2H, CH_2, isomer 2), 2.95 (m, 2H, CH_2, isomer 2), 2.65 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 1), 2.37 (s, 3H, CH_3, isomer 1+2), (2.10 (quin, *J* = 6.8 Hz, 2H, CH_2, isomer 1+2).

Only the majoritary isomer is visible in ^{13}C NMR.

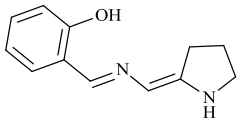
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 167.3 (C_{IV}), 153.3 (CH), 139.8 (C_{IV}), 132.8 (C_{IV}), 129.2 (2 CHAr), 127.4 (2 CHAr), 44.9 (CH_2), 28.9 (CH_2), 21.8 (CH_3), 21.4 (CH_2).

IR ν (cm^{-1}): 2917, 2852, 1639, 1582, 1304, 1039.

Anal. Calcd for $C_{12}H_{15}N_3$: C, 71.61; H, 7.51; N, 20.88.

2-((pyrrolidin-2-ylidenehydrazono)methyl)phenol – 195

HEI 3424



Ratio isomer 1/isomer 2: 68/32

Following the general procedure, 2-((pyrrolidin-2-ylidenehydrazono)methyl)phenol was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 2-hydroxybenzaldehyde (5 mmol, 0.6 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a lemon off white solid (0.45 g, 44% yield).

M.p. 161-162°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 12.1 (s, 1H, O*H*, isomer 2), 11.7 (s, 1H, OH, isomer 1), 8.41 (s, C*H*, isomer 1), 8.27 (s, C*H*, isomer 2), 7.34-7.21 (m, 2H, Ar*H*, isomer 1+2), 6.97-6.86 (m, 2H, Ar*H*, isomer 1+2), 5.67 (s, 1H, N*H*, isomer 1), 5.26 (s, 1H, N*H*, isomer 1), 3.52 (t, *J* = 7.4 Hz, 2H, CH_2, isomer 1), 3.51 (t, *J* = 7.4 Hz, 2H, CH_2, isomer 2), 2.86 (t, *J* = 7.7 Hz, 2H, CH_2, isomer 2), 2.66 (t, *J* = 7.7 Hz, 2H, CH_2, isomer 1), 2.13 (quin, *J* = 7.7 Hz, 2H, CH_2, isomer 1+2).

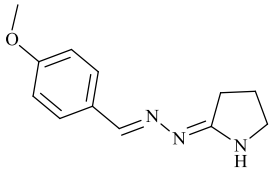
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 172.0 (C_{IV}, isomer 2), 165.7 (C_{IV}, isomer 1), 158.7 (C_{IV}, isomer 2), 158.7 (C_{IV}, isomer 1), 156.9 (CH, isomer 1), 154.0 (CH, isomer 2), 131.1 (CHAr, isomer 1), 131.0 (CHAr, isomer 1), 130.5 (CHAr, isomer 2), 130.3 (CHAr, isomer 2), 119.3 (CHAr, isomer 1), 119.1 (C_{IV}, isomer 2), 119.0 (C_{IV}, isomer 1), 118.7 (CHAr, isomer 2), 116.3 (CHAr, isomer 1), 116.3 (CHAr, isomer 2), 45.4 (CH_2, isomer 1), 44.4 (CH_2, isomer 2), 29.2 (CH_2, isomer 1), 26.6 (CH_2, isomer 2), 21.8 (CH_2, isomer 1), 21.5 (CH_2, isomer 2).

IR ν (cm^{-1}): 3230, 2923, 2857, 1627, 1398, 1267, 1200.

Anal. Calcd for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85.

2-((4-methoxybenzylidene)hydrazono)pyrrolidine – 196

HEI 3434



Ratio isomer 1/isomer 2: 94/6

Following the general procedure, 2-((4-methoxybenzylidene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 4-methoxybenzaldehyde (5 mmol, 0.7 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a white solid (0.9 g, 82% yield).

M.p. 143-144°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H, C*H*, isomer 1), 8.13 (s, 1H, C*H*, isomer 2), 7.62 (d, 2H, *J* = 9.2 Hz, Ar*H*, isomer 1+2), 6.87 (d, 2H, *J* = 8.8 Hz, Ar*H*, isomer 1+2), 6.21 (bs, N*H*, isomer 1+2), 3.81 (s, 3H, CH_3, isomer 2), 3.80 (s, 3H, CH_3, isomer 1), 3.46 (t, *J* = 7.4 Hz, 2H, CH_2, isomer 1), 3.40 (m, 2H, CH_2, isomer 2), 2.91 (t, *J* = 8.0 Hz, 2H, CH_2, isomer 2), 2.60 (t, *J* = 7.6 Hz, 2H, CH_2, isomer 1), 2.27 (m, 2H, CH_2, isomer 2), 2.07 (quin, *J* = 8.0 Hz, 2H, CH_2, isomer 1).

Only the majoritary isomer is visible in ^{13}C NMR.

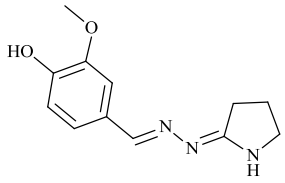
$^{13}C\{^{1}H\}$NMR (100 MHz, $CDCl_3$): δ 167.1 (C_{IV}), 160.8 (C_{IV}), 152.9 (CH), 128.9 (2 CHAr), 128.3 (C_{IV}), 113.9 (2 CHAr), 55.3 (CH_3), 44.8 (CH_2), 28.8 (CH_2), 21.9 (CH_2).

IR ν (cm^{-1}): 3136, 2921, 2860, 1634, 1587, 1295, 1243.

Anal. Calcd for $C_{12}H_{15}N_3O$: C, 66.34; H, 6.96; N, 19.34.

2-methoxy-4-((pyrrolidin-2-ylidenehydrazono)methyl)phenol – 197

HEI 3446



Ratio isomer 1/isomer 2: 93/7

Following the general procedure, 2-methoxy-4-((pyrrolidin-2-ylidenehydrazono) methyl) phenol was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with vanillin (5 mmol, 0.8 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a white solid (0.7 g, 70% yield).

M.p. 146-147°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, C*H*, isomer 1), 8.13 (s, 1H, C*H*, isomer 2), 7.34 (s, 1H, Ar*H*, isomer 1+2), 7.13 (d, *J* = 8.1 Hz, 2H, Ar*H*, isomer 1+2), 6.91 (d, *J* = 8.1 Hz, 2H, Ar*H*, isomer 1+2), 6.23 (s, 1H, N*H*, isomer 1+2), 3.94 (m, 3H, CH_3, isomer 1+2), 3.50 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 1+2), 2.63 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 1+2), 2.11 (quin, *J* = 6.8 Hz, 2H, CH_2, isomer 1+2).

Only the majoritary isomer is visible in ^{13}C NMR.

