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Cannabinoid system combined to classic targets for a new MTDL strategy: design and synthesis of natural inspired molecules for Alzheimer's disease

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CHAPTER 1

1- INTRODUCTION

1.1 Alzheimer's disease

First described by Alois Alzheimer (1864-1915) in 1907, the disease that bears his name largely remained an enigma until the twilight of the 20th century. During the Psychiatric Convention Tübingen Alzheimer presented the case of a 51 years old woman, Auguste Deter, with an unknown form of dementia; the psychiatrist E. Kraepelin four years later, in his treatise, named this form of dementia, **Alzheimer's disease (AD)**.

Alzheimer's disease is the leading and common cause of dementia in the elderly and it is the most common cause of adult-onset dementia in Western world. It is a progressive neurological condition characterized at the beginning by short-term memory impairment and it evolves to profound cognitive and physical disability.[1] AD is a multifactorial disorder caused by genetic, environmental and endogenous factors that include excessive protein aggregation, oxidative stress, free radical formation, mitochondrial abnormalities and neuro-inflammatory processes that involve the activation of astrocytes and microglia.[2] More in detail, environmental factors, such as trauma or exposure to toxic substances (aluminum, aromatic hydrocarbons), may play an important role.

Aging is a major risk factor for neurodegenerative disorders such as AD. Analyses suggest that the number of Americans with Alzheimer's disease in 1997 was 2.32 million (range: 1.09-4.58 million) and that this number will nearly quadruple in the next 50 years, at which time 1 in 45 Americans will be living with the disease. The growth in the prevalence of Alzheimer's disease results from the aging of the US population. In 1997, the percentages of Americans more than 65, 75, and 85 years of age were 13%, 6%, and 1.4%, respectively. By the year 2047, the corresponding percentages of such individuals will increase to approximately 20%, 11%, and 4%. The percentages of persons living with Alzheimer's disease among 75, 80, 85, and 90-year-olds are 4.3%, 8.5%, 16.0%, and 28.5%, respectively.[3] The incidence of AD is increasing at an alarming rate along with aging of populations of industrialized countries. United Nation population projections estimate that the number of people older than 80 years will approach 370 million by the year 2050. Therefore, if these statistics hold true, in 50 years, more than 100 million people worldwide will suffer from dementia. The vast number of people requiring constant care and other services will severely strain medical, monetary, and human resources. High level of education and jobs that require a high level of cognitive activity appear a protective effects on the onset of dementia, due to increased efficiency of neuronal circuits and to the ability of the brain to activate alternative neural circuits.

Diagnosis of AD is based on clinical features, although it should be confirmed by brain histopathological examination. There are three clinical stages of AD with cognitive and functional decline stretching over 5–8 years: mild, moderate and severe (fig. 1).

The initial stage usually lasts 2–3 years and is characterized by short-term memory impairment often accompanied by symptoms of anxiety and depression. In a small proportion of them, slurred speech, executive functions, perception, or movement disfunctions are more prominent than memory problems. Episodic memory, semantic memory and implicit memory are affected to a lesser degree

than new facts or recent memories. In this stage, patients are able of adequately communicating basic ideas. As the disease progresses, people with AD can often continue to perform many tasks independently, but may need assistance or supervision with the most cognitively demanding activities.

In the moderate stage, these symptoms appear to abate as neuropsychiatric manifestations, such as visual hallucinations, false beliefs and reversal of sleep patterns. Speech difficulties become evident and frequently they use incorrect word substitutions. Reading and writing skills are also progressively lost. Complex motor sequences are less coordinated. Memory problems worsen, and the person could not recognize relatives. Long-term memory becomes impaired. Common neuropsychiatric manifestation are irritability and labile effect, depression and aggression.

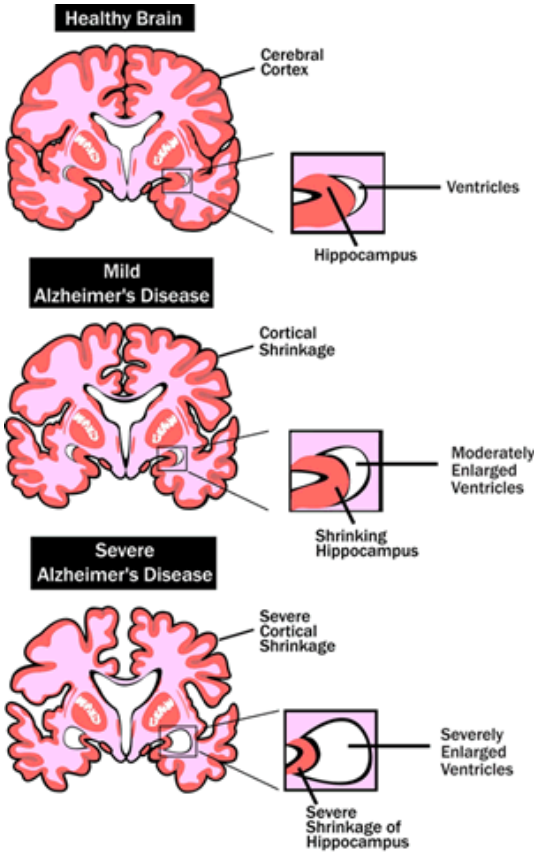


Figure 1: morphological changes of healthy brain during the progression of AD.[4]

The severe and final stage is characterized by motor signs, such as motor rigidity and prominent cognitive decline. Patients are completely dependent upon caregivers. Language is reduced to simple phrases or even single words and, at the end, there is a complete loss of speech. Usually extreme apathy and exhaustion are much more common. Patients will ultimately not be able to perform even the most simple tasks without assistance. Finally comes death, usually caused by some external factor such as pressure ulcers or pneumonia, not by the disease itself. The mean life expectancy following diagnosis is approximately seven years. Less than 3% of individuals lives more than fourteen years after diagnosis. Cognitive and functional decline tend to be linear throughout

the three stages of the disease, whereas caregiver burden peaks with the onset of neuropsychiatric symptoms and declines somewhat during the final stage, when the patient is more sedentary. This is the result of neuronal degeneration in the cortex, particularly in the temporal lobes, hippocampus and basal ganglia, which leads to reduction of the weight of the brain, dilation of ventricular cavities and cortex and the thinning of the convolutions. Linear correlation between the stages of AD and volume of brain structures, suggest that successful therapeutic intervention might stop the progress of pathological changes at any stage of disease.

The disease can be caused by a “sporadic” or a "familiar" form with early onset;[5] the latter is transmitted through a dominant autosomal mechanism. 99% of cases of AD is sporadic and occurs in people who do not have a clear familiarity. Only 1% of cases of AD is caused by an altered gene that determines the transmission from generation to generation.

Generally, hereditary forms have a high penetrance, in fact many people of a family (3 or more) are affected by the disease. Furthermore, most of the hereditary forms appear in relatively early age (before 65-70 years) and the age of onset of the first symptoms is relatively stable within the same family.

To date, mutations identified relate to chromosomes 21, 4, 1. The mutations located on chromosome 21 are responsible for the synthesis of β-amyloid, those in the chromosomes 14 and 1 are responsible for the synthesis of abnormal presenilins (the neuronal membrane proteins that control the phosphorylation of τ protein). In addition, a mutation not related to familiar form was discovered: it affects the chromosome 19, which encodes proteins responsible for the transport of fat in the blood. Its aberration causes the production of ApoE 4, an apolipoprotein (Apo) that is not efficient for the transport of cholesterol; this prevents cholesterol to reach the damaged neuronal membranes and to repair them. Moreover, a recently emerged and still quite unknown system that could be involved in the disease was found to be the endocannabinoid system. Some studies showed that cannabinoids play a protective role against the toxicity of amyloid peptide.

1.2 Ethiopathology

Data show that the accumulation of abnormal proteins during aging of the brain is connected to oxidative and inflammatory damage and to a deficit of neurotransmitter systems, among which the most affected appears to be the cholinergic system. Moreover, a reduction of various other neurotransmitters such as dopamine, glutamate and serotonin was revealed. For this reason, several theories were formulated with the aim of defining the molecular mechanisms of the disease:

- Cholinergic hypothesis
- β -Amyloid peptide hypothesis
- Tau protein thesis
- ApoE
- Others causes

1.2.1 The cholinergic hypothesis

In the past decade, treatment for AD has largely involved the replacement of neurotransmitters that are known to be lacking in AD, mostly based on the “cholinergic hypothesis” of AD. A deficit in central cholinergic transmission caused by degeneration of the basal forebrain nuclei is an important pathological and neurochemical feature of AD.

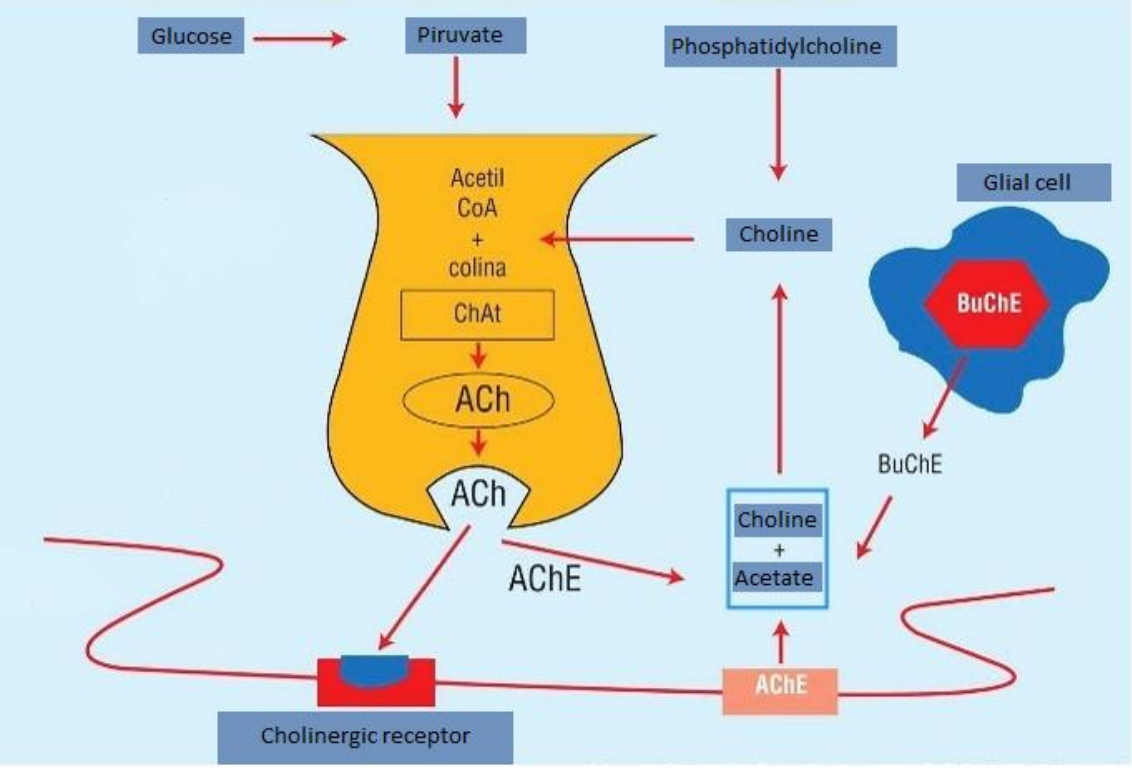


Figure 2: acetylcholine methabolism

The deficit shows reduced activity of choline acetyl transferase (ChAT) enzyme, decrease of axonal transport, synthesis of acetylcholine (ACh) and choline reuptake (fig. 2). There is also a reduction in the number of cholinergic receptors in the hippocampus and cortex.[6] Presynaptic nicotinic receptors control the release of neurotransmitters (acetylcholine, glutamate, serotonin and norepinephrine) that are important for memory and behavior. Experimental studies showed that Aβ binds presynaptic nicotinic α-7 receptors, essential for cognitive processes, reducing in this way the release of ACh and that, levels of muscarinic receptors is reduced in patients with Alzheimer's disease.[7] The therapeutic approach aims to reduce the neuronal decline and symptoms through the reintegration of lacking neurotransmitters. This is demonstrated with the use in therapy of inhibitors of acetylcholinesterase (AChE), the enzyme that hydrolyzes ACh forming acetic acid and choline. To date, this is the most important therapeutic strategy for the Alzheimer's disease treatment. Unfortunately, it is only a symptomatic treatment considering that these inhibitors do not prevent or reverse the progression of the disease.

AChE is an enzyme with a very fast action and it is able to hydrolyze approximately 5.000-10.000 molecules of ACh per second. AChE is localized in synaptic or junctional space. In brain, AChE hydrolyzes neurotransmitters such as P substance and encephalins and it was found in association with glial cells and with the blood brain barrier. It is also present in the blood and in peripheral organs. The AChE is the best-known representative of a group of enzymes designated with the name of cholinesterase and which have the function to catalyze the hydrolysis of choline esters of various carboxylic acids. In 1991, Sussmann et al. determined the three-dimensional structure at the AChE through X-ray analysis of a crystal of the homodimeric form of the enzyme obtained by electric organ of a race (Torpedo californica). Previously, it was showed that the AChE oligomeric forms present in the electric organs of fish (electrophorus and torpedo) were structurally homologous to those coming from the muscles and nerves of vertebrates. The monomer studied by Sussmann is a polypeptide of 537 amino acids with ellipsoidal shape, belonging to the class of α/β protein; the secondary structure of the molecule has 12 segments in β-sheet and 14 α-helices. The most typical feature of the structure is the presence of a deep groove (gorge) that extends for about 20 Å within the molecule (fig. 3). At the base of this cavity, initially narrow but gradually larger, there is the so-called catalytic triad of the AChE.

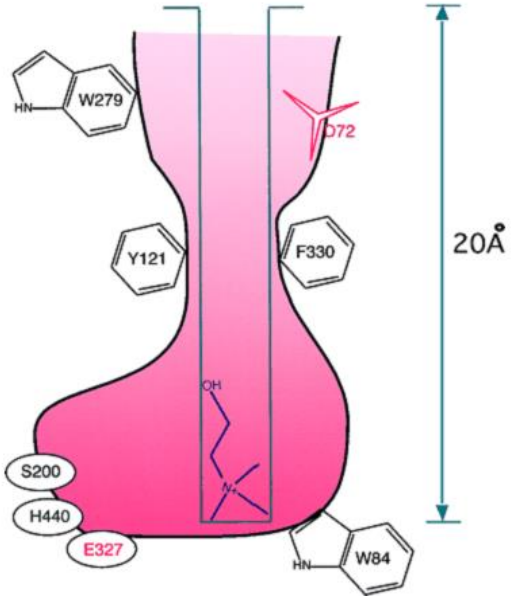


Figure 3: aromatic gorge of AChE

It consists of the three residues (Ser^{200}, His^{440}, $Glu^{327)}$ responsible for the hydrolytic action of the enzyme. Another important residue located in the bottom of the gorge is the Trp84; to its indole ring was assumed the bond of cationic head of ACh with a cation-π bond. The mechanism of hydrolysis of ACh involves the nucleophilic attack of a Ser 200 hydroxyl of the enzyme to the carbonyl carbon of the ACh, which forms the acetylated enzyme and the choline. Subsequently, the ester bond of the complex is hydrolyzed, allowing the regeneration of the AChE and the formation of acetate ion. In order to improve cholinergic neurotransmission, many strategies have been investigated including the increasing of acetylcholine synthesis, the augmentation of presynaptic acetylcholine release and

the stimulation of cholinergic postsynaptic muscarinic and nicotinic receptors. Current data do not support the use of precursors of acetylcholinesterase, presynaptic releasing agents, or muscarinic agonists because of a lack of efficacy and unacceptable side effects.[8] It is important to underline that the AChE can accelerate the deposition of Aβ. In fact, a peripheral anionic site (PAS) was identified. It is approximately situated at 14 Å from the active site, with which the Aβ can interact, contributing, in this way, to the formation of senile plaques.[9,10.] In fact, the interaction of Aβ with the peripheral anionic site (PAS) of AChE, accelerates the aggregation of this toxic peptide, since it catalyzes the conformational conversion of the fibrils in β-sheet.

Butyrylcholinesterase is also known as pseudocholinesterase, non-specific cholinesterase or simply cholinesterase. It preferentially acts on butyrylcholine, but also hydrolyzes ACh. BuChE exhibits the substrate activation in excess substrate and has higher activity in liver, intestine, heart, kidney and lung.[11] In AD BuChE is found as well as AChE in CNS. In fact, many AChEIs also inhibit BuChE, because both AChE and BuChE enzymes are found in the CNS. Moreover, AChE and BuChE share 65% amino acid sequence homology even though being encoded by different genes on human chromosomes. The BuChE inhibition can lead to adverse peripheral side effects. Functionally, both enzymes hydrolyze acetylcholine efficiently but at different rate, that is, at the same temperature and pH, AChE has higher hydrolytic acetylcholine activity than BuChE. The physiological role of BuChE is still unclear. Moreover, BuChE did not affect amyloid formation because three aromatic residues of the AChE PAS are missing in the PAS of BuChE. The PAS of BuChE had weaker affinity than AChE, which mediates substrate activation. Nevertheless, BuChE may play a compensatory role in the hydrolysis of acetylcholine in brain with degenerative changes; in fact, AChE activity decreases in certain brain regions as AD progresses, while BuChE activity is not affected or even increases, making BuChE available in neuritic plaques. Considering this assertion, mixed inhibition of AChE/BuChE enzymes could lead to an improved AD therapeutic benefit but the inhibition of BuChE, more than the AChE, can give adverse peripheral side effects. The differences in the enzyme kinetic properties and locations of brain of AChE and BuChE suggested that, in the normal brain, AChE is the main enzyme responsible for acetylcholine hydrolysis, while BuChE plays a secondary functional role.[12]

More in detail, the difference between the two enzymes can be explained by the different structures around the main entrance area. In AChE, there are eight aromatic residues associated with the main door: three of them are located on the entrance of the active site (Tyr 72, Trp 286, and Tyr 341); five of them are located almost the same depth inside the gorge (Tyr 124, Phe 295, Phe 297, Tyr 337, and Phe 338), (fig. 4, A).

In BuChE, however, six of these residues are changed to smaller ones, including Tyr 72 to Asn 68, Tyr 124 to Gln 119, Trp 286 to Ala 277, Phe 295 to Leu 286, Phe 297 to Val 288, and Tyr 337 to Ala 328, with both sets of residues (fig. 4, B, C). These smaller residues will significantly enlarge the entrance radius, which makes it possible for larger substrates to enter the active site. [13]

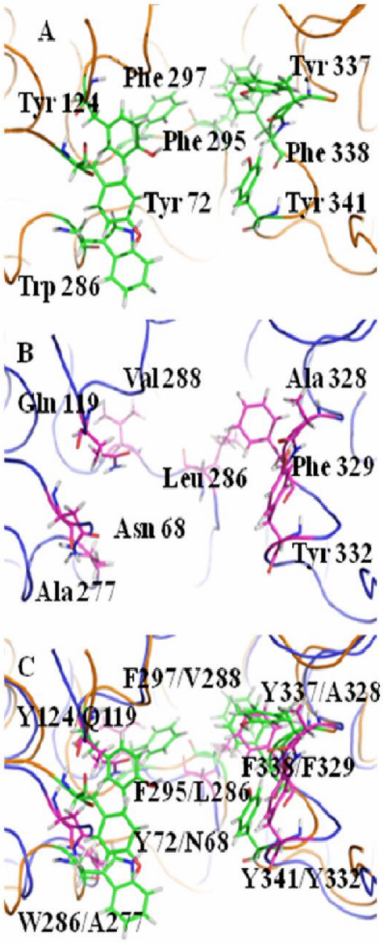


Figure 4: (A, top) The eight residues with aromatic or indole side chains in the gorge of AChE. (B, middle) The eight residues in the gorge of BuChE. (C, bottom) Superimposing of the gating residues in AChE and BuChE.

1.2.2 β-Amyloid peptide hypothesis

A neurological macroscopic point of view shows that the main brain lesions that characterize the disease are accumulations of: extracellular beta-amyloid protein (Aβ) that constitutes the so-called "senile plaques", intracellular neurofibrillary tangles and neuronal degeneration. The amyloid cascade theory proposes Aβ as the central trigger of the pathological changes observed in the brains of AD patients, such as synapse loss, activation of inflammatory processes, the induction of neurofibrillary changes leading to the formation of paired helical filaments (PHF) and, ultimately, neuronal death (fig. 5). It formed the basis for numerous research activities, which significantly contributed to our understanding of AD. [14] "Amyloid" means "starch-like", and it is due to the property to react with iodine and indicates the protein material rich of glycosaminoglycans, which is extra-cellular deposited. Several proteins with amyloid characteristics are associated with many diseases. Among these, is included the β-amyloid protein, that is involved in the AD pathology. The main hypothesis about the causes of AD is called “amyloid cascade hypothesis” that defines the Aβ as the initiator. This hypothesis states that the increase in production, aggregation and accumulation

of Aβ in the brain cause a cascade of neurotoxic events that can lead to widespread neuronal degeneration and the clinical symptoms of dementia. The β amyloid protein is the main constituent of senile plaques and extracellular aggregates formed by a conformational change of the protein. It is produced starting from the “amyloid precursor protein” (APP), a membrane protein encoded by a gene located in the central portion of the long arm of chromosome 21.

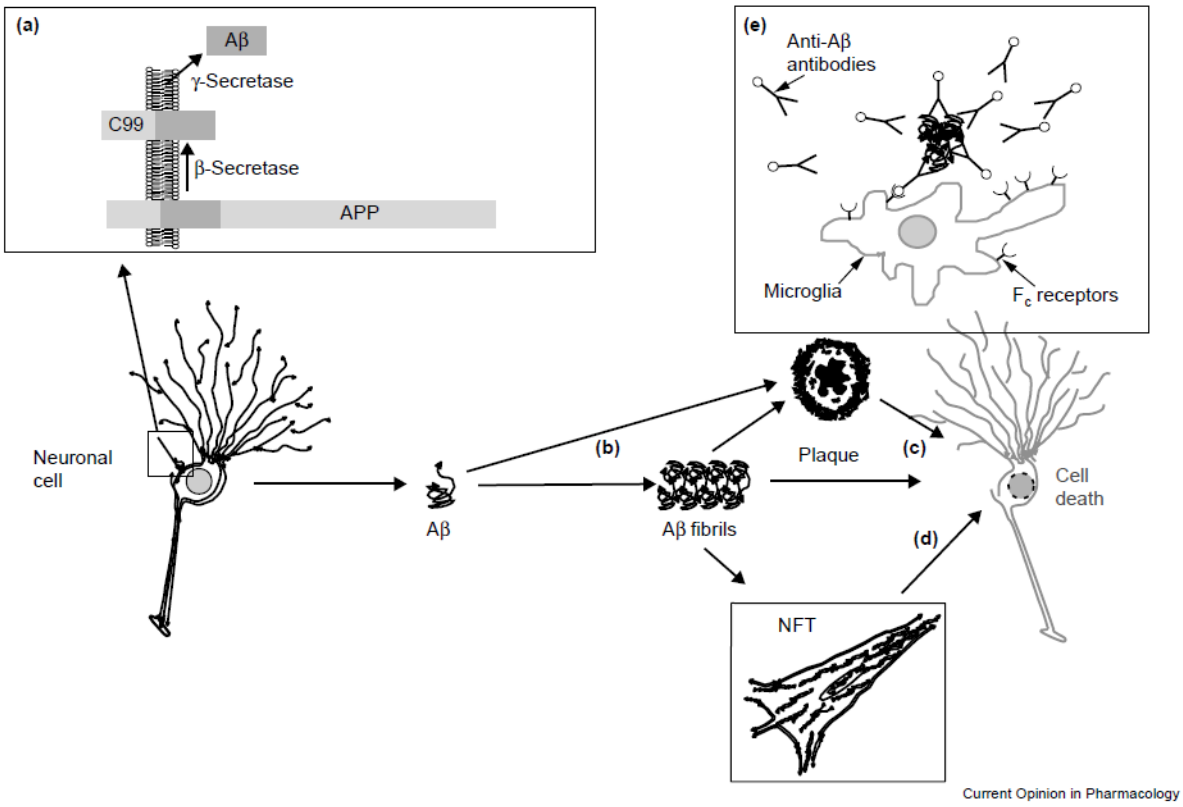


Figure 5: β-amyloid cascade

It consists of three domains: the extracellular hydrophilic N-terminal, the hydrophobic transmembrane and the intracellular C-terminal. It is expressed in different cells, particularly in brain, heart, spleen and kidneys. Until today at least 10 isoforms have been identified; the three most common are called C (mainly located in neurons), B (located in T cells) and A; them are respectively composed of 695, 751 and 770 aminoacids. Although the physiological role of APP and its isoforms has to be clarified, it seems involved in various physiological processes. I. e., it demonstrates neuroprotective properties; moreover, its addition to cell cultures decreases intracellular calcium levels. APP is synthesized in ribosomes then, it is transported into the endoplasmic reticulum and in the Golgi apparatus where it undergoes several changes such as phosphorylation, glycosylation and sulphurization. Most of it is degraded during the transport to the cell membrane. This protein can undergo the action of three different enzymes, present at the cellular level: α-, β- and γ-secretase. At physiological conditions, the α-secretase enzyme acts at residue 687, between residues Lys and Leu, and, in this way, allows to the not amyloidogenic process. This process forms a large soluble N-terminal fragment, called α-APPs, and a C-terminal fragment of 83 residues, which

undergoes the action of γ-secretase, forming two other fragments, P-3_{40} and P-3_{42}, which are soluble and thus harmless and are released in the extracellular.

On the other hand, the amyloidogenic pathway begins with the action of β-secretase (BACE-1, beta site amyloid precursor protein-cleaving enzyme 1) which releases a soluble N-terminal fragment, called β-APPs, shorter if compared to α-APPs. The remaining C-terminal fragment, with 99 residues, is a substrate for the γ-secretase that forms $Aβ_{40}$, a peptide fragment consisting of 40 amino acids, soluble and harmless, and $Aβ_{42}$, which it is insoluble, toxic and has a tendency to aggregate (fig. 6).

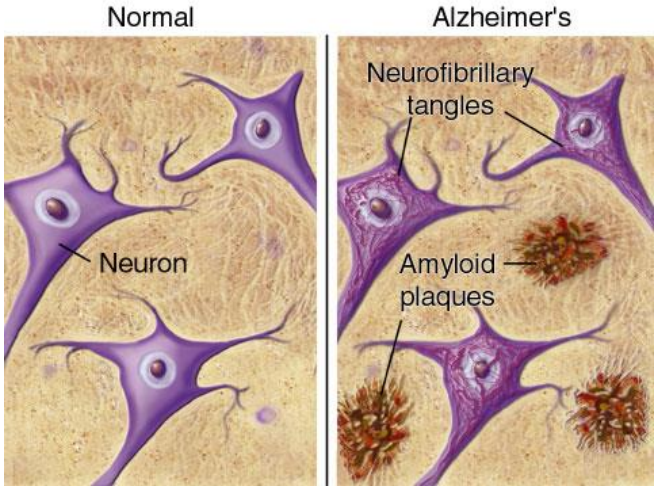


Figure 6: β-amyloid plaques and neurofibrillary tangles in AD[15]

The action of γ-secretase occurs within the cell membrane and further to its action a short tail (AICD) consisting of about 50 amino acids, is also formed; it is released into the cytoplasm and it has as its cellular target the nucleus, where it activates gene transcription.

$Aβ_{42}$ accumulates in patients with AD or Down syndrome; recent studies demonstrate that individuals with trisomy 21 faster progress the disease and show signs of mitochondrial alterations, causing premature aging, which appear to be due to an accumulation of APP, codified precisely on chromosome 21. The β-amyloid peptide aggregates spontaneously in many forms. One of these consists of oligomers (from 2 to 6 peptides) which merge into intermediate forms.[16,17] It can grow into fibrils, which are arranged in β-sheets in order to form β-amyloid plaques. It is not yet clear if the formation of insoluble peptides is the event that triggers the process of neuronal death or if it is a consequence of the damage.[18] Although $Aβ_{42}$ seems to be the responsible of AD, also soluble forms could be correlated with the different neuropathological aspect of the disease.

1.2.3 Tau protein

The formation of aggregates of tau protein is very important in AD. In normal conditions, in healthy subjects, Tau is a soluble protein and it acts as a stabilizer of the cytoskeleton of neurons, promoting the assembly, the microtubule stability and the transport of vesicles. In fact, the cytoskeleton is formed by microtubules, in particular of polymers of tubulin. These microtubules are

linked to associated proteins that regulate their stability. The most important of these proteins at axonal level is tau protein, a very hydrophilic protein that contains several phosphorylation sites. A high degree of phosphorylation by the kinase causes a detachment of the protein from microtubules and induces neuronal damage. More in details, in patients affected by AD and other neurodegenerative disorders, called "tauopathies", tau protein undergoes a series of changes leading to the formation of neurofibrillary tangles (NTFs), filamentous inclusions that are the main microscopic changes along with senile plaques (fig. 7). Six isoforms are expressed in human brain, originating from the alternative splicing of a single gene, located on chromosome 17.

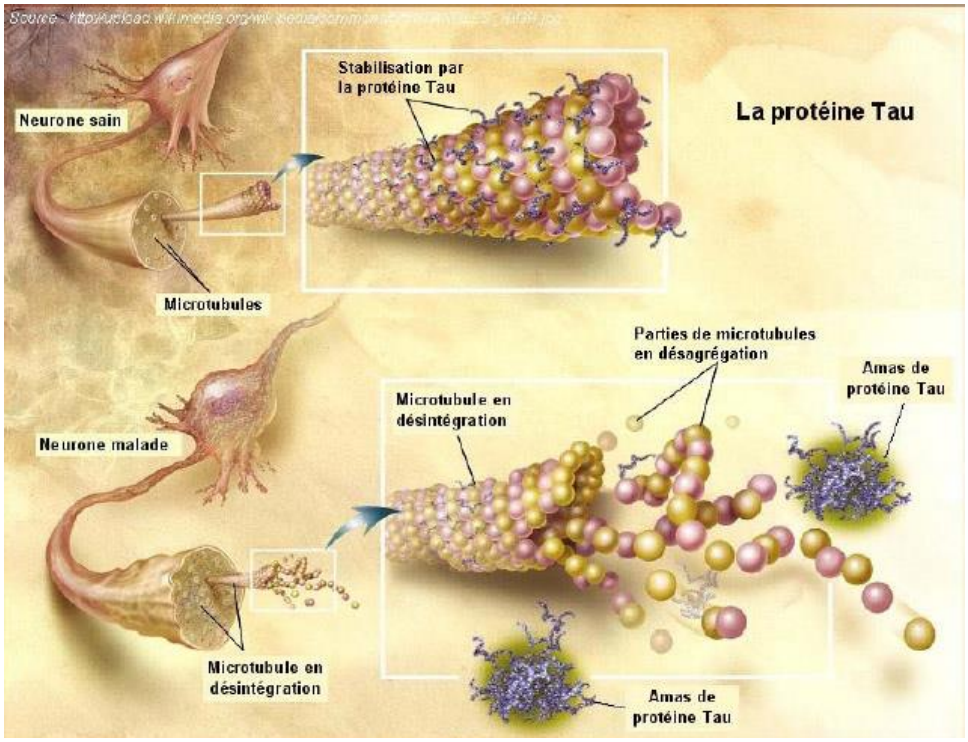


Figure 7: neurofibrillary tangles

Its physiological location is in the cytoskeleton of axons, but in conditions of neurodegenerative disease is also found in cell bodies and dendrites. The main component of tangles is a hyperphosphorylated and aggregated form of tau. Four repeated sequences constitute the binding site of tau to microtubules. Standard tau phosphorylation occurs at serine and threonine; when followed by proline, these aminoacids are phosphorylated by GSK-3β (Glycogen Synthase Kinase 3β), cdk5 (cyclind-dependent kinase) or by MAPK (mitogen-activated protein kinase). The hyperphosphorylated form is insoluble, lacks affinity for microtubules and it is associated in pairs of helical filaments; finally, it forms intracellular aggregates in order to obtain neurofibrillary tangles. In this way, axonal transport and cell vital processes are damaged.

The amount of tangles is a pathological marker of the severity of the disease; in fact, increased levels of phosphorylated protein in the cerebrospinal fluid are correlated with the reduction in scores on cognitive tests.[19] Tau hyperphosphorylation could be induced by Aβ or it could come from by a mutation of the PS-1 gene that encodes for presenilin-1 protein; the latter is responsible for the

phosphorylation of tau by kinase GSK-3β.[20] However, tau protein if dephosphorylated is able to recover the ability to bind microtubules. Recently, a gene encoding a protein involved in cholesterol transport, the apolipoprotein E (ApoE) has been identified as a molecular factor implicated in the pathogenesis of AD. In particular, ApoE4 genotype is a risk factor that can lead to early onset of the disease. The general idea is that ApoE4 activity increases the concentration of the peptide Aβ decreasing in this way its clearance. At the same time, ApoE increases the activity of glycogen synthase kinase 3β (GSK3β), the kinase that cause tau hyperphosphorylation.

1.2.4 Neuroinflammatory hypothesis

The neuroinflammatory hypothesis states that inflammation plays a critical role in promoting the degenerative process of AD. Although inflammation represents the first line of defense against dangerous agents, a disproportionate response can increase the damage to neuronal cells and, thereby, allow to the progression of the disease.[21] Extracellular Aβ deposits and tau protein intracellular tangles induce microglial cells and astrocytes to the inflammatory response through the production of factors, such as cytokines (IL-1, IL-2, TNF), chemokines and ROS. Initially the microglial cells phagocytize and degrade Aβ and also astrocytes are involved in its elimination through receptor-mediated internalization and facilitating its transport out of the central nervous system in the bloodstream. Aβ plaques and activation of glial cells too, stimulate the intervention of the classical complement pathway.[22] Activated cells release proteins of the acute phase, alpha1-antichymotrypsin, alpha2-macroglobulin, C-reactive protein, which aggravate the disease.[23] The contradictory role of microglia (elimination of Aβ and release of pro-inflammatory molecules) complicate the treatment; it was reported that the use of nonsteroidal anti-inflammatory agents could reduce risk of the onset of the disease and will slow down the progression.[24] Their mechanism of action would include the reduction of $A\beta_{42}$, the inhibition of cyclooxygenase-2 or the receptor of prostaglandin E2, the stimulation of phagocytosis by microglia, and the activation of PPARγ (peroxisome proliferator-activated receptor gamma), which regulates the deposit of fatty acids and the glucose metabolism. [25]

1.2.5 The oxidative stress

Oxidative stress is a cause of neuronal death in AD (fig. 8). This is related to a mitochondrial dysfunction, caused by exposure to Aβ, which inhibits key mitochondrial enzymes and in particular seems to be affected cytochrome c-oxidase, the last enzyme complex involved in the electron transport, in addition to enzymes key of the Krebs cycle (α-ketoglutarate and pyruvate dehydrogenase) and mitochondrial DNA, which is damaged.[26] In patients, high concentrations of reactive oxygen species (ROS) are observed. Abnormal mitochondria lead to an increase in the concentration of hydrogen peroxide in the cytoplasm, the interaction of this with iron induces the production of free radicals.

This interaction is possible thanks to the higher concentration of free iron resulting from a decrease in the concentrations of ferritin observed in patients. Free radicals oxidize lipids and damage the membranes of the brain. The products of lipid peroxidation, e.g. the aldehydes formed by oxidation of polyunsaturated fatty acids, have a half-life longer than radicals. Thus, aldehydes can spread to other sites and react.

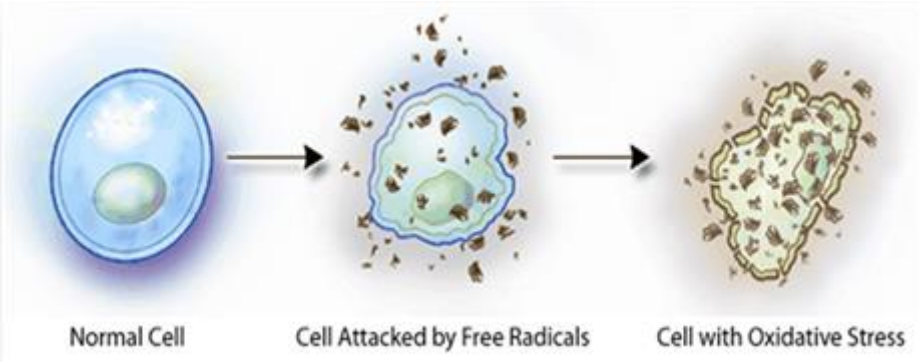


Figure 8: the oxidative stress

Free radicals oxidize proteins causing the loss of activity that leads to destabilization of the various systems and balances into neurons. High levels of oxidation products of DNA and RNA, that may have serious consequences on the function of ribosomes and protein synthesis, were also observed in neurons of patients. In addition to the disorder of iron homeostasis, there is also a change in the concentration of zinc and copper. Aβ peptide binds copper ions with high affinity, and after several redox exchanges, Cu^{2+} is able to reduce the oxygen and to generate H_2O_2.

1.2.6 Interconnected pathways in Alzheimer's disease

The Aβ monomers that are released outside neurons can be removed by microglia, which release insulin-degrading enzyme (IDE) that destroys them (fig. 9). This aberrant peptide increases the influx of Ca^{2+} by forming Ca^{2+} permeable channels in the plasma membrane. Aβ42 has high potential to aggregate to form Aβ oligomers, which rate of aggregation is also increased by the interaction of Aβ with the peripheral anionic site of AChE. Aβ oligomers can interact with metal ions Fe^{2+} or Cu^{+} activating the generation of reactive oxygen species (ROS).

These species damage the plasma membrane facilitating the influx of different ions resulting in membrane depolarization. Membrane depolarization and sub products of lipid peroxidation affect the function of different receptor and channels such as N-methyl D—aspartate receptors (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) voltage-dependent calcium channels (VDCCs). Aβ oligomers can affect also the activity of these receptors and channels directly. Presenilins (PS) function as Ca^{2+} leak channels at the endoplasmic reticulum (ER), mutated PS are highly related to familial AD. PS were found at ER membranes. Mutations in PS modify their ability to regulate Ca^{2+} in the ER enhancing Ca^{2+} release through ryanodine receptors (RyR) and

inositol triphosphate receptors (InsP3R) channels. There is also evidence that PS can interact directly with InsP3Rs, RyRs, and the SERCA pump to alter ER Ca^{2+} release and uptake.