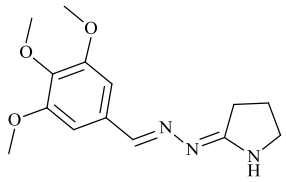
$^{13}C\{^{1}H\}$NMR (100 MHz, $CDCl_3$): δ 166.9 (C_{IV}), 153.5 (CH), 147.5 (C_{IV}), 146.8 (C_{IV}), 128.0 (C_{IV}), 122.7 (CHAr), 114.3 (CHAr), 108.2 (CHAr), 55.9 (CH_3), 44.9 (CH_2), 28.8 (CH_2), 21.9 (CH_2).

IR ν (cm^{-1}): 3136, 2921, 2860, 1634, 1587, 1295, 1243.

Anal. Calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.01.

((3,4,5-trimethoxybenzylidene)hydrazono)pyrrolidine – 198

HEI 3430


Ratio isomer 1/isomer 2: 93/7

Following the general procedure, (3,4,5-trimethoxybenzylidene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 3,4,5-trimethoxybenzaldehyde (5 mmol, 1 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a white solid (1.3 g, 93% yield).

M.p. 180-181°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (s, 1H, C*H*, isomer 1), 8.11 (s, 1H, C*H*, isomer 2), 6.94 (s, 2H, Ar*H*, isomer 1+2), 6.28 (s, 1H, N*H*, isomer 1+2), 3.90 (s, 6H, CH_3, isomer 1+2), 3.87 (s, 3H, CH_3, isomer 1+2), 3.50 (t, *J* = 7.4 Hz, 2H, CH_2, isomer 1), 3.40 (t, *J* = 7.4 Hz, 2H, CH_2, isomer 2), 2.66 (t, *J* = 7.9 Hz, 2H, CH_2, isomer 1+2), 2.30 (m, CH_2, isomer 2), 2.15 (quin, *J* = 7.4 Hz, 2H, CH_2, isomer 1).

Only the majoritary isomer is visible in ^{13}C NMR.

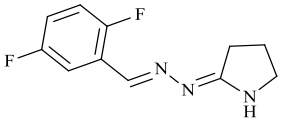
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 167.3 (C_{IV}), 153.3 ($2C_{IV}$), 153.1 (CH), 131.0 (C_{IV}), 104.4 (2CHAr), 60.9 (CH_3), 56.1 ($2CH_3$), 44.9 (CH_2), 28.9 (CH_2), 21.8 (CH_2).

IR ν (cm^{-1}): 3207, 3097, 2926, 1639, 1410, 1300, 1229, 1129.

Anal. Calcd for $C_{14}H_{19}N_3O_3$: C, 60.63; H, 6.91; N, 15.15.

2-((2,5-difluorobenzylidene)hydrazono)pyrrolidine – 199

HEI 3429


Ratio isomer 1/isomer 2: 89/11

Following the general procedure, 2-((2,5-difluorobenzylidene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 2,5-difluorobenzaldehyde (5 mmol, 0.7 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a lemon yellow solid (0.75 g, 66% yield).

M.p. 130-131°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (d, *J* = 1.2 Hz, 1H, C*H*, isomer 1), 8.38 (s, 1H, C*H*, isomer 2), 7.62 (m, 1H, Ar*H*, isomer 1+2), 6.97 (m, 2H, Ar*H*, isomer 1+2), 6.29 (s, N*H*, isomer 1+2), 3.53 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 1), 3.45 (m, CH_2, isomer 2), 2.96 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 2), 2.64 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 1), 2.30 (m, CH_2, isomer 2), 2.13 (quin, *J* = 6.8 Hz, 2H, CH_2, isomer 1).

Only the majoritary isomer is visible in ^{13}C NMR.

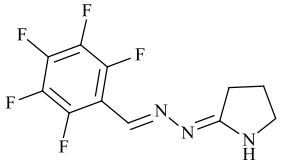
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 168.6 (C_{IV}), 158.7 (d, J_{C-F} = 244.8 Hz, C_{IV}), 157.5 (d, J_{C-F} = 244.8 Hz, C_{IV}), 145.3 (d, J_{C-F} = 2.8 Hz, CH), 142.9 (CH), 124.8 (d, J_{C-F} = 4.6 Hz, C_{IV}), 117.2 (m, 2 CHAr), 112.6 (dd, J_{C-F} = 24.1 Hz, J_{C-F} = 3.9 Hz, CHAr), 45.1 (CH_2), 29.0 (CH_2), 21.8 (CH_2).

IR ν (cm^{-1}): 3049, 2924, 2863, 1634, 1553, 1482, 1407, 1290.

Anal. Calcd for $C_{11}H_{11}F_2N_3$: C, 59.19; H, 4.97; N, 18.82.

(((perfluorophenyl)methylene)hydrazono)pyrrolidine – 200

HEI 3437



Ratio isomer 1/isomer 2: 93/7

Following the general procedure, (((perfluorophenyl)methylene)hydrazono) pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 3,4,5-2,3,4,5,6-pentafluorobenzaldehyde (5 mmol, 1.0 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a white solid (0.65 g, 47% yield).

M.p. 166-167°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (s, 1H, C*H*, isomer 1), 8.22 (s, 1H, C*H*, isomer 2), 6.34 (s, 2H, N*H*, isomer 1+2), 3.54 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 1), 3.49 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 2), 2.96 (m, 2H, CH_2, isomer 2), 2.68 (t, *J* = 8.1 Hz, 2H, CH_2, isomer 1+2), 2.15 (quin, *J* = 7.4 Hz, 2H, CH_2, isomer 1+2).

Only the majoritary isomer is visible in ^{13}C NMR.

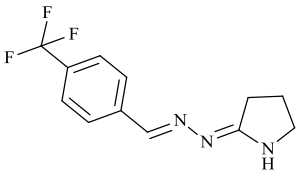
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 169.6 (C_{IV}), 141.3 (CH), 145.3 (m, *J* = 259.1 Hz, 2 C_{IV}), 141.0 (m, J_{C-F} = 259.1 Hz, C_{IV}), 137.7 (m, J_{C-F} = 259.1 Hz, 2C_{IV}), 110.8 (m, C_{IV}), 45.1 (CH_2), 28.9 (CH_2), 21.7 (CH_2).

IR ν (cm^{-1}): 3057, 2924, 2863, 1641, 1557, 1406, 1298.

Anal. Calcd for $C_{11}H_8F_5N_3$: C, 47.66; H, 2.91; N, 15.16.

2-((4-(trifluoromethyl)benzylidene)hydrazono)pyrrolidine – 201

HEI 3435



Ratio isomer 1/isomer 2: 93/7

Following the general procedure, 2-((4-(trifluoromethyl)benzylidene)hydrazono) pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 4-(trifluoromethyl)benzaldehyde (5 mmol, 0.9 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as an off white solid (1 g, 78% yield).

M.p. 157-158°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, C*H*, isomer 1), 8.24 (s, 1H, C*H*, isomer 2), 7.82 (d, *J* = 7.9 Hz, 2H, Ar*H*, isomer 1+2), 7.61 (d, *J* = 7.9 Hz, 2H, Ar*H*, isomer 1+2), 6.36 (bs, 1H, N*H*, isomer 1+2), 3.53 (t, *J* = 7.2 Hz, 2H, CH_2, isomer 1+2), 2.97 (m, 2H, CH_2, isomer 2), 2.67 (t, *J* = 7.2 Hz, 2H, CH_2, isomer 1), 2.15 (quin, *J* = 7.6 Hz, 2H, CH_2, isomer 1+2).

Only the majoritary isomer is visible in ^{13}C NMR.

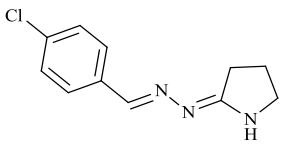
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 168.6 (C_{IV}), 151.4 (CH), 138.9 (C_{IV}), 130.0 (q, C_{IV}, J_{C-F} = 32 Hz), 127.4 (2 CHAr), 125.4 (q, C_{IV}, J_{C-F} = 4 Hz), 123.8 (q, CF_3, J_{C-F} = 270 Hz), 45.1 (CH_2), 29.0 (CH_2), 21.7 (CH_2).

IR ν (cm^{-1}): 3095, 2935, 2833, 1644, 1584, 1309.

Anal. Calcd for $C_{12}H_{12}F_3N_3$: C, 56.47; H, 4.74; N, 16.46.

2-((4-chlorobenzylidene)hydrazono)pyrrolidine – 202

HEI 3426



Ratio isomer 1/isomer 2: 90/10

Following the general procedure, 2-((4-chlorobenzylidene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 4-chlorobenzaldehyde (5 mmol, 0.7 g), in 30 mL of ethanol. The crude product was purified

through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a white solid (0.6 g, 54% yield).

M.p. 165-166°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, C*H*, isomer 1), 8.15 (s, 1H, C*H*, isomer 2), 7.64 (d, 2H, *J* = 8.0 Hz, 2H, Ar*H*, isomer 1+2), 7.33 (d, 2H, *J* = 8.0 Hz, 2H, Ar*H*, isomer 1+2), 6.23 6.27 (s, 1H, N*H*, isomer 1+2), 3.51 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 1), 3.40 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 2), 2.95 (m, 2H, CH_2, isomer 2), 2.65 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 1), 2.30 (t, *J* = 6.8 Hz, 2H, CH_2, isomer 2), 2.14 (quin, *J* = 6.8 Hz, 2H, CH_2, isomer 1).

Only the majoritary isomer is visible in ^{13}C NMR.

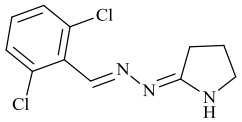
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 168.0 (C_{IV}), 151.9 (CH), 135.3 (C_{IV}), 134.1 (C_{IV}), 128.5 (2 CHAr), 128.5 (2 CHAr), 45.0 (CH_2), 29.0 (CH_2), 21.8 (CH_2).

IR ν (cm^{-1}): 3073, 2931, 1638, 1578, 1394, 1286.

Anal. Calcd for $C_{11}H_{12}ClN_3$: C, 59.60; H, 5.46; N, 18.95.

2-((2,6-dichlorobenzylidene)hydrazono)pyrrolidine – 203

HEI 3436



Following the general procedure, 2-((2,6-dichlorobenzylidene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 2,6-dichlorobenzaldehyde (5 mmol, 0.9 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 90% ethyl acetate, as a yellowish solid (0.3 g, 23% yield).

M.p. 130-131°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, C*H*, isomer 1), 8.41 (s, 1H, C*H*, isomer 2), 7.31 (d, *J* = 8 Hz, 2H, Ar*H*, isomer 1+2), 7.13 (t, *J* = 7.3 Hz, *J* = 0.8 Hz, 1H, Ar*H*, isomer 1+2), 6.26 (bs, 1H, N*H*, isomer 1+2), 3.49 (t, *J* = 7.7 Hz, 2H, CH_2, isomer 1), 2.95 (m, 2H, CH_2, isomer 2), 2.65 (t, *J* = 7.7 Hz, 2H, CH_2, isomer 1), 2.52 (m, 2H, CH_2, isomer 2), 2.12 (quin, *J* = 7.6 Hz, 2H, CH_2, isomer 1+2), 2.07 (m, 2H, CH_2, isomer 2).

Only the majoritary isomer is visible in ^{13}C NMR.

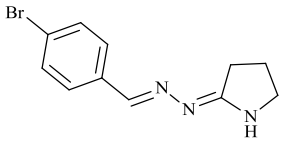
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 168.8 (C_{IV}), 148.3 (CH), 135.0 (C_{IV}), 131.4 (C_{IV}), 129.4 (CHAr), 128.9 (2 CHAr), 127.6 (C_{IV}), 45.0 (CH_2), 28.8 (CH_2), 21.8 (CH_2).

IR ν (cm^{-1}): 2929, 2857, 1642, 1569, 1548, 1402, 1293.

Anal. Calcd for $C_{12}H_{12}F_3N_3$: C, 56.47; H, 4.74; N, 16.46.

2-((4-bromobenzylidene)hydrazono)pyrrolidine – 204

HEI 3441



Ratio isomer 1/isomer 2: 87/13

Following the general procedure, 2-((4-bromobenzylidene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (10.1 mmol, 1 g) with 4-bromobenzaldehyde (10.1 mmol, 1.8 g), in 50 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a lemon yellow solid (1.5 g, 60% yield).

M.p. 155-156°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.59 (s, 1H, C*H*, isomer 2), 8.26 (s, 1H, C*H*, isomer 1), 7.71 (d, 2H, *J* = 8.6 Hz, Ar*H*, isomer 2), 7.59 (m, 2H, Ar*H*, isomer 2), 7.57 (d, 2H, *J* = 8.6 Hz, Ar*H*, isomer 1), 7.49 (d, 2H, *J* = 8.6 Hz, Ar*H*, isomer 1), 6.28 (s, N*H*, isomer 1+2), 3.51 (t, *J* = 8.3 Hz, 2H, CH_2, isomer 1), 3.37 (m, CH_2, isomer 2), 2.96 (t, *J* = 8.3 Hz, 2H, CH_2, isomer 2), 2.65 (t, *J* = 8.3 Hz, 2H, CH_2, isomer 1), 2.29 (t, *J* = 8.3 Hz, 2H, CH_2, isomer 2), 2.12 (t, *J* = 8.3 Hz, 2H, CH_2, isomer 1).