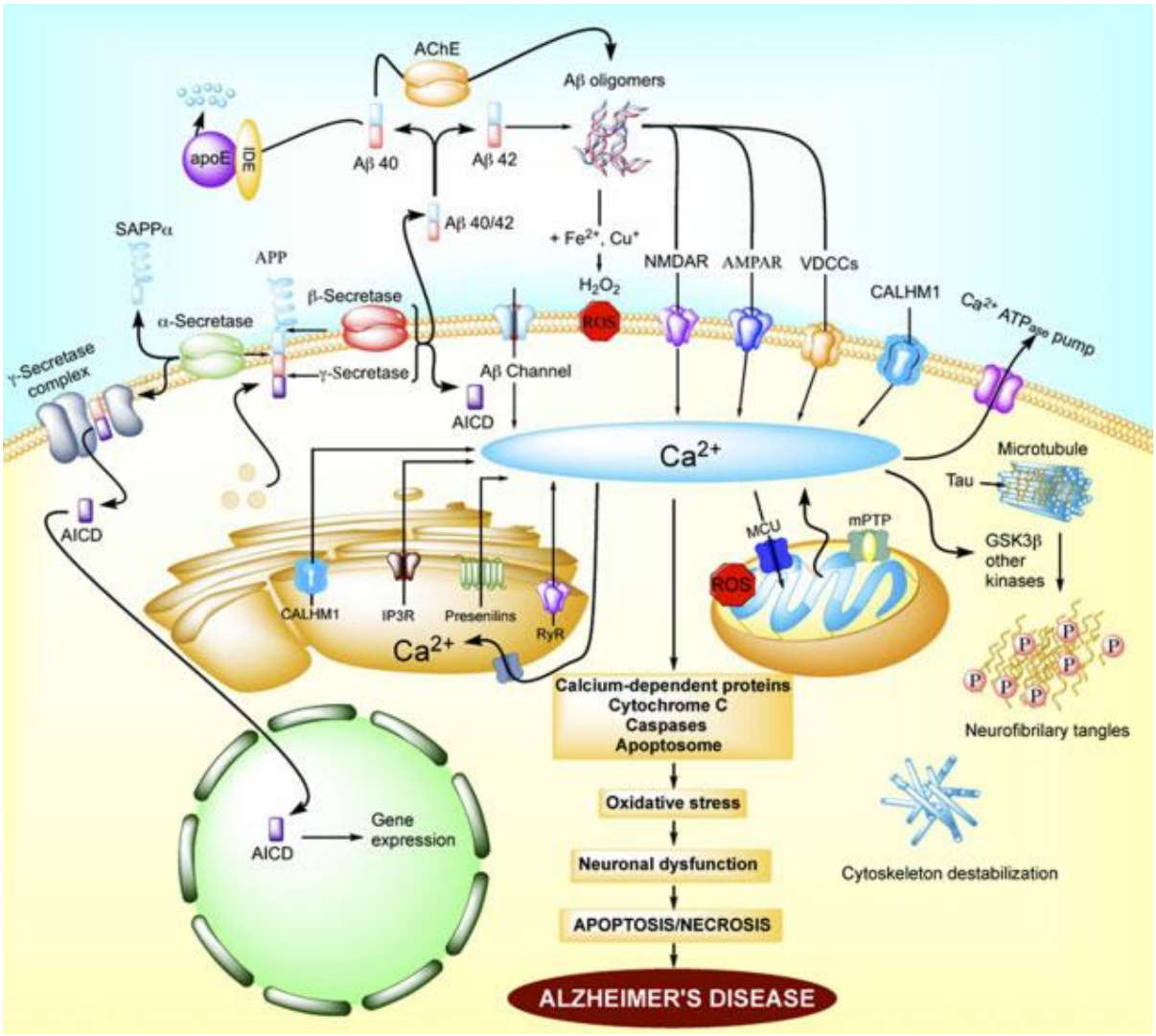


Figure 9: interconnected pathways

AICD migrates to the nucleus, interacts with transcription regulators, and modifies gene transcription to disrupt Ca^{2+} homeostasis.

Elevated Ca^{2+} can also affect the attachment/detachment equilibrium of protein Tau (τ) to tubulin to form the cytoskeleton. GSK3β and other kinases are involved in this equilibrium; Ca^{2+} can modify their activity inducing τ hyperphosphorylation to generate, after its aggregation, the neurofibrillary tangles. Aβ also affect mitochondria inducing oxidative stress and Ca^{2+} dysregulation resulting in an increase in the production of radicals and decreased production of ATP. High concentrations of Ca^{2+} can be stored by mitochondria through mitochondrial Ca^{2+} uniporter (MCU), resulting in Ca^{2+} overload of mitochondria leading to opening of mitochondrial permeability-transition pore (mtPTP) and apoptosis." [27]

1.3 Therapeutic approaches

Most of the drugs on the market acts on the cholinergic system that appears to be the most affected. First of all, considering that there is a decrease in ACh levels, the first approach was to restore physiological levels or, otherwise, to mimic the action at receptor level of this neurotransmitter. Cholinergic receptors in central nervous system are located in presynaptic and postsynaptic levels and they can be either of nicotinic or muscarinic type. These differ not only for the various types of ligands that are able to interact with them, but also for many other basic aspects, as a function, molecular architecture, localization and biological mechanisms associated with their activation. Nicotinic receptors are ionotropic and pentamers, constituted, in most cases, of different subunits classified as α and β. Presynaptic nicotinic receptors control the release of ACh and also of glutamate, serotonin and norepinephrine, that are important neurotransmitters for memory and behavior. Thus, the presence of agonists able to interact with these receptors would be pharmacologically useful.

Studies concerning the various receptor subtypes present in the central nervous system, show that best results were obtained with active substances against subtypes α4β2 and α7, designed by modifying the pyridine ring and pyrrolidine nicotine, specific agonist of nicotinic receptors. Despite their potential function, these are not used in therapy for several reasons, including lack of selective ligands for the various receptor subtypes, with consequent difficulty of differentiate therapeutic effects. Another potential approach is to modulate the action of metabotropic muscarinic receptors. Therefore they are membrane proteins associated with their effectors via a functional unit including a G protein. They are also characterized by a single polypeptidic chain that crosses the membrane seven times, forming in this way seven transmembrane domains. Five receptor subtypes, named M1, M2, M3, M4, are pharmacologically characterized. Subtype M5, has not yet been characterized from a pharmacological point of view, due to a lack of selective ligands and knowledge on its specific location. Among different compounds that can modify cholinergic neurotransmission, the only class of drugs that obtained from regulatory authorities the indication for the symptomatic treatment of AD are the cholinesterase inhibitors (ChEIs).[28] These drugs act by slowing the biochemical breakdown of acetylcholine and thereby, prolonging cholinergic neurotransmission. Of interest is that humans have two types of cholinesterases, AChE and BuChE. The physiological role of BuChE is unknown, but levels of this enzyme have been shown to increase as AD progresses and it contributes at the hydrolysis of acetylcholine while levels of AChE decrease. In AD patients, both enzymes are located in neuritic plaques, and their inhibition with ChEIs may modify the deposition of Aβ, a key component of the pathophysiology of AD. Tacrine was the first approved drug (1993) able to inhibit AChE. Thereafter, donepezil, galantamine and rivastigmine were approved (fig. 10). The latter three drugs are currently the most widely used for the treatment of mild to moderate stages of Alzheimer's although some studies suggested a small benefit also in patients suffering from more advanced stages of the disease. However, their clinical use is limited by many side effects such as confusion, hallucinations, mood swings and nausea, resulting from excessive activation of the cholinergic system. Donepezil is a reversible AChE inhibitor. It also binds PAS and decreases the beta amyloid

aggregation induced by AChE. Donepezil is metabolised in the liver by the cytochrome P450 isoenzymes CYP2D6 and CYP3A4 and byuridine-diphosphate glucuronosyl transferase, with extensive first-pass metabolism. It has a long half-life of elimination (70 h) and is assumed once daily. Rivastigmine is a slowly reversible (pseudoirreversible) inhibitor of both AChE and BuChE, has low plasma-protein binding (40%), and is hydrolyzed by esterases. It is not metabolized by cytochrome P450 and its short elimination half-life necessitates two doses daily. It can be orally or transdermally administered and it has good capacity to cross the blood-brain barrier; then, it performs its pharmacological action on both the central and the peripheral acetylcholinesterase.

Galantamine is a selective reversible AChE inhibitor and a positive allosteric modulator of nicotinic receptors on pyramidal neurons increasing the release of ACh. It is poorly bound to plasma protein (18%) and is metabolized in the liver via cytochrome P450 isoenzymes (mainly CYP2D6 and CYP3A4). With its terminal elimination of about 5h, two doses are required daily. To date the clinical relevance of this pharmacological heterogeneity is unknown.

Memantine was recently approved; it is a non-competitive NMDAR (postsynaptic receptors) antagonist and reduces glutamatergic excitotoxicity; it was developed considering the role of Ca^{2+} in the pathogenesis of Alzheimer's. Glutamate is an excitatory neurotransmitter in the brain and it is implicated in neurodegenerative disorders. However, excessive glutamatergic stimulation can cause neuronal damage, which leads to a pathological condition of neurotoxicity. Such excitotoxicity ultimately leads to neuronal calcium overload and has been implicated in neurodegenerative disorders. Glutamate stimulates various postsynaptic receptors, including the NMDA receptor, which has been particularly implicated in memory processes, dementia, and the pathogenesis of AD. However, it is not a ChEI and therefore, is different from other drugs currently used for AD treatment. The mechanism of action of memantine is a voltage-dependent, low-moderate affinity, uncompetitive NMDA receptor antagonism with fastblocking/ unblocking kinetics. The low-moderate affinity is important because other NMDA receptor antagonists, such as ketamine and amantadine, are high-affinity compounds with neuropsychiatric side effects. The fast on/off kinetics are also important because this means that memantine sits on the receptor just long enough to prevent pathologic activation of the glutamate receptors and then quickly goes away when physiologic activation of the glutamate receptors is needed.

Memantine blocks the effects of abnormal glutamate activity that may lead to neuronal death and cognitive dysfunction. The fast on/off kinetics and low-moderate affinity are the key of memantine action because it blocks the effects of excessive glutamate while preserves physiologic activation of NMDA receptors required for learning and memory. Like other NMDA receptor antagonists, memantine at high concentrations can inhibit mechanisms of synaptic plasticity that are supposed to underlie learning and memory. However, at lower, clinically relevant concentrations memantine can promote synaptic plasticity and preserve or enhance memory in animal models of AD. Moreover, memantine can protect against the excitotoxic destruction of cholinergic neurons.

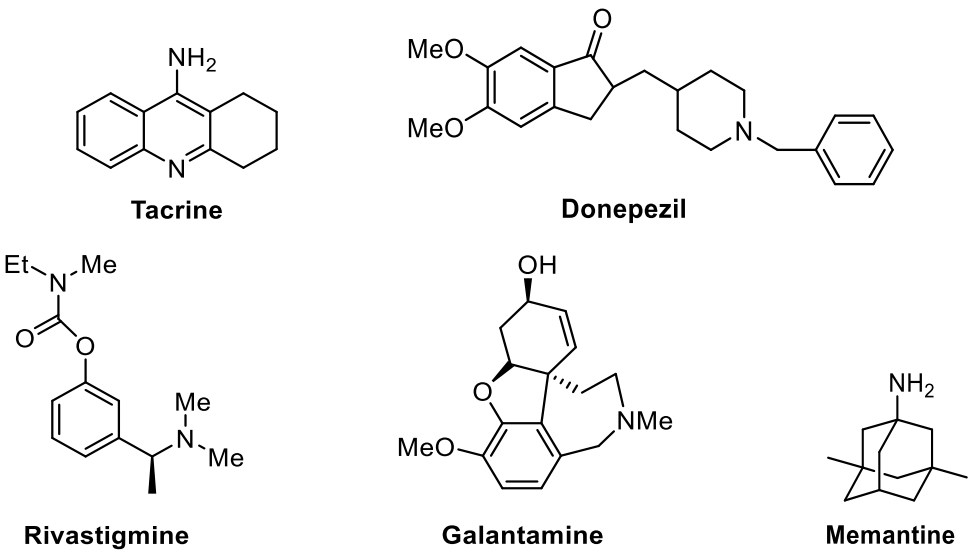


Figure 10

Inflammation and glial activation observed in patients of AD led to the idea that NSAIDs (nonsteroidal antiinflammatory drugs) might be important for the Alzheimer's treatment and especially due to its prevention. Other targets such as the GSK3β, BACE-1, γ-secretase were investigated for the development of neuroprotective and neurotrophic agents. In fact, other pharmacotherapeutic treatment of AD should be directed to the prevention of Aβ production, aggregation, toxicity or degradation, or a combination. Two main classes of disease-modification approaches can be defined, namely, the neurotrophic and neuroprotective strategies. Neurotrophic strategy includes hormone replacement therapy and drugs acting on insulin signal transduction. Neuroprotective (anti-amyloid strategies) include secretase inhibition, statins to lower cholesterol, neprilysin to promote Aβ degradation, metal chelators that block Aβ aggregation. and NSAIDs that reduce Aβ production. Some studies in cultured cells and Aβ precursor protein transgenic mice suggest that some NSAIDs associated with protection from AD (such as ibuprofen) can actually modulate γ-secretase processing of Aβ precursor protein to decrease Aβ protein 42 production without lowering overall Aβ protein levels or interfering with Notch cleavage.[29]

This unexpected finding could lead to derivatives of certain NSAIDs being used as a special type of ‘‘γ-secretase. Agents promoting clearance of Aβ from brain may also represent a promise as therapeutic intervention for AD. For instance, the removal of Aβ by active and passive immunization strategies results in clearance of Aβ from brain and improves performance in behavioral tests.

Various antioxidants, free radical scavengers, calcium-channel blockers, metal chelators, or modulators of certain signal transduction pathways might protect neurons from the downstream effects of accumulation of Aβ protein. Some studies suggest that high intake of vitamins C, E, B6, and B12, and folate, unsaturated fatty acids, and fish are related to a low risk of AD, but the results of different reports are inconsistent. Modest to moderate alcohol intake, particularly wine, may be related to a low risk of AD. Alpha-tocopherol (Vitamin E) is a lipid-soluble vitamin that interacts with cell membranes, traps free radicals, and interrupts the chain reaction that damages cells. Vitamin E is an antioxidant and it is thought to mitigate the inflammatory effects of plaque formation in the

brain. Selegiline, a monoamine oxidase inhibitor, similarly to alpha-tocopherol may have beneficial effects in patients with AD. Selegiline also increases levels of catecholamines, and adrenergic stimulation may improve cognitive deficits associated with AD. A number of studies have examined evidence for the use of selegiline, a selective monoamine oxidase inhibitor, in the treatment of AD.[30] As demonstrated by several hypotheses that were formulated with the attempt to define the molecular mechanisms of the disease, the multifactorial nature of AD leads to not focus the attention on a single therapeutic target. From a pharmacological point of view, the actual problem is that there are no drugs able to stop progression and worsening of the disease but, nowadays, all available treatments can only relieve symptoms.

1.4 New directions: MTLD strategy

Although the etiology of AD is not very clear, multiple factors, such as β-amyloid and τ protein aggregation, excessive metal ions (e.g. Cu^{2+}, Zn^{2+}, Fe^{3+}), oxidative stress and reduced acetylcholine level, have been considered to play important roles in the pathogenesis of AD. This provides various targets for screening AD-modifying drugs. Indeed, numerous synthetic or natural molecules have been screened to decrease Aβ production to prevent Aβ or τ aggregation, to chelate transition metals, to scavenge reactive oxygen species (ROS) or to inhibit acetylcholinesterase or monoamine oxidase (MAO). However, the success of the one-drug-one-target strategy (fig. 11, i.e. 1) is limited considering that it is not sufficient to effectively treat a multifactorial disease, which has stimulated the search for more efficient combined weapons to combat AD. [31] Moreover, at present, as assumed before, all treatments approved by the FDA are only palliative and they do not heal the disease.

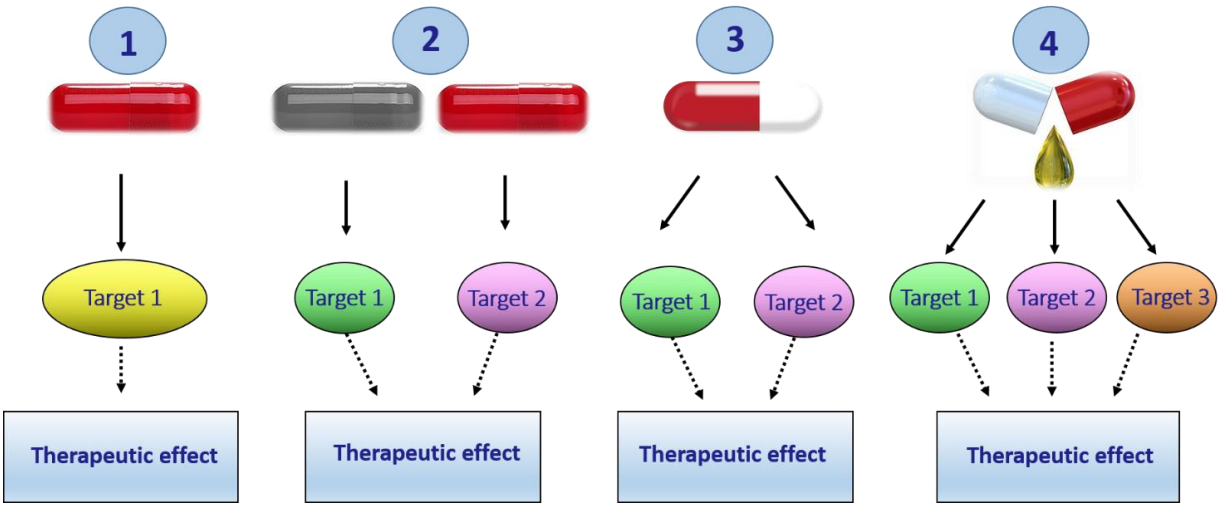


Figure 11: pharmaceutical strategies

To hit the multiple targets implicated in the complex diseases, three strategies are conceivable. One is called “MMT” (*multiple-medication therapy*) and it consists in a “cocktail” or “combination of drugs”;

the MMT (fig. 11, i.e. 2) can be formed by two or three different drugs combining different therapeutic mechanisms.[32]

However, this approach could be disadvantageous for patients with compliance problems; moreover, also pharmacokinetic interferences might appear. Another strategy is the “MCM” (*multiple-compound medication*), which incorporates two or more active ingredients in one single-pill drug combination (fig. 11, i.e. 3) which involves the incorporation of different drugs into the same formulation in order to simplify dosing regimens and improve patient compliance. This strategy has been successfully used in traditional medicine (in China and many other countries) for thousands of years and in current drug cocktails as well to suppress the spreading of HIV. The other attempts is to employ one compound to hit multiple targets: the multi-target-directed ligands (MTDL) strategy (fig. 10, i.e. 4). More in detail, the MTDL strategy consists in the combination of various pharmacophores of different already known drugs in order to obtain a new hybrid molecule able to interact with all selected targets. Although the latter strategy seems more convenient than the former, it is more difficult to be fulfilled. Nevertheless, the accumulating experience gained in the battle against AD displays the feasibility of the latter strategy. A therapy with a single drug with multiple biological properties would have several advantages over MMT or MCM that have different bioavailability, pharmacokinetics, and metabolism. In fact, if a single molecular species can show a complex ADMET profile, an MMT/MCM approach might be unpredictable. Considering pharmacokinetic and ADMET optimization, the clinical development of a drug able to hit multiple targets should not be different from the development of any other single lead molecule, offering a simpler approach than MMT/MCM; moreover, the risk of possible drug-drug interactions would be eluded.

Despite the in vitro success of synthetic multifunctional agents, the potential risks in safety, absorbability and pharmacokinetics is a big hurdle in their further development. Hence, it is exciting to note that some natural products also hold multiple functions. I. e. the beneficial effect of curcumin to prevent AD has been shown by transgenic mouse experiment and epidemiologic investigation that AD prevalence is only 1% in people over age 65 of rural India, where turmeric is commonly used in food. The AD-preventing mechanism of curcumin was naturally related to its well known antioxidant and anti-inflammatory activities. Besides curcumin, flavonoids, such as gossypetin, (-)-epicatechin- 3-gallate and myricetin, are pleiotropic natural products too. They have long been known as excellent ROS scavengers endowed with high metal-chelating ability. The different structures of curcumin and flavonoids suggest that the structural requirements to fulfill the multifunction are diverse. Thus, it can be expected to find more candidates from natural product libraries that can be regarded as a multifunctional pharmacophore, which means that the multiple targets in AD can be hit not only by one compound but also by one pharmacophore.

1.5 New targets: the endocannabinoid system

The endocannabinoid system (ECS) is a complex endogenous system of communication among cells. It is composed of cannabinoid receptors, their endogenous ligands and proteins

involved in the metabolism and transport of endocannabinoids themselves. This system is essential for the normal functioning of the body and it has been identified in mammals, fish, shellfish, amphibians, birds and leeches. It plays a key role in neuronal development as well as in neurodegenerative processes. Endocannabinoids are a class of bioactive lipids, which includes amines, esters, and ethers of long chain fatty acids with the mutual ability to bind to cannabinoid receptors. Phytocannabinoids indicate a class of chemical compounds present in Cannabis sativa from which is named the entire system. Natural phytocannabinoids are a class of aromatic hydrocarbons containing oxygen and until today over 60 of them have been identified; their distribution varies in different cannabis strains and usually only three or four cannabinoids are found in a plant at a concentration of 0.1%. ECS constitutes a neuromodulator system that can regulate neuronal excitability by inhibiting communication through tight junctions or through interactions with GABA, serotonergic, glutamatergic and dopaminergic transmissions. According to the localization of cannabinoid receptors it has been hypothesized that the system is involved in a large number of physiological processes[33] especially in the central nervous system (CNS), where it acts as a neuromodulator mediating the effects of psychoactive constituent of Cannabis Δ^9-tetrahydrocannabinol (THC), but also in the immune, cardiovascular and gastrointestinal systems.[34] Nowadays, THC and its analogs are used to treat nausea and vomiting caused by chemotherapy and radiotherapy and also in patients with AIDS.

Regarding endogenous compounds, the first to be identified in 1992, is anandamide (AEA), followed by 2-arachidonoylglycerol (2-AG). More recently at least three other endogenous cannabinoids have been identified: the 2-arachidonyl glyceryl ether (noladin, 2-AGE), a structural analogue of 2-AG, the Virodhamine and N-Arachidonoyl dopamine (NADA). AEA and 2-AG (fig. 12) mimic some of the pharmacological effects of THC. Cannabinoid receptors CB_1 and CB_2, the endogenous ligands endocannabinoid (EC) anandamide and 2-arachidonoylglycerol and their degradative enzymes fatty acid amide hydrolase (FAAH) and monoacylglyceride lipase (MAGL) are key elements of the ECS implicated in several physiological functions including cognitive, motor activities and immune response.

Endocannabinoids are produced inside cells through multiple synthetic routes and differ from other neurotransmitters: they are not stored inside the vesicles, but are produced on-demand from membrane phospholipid precursors. The process of biosynthesis is activated by a stimulus that causes the depolarization of the cell membrane. The lipid precursor that is processed for the synthesis of anandamide (AEA) is the N-arachidonoyl-phosphatidylethanolamine (NArPE), on which, first the N-acetyltransferase and then a phospholipase D type act in a sequential manner; the process ends with the release of AEA and phosphatidic acid (PA). The biosynthetic pathways that lead to the formation of 2-arachidonoylglycerol (2-AG), can follow different mechanisms, but the most probable hypothesis consists in the formation of di-acyl-glycerol that then is hydrolyzed to 2-AG through the action of a phospholipase C-type. Synthesized endocannabinoids are immediately released from the cell in order to bind receptors on neighboring cells or on the same cell that produced them, thus acting as both paracrine and autocrine mediators. In particular, it has been

suggested that endocannabinoids behave as retrograde messengers: after being synthesized in the postsynaptic cell, they would activate the receptors of the axons of the presynaptic cell.

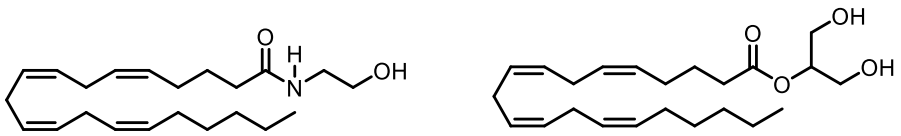


Figure 12: AEA and 2- AG.

The inactivation of these compounds involves different enzymatically regulated mechanisms: their reuptake can be done thanks a passive diffusion across the membrane or through a specific carrier or they may undergo hydrolysis mediated by a specific enzyme. The AEA is inactivated by a reuptake mediated by the AMT (AEA membrane transporter) and it is subsequently enzymatically degraded by the action of FAAH, which releases arachidonic acid and ethanolamine. In addition to hydrolysis mediated by FAAH, the AEA is also metabolized by other enzymes such as COX-2 and the complex of cytochrome P450.[35] The 2-AG too is inactivated by reuptake mediated by the AMT or by a passive diffusion across the membrane and by subsequently enzymatic degradation caused by MAGL, like the other monoacylglycerols, which releases arachidonic acid and glycerol. As for the AEA, other enzymes such as COX2 can also intervene.

1.5.1 CBs receptors

Endocannabinoids and cannabinoid receptor type 1 and 2 (CB1, CB2) play a significant role in physiological and pathological processes, including cognitive and immune functions.[36] There is a growing appreciation of the therapeutic potential of cannabinoids in many pathological conditions involving the chronic inflammation. The two receptors have different physiological properties. The psychoactive effect is associated with cannabinoid CB1 receptor, while CB2 receptor has primarily anti-inflammatory, and immunomodulatory actions. CB1 receptor was discovered by Devane and coworkers in 1988. It is widely distributed in regions of the brain, particularly the frontal cortex, hippocampus and amygdala, sensory and motor areas and hypothalamus. Instead, CB2 receptor was isolated from human myeloid cells in 1992; between the two receptors, there is a structural homology of 44%. The endocannabinoid receptors belong to the superfamily of G protein-coupled receptors (GPCRs); they have an amino terminal extracellular extremity, seven transmembrane α-helices and a carboxy terminal intracellular extremity. The most important difference between the two receptors is their interaction with the lipid-surrounding environment which is in the TM7domain. CB1 receptor has a hydrophobic surface that is suitable for the specific interaction with cholesterol and palmitic acid, while CB2 receptor shows a region of negative charge which is quite unfavorable to any lipid interaction, although they present the ability to be activated by the same endocannabinoids and they may cause the same signal. The differences between the two receptors could be exploited to develop specific ligands.

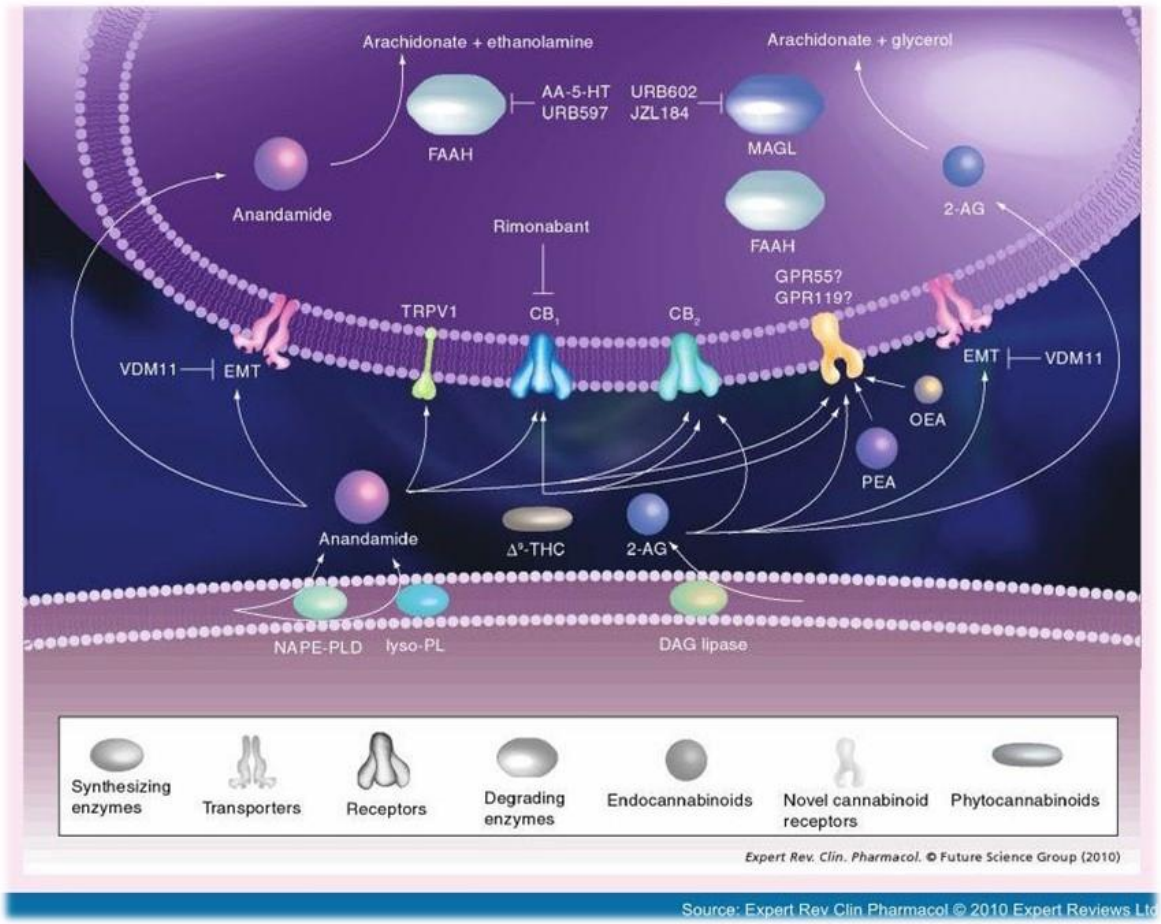


Figure 13: cannabinoid system[37]

It was also identified a third type of receptor, CB3, present in the brain and endothelial tissues but it still has not been well characterized. CB1 cannabinoid receptors are the most abundant and widely distributed GPCRs in brain especially in the basal ganglia, globus pallidus and substantia nigra and, to a lesser extent, in the cerebellum, hippocampus, caudate nucleus, putamen, hypothalamus and amygdala. They have also been identified, but with lower density, in lungs, liver, kidneys, and in both male and female cells of the reproductive system. CB1 receptors are absent in the medulla oblongata, the part of the nervous system that governs the control of respiratory and cardiovascular functions. The role of the receptors is essentially to regulate the release of other neurotransmitters, in fact, the activation of CB1 protects the CNS from overstimulation or extra-inhibition caused by other neurotransmitters. As for the CB2, it was thought that they were almost exclusively on T cells of the immune system, with the highest density in the spleen. However, recent studies have shown their presence also at the level of the central nervous system, in particular at the level of glial and microglial cells.

The stimulation of CB2 receptors appears to be primarily responsible for the anti-inflammatory and immunomodulatory activity of cannabinoids, through the regulation of cytokine release. Anandamide and NADA do not only bind to cannabinoid receptors but as capsaicin, an active ingredient in hot peppers, have the ability to bind the vanilloid receptors (TRPV1) (fig. 13).

As mentioned before, CB1 and CB2 receptors are G-protein coupled inhibitory; in fact their activation involves inhibition of adenylate cyclase, ion channel voltage-dependent Ca^{2+} N, P/Q and L type. The action on these channels and on adenylate cyclase is mediated by the α subunit of the G protein. In particular, the activation of CB1 receptors blocks N type presynaptic calcium channels reducing the synaptic transmission. It is also probable that the THC induces some of its symptoms at cognitive level thanks this molecular mechanism.[38] It was then suggested that activation of these receptors plays a role in neurogenesis, synaptogenesis and axonal growth even if the molecular mechanisms involved in these processes are not yet completely understood. [39] The adenylate cyclase inhibition and the consequent decrease of cAMP also leads to a decrease in the activity of the protein kinase A (PKA), which leads to a decrease in the phosphorylation of the type D potassium channels. The effects of the activation of these receptors include, depending on the dose, the cell type and the state of the organism, euphoria, anxiety, dry mouth, muscle relaxation, hunger and pain reduction. This means that they not only block the effects of endocannabinoids but they also produce opposite effects compared to agonists, such as an increased sensitivity to pain or nausea, suggesting that the cannabinoid system is tonically active. This tonic activity may be due to a constant release of endocannabinoids by a portion of the receptor that is in a constantly active state. CB receptor antagonists are in clinical use for the treatment of obesity and are being tested for the treatment of addiction to nicotine and other. CB1 receptor agonists should not overcome the blood-brain barrier, in order to avoid the psychotropic action, and the development of compounds that influence the levels of endocannabinoids through the inhibition of their membrane transport or hydrolysis (as inhibitors of FAAH). For example, blockers of anandamide hydrolysis are able, in animal tests, to reduce anxiety, pain, tumor growth and colitis. Currently, for the medical use, two cannabinoid receptor agonists are available, dronabinol and nabilone and a cannabis extract (Sativex®). In some countries, the possession of small amounts of cannabis for recreational use or treatment is allowed or tolerated, as in the Netherlands, Spain, Belgium and some regions of Switzerland. Dronabinol is a synthetic Δ^9-THC, the primary psychoactive cannabis which is used to treat nausea and vomiting associated with cancer chemotherapy who do not respond adequately to conventional antiemetic treatments. Rimonabant, with Acomplia trade name, a cannabinoid receptor antagonist, is instead used for the treatment of obesity but it was withdrawn from the market owing to its side-effects. The high concentration of CB1 and the presence of AEA and 2-AG in regions of the brain involved in memory processes are in line with the idea that ECs modulate cognitive processes. The activation of these receptors reduces the ACh levels in the hippocampus, followed by a cognitive decline, suggesting a function of modulation on ACh release by CB1 receptors.

Many neurodegenerative diseases are associated with chronic inflammation from the activation of microglial cells. Considering that increased proliferation of microglial cells in the brain of patients affected by AD, has deleterious effects on surrounding neurons, many studies focused the attention on factors that mediate the activation of microglial cells. In this scenario, CB2 receptors seem to play a crucial role in inflammatory processes that affect the brain. It is proved that activation of CB2 receptors causes immunomodulatory effects that lead to many changes in the production of substances related to inflammation. The ability of compounds selective for the CB2 receptors in

reducing inflammation, can be explained by increasing proliferation and recall of immune cells involved in immune-mediated repair of damaged tissues. Thus, CB2 receptors expressed on leukocytes in the SCN may be activated by EC which modulates the progression of neuroinflammation. Cannabinoids modulate the immune response during inflammatory processes. Several animal studies showed that cannabinoids exert their immunomodulatory properties in four different ways: the induction of apoptosis, suppression of cell proliferation, inhibition of the production of pro-inflammatory cytokines and the increase of anti-inflammatory cytokines and the induction of T regulatory cells. The activation of CB2 triggers apoptosis in immune cells; this suggests that this receptor may be the target of a new therapeutic approach for the treatment of inflammatory and autoimmune diseases. Several studies demonstrated that the expression of CB2 receptor is upregulated during neuroinflammation and that agonists of this receptor reduce the permeability of the blood-brain barrier through the increase in the expression of tight junctions.

1.5.2 Endocannabinoid system and Alzheimer disease

The probable neuroprotective role of ECs and their modulating action on neurotransmitter systems affected by neurodegenerative diseases such as Alzheimer's, Huntington's disease and multiple sclerosis are under investigation. Increasing data show an imbalance in the system CEs (for example, a decrease of neuronal CB1 receptors, an increase in glial CB2 receptors and an over expression of the enzyme FAAH in astrocytes) in experimental models of AD and in post-mortem brain tissue of AD patients. The central role of ECs in several diseases of the CNS, such as mood disorders and neurodegenerative diseases is confirmed by the high expression of cannabinoid CB1 receptors in brain areas such as the cortex, cerebellum, hippocampus and basal ganglia affecting cognition, motor activities and satiety.[40] The EC system plays a dual role in the pathophysiology of AD, the first related to its inhibitory action on the release of acetylcholine and the second related to its anti-inflammatory action in the CNS.

Recent studies focused the attention on the possible protective role of ECs in limiting microglia activation and inflammation in AD in fact microglia activation leads to in vivo and in vitro neurodegeneration and also AD transgenic mouse models develop plaques characterized by deposits of Aβ and activated microglia. The role of ECs is important in the modulation of immune function and inflammation; endocannabinoids and Δ^9-THC affect memory as well as neurochemical substrates of the acquisition and consolidation of memory, such as long-term potentiation. Cannabinoid receptors are located in immune cells where their expression is modulated by stimuli that induce immune activation, although it has been suggested a cannabinoid-independent mechanism. Recently, Esposito et al. showed that CB2 is able to attenuate the inflammatory responses caused by Aβ in neurotoxicity Aβ models, suppressing IL-1β and inducible nitric oxide synthase (iNOS). Although there are still doubts, all components of the EC system that act on different nervous cells or neurons and astrocytes, may play a role in normal and pathological conditions.