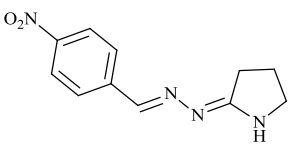
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 168.0 (C_{IV}, isomer 1), 161.2 (CH, isomer 2), 151.9 (CH, isomer 1), 149.9 (C_{IV}, isomer 2), 134.5 (C_{IV}, isomer 1), 132.1 (2 CHAr, isomer 2), 131.7 (2 CHAr, isomer 1), 131.6 (2 CHAr, isomer 2), 129.9 (2 CHAr, isomer 2), 128.5 (2 CHAr, isomer 1), 125.8 (C_{IV}, isomer 2), 125.6 (C_{IV}, isomer 1), 45.0 (CH_2, isomer 1), 44.3 (CH_2, isomer 2), 29.0 (CH_2, isomer 2), 26.6 (CH_2, isomer 1), 21.8 (CH_2, isomer 1), 20.9 (CH_2, isomer 2).

IR ν (cm^{-1}): 3089, 2933, 1638, 1575, 1390, 1294.

Anal. Calcd for $C_{11}H_{12}BrN_3$: C, 49.64; H, 4.54; N, 15.79.

((4-nitrobenzylidene)hydrazono)pyrrolidine – 205

HEI 3428



Mixture of isomers

Following the general procedure, ((4-nitrobenzylidene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 4-nitrobenzaldehyde (5 mmol, 0.8 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a lemon yellow solid (0.8 g, 65% yield).

M.p. 174-175°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8..69-8.22 (s, 1H, C*H*, all isomers), 8.22-8.17 (d, *J* = 8.9 Hz, 2H, Ar*H*, all isomers), 7.86-7.79 (dd, *J* = 8.9 Hz, *J* = 2.1 Hz, 2H, Ar*H*, all isomers), 6.45-6.36 (s, N*H*, all isomers), 3.55-3.37 (t, *J* = 7.2 Hz, 2H, CH_2, all isomers), 2.96-2.65 (t, *J* = 7.2 Hz, 2H, CH_2, all isomers), 2.32-2.10 (t, *J* = 8.5 Hz, 2H, CH_2, all isomers).

Only the major product is described.

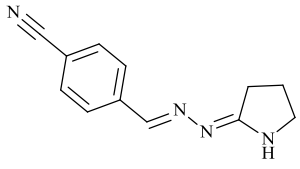
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 169.3 (C_{IV}, isomer 1), 150.4 (CH, isomer 1), 141.7 (CH, isomer 1), 127.8 (2CHAr, isomer 1), 123.9 (2CHAr, isomer 1), 45.2 (CH_2, isomer 1), 29.1 (CH_2, isomer 1), 21.7 (CH_2, isomer 1).

IR ν (cm^{-1}): 3430, 3107, 1682, 1624, 1593, 1507, 1331, 1292.

Anal. Calcd for $C_{11}H_{12}N_4O_2$: C, 56.89; H, 5.21; N, 24.12.

((pyrrolidin-2-ylidenehydrazono)methyl)benzonitrile– 206

HEI 3440



Mixture of isomers

Following the general procedure, ((pyrrolidin-2-ylidenehydrazono)methyl)benzonitrile was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 4-formylbenzonitrile (5 mmol, 0.7 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a lemon yellow solid (1 g, 93% yield).

M.p. 171-172°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64-8.14 (s, 1H, C*H*, all isomers), 7.97-7.72 (d, *J* = 8.5 Hz, 2H, Ar*H*, all isomers), 7.63 (d, *J* = 8.5 Hz, 2H, Ar*H*, all isomers), 6.36 (s, N*H*, all isomers), 3.54 -3.35 (t, *J* = 7.1 Hz, 2H, CH_2, all isomers), 2.99-2.63 (t, *J* = 7.1 Hz, 2H, CH_2, all isomers), 2.30-2.07 (t, *J* = 7.1 Hz, 2H, CH_2, all isomers).

Only the major product is described.

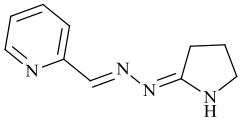
$^{13}C\{^{1}H\}$NMR (100 MHz, $CDCl_3$): δ 169.0 (C_{IV}, isomer 1), 150.9 (CH, isomer 1), 139.9 (C_{IV}, isomer 1), 132.3 (CHAr, isomer 1), 127.7 (CHAr, isomer 1), 118.8 (C_{IV}, isomer 1), 112.4 (C_{IV}, isomer 1), 45.1 (CH_2, isomer 1), 29.1 (CH_2, isomer 1), 21.7 (CH_2, isomer 1).

IR ν (cm^{-1}): 3107, 2889, 2220, 1682, 1635, 1568, 1530, 1463, 1397, 1287.

Anal. Calcd for $C_{12}H_{12}N_4$: C, 67.90; H, 5.70; N, 26.40.

((pyrrolidin-2-ylidenehydrazono)methyl)pyridine – 207

HEI 3438



Ratio isomer 1/isomer 2: 87/13

Following the general procedure, ((pyrrolidin-2-ylidenehydrazono)methyl)pyridine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with picolinaldehyde (5 mmol, 0.5 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 60% ethyl acetate, as an off-white solid (0.6 g, 60% yield).

M.p. 147-148°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (dd, *J* = 4.7 Hz, *J* = 1.0 Hz, 1H, Ar*H*, isomer 1), 8.60 (d, *J* = 4.7 Hz, 1H, Ar*H*, isomer 2), 8.40 (s, 1H, C*H*, isomer 1), 8.28 (s, 1H, C*H*, isomer 2), 7.98 (d, *J* = 7.7 Hz, 1H, Ar*H*, isomer 2), 7.92 (d, *J* = 7.7 Hz, 1H, Ar*H*, isomer 1), 7.70 (td, *J* = 7.7 Hz, *J* = 1.9 Hz, 1H, Ar*H*, isomer 1+2), 7.21 (td, *J* = 4.7 Hz, *J* = 1.2 Hz, 1H, Ar*H*, isomer 1), 7.19 (t, *J* = 4.7 Hz, 1H, Ar*H*, isomer 2), 6.45 (s, N*H*, isomer 1+2), 3.52-3.36 (t, *J* = 7.4 Hz, 2H, CH_2, all isomers), 2.98-2.63 (t, *J* = 7.5 Hz, 2H, CH_2, all isomers), 2.29-2.13 (t, *J* = 7.5 Hz, 2H, CH_2, all isomers).

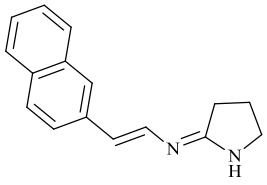
$^{13}C\{^{1}H\}$NMR (100 MHz, $CDCl_3$): δ 174.2 (CH, isomer 2), 168.7 (C_{IV}, isomer 1), 154.5 (CH, isomer 1), 155.1 (C_{IV}, isomer 2), 153.3 (C_{IV}, isomer 1), 151.4 (C_{IV}, isomer 2), 149.6 (CH, isomer 1), 149.3 (CH, isomer 2), 136.1 (CHAr, isomer 1), 136.0 (CHAr, isomer 2), 123.5 (CHAr, isomer 1), 123.1 (CHAr, isomer 2), 121.1 (CHAr, isomer 1), 120.5 (CHAr, isomer 2), 45.0 (CH_2, isomer 1), 44.4 (CH_2, isomer 2), 29.1 (CH_2, isomer 1), 29.7 (CH_2, isomer 2), 21. (CH_2, isomer 1), 20.9 (CH_2, isomer 2).

IR ν (cm^{-1}): 3131, 3078, 1682, 1638, 1556, 1401, 1285.

Anal. Calcd for $C_{10}H_{12}N_4$: C, 63.81; H, 6.43; N, 29.77.

2-((naphthalen-2-ylmethylene)hydrazono)pyrrolidine – 208

HEI 3431



Ratio isomer 1/isomer 2: 92/7

Following the general procedure, 2-((naphthalen-2-ylmethylene)hydrazono)pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 2-naphthaldehyde (5 mmol, 0.8 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a white solid (0.85 g, 71% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (s, C*H*, isomer 1), 8.15 (s, 1H, C*H*, isomer 2), 8.01 (d, 2H, *J* = 9.1 Hz, 2H, Ar*H*, isomer 1+2), 7.96 (s, 1H, Ar*H*, isomer 1), 7.92 (s, 1H, Ar*H*, isomer 2), 7.85 (m, 1H, Ar*H*, isomer 1+2), 7.80 (m, 1H, isomer 1+2), 7.48 (m, 1H, Ar*H*, isomer 1+2), 6.35 (s, 1H, N*H*, isomer 1+2), 3.53-3.35 (t, *J* = 7.0 Hz, 2H, CH_2, all isomers), 3.01-2.64 (t, *J* = 7.0 Hz, 2H, CH_2, all isomers), 2.30-2.07 (t, *J* = 7.0 Hz, 2H, CH_2, all isomers).

Only the major

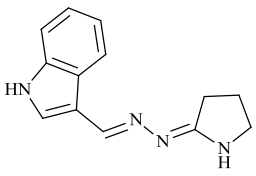
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 167.7 (C_{IV}, isomer 1), 153.3 (CH, isomer 1), 134.1 (C_{IV}, isomer 1), 133.3 (C_{IV}, isomer 1), 133.2 (C_{IV}, isomer 1), 128.9 (CHAr, isomer 1), 128.3 (CHAr, isomer 1), 128.2 (CHAr, isomer 1), 127.8 (CHAr, isomer 1), 126.6 (CHAr, isomer 1), 126.4 (CHAr, isomer 1), 126.5 (CHAr, isomer 1), 45.0 (CH_2, isomer 1), 28.9 (CH_2, isomer 1), 21.8 (CH_2, isomer 1).

IR ν (cm^{-1}): 2932, 2864, 1685, 1637, 1560, 1400, 1287, 1036.

Anal. Calcd for $C_{16}H_{16}N_2$: C, 81.32; H, 6.82; N, 11.85.

((pyrrolidin-2-ylidenehydrazono)methyl)-1H-indole – 209

HEI 3439



1 major isomer

Following the general procedure, ((pyrrolidin-2-ylidenehydrazono)methyl)-1*H*-indole was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 1*H*-indole-3-carbaldehyde (5 mmol, 0.7 g), in 30 mL of ethanol. The crude product was purified through

flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a yellowish solid (1.1 g, 95% yield).

M.p. 176-178°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.75 (bs, N*H*, isomer 1), 8.59 (s, 1H, C*H*, isomer 1), 8.28 (d, *J* = 8.4 Hz, 1H, Ar*H*, isomer 1), 7.45 (s, 1H, Ar*H*, isomer 1), 7.40 (d, *J* = 8.4 Hz, 1H, Ar*H*, isomer 1), 7.24 (m, 3H, Ar*H*, isomer 1), 6.25 (s, 1H, N*H*, isomer 1), 3.54 (t, *J* = 7.1 Hz, 2H, CH_2, isomer 1), 2.65 (t, *J* = 7.1 Hz, isomer 1), 2.14 (quin, *J* = 7.1 Hz, 2H, CH_2, isomer 1).

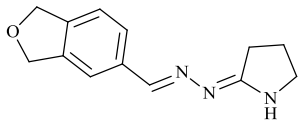
^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 165.8 (C_{IV}, isomer 1), 149.1 (CH, isomer 1), 136.9 (C_{IV}, isomer 1), 128.0 (CHAr, isomer 1), 125.1 (C_{IV}, isomer 1), 123.2 (CHAr, isomer 1), 122.3 (CHAr, isomer 1), 121.0 (CHAr, isomer 1), 114.4 (C_{IV}, isomer 1), 111.3 (CHAr, isomer 1), 44.9 (CH_2, isomer 1), 28.8 (CH_2, isomer 1), 22.0 (CH_2, isomer 1).

IR ν (cm^{-1}): 3445, 3184, 1626, 1440, 1293, 1244, 1194, 1009.

Anal. Calcd for $C_{13}H_{14}N_4$: C, 69.00; H, 6.24; N, 24.76.

2-((1,3-dihydroisobenzofuran-5-yl)methylene)hydrazono)pyrrolidine – 210

HEI 3442



Ratio isomer 1/isomer 2: 94/6

Following the general procedure, 2-((1,3-dihydroisobenzofuran-5-yl)methylene) hydrazono) pyrrolidine was obtained from the reaction of 2-hydrazonopyrrolidine (5 mmol, 0.5 g) with 4-bromobenzaldehyde (5 mmol, 0.8 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 90% ethyl acetate, as a white solid (0.6 g, 52% yield).

M.p. 139-140°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (s, C*H*, isomer 1), 8.15 (s, 1H, C*H*, isomer 2), 7.34 (d, *J* = 1.2 Hz, 1H, Ar*H*, isomer 1+2), 7.07 (dd, *J* = 8.0 Hz, *J* = 1.2 Hz, 1H, Ar*H*, isomer 1+2), 6.79 (d, *J* = 8.0 Hz, 1H, Ar*H*, isomer 1+2), 6.22 (bs, 1H, N*H*, isomer 1+2), 5.98 (s, 2H, CH_2, isomer 1+2), 3.50 (t, *J* = 7.6 Hz, 2H, CH_2, isomer 1+2), 3.40 (t, *J* = 7.6 Hz, 2H, CH_2, isomer 2), 2.96 (m, 2H, CH_2, isomer 2), 2.64 (t, *J* = 7.6 Hz, 2H, CH_2, isomer 1), 2.12 (t, *J* = 7.6 Hz, 2H, CH_2, isomer 2), 2.13 (quin, *J* = 7.6 Hz, 2H, CH_2, isomer 1).