The central role of ECs in several diseases of the CNS, such as mood disorders and neurodegenerative diseases, is confirmed by the high expression of cannabinoid CB1 receptors in brain areas such as cortex, cerebellum, hippocampus and basal ganglia that affect cognition, motor activities and satiety. Endocannabinoids, showing an anti-inflammatory and antioxidant action, as well as a protective action against excitotoxicity and improvement of neurogenesis, may have beneficial effects on Alzheimer's. The involvement of the endocannabinoid system in AD is supported by a number of studies done on post-mortem brains of patients that showed a nitrosylation and up-regulation of cannabinoid receptors in microglia. Some studies demonstrated that cannabinoids can contribute for a neuroprotective effect against toxicity induced by Aβ considering that the CB1 receptors are related to the processes of learning and memory and the block of the latter by SR141716A, CB1 antagonist, seems to reduce amnesia induced by Aβ plaques in mice.[41]
In addition, also membrane transport or FAAH and MAGL enzymes inhibitors (that regulate the levels of cannabinoids in the brain) may play an important role for the protection against neurodegeneration induced by Aβ. Recent studies showed that Δ^9-THC inhibits AChE and prevents the aggregation of the peptide induced by the enzyme with a link with the peripheral site. The neuroprotective effect is explained by the anti-inflammatory action that these compounds exert in the CNS. In fact, CB receptors are located on the membrane of cells belonging to the immune system, such as leukocytes; therefore, the protective effect is supported by the fact that CB1 receptor agonists, such as anandamide and noladin are able, in vitro, to reduce the toxicity induced by the peptide and that this effect is reverted by receptor antagonists. The high concentration of CB1 receptors and the presence of AEA and 2-AG in regions of the brain involved in memory processes leads to the hypothesis that this system could play an important role in cognitive processes. The activation of these receptors reduces the levels of ACh in the hippocampus, followed by a cognitive decline, therefore their block has a positive effect, as demonstrated by studies on rats which rimonabant and donepezil were administered.
In AD the expression of CB1 receptor is reduced and it plays a role in the preservation of cognitive function. In addition it was found that the levels of anandamide and its precursor lipid are reduced in the frontal and temporal cortices.
The neuroprotective effect of CB2 agonists is associated with suppression of the activation of microglia, inhibiting the release of neurotoxic factors and decreasing neuronal damage. The expression of the CB2 is connected with cortical levels of Aβ42 and with the rate of senile plaques; they are upregulated in both the brain treated with Aβ, both in the brain suffering from AD. So, it has been hypothesized that, in people affected by AD, overexpression of CB2 in microglial cells can be regarded as an anti-inflammatory response of the CNS in order to protect neurons from degeneration inducing an increase in the proliferation and recruitment of immune cells.
Regarding the modulation of FAAH, both AEA that 2-AG, even if to a lesser degree, are substrates for the enzyme and its degradation leads to the release of arachidonic acid which itself can be metabolized to obtain pro-inflammatory molecules, such as prostaglandins. Therefore, inhibition of this enzyme may be beneficial in the prevention inflammatory processes associated with the

deposition of Aβ. Is important to underline that in brain areas rich in amyloid plaques, levels of FAAH are increased.

1.5.3 FAAH enzyme

Fatty acid amide hydrolase (FAAH) enzyme, responsible for the hydrolysis of endogenous ligands of the CB1 and CB2 receptors, was purified and sequenced by Scripps group in 1996; it was first isolated from rat liver through its ability to catalyze the hydrolysis of the sleep-inducing lipid, oleamide. FAAH is a membrane-bound enzyme and it belongs to the family of amidase proteins. They are characterized by a highly conserved region that is rich in serine, glycine and alanine residues. This region is common for more than 80 amidases and it corresponds to amino acids 215–257 in mammalian FAAH.

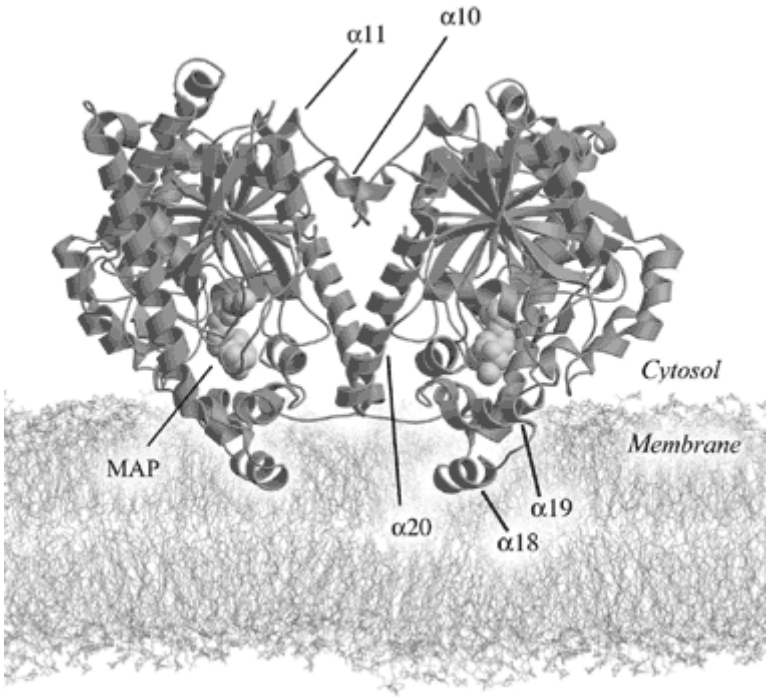


Figure 14: Structure of the integral membrane protein FAAH[42]

FAAH is generally required for inactivation of endocannabinoids in the brain. The mouse and human FAAH genes, each composed of 15 exons, are localized on chromosomes 1 and 4, respectively. Recently, the first 2.8 Å X-ray crystal structure of FAAH in a complex with the irreversible inhibitor methoxy arachidonyl fluorophosphonate revealed several unusual features of the enzyme. First, the catalytic core of FAAH is composed of a serine–serine–lysine catalytic triad (Ser^{217}, Ser^{241}, Lys^{142}), in contrast to the serine–histidine–aspartate triad typical of most serine hydrolases. These catalytic residues are located deep within the enzyme and can be reached through two narrow channels; one

of these is wider and more hydrophilic, thanks to the presence of polar residues, instead the other one is longer, narrower and more hydrophobic.

These results are consistent with previous enzymological studies indicating that S241 and K142 play key catalytic roles as the nucleophile and acid/base, respectively. The structure of FAAH (fig. 14) also revealed that this enzyme possesses a significant collection of channels that could allow simultaneous access to both the membrane and cytoplasmic compartments of the cell, probably to facilitate product release, substrate binding, and catalytic turnover. [43]

These uncommon mechanistic and structural features of FAAH inspired new strategies for the design of specific inhibitors (i.e. URB 597) that display high selectivity for this enzyme relative to the hundreds of serine hydrolases present in the human proteome. Despite biochemical and cell biological studies supporting a role for AEA as an endogenous CB1 agonist, the behavioral effects caused by AEA are weak and brief compared with those produced by Δ9-THC. The limited pharmacological activity of AEA is may be due to its rapid catabolism *in vivo*, considering that it is hydrolyzed to arachidonic acid within minutes of exogenous administration. However, the relative contribution made by FAAH to the hydrolysis of anandamide *in vivo* was largely unclear until it was cloned and a mouse model was generated in which this enzyme was genetically deleted (FAAH-knockout).

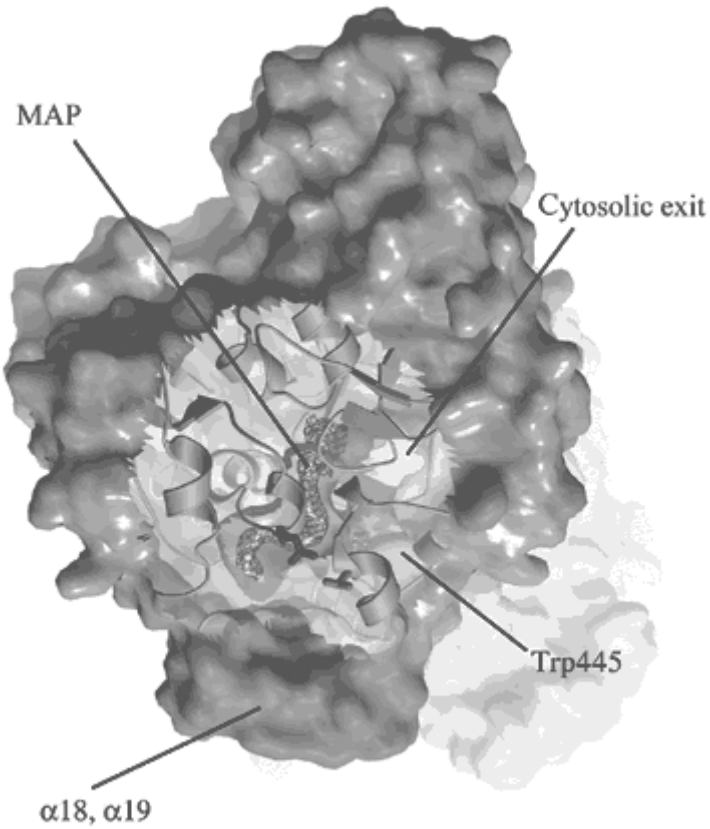


Figure 15: Active site of FAAH in complex with the arachidonyl inhibitor methoxy arachidonyl fluorophosphonate (MAP)

Tissues from FAAH- KO mice were found to display a 50–100-fold reduction in hydrolysis rates for AEA and related FAAs. In contrast to FAAH wild-type mice, in which administered AEA failed to produce important behavioral effects, FAAH-KO mice showed significant responses to AEA, becoming hypomotile, analgesic, cataleptic, and hypothermic.

Additionally, all of the behavioral effects of AEA in FAAH-KO mice were blocked by pre-treatment with the CB1 receptor antagonist SR141716A (rimonabant), indicating that AEA acts as a potent and selective CB1 agonist in these animals. Moreover, AEA in brain homogenates from FAAH-KO mice showed about 15-fold higher apparent binding affinity for the CB1 receptor. These FAAH-KO mice have been shown to have elevated levels (10–15-fold) of AEA and other *N*-acylethanol-amines (NAE) in several brain regions, including the hippocampus, cortex, and cerebellum; those levels are correlated with a CB1-dependent reduction in pain sensation in FAAH-KO mice. Considering this information, FAAH is a key enzyme involved in the catabolism of AEA and other NAEs *in vivo* and suggest that pain pathways are under the influence of a FAAH-regulated endocannabinoid tone. In mouse brain, FAAH is mostly associated with neurons. FAAH appears not to be limited to neuronal somata but also extends into the areas surrounding the neural tissue. This is most clearly seen in the hippocampal formation where, although the highest concentration of FAAH is clearly located in the somata of hippocampal pyramidal cells and granule cells of the dentate gyrus, there is also a widespread presence of FAAH in the adjacent layers that contain their axons and dendrites.[44]

Recently, there has been an explosion in number and quality of FAAH inhibitors available for pharmacological evaluations. The majority of evidence from animal models indicate a pharmacological analgesic action associated with these compounds, with different structures and mechanisms in different models of pain. It was also demonstrated that the analgesic effect can be achieved at lower doses than those required to produce sedation and catalepsy, typically associated with the CB1 agonists with analgesic action. Other potential indications for these compounds are still being discussed and we were still unsure about the models of anxiety and depression.

FAAH is present in neuronal and glial elements and shows significant overlap with CB1 receptors, especially in areas related to cognitive processes which are involved in extinction learning. The FAAH enzymes along with the CB2 receptors modulate inflammation because the protein and the FAAH activity, along with the CB2 receptors are overexpressed in glial cells related to inflammatory processes typical of AD. Since AEA and 2-AG are partially FAAH substrates, and are converted to arachidonic acid, the high presence of FAAH in astrocytes surrounding the neuritic plaques suggests that astrocytes, through FAAH, could be a significant source of arachidonic acid and correlated proinflammatory substances around these plaques. Thus, inhibition of FAAH may be beneficial in the prevention of inflammatory processes associated with the deposition of Aβ.

1- PROJECT 1

2.1 Aim of the project: coumarin-based molecules

The mechanisms underlying AD are not completely clear yet, but genetic, pathological and biochemical clues suggest that dysfunction of the basal acetylcholine (ACh) forebrain signaling, deposits of β-amyloid protein, τ hyperphosphorylation, oxidative damage and neuroinflammation play a significant role in the disease. Considering the multifactorial nature of Alzheimer disease and that only palliative treatments are available to date,[45,46] we decided to apply a multi-target-directed ligand strategy in order to tackle the disease from different paths, considering AChE, BuChE, BACE-1, FAAH, Aβ aggregation and CB receptors as targets.

The first strategic line that we adopted concerns carbamates that are usually recognized as cholinesterases (AChE and BuChE) inhibitors and are also well known in literature as FAAH inhibitors. The research group with whom I did my PhD, has been involved for many years in the development of carbamate ChEIs.

Taking into account this information and considering the recent finding that the eCB anandamide and some of its congeners potently inhibit human plasma BuChE, a library of carbamates with the general formula shown in figure 16, that was already proven as AChE inhibitors and that was already available in house, was tested on the new target FAAH.

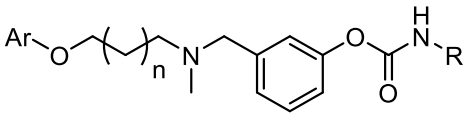


Figure 16

Tested molecules were constituted of various natural inspired heterocyclic rings like cumarine, chromone, flavone, xanthone, azaxanthone; they had different lengths of the spacer chain (from 2 to 12 carbon atoms) and the carbamic function was formed ranging from a methyl group to a long chain and a morpholine was also inserted.

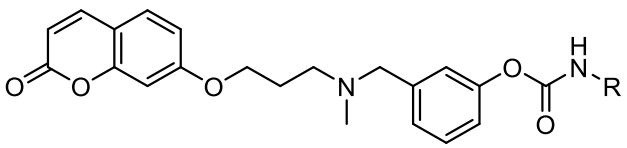
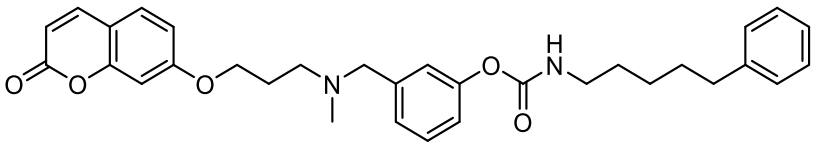


Figure 17

The biological results allowed to establish some important structure−activity relationships (fig. 17): among the different aryl groups (Ar), coumarin, azaxanthone, and xanthone were preferred; the optimal chain length (n) seemed to be of three methylene units; the variation of the length of the carbamic N-substituent has a significant effect on the activity of the inhibitors, longer chains producing the most active compounds.

Starting from these results, considering compounds with the general formula showed in figure 17 (with the cumarine nucleous and the three methylenes spacer) as the most active ones, the main goal was to find the optimal carbamic group for FAAH. Focused on this issue, a lead (fig. 18) with activity in the nanomolar range on AChE and FAAH was obtained. Furthermore, it showed to be a selective BuChE inhibitor and one of the most potent compounds known to date.



LEAD

AChE IC_{50}= 74.9 nM BuChE IC_{50}= 1.57 nM FAAH IC_{50}= 50 nM

Figure 18

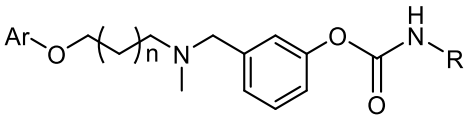
This dual activity could make it suitable to effectively counteract the progression of AD or disorders characterized by defective cholinergic and endocannabinoid signaling.

On the basis of previous research work, focused on the development of FAAH and cholinesterase inhibitors[47] combining all these information and the first promising results, the aim of this thesis was the optimization of the activity of the lead in order to both increase the potency and balance the biological activities towards the three targets.

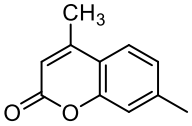
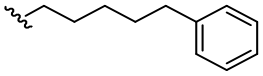
2.2 Series 1

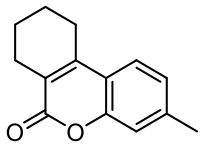
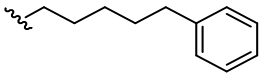
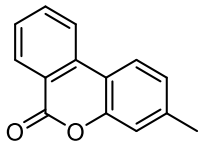
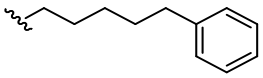
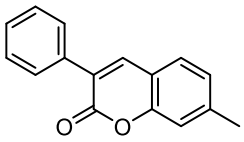
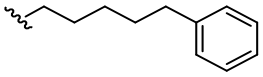
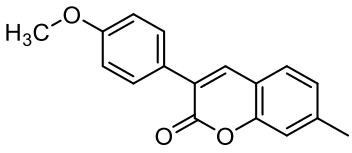
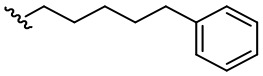
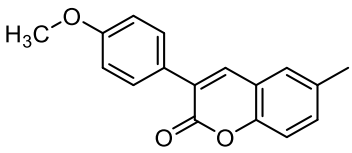
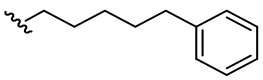
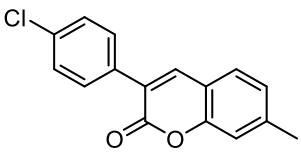
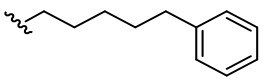
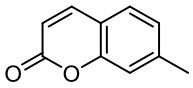
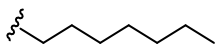
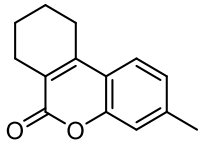
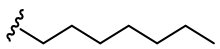
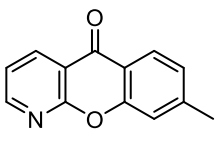
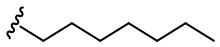
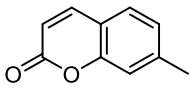
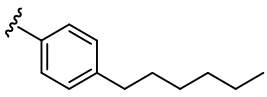
New carbamate-based molecules, able to contrast the three enzymes (cholinesterases and FAAH), were designed and synthesized, with the aim of obtaining multi-target compounds. A library of 16 compounds (table 1) substituted with natural inspired heterocycles, different lengths of the spacer chain and various carbamates has been synthesized, in order to explore the chemical space of the enzymes and determine the structural requirements necessary for high activities.

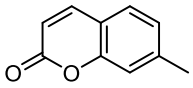
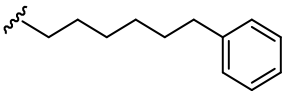
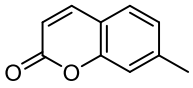
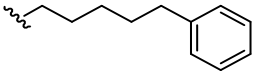
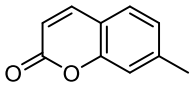
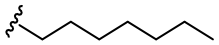
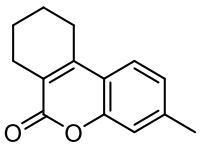
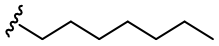
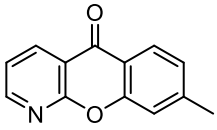
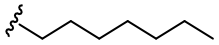
TABLE 1



1-16

	Ar	n	R
1		**1**	

2		**1**	
3		**1**	
4		**1**	
5		**1**	
6		**1**	
7		**1**	
8		**1**	
9		**1**	
10		**1**	
11		**1**	

12		**1**	
13		**3**	
14		**3**	
15		**3**	
16		**3**	

2.3 Biological results series 1 and discussion

Biological results of inhibitory activities against AChE, BuChE and FAAH are collected and showed in table 2.