Only the majoritary isomer is visible in ^{13}C NMR.

^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 167.3 (C_{IV}), 152.8 (CH), 149.0 (C_{IV}), 148.1 (C_{IV}), 130.2

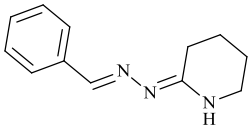
(C_{IV}), 123.6 (CHAr), 108.2 (CHAr), 105.9 (CHAr), 101.2 (CH_2), 44.9 (CH_2), 28.9 (CH_2), 21.9 (CH_2).

IR ν (cm^{-1}): 2925, 2862, 1642, 1500, 1445, 1248.

Anal. Calcd for $C_{13}H_{15}N_3O$: C, 68.10; H, 6.59; N, 18.33.

(benzylidenehydrazono)piperidine – 211

HEI 3443



(Benzylidenehydrazono)piperidine was obtained from the reaction of 2-hydrazonopiperidine (4.4 mmol, 0.5 g) with benzaldehyde (4.4 mmol, 0.5 g), in 30 mL of ethanol. The crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a yellow solid (0.47 g, 56% yield).

M.p. 72-73°C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (s, 1H, C*H*), 7.70 (d, *J* = 6.4 Hz, 2H, Ar*H*), 7.36 (d, *J* = 6.4 Hz, 2H, Ar*H*), 6.78 (s, 1H, N*H*), 3.31 (s, 2H, CH_2), 2.50 (s, 2H, CH_2), 1.81 (s, 4H, CH_2).

^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 161.0 (C_{IV}), 152.5 (CH), 135.6 (C_{IV}), 129.5 (CHAr), 128.5 (2CHAr), 127.5 (2CHAr), 41.8 (CH_2), 28.3 (CH_2), 23.4 (CH_2), 20.7 (CH_2).

IR ν (cm^{-1}): 3056, 2938, 1678, 1632, 1588, 1440, 1274.

Anal. Calcd for $C_{12}H_{15}N_3$: C, 71.61; H, 7.51; N, 20.88.

2 Analytical studies

2.1 Materials and methods – capillary electrophoresis

2.1.1 Capillary electrophoresis apparatus

Capillary zone electrophoresis experiments were performed on a Beckman P/ACE MDQ Capillary Electrophoresis system with an on-column diode-array UV detector, the whole system being driven by a computer with the ^{32}Karat software package (Beckman Coulter France S. A., Villepinte, France) for system control, data collection and analysis. A 50.1 cm x 50µm i.d untreated fused silica capillary was used (Composite Metal Services LTD., Silsden, West Yorkshire, U.K.). A hydrodynamic injection was made with a 5 s injection time at 1 psi, unless otherwise specified. In the screening conditions the applied field was 0.40 kV/cm (corresponding to 20 kV). long-end (LE) injection corresponds to an effective length of 40 cm and short-end (SE) injection corresponds to an effective length of 10.1 cm. Normal or reverse polarity mode was used to polarize the two electrodes. The capillary was mounted in a cartridge and thermostated for screening at 20°C. Compounds were detected at 210 nm. New capillaries were conditioned for 20 min with 0.1 M NaOH (P = 20 psi) and 5 min with water (P = 20 psi). Each day, at the beginning of the analyses, the capillary was flushed successively with NaOH (5 min, 20 psi), water (1 min, 20 psi), polyethylene oxide (PEO) (1 min, 25 psi), water (1 min, 25 psi) and then with background electrolyte (BGE) (3 min, 25 psi). Between each run, the capillary was treated with water (1 min, 20 psi) and BGE (1 min, 20 psi). The same procedures were used for basic buffer, but the PEO treatment was omitted. All injections were run three times for the method development. The pH of the buffer solutions was measured using a combination pH electrode (Hanna Instruments, Rhode Island, USA).

2.1.2 Chemicals

SBE-β-CD Captisol® was freely purchased from CyDex Pharmaceuticals (Lawrence, USA). Highly S-γ-CD (HS-γ-CD; Mw = 2538; aqueous solutions containing 20 % *w/v* of CD which correspond to 78.8 mM respectively) was purchased from Beckman (Beckman Coulter France, Villepinte, France). Characterization of these CDs indicated relatively good homogeneity in terms of degree of sulfation. Elemental analysis of the HS-γ-CD showed that

the average sulfate content was 13 per CD molecule, respectively.[255] Either the molar concentration unit or percentage (*w/v*) are used when relevant, to permit the comparison of the different selectors used. Polyethylene oxide (PEO; 0.4%; Mw = 300000) was purchased from Beckman-Coulter. Phosphoric acid (d=1.71, 85% *w/w*), triethanolamine (TEA) (d= 1.12, 98 % w/w) and sodium hydroxide (NaOH) were purchased from Baker (Paris, France). Deionized (DI) water was obtained from a Milli-Q system (Millipore, Saint Quentin-en-Yvelines, France).

2.1.3 Solutions

For chiral studies a 150 mM phosphate buffer was prepared from a H_3PO_4 solution adjusted to pH 2.5 by addition of TEA then diluted to give 25 and 2.5 mM solutions. For the method development, stock solutions of samples were prepared in methanol (2 mM) and diluted to 0.100 mM with 2.5 mM phosphate buffer. A 150 mM borate buffer was prepared from a H_3BO_3 solution adjusted to pH 10 by addition of NaOH (5N) then diluted to give 50 and 5 mM solutions. Stock solutions of samples were prepared in ethanol (2 mM) and diluted to 0.100 mM with 5 mM borate buffer.

2.2 Materials and methods – supercritical fluid chromatography

2.2.1 Supercritical fluid chromatography apparatus

The chromatographic system used was an SFC-PICLAB hybrid 10–20 apparatus (PIC Solution, Avignon, France) equipped with an autosampler comprised a 48-vials plate (model Alias, Emmen, Netherlands), three model 40 P pumps: two for CO_2 and a third for the modifier (Knauer, Berlin, Germany), a column oven with a Valco ten-position column selection valve, and a Valco six-position solvent switching valve. The pump head used for pumping the CO_2 was cooled to -8 °C by a cryostat (model Minichiller, Huber, Offenburg, Germany. The detector is a Smartline 2600 diode array detector (DAD) (Knauer, Berlin, Germany). After the detector, the outlet pressure was controlled by a back-pressure regulator (BPR). The outlet regulator tube was heated to 55°C to avoid ice formation during the CO_2 depressurization. The system was controlled and the data were acquired with the SFC PicLab Analytic Online v.3.1.2 software (PIC Solution, Avignon, France). During the separation optimization, the mobile phase was

[255] Chen F.T.A., Shen G., Evengelista, R.A. Characterization of highly sulfated cyclodextrins. *J. Chromatogr A* 2001, 924, p. 523-532.

always CO_2-modifier mixture with a proportion of an organic solvent with a ranging percentage, at a ranging flow rate. The column oven temperature was 40 ˚C and the outlet pressure was 150 bar. The wavelength was equal to 210 nm.