TABLE 2

Compound	IC_{50} hAChE (nM)	IC_{50} hBuChE (nM)	IC_{50} on FAAH (nM) n≥2
1	80.5 ± 4.7	2.75 ± 0.13	134.93±5.49
2	89.9 ± 1.8	1.44 ± 0.06	49.09±38.22
3	390 ± 24	1.19 ± 0.03	312.17±60.34
4	125 ± 15	2.06 ± 0.08	249.54±91.49
5	312 ± 23	1.30 ± 0.07	322.09±36.21
6	179 ± 12	1.15 ± 0.06	210.69±21.62
7	900 ± 49	3.51 ± 0.16	265.53±112.49
8	37.4±1.9	1.36±0.07	28.55±9.74
9	21.6 ± 0.7	0.271 ± 0.007	306.41±65.96
10	13.0 ± 0. 6	0. 696 ± 0.020	202.14±1.14
11	3800 ± 540	32.1 ± 2.9	>50000
12	109 ± 5	2.85 ± 0.14	529.69±166.48
13	88.1 ± 4.0	1.23 ± 0.10	120.19±38.49
14	11.3 ± 4.9	0.787 ± 0.044	241.90±38.00
15	18.3 ± 0.8	0.277 ± 0.010	109.07±6.12
16	6.92 ± 0.58	0.534 ± 0.032	178.35±15.13

These data highlight some important structure−activity relationships. Regarding AChE, the best results were obtained, in ascending order, with the nuclei of the basic coumarin (**8**, **13**, **14**), 7,8,9,10-tetrahydro-6H-benzo [c] chromen-6-one (**15**) and azaxanthone (**16**). Undoubtedly, the best carbamic function was the one with long linear chain (**8-10**, **14-16**); in addition, the spacer chain with five methylene units provided greater activity, probably allowing for the compounds to bind into the gorge (**14-16**). The same goes for BuChE, towards which compounds showed highs activity than AChE probably due to the physical properties of the enzyme that, with the channel of greater size, allows the molecules to more easily reach the catalytic site. In particular, compounds **9-10** and **14-16** showed an inhibitory activity toward BuChE in the subnanomolar range.

Regarding FAAH, all compounds are less potent than the lead, except compound **8**, with the coumarin core and the carbamate with long linear chain; perhaps these features allow for an easier entry of the carbamate in the binding pocket.

Resuming, **8** boosted the activity at each selected target showing balanced inhibitory activity and increased potency against AChE and FAAH by about a half more if compared to the starting lead compound.

For these reasons, we decided to consider compound **8** as the new lead and to continue studies about activity optimization.

2.4 FAAH docking studies series 1

Thanks to the group of Marco Mor in Parma, a docking study of the most active compound **8** on FAAH was performed, taking into account both the neutral and the charged form of the amino group. Different conformations of the compound generated through a conformational search have been docked into the B chain of FAAH dimer (PDB ID: 1MT5; chain A blue cartoon; chain B white cartoon).

The docking simulation was performed using the Induced Fit Docking protocol of the Schrödinger suite. Figure 19 represents one of the best poses obtained for compound **8** in its neutral form (green sticks). Docking of **8** showed that the position of the carbamate group is stabilised by polar interactions within the oxyanion hole, in proximity of the catalytic triad. While the alkyl moiety of **8** occupies a portion of region corresponding to that of the arachidonyl chain of MAP, co-crystallised with FAAH, the chromenonic head of the molecule spans within the so called "cytosolic port", a cavity characterised by polar amino acids situated at the interface of chain A and chain B.

The figure on the left clearly shows that in the docking pose the carbamate group of **8** makes hydrogen bonds with residues I238 and M191, while the neutral amino group interacts with T236 forming a hydrogen bond.

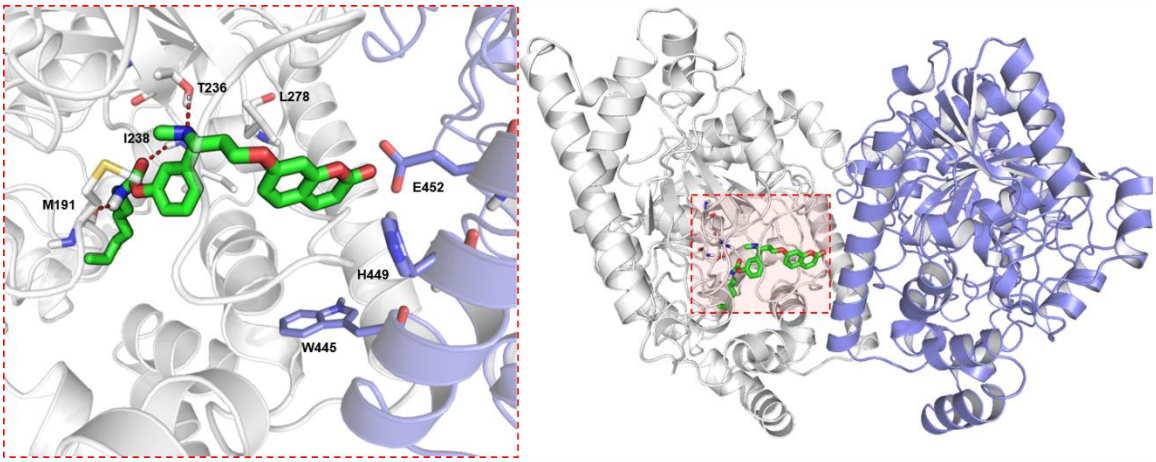


Figure 19: FAAH dimer is represented with cartoon (chain B –white cartoon; chain A –blue cartoon). In the docking pose of **8** (green sticks) the carbamate group is situated in proximity of the catalytic triad, while the neutral amino group points towards the side chain of Thr238 forming polar interactions with it.

2.5 BuChE docking studies series 1

For each compound a conformational analysis was performed, in order to obtain different conformations that can be used for the docking study. Starting from the structure of hBuChE, co-crystallized in presence of a reversible inhibitor (PDB ID: 4TPK), the docking grid was prepared to be centered on the position of the inhibitor located in the crystallized complex, with an amplitude of 14 Å in each direction.

Compound **9** (IC_{50}= 0.271) turned out to be the most active on BuChE. Docking poses of the latter showed that the carbamic group makes polar interactions with backbone nitrogen atoms of the oxyanion hole (Gly116 and Gly117). The N-heptyl substituent was localized in the region occupied by tacrine in the crystallized complex tacrine-hBuChE (4BDS). O-phenyl ring was located in the lipophilic pocket delimited by Leu286, Val288 and Trp231 residues, and was perfectly superimposed on the second naphthalene ring of the inhibitor of the crystallized complex used for the docking study.

The basic nitrogen and the trimethylene linker were located within the access channel to the catalytic site, while the aryl group was located at the entrance of the channel. This conformation was further stabilized by the formation of a hydrogen bond between the oxygen of the linker and the side chain of Asn289 residue.

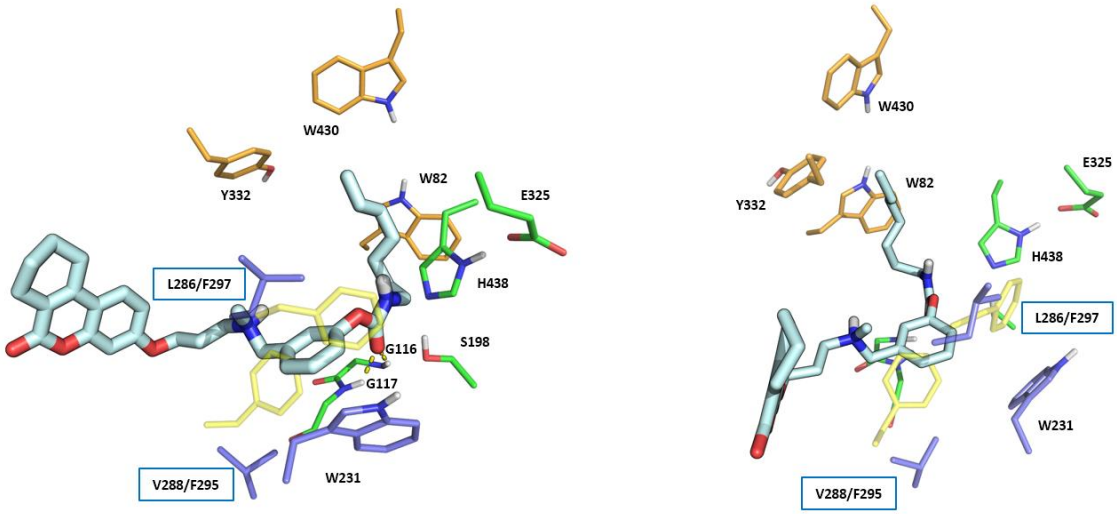


Figure 20: Binding mode of compound **9** (blue ligand), with two rotated representations along the vertical axis. The residues of the catalytic triad and oxyanion hole were represented in green, the residues of the acyl binding pocket was blue and the choline binding site residues were orange. F297 and F295 residues of hAChE were represented in yellow.

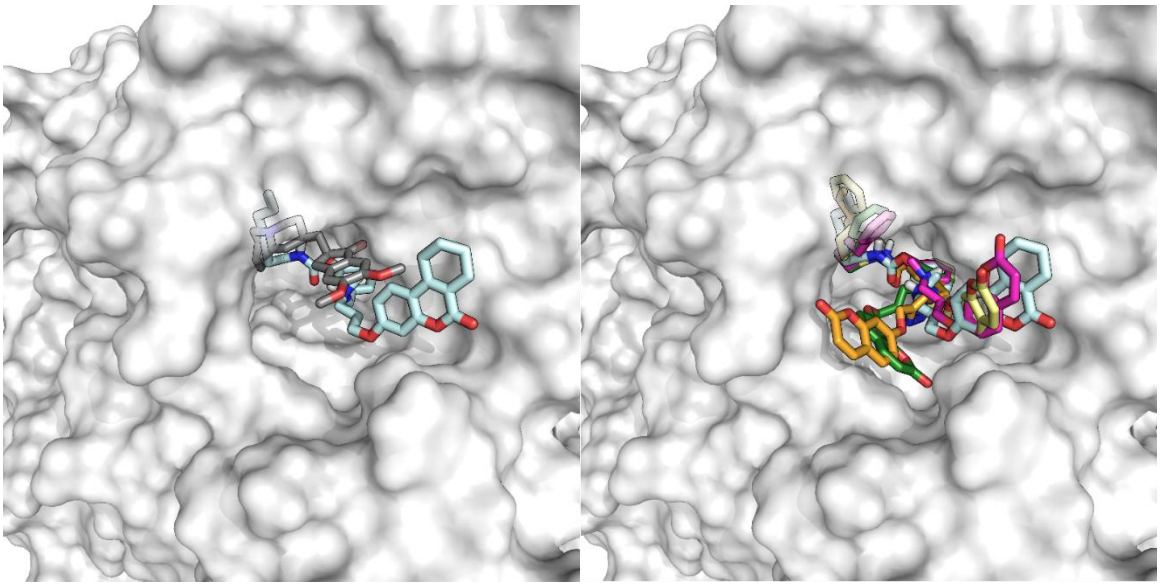


Figure 21: Representation of the access channel to the catalytic site of hBuChE. Left panel: the ligand **9** occupied a more external portion of the channel than that occupied by donepezil in hAChE (PDB: 4EY7). Right panel: the entrance width of the access channel allowed ligands to adopt alternative binding modes.

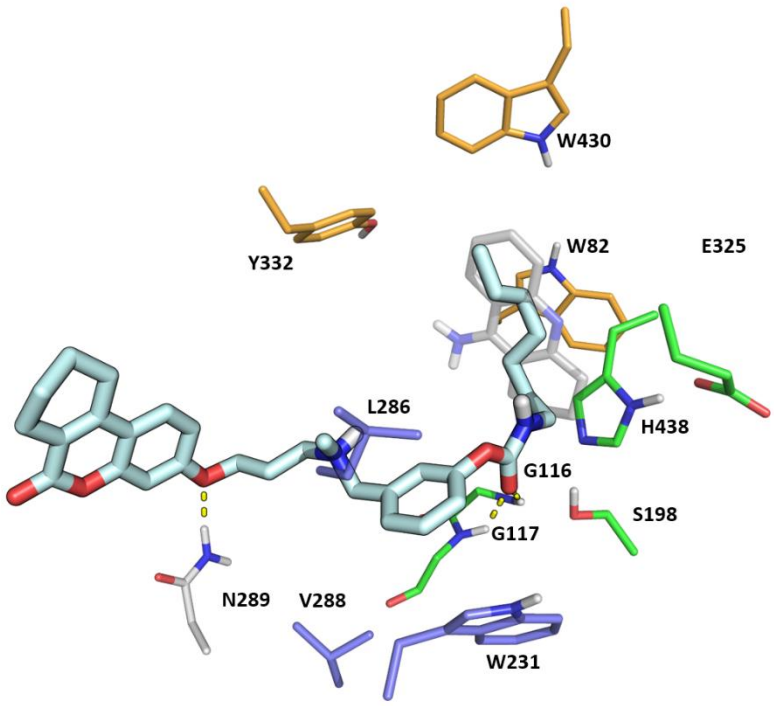


Figure 22: Binding mode of **9**; the N-heptyl substituent was located in the pocket occupied by tacrine (PDB: 4BDS)

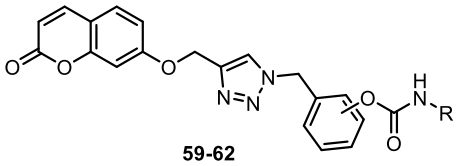
Docking poses obtained for the other compounds of the series showed similar elements with the binding mode of **9**. The lipophilic substituents of the carbamic nitrogen tended to occupy the binding pocket of tacrine, while the carbamic group was oriented in order to form polar interactions with residues of the oxyanion hole.

A single element of variability was related to the position occupied at the entrance of the access channel to the catalytic site by different O-aryl groups. This variability was mainly related to the width of this region.

2.6 Series 2

In order to improve the activity, pooling all the data and considering the docking studies regarding compound **8** showing that the best pose is in the neutral form, a new series of coumarin derivatives (table 3, 4) bearing a triazole moiety as spacer was synthesized.

TABLE 3



59-62

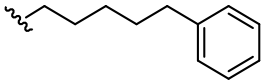
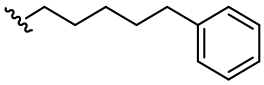
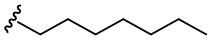
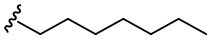
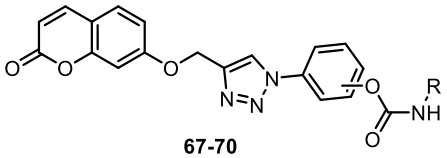
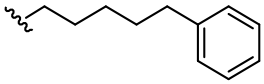
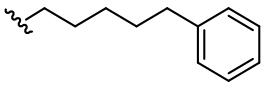
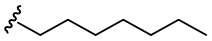
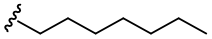
	Chain position	R
59	**para**	
60	**meta**	
61	**para**	
62	**meta**	

TABLE 4



67-70

	Chain position	R
67	**para**	
68	**meta**	

69	**para**	
70	**meta**	

2.7 FAAH docking studies series 2

Docking studies of compound **68** on FAAH enzyme were performed. As shown in picture 23, the triazole ring of **68** is located in proximity of the side chain of T236, resulting in a different arrangement of the cromenonic portion with the formation of a hydrogen bond in the channel that leads to the cytosolic port; the terminal phenyl ring is located in the ACB pocket.

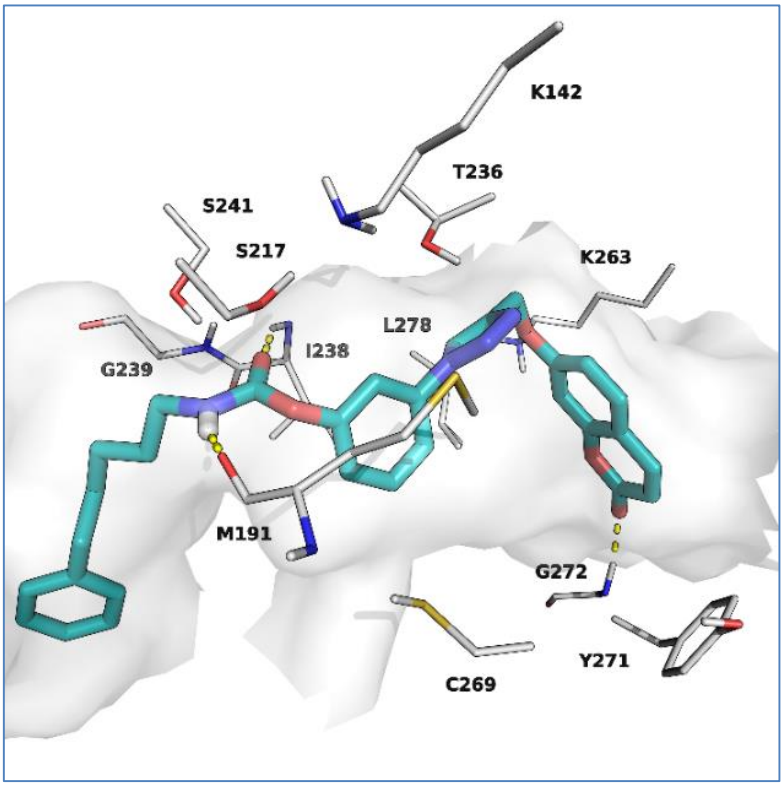


Figure 23: docking of compound **68**

2.8 BuChE docking studies series 2

Docking study on triazole derivatives revealed some important information on the effect produced by the presence of the phenyl or benzyl-triazole group, and the meta or para position of these groups on the O-phenyl ring of the phenyl-carbamate.

Benzyl-triazole derivatives:

Benzyl-triazole derivatives, if para-substituted (**59** e **61**), showed higher power. The resulting docking poses of compounds **59** and **61** showed a good overlap of the O-position of the phenyl ring with that obtained for compound **9**. Docking poses characterized by the positioning of the O-phenyl ring in the lipophilic pocket of the acyl binding pocket and by the correct positioning of the carbamic group close to the catalytic serum, were also observed for meta-substituted compounds. This result suggested that the m-triazolyl-methyl derivatives were less effective at ensuring an optimum interaction between carbamate and catalytic triad.

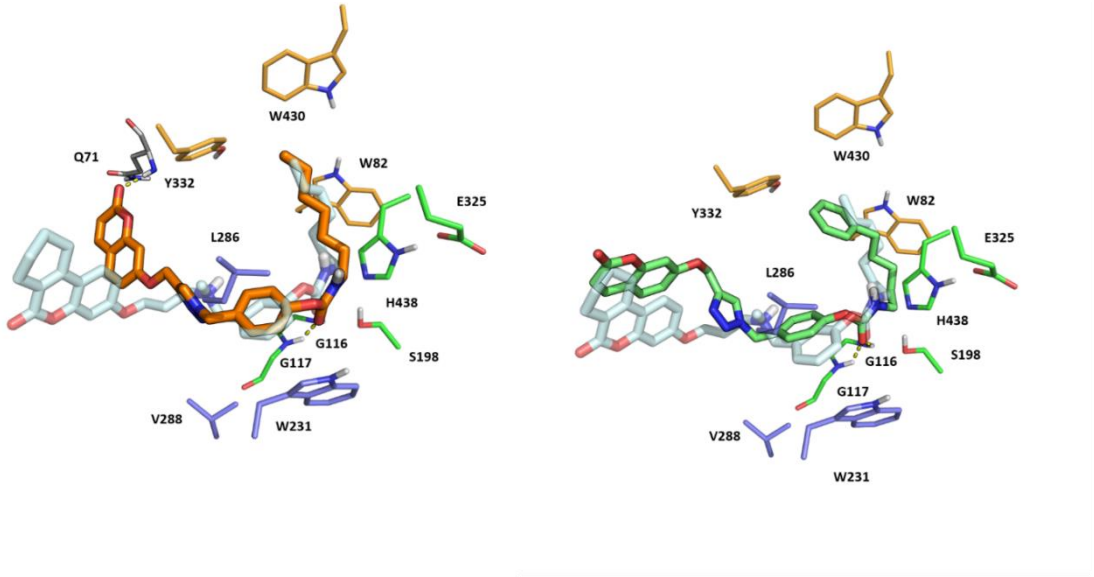


Figure 24: Binding mode of **61** (left panel, orange ligand) and **59** (right panel, green ligand) compared with docking of **9** (blue ligand)

Phenyl-triazole derivatives:

Unlike what was observed for benzyl-triazole compounds, the meta-substituted phenyl-triazole derivatives are more powerful than the para-substituted derivatives. Coherently, the docking study showed that for **68** and, to a lesser degree, **70**, the most frequent and with best score poses located the O-phenyl ring in the hydrophobic pocket of the acyl binding pocket.

On the contrary, this binding mode was not found for compounds **67** and **69**, in which the dimensions of the triazole ring and the greater stiffness conferred by the absence of methylene bridge prevented this type of ligand-protein interaction.

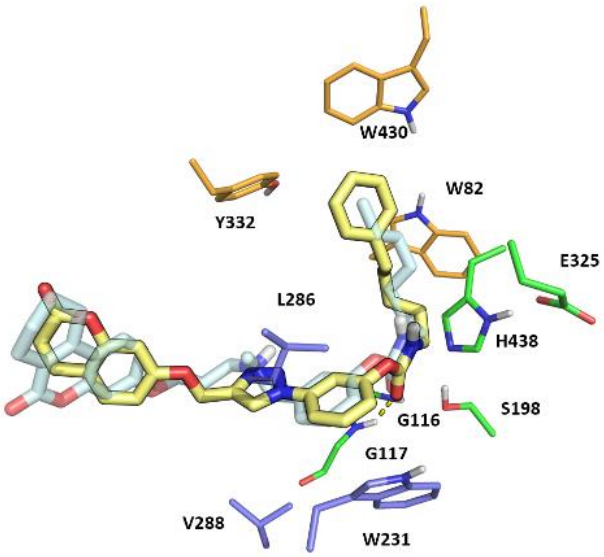


Figure 25: Binding mode of **68** (yellow ligand) compared with the laying of **9** (blue ligand)

2.9 Biological results series 2 and discussion

TABLE 5

Compound	AChE IC_{50} (nM)	BuChE IC_{50} (nM)	FAAH IC_{50} (nM) n≥2	FAAH % inhibition at 10 μM
59	255 ± 12	1.17 ± 0.12	55.44 ± 10.64	86.44%
60	941 ± 106	42.7 ± 1.5	51.18 ± 10.65	94.87%
61	214 ±19	15.9 ± 0.5	6290.30 ± 82.60	80.15%
62	1044± 82	78.2 ± 4.8	317.55 ± 153.15	94.42%
67	47900 ± 1400	1230 ± 90	565.97 ± 248.47	83.85%
68	1175 ± 145	15.2 ± 1.2	12.80 ± 1.33	95.13%
69	157550 ± 26800	122000 ± 23000	153.15 ± 24.25	86.61%
70	702 ± 18	71.2 ± 2.9	48.07 ± 14.90	92.35%

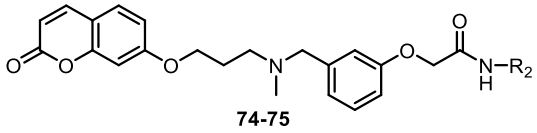
Biological data, shown in table 5, demonstrated a loss of activity on AChE, probably due to the rigidity of the structure that could not allow to enter into the narrow gorge, and a decrease in inhibitory activity on BuChE, although it is still in the nanomolar range (except for 67 and 69). Probably this is due to the fact that the radius of BuChE is wider and it allows the molecules to better fit into the gorge. Some compounds improved their activity on FAAH; in particular, compound **68,** although with limited mobility capabilities, thanks to its spatial arrangement was able to get into the binding pocket and interact with FAAH. Moreover, it showed an interesting and balanced activity on BuChE and FAAH. Indeed, this is an essential peculiarity for a multi-target molecule.

2.10 Series 3

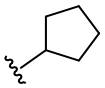
We then decided to synthesize a new small series of compounds shown in table 6, in which the carbamic function was replaced by an amide function. This modification could be suitable to obtain reversible cholinesterase and FAAH inhibitors. Moreover, the long chain is closed in a 5 or 6-membered ring which, could maybe retain the features required to give hydrophobic interactions with the binding pockets of the selected enzymes.

The biological profile of compounds **74** and **75** is now under evaluation.

TABLE 6



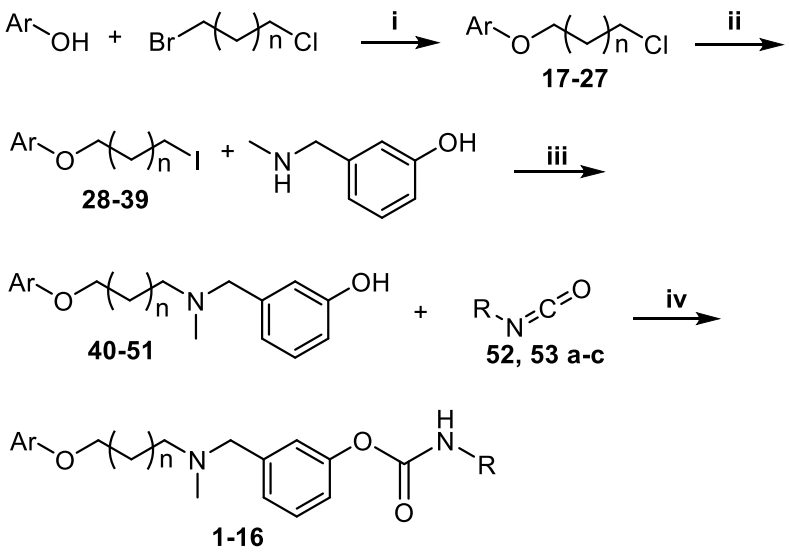
74-75

	R_2
74	
75	

2.11 Chemistry

Compounds **1-16** were prepared according to this scheme:

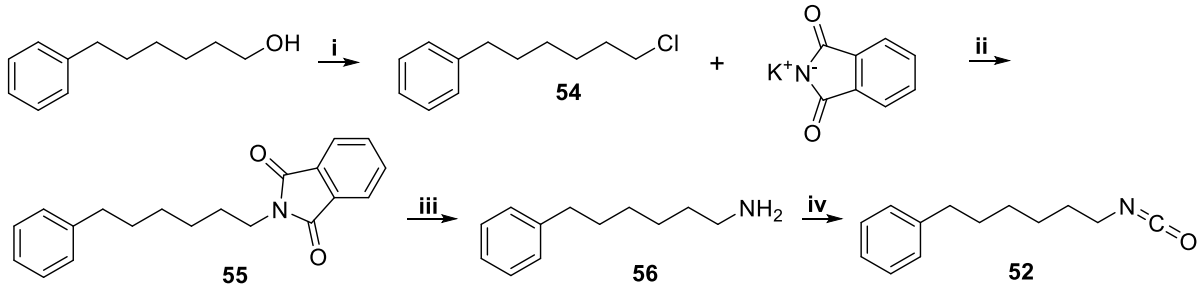
SCHEME 1



Reagents and conditions: i) K_2CO_3, toluene, reflux; ii) NaI, methyl ethyl ketone, reflux; iii) TEA, toluene, reflux; iv) NaH, toluene, r.t.

The hydroxyl group of the natural inspired heterocycles was alkylated with bromochloroalkane of various length, refluxing the mixture in acetone with K_2CO_3 in order to achieve the chloro--derivatives. Compounds 17-27 were treated with NaI, refluxing in methylethylketone, with the purpose of obtain more reactive compounds for the next step of nucleophile substitution. The latter was conducted refluxing 3-((methylamino)methyl)phenol in toluene with compounds 40-51; then thanks to a carbamylation achieved in presence of NaH and with appropriate isocyanates, we reached final compounds from 1 to 16.

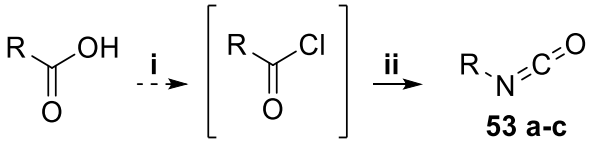
SCHEME 2



Reagents and conditions: i) $SOCl_2$, TEA, DCM, reflux; ii) DMF, reflux; iii) hydrazine monohydrate, ethanol, reflux; HCl 37%, reflux; iv) $COCl_2$, TEA, toluene, reflux

The synthesis starts with an activation of the alkyl alcohol obtained with $SOCl_2$. In order to obtain the amino compound 56, thanks to Gabriel synthesis, 54 was treated with potassium phthalimide refluxing in DMF, then 55 was refluxed in ethanol with hydrazine monohydrate. The last step, refluxing in toluene with phosgene, allowed to the formation of the isocynate,.

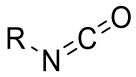
SCHEME 3



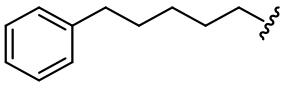
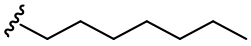
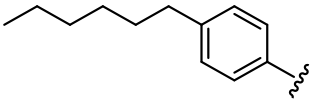
53 a-c

Reagents and conditions: i) $SOCl_2$, reflux; ii) NaN_3, acetone, H_2O; benzene, 60°C

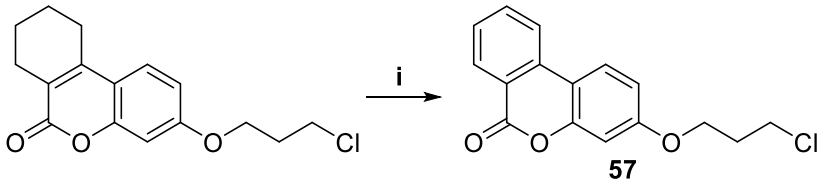
Thanks to its previously activation with $SOCl_2$, the acid, combined with NaN_3, was heated giving the formation of various isocyanates.



53 a-c

	R
53a	
53b	
53c	

SCHEME 4

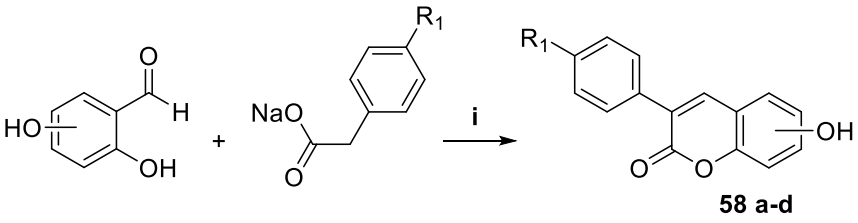


57

Reagents and conditions: i) DDQ, dioxane, reflux

The 3-(3-chloropropoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one was dehydrogenated using DDQ (2,3-dichloro-5,6-dicyano-p-benzoquinone) in dioxane, refluxing for 24h.

SCHEME 5

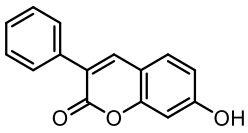
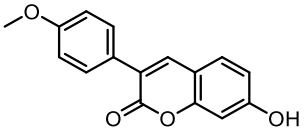
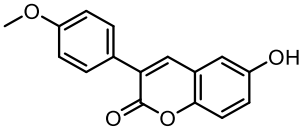


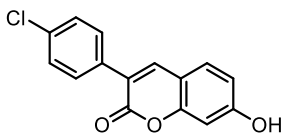
58 a-d

R= H, Cl, OCH_3

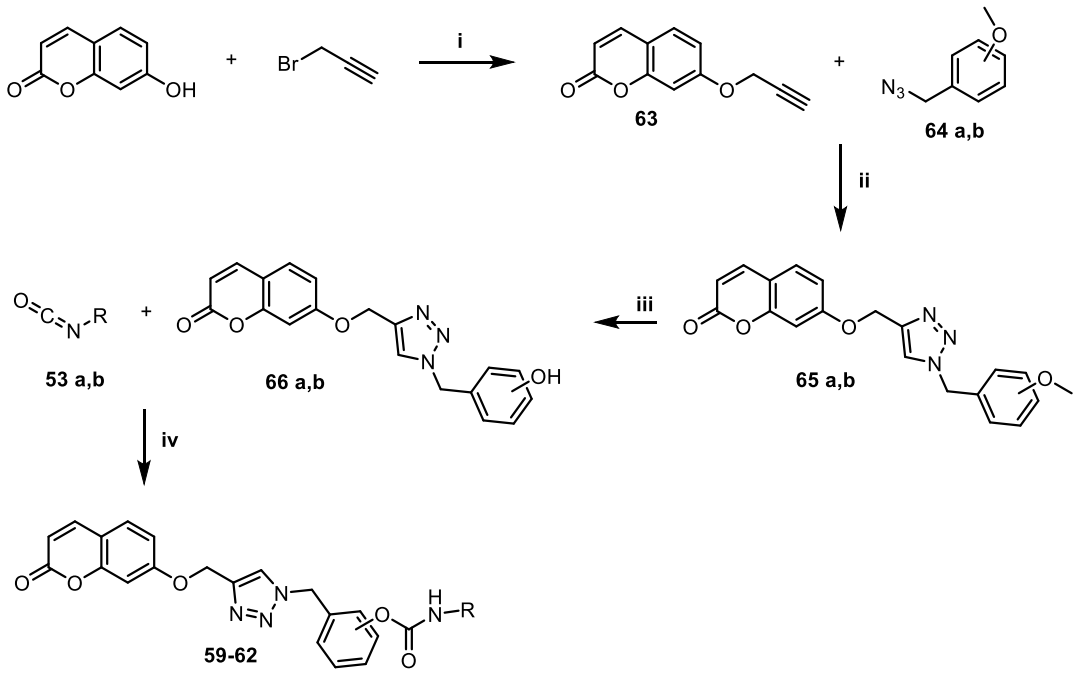
Reagents and conditions: i) $(CH_3CO)_2O$, 180°C; K_2CO_3; ethanol, reflux

The hydroxyphenyl coumarines are obtained thanks to the classic method of Perkin and Pechmann. The selected 2,4-dihydroxybenzaldehyde is condensed with the sodium salt of selected phenylacetic acid in acetic anhydride. Then, acetylated phenylcoumarines are hydrolyzed with K_2CO_3 in order to obtain desired compounds **58 a-d**.

58a	
58b	
58c	

58d	

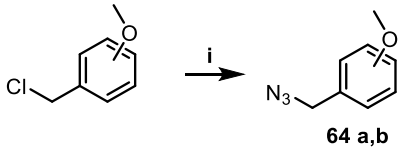
SCHEME 6



Reagents and conditions: i) K_2CO_3, acetone, reflux; ii) TEA, $CuSO_4$, Sodium ascorbate, DMSO, r.t.; iii) BBr_3, DCM, N_2 atmosphere, r.t. ; iv) NaH, toluene, r.t.

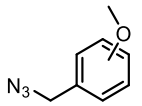
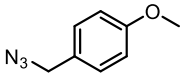
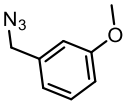
The 7-OH-coumarin was combined with propargyl bromide obtaining 63 then, thanks to a click reaction conducted in presence of $CuSO_4$ and sodium ascorbate the triazole was formed. Subsequently, the methoxyl group was demethylated with BBr_3; finally compounds 66a,b were combined with properly isocyanate reaching the various carbamates.

SCHEME 7

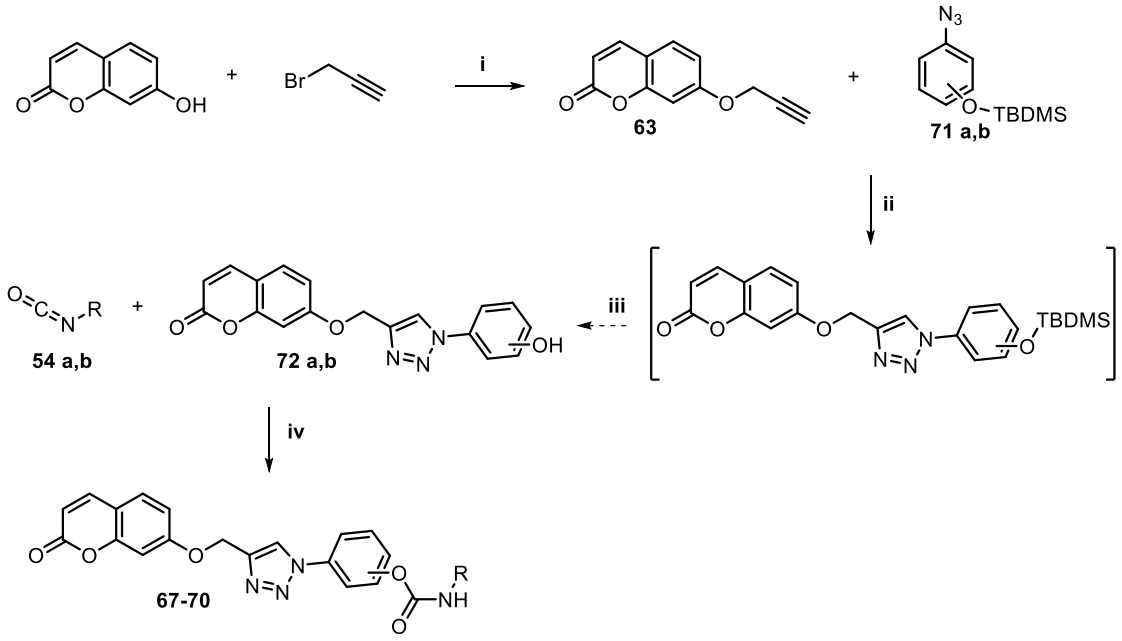


Reagents and conditions: i) NaN_3, DMF, 80°C

Azido compounds were obtained combining chlorine derivatives with sodium azide in DMF and heating at 80°C.

	 **64 a,b**
64a	
64b	

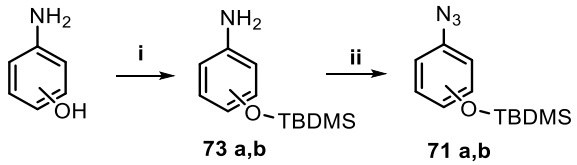
SCHEME 8



Reagents and conditions: i) K_2CO_3, acetone, reflux; ii) TEA, $CuSO_4$, Sodium ascorbate, DMSO, r.t.; iii) flash chromatography ; iv) NaH, toluene, r.t.

The 7-OH-coumarin was combined with propargyl bromide obtaining 63 then, thanks to a click reaction, combining the alkyne-derivative with the azidophenoxy tert-butyl dimethylsilane derivative in presence of $CuSO_4$ and sodium ascorbate the triazole was formed. Subsequently, the oxygen was deprotected thanks a flash chromatography; finally compounds 72a,b were reacted with properly isocyanate reaching the various carbamates.