2.3 Materials and methods – Solid state analysis

2.3.1 Differential Scanning Calorimetry (DSC) Thermal Gravimetric Analysis (TGA)

The thermal decomposition curve of the compounds were measured on a DSC Q2000 V24.11. DSC samples were prepared by weighing about 5 mg of the compound in a standard 40 μL non-hermetic aluminium pan. The heating rate was set at 5°C/min and two cycles of scanning ranging from -40 °C to the melting point observed from the TGA, were performed under nitrogen atmosphere with a flow rate of 50 mL/min. Point calibrations using indium were carried out to check the temperature axis and heat flow of the equipment.

2.3.2 Thermal Gravimetric Analysis (TGA)

The thermal decomposition curve of the compounds were measured on a NETZSCH TG 209F1 Libra TGA209F1D-0240-L.TGA samples were prepared by weighing about 15 mg of the compound in a standard alumina pan. The heating rate was set at 5°C/min, the analysis were performed under air atmosphere.

2.3.3 Dynamic vapor sorption (DVS)

The water sorption and desorption processes were performed on a DVS Q5000 SA. The samples were mounted on a balance and studied over a humidity range from 0 to 100% RH, and then decreasing the humidity to 0% RH at 25 °C. Each humidity step was performed if less than 0.02% weight change occurred over 10 min.

3 Antibacterial *in vitro* biological screening tests

3.1 Activity assay

A sampling of the products of the present invention were tested for their activity against *Acinetobacter baumannii* (*A. baumannii*) as well as against the following bacteria: *Staphylococcus aureus (S. aureus), Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E. coli),* and *Pseudomonas aeruginosia (P. aeruginosa).*

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) for *Acinetobacter baumannii (*LMG 1025, ATCC 17978), was determined using the colorimetric resazurine microtitre assay (REMA). Briefly, ampules of frozen bacterial stocks were thawed, and bacteria diluted into Cation Adjusted Mueller Hinton Broth (CAMHB, Difco), to a final working concentration (OD600, optical density at 600 nm, of 0.0003 for *A. baumannii).* Working bacterial cultures were then added to the wells of a 96-well plate using a repeater pipette (100 µL in all wells, except first column with 200 µL). To the wells of the first column the plate (containing the 200 µL of culture), 2 µL of test compound was added at a concentration of 10 mg/mL in DMSO, giving a final concertation of 100 µg/mL (lower concentrations were used for more potent compounds). Eleven serial 1-in-2 dilutions were then made down the plate by transferring 100 µL between wells. The last column of the plate remained without compound as control. Plates were then incubated at 37°C for 5 hrs. To evaluate bacterial viability, 10 µL of 0.025% resazurin was added to the wells and left to incubate for up to 2 hours. Bacterial resazurin turnover to fluorescent resorufin was measured by fluorescence (Ex, 530 nm, Em: 590 nm). The MIC was defined as the concertation of compound that prevented the turnover of resazurin (less than 10 % resazurin turnover compared to the non-treated control).

3.2 Cytotoxicity assay

HepG2 cells (human hepatocellular cell line, ATCC HB-8065) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained in Dulbecco's modified Eagle's Medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Thermo Fisher Scientific) in a humidified atmosphere of 5% CO2, 95% air at 37°C.

HepG2 cells were seeded at a density of 2500 cells/well on a 96-well plate in 100 µL of DMEM without red phenol supplemented with 10% FBS. Cells were allowed to attach overnight. Thereafter, 1 µL of two-fold serial dilutions of 100x compound in DMSO were added to the cell culture (final concentration 100 µg/ml to 0.4 µg/ml). As a positive control, 1 µM staurosporine was used (final concentration, Biaffin, Kassel, Germany).

Plates were incubated for 3 days (37°C 5% CO_2) before the addition of 10 µL of 0.04% resazurin (Thermo Fisher Scientific). After 4h of culture, the fluorescence of the resazurin metabolite resofurin was determined (excitation, 530 nm; emission, 590 nm; gain, 850) using FLUOstar OPTIMA microplate reader (BMG Labtech, Ortenberg, Germany). Cytotoxicity was evaluated as a concentration of compound that prevented 50% of resazurin turnover (Cytotoxic IC_{50}) compared to staurosporine.

4 Antifungal *in vitro* biological screening tests

4.1 Preparation of the nutrient agar media and growing of bacterial cultures

Commercial nutrient agar was dissolved in distilled water to make one litre of the solution and placed in an autoclave at for 60 minutes for sterilization. Portions of the sterilized nutrient agar medium (10 ml) were dispensed into 90 mm diameter Petri-dishes to obtain a uniform depth of approximatively 30 mm under septic conditions in a laminar flow. After, the Petri dishes were carefully covered and allowed to cool at room temperature until the culture media completely solidified. With a sterile wire loop under septic conditions, bacteria cultures from stock cultures were spread on the nutrient agar surface and incubated aerobically at 37°C for 24 hours. After, one loopful of the bacterial strain from the 24 hours culture was added into the sterile nutrient broth medium and incubated at 37°C for 24 hours in a rotator shaker.

4.2 Preparation of the nutrient agar media and growing of fungi and yeast cultures

Commercial potato dextrose agar powder was dissolved in distilled water to make a litre of the solution followed by steam sterilization in an autoclave for 60 min. After cooling down, small portions of this solution (10ml) were carefully dispensed into sterile Petri dishes under septic conditions in a laminar flow and left to solidify, providing the medium for growing the fungal strains. Pure culture for the concerned fungus was made on the PDA surface in the Petri dishes

from stock culture and incubated to 22^0C for seven days to produce a good crop of spores, with periodic subculturing. The antifungal activities of the synthesized compounds were tested in vitro against a total of 12 species of fungi and yeasts from the collection of TIMR (UTC) laboratory. The strains Aspergillus oryzae (MUCL19009), Alternaria alternata (MUCL53651), Paecilomyces variotii (MUCL 39890), Penicillium ochrochloron (MUCL 38775), Botrytis cinerea (MUCL 000399), Sclerotinia sclerotiorium (MUCL 011553), Fusarium solani (MUCL 035016), Cladosporium cladosporioides (Laboratory's isolate), and yeasts Geotrichum candidum (Laboratory's isolate), Candida krusei (Laboratory's isolate), Candida preudotropicalis (MUCL46196), Candida tropicalis (MUCL29893). Strains were subcultured every week on Petri dishes containing potato dextrose agar and incubated at 22° C under a 12h:12h photoperiod.

4.3 Culture procedures

The mineral medium used for antifungal tests was composed of following macroelements: KCl (0.250 g/L), NaH_2PO_4 (1.544 g/L), $Na_2HPO_4 \cdot 2H_2O$ (0.008 g/L), MgSO4 (0.244 g/L), NO_3NH_4 (1 g/L) and trace-elements consisting of: $ZnSO_4.7H_2O$ (0.01 mg/L), $MnCl_2.4H_2O$ (0.001 mg/L), $FeSO_4.7H_2O$ (0.01 mg/mL), $CuSO_4.5H_2O$ (0.005 mg/mL), $CaCl_2$.2H2O (0.001 mg/mL), MoO_3 (0.003 mg/mL), pH 5.5. The non-sporulating fungi such as A. alternata, B. cinerea, S. sclerotiorium and the yeasts G. candidum, C. krusei, C.preudotropicalis, C. tropicalis were grown in Erlenmeyer flasks (500ml), to which a volume of 50 mL of mineral medium and glucose (5 g/L) were added, for one week at 23° C under a 12h :12h photoperiod on orbital shaker at 140 rpm. OD at 600 nm of pre-culture is measured (100th dilution).

Considering the non-sporulating fungi, the inoculum is prepared using the Buchner filtration method. The preculture incubated one week at 22° C is poured into the vacuum filtration system composed of a vacuum flask, a filter paper and a Buchner funnel previously sterilized by autoclaving, as well as a water pump system. The biomass cake which is recovered by filtration is then chopped until is homogeneous, then 50 mg are added in 20 mL of mineral medium to which glucose was added to rich a 5 g/L concentration.

The yeast inoculum, on the other hand is prepared from the pre-culture incubated for under agitation by using a turbidity measurement. The OD at 600 nm of inoculum suspension containing mineral medium, glucose (5 g/L) and yeast has to be equal to 0.2.Considering the sporulating fungi, the inoculum was prepared as following. The spores are recovered directly

from the fungi culture petri box by pouring water with sterile Tween 80 directly on the filaments. Spore counting was performed using a Malassez.cell to obtain an inoculum concentration of spores of 103 CFU/mL in mineral water and glucose (5 g/L).

Fungal precultures or spore suspensions were used as inoculum for antifungal tests in 96-well microtiter plates. An inoculum suspension (100 µL) was added to each well. For the assay, compound test wells (CTWs) were prepared with 2 µL of stock solutions of each compound in DMSO. The final proportions being 100 µL inoculum, 98 µL of mineral medium having a concentration of 5 g/L glucose and the 2 µL of compound, to reach a total volume of 200 µL per well. The compounds possessing good activity (inhibitory rate> 70% at 100 µg/mL) were further evaluated using different concentrations by diluting the mother solution to reach final concentrations from 1 to 100 µg/mL. A growth control well (GCW) (containing medium, inoculum and the same amount of DMSO used in a CTW) were included for each tested fungus. Non inoculated mineral medium served as the negative control. Microtiter trays were incubated in a moist chamber at 22° C under 12h : 12h photoperiod for 5 to 7 days. and the optical density was determined every day using a microplate UV reader (Ultrospec10 Amersham Bioscience), at 600 nm. Tests were performed in triplicate.

4.4 Cytotoxicity screening of selected antifungal molecules

The human embryonic kidney 293 cell line (HEK293) was cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, Waltham, MA) supplemented with 2 mM L-glutamine, 100 IU/ml penicillin/strepomycin, non-essential amico acid solution (1/100) and 5% (v/v) heat-inactivated foetal bovine serum (Sigma-Aldrich, Saint-Louis, MO), and grown at 37 °C in a humidified incubator with 5% CO2. Cells were seeded at 3000 cells per well onto 96-well plates in DMEM medium. Cells were incubated in a culture medium that contained 100 µM of the different test compounds and 2µM of the references, each dissolved in less than 1% DMSO. After 72 h of incubation, cell viability (in proliferation and cytotoxicity) was estimated by the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay.

5 Antioxidant test using DPPH

Protocol

A solution of DPPH in ethanol is freshly prepared, having a concentration of 25 mg/L. Its absorbance is measured at 517 nm by UV. For this concentration, the absorbance is normally around 0.590. The reference of the measurement is the pure ethanol. The solution is stocked in an amber flask, the solution being stable for hours.

The used concentration for tested compounds was 1mM and sequential dilution 1:2 were performed to evaluate the activity.The radical scavenging activity of each diluted solution is measured by addition of the antioxidant solution to the DPPH sample.

In 10 mL vials, 1.9 mL of DPPH solution and 100 µL of solution compound are mixed and slowly stirred. The absorbance is measured after 30 minutes of room temperature incubation, in natural light. A colour transition from purple to pale yellow is observed whether the compound is radical scavenger or not. When plotting the inhibition pourcentage versus the concentration, the IC50 for the radical scavenging potency can be determined.

Inhibition percentage calculation: $\%INH = 100*(1-ABS_{compound}/ABS_{DPPH})$.

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Abstract

The lack of therapeutic innovations has recently reached a critical level, considering antibiotic treatments. This issue, correlated with the appearance of multidrug-resistant bacteria underlined the need for new approaches towards the discovery of innovative antibiotics. As a consequence, the BIOANTIBIO project was proposed.

The two main initially envisioned applications for the most active compounds being:

• Additives for the formulation of innovative materials (*eg* paints and coatings) in order to guarantee their durability in storage (working in collaboration with IFMAS).

• Drugs: antibacterial and antifungal agents, particularly for the development of antimicrobial agents against drug-resistant bacteria for human health protection.

Employing natural molecules to develop novel antifungal or antimicrobial classes can be challenging. Nevertheless, nature is known to inspire chemists in the synthesis of molecules with improved biological properties and within this project we have demonstrated that even the well known pyroglutamic acid is still to be exploited as a scaffold.

Résumé

Le manque d'innovations thérapeutiques a récemment atteint un niveau critique par rapport aux traitements antibiotiques. Cette question, corrélée à l'apparition de bactéries multirésistantes, a mis en évidence la nécessité de nouvelles approches pour la découverte d'antibiotiques innovants. En conséquence, le projet BIOANTIBIO a été proposé.

Les deux principales applications initialement envisagées pour les composés les plus actifs sont:

• Additifs pour la formulation de matériaux innovants (par exemple, peintures et revêtements) afin de garantir leur durabilité en stockage (en collaboration avec IFMAS).

• Médicaments : agents antibactériens et antifongiques, en particulier pour le développement d'agents antimicrobiens contre les bactéries résistantes aux médicaments, destinés à la protection de la santé humaine.

L'utilisation de molécules naturelles pour développer de nouvelles classes d'antifongiques ou d'antimicrobiens peut être difficile. Néanmoins, on sait que la nature inspire les chimistes dans la synthèse de molécules aux propriétés biologiques et dans le cadre de ce projet, nous avons démontré que même l'acide pyroglutamique, composé biosourcé bien connu, devait encore être exploité en tant que motif phare de la chimie du laboratoire.