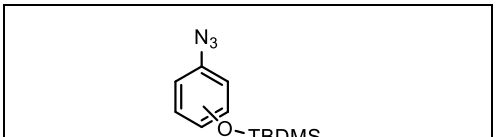
SCHEME 9



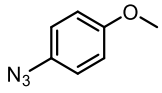
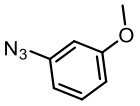
73 a,b 71 a,b

Reagents and conditions: i) imidazole, TBDMS-Cl, DMF, r.t.; ii) H_2O, HCl 37%, $NaNO_2$, NaN_3

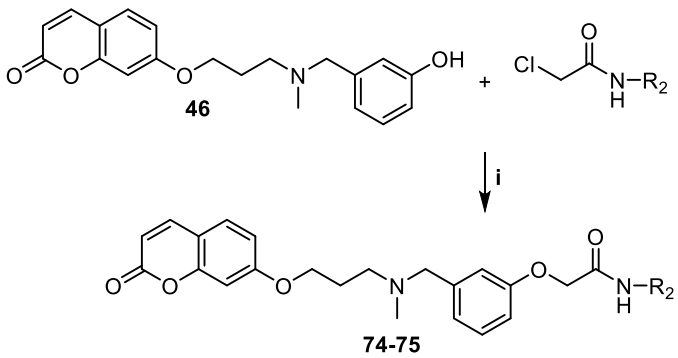
The aminophenol was protected with TBDMS-Cl at room temperature in DMF; at that time, previously the diazonium salt was formed adding 73 a,b to $NaNO_2$ in H_2O and HCl 37%, then, adding NaN_3 and the azide was formed.



71 a,b

	R
71a	
71b	

SCHEME 10



74-75

Reagents and conditions: i) K_2CO_3, acetone, reflux

The last step of synthesis is an alkylation of the phenol with the chloro-derivative in acetone–in presence of K_2CO_3.

2.12 Conclusions

As first, we interesting improved the activity of coumarin-core molecules. This study led to the identification of a series of compounds showing an inhibitory potency on selected target up to nanomolar and subnanomolar range.
To strongly enhance activity and selectivity toward each target, one derivative was recognized as possessing all the structural requirements identified as:

- basic coumarin core
- spacer chain of three units
- long seven methylenes carbamic tail

We found a new lead (compound **8**) with increased activity on AChE, BuChE and FAAH. Thus, this compound was selected for further development and on this basis, considering docking studies too that demonstrated that the best poses were obtained in the neutral form, we projected and synthesized second series of compounds. At a first sight the inhibitory activity on AChE and on BuChE decreased and maybe it was due to the rigidity caused by the triazole ring; although the inhibition of BuChE was reduced, it was still satisfactory. In particular, compound **68** showed potent and balanced activity both on BuChE and on FAAH, a researched and fundamental feature for a multi-target compound that bodes well for the future potentialities of this molecule.

4- PROJECT 2

3.1 Aim of the project: benzofuran-core molecules

As previously explained, the endocannabinoid system is involved in AD in neuroprotective, anti-inflammatory and neurotrophic actions, which can be obtained by activating type 2 cannabinoid receptors (CB_2), either directly or by endocannabinoid cellular reuptake inhibition.[48] It has been demonstrated that CB_2 receptors are overexpressed in the area of neural plaques; in particular, they are located in microglia and astrocytes, that are activated by neuronal damage and beta-amyloid plaques. Indeed, an activation of CB_2 receptors could reduce microglia activation and prevent the inflammatory process. In addition, CB_1 receptors activation or inhibition, depending on the stage of the disease, was also suggested to be beneficial for AD. Moreover, the inhibition of FAAH could be successfully considered as physiologic receptor activation. Considering all this, the second project of this thesis had the intention to hit multiple target: AChE, BuChE, BACE-1, FAAH, Aβ aggregation and CB receptors.

The design of this MLTD strategy considers the benzofuran heterocyclic moiety. It is a privileged structure and this scaffold is present in many biologically active natural products and therapeutics, and thus represents a very important pharmacophore.

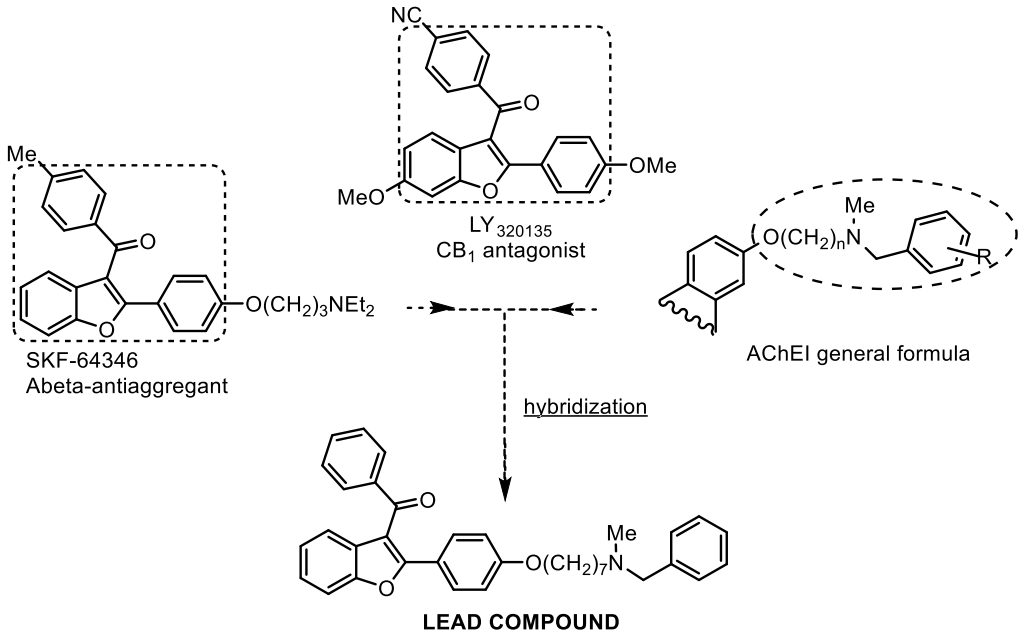


Figure 26

In previous studies a lead molecule was identified, combining the benzofuran moiety of in SKF-64346, an inhibitor of Aβ fibril formation, with an AChEI methylbenzylamino fragment previously optimized by the research group were I did the PhD.

The lead compound had a remarkable inhibitory activity: besides the important micromolar activity on AChE and BuChE, it also showed significant activity for inhibition of the formation of $A\beta_{25\text{-}35}$ ([a]) fibrils to 50% of the control value, for the inhibition of $A\beta_{25\text{-}35}$ ([b]) peptide toxicity in human neuronal SH-SY5Y and for the loss of neuronal viability evoked by Aβ, thus providing neuroprotection. Taking into account that the benzofuran framework was present in LY320135 (fig. 26) a high affinity antagonist/ inverse agonist at CB1 receptor, the lead compound was also tested on CB receptors, demonstrating moderate affinity and good selectivity for CB1 receptors.

TABLE 7

Lead compound biological activities						
***h*AChE IC_{50}**	***h*BuChE IC_{50}**	**$A\beta_{25\text{-}35}$ IC_{50}[a]**	**$A\beta_{25\text{-}35}$ IC_{50}[b]**	**SH-SY5Y cells PI**	**CB_1 EC_{50}**	**CB_2 EC_{50}**
40.7 µM	38.1 µM	12.5 µM	5.6 µM	58.16 %	0.79 µM	>10µM

My aim for this project was to optimize and improve the biological activities of this lead, focusing on the inhibition of cholinesterases, Aβ aggregation, BACE-1 and FAAH, and on the interaction with CB_1 and CB_2 receptors. Five new series of compounds, depicted in the scheme below, have been synthesized (fig. 27).

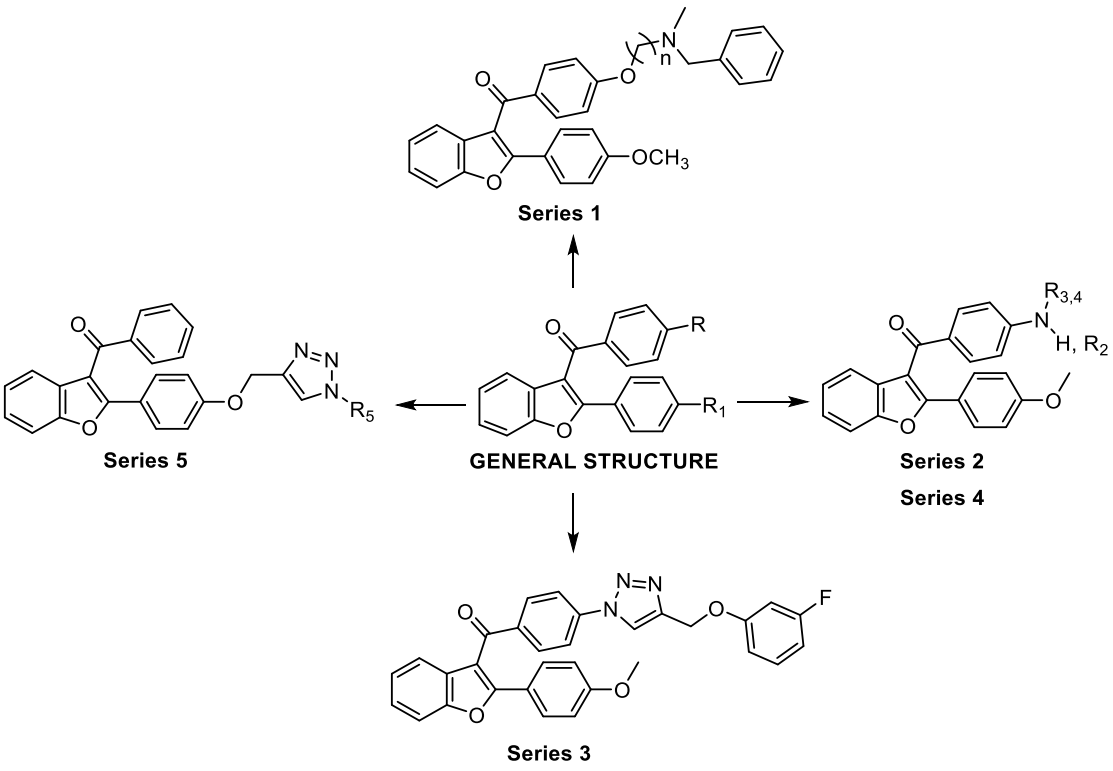
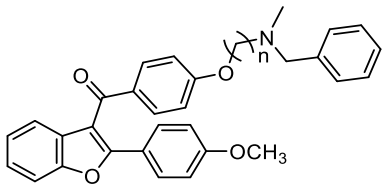


Figure 27

3.2 Series 1, 2, 3, 4, 5: lead optimization

SERIES 1

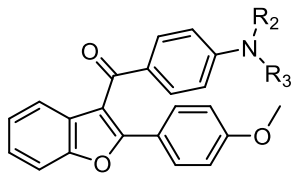


76-79

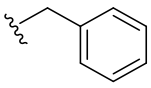
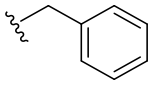
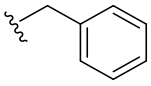
	n
76	**5**
77	**4**
78	**3**
79	**2**

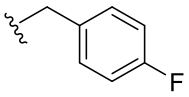
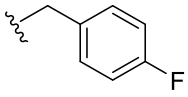
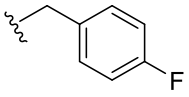
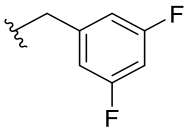
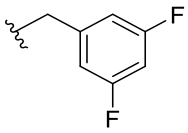
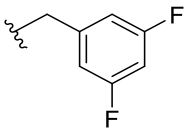
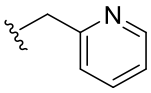
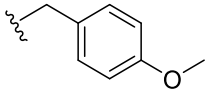
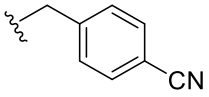
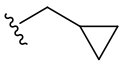
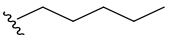
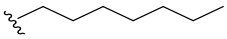
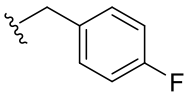
In **Series 1** the R in the general structure was replaced with an N-methyl benzyl amine residue with an ethereal chain of various lengths and R_1 was substituted with a methoxyl group.

SERIES 2



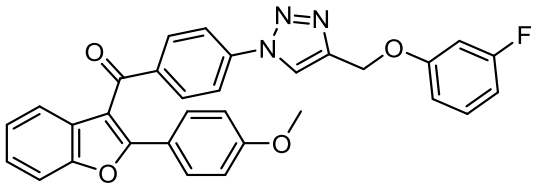
100-112

	R_2	**R_3**
100	H	
101		

102	H	
103		
104	H	
105		
106	H	
107	H	
108	H	
109	H	
110	H	
111	H	
112	CH_3	

Series 2, allowed the formation of mono or di-alkylated amine on R, while R_1 was still a methoxyl group.

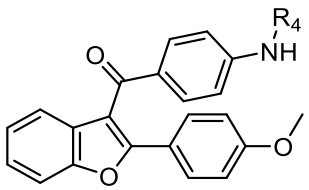
SERIES 3



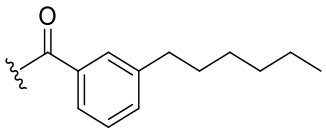
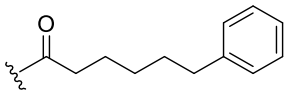
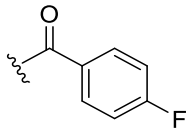
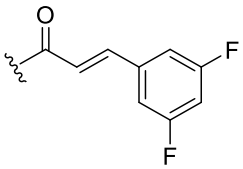
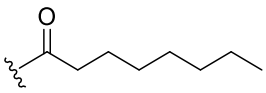
113

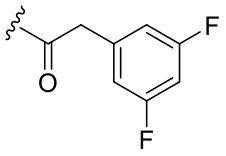
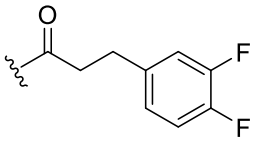
Series 3 is in fact constituted of only one compound where R was transformed in a substituted triazole and R_1 was again a methoxyl group.

SERIES 4



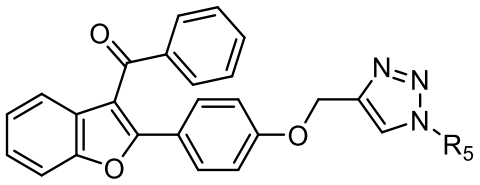
117-123

	R_4
117	
118	
119	
120	
121	

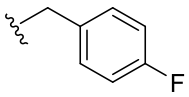
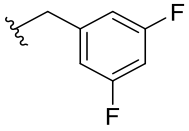
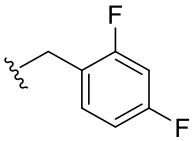
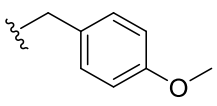
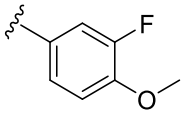
122	
123	

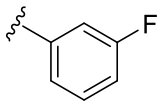
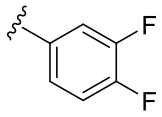
In **series 4** various amides were inserted on R, that could contribute for a better affinity with BACE-1 or FAAH.

SERIES 5



124-130

	R_5
124	
125	
126	
127	
128	

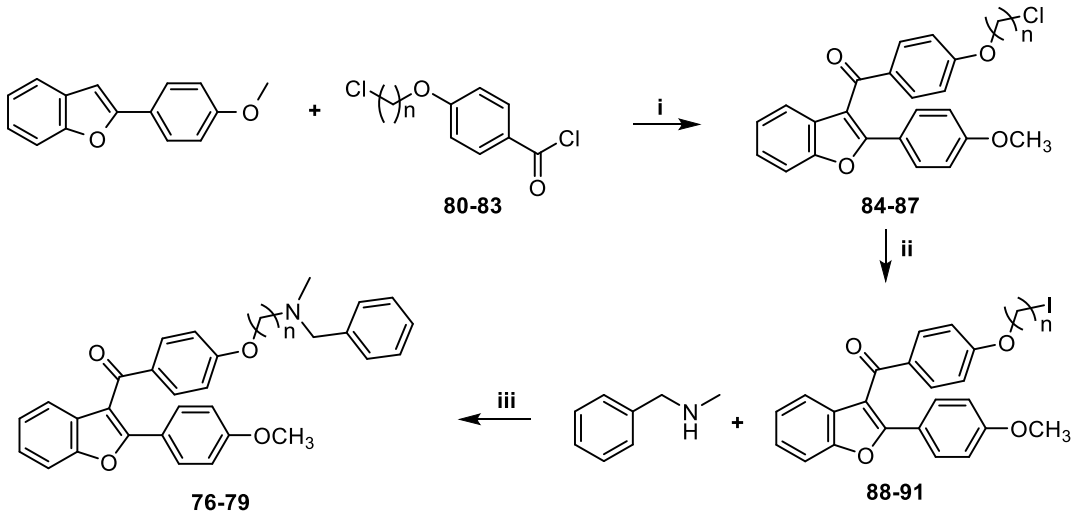
129	
130	

In **series 5** the triazole was moved from R to R_1 and substituted with a benzyl moiety.

.

3.3 Chemistry

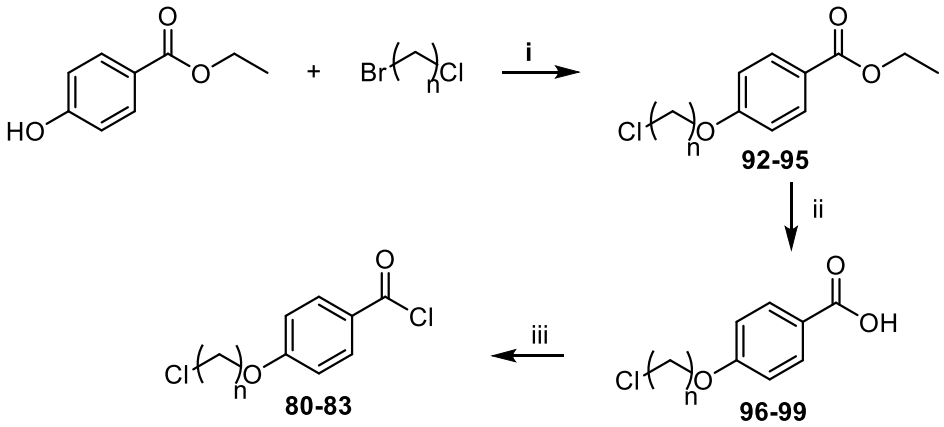
SCHEME 11



Reagents and conditions: i) $SnCl_4$, DCM, 0°C, r.t.; ii) NaI, methylethyl ketone; iii) toluene, reflux

The benzofuran undergoes Friedel Craft acylation, using 80-83 in order to obtain compounds 84-87, which were treated with NaI to replace the chlorine atom by introducing a better leaving group to facilitate the subsequent nucleophilic substitution; the latter was obtained refluxing with methylbenzylamine in toluene consequently reaching compounds 76-79.

SCHEME 12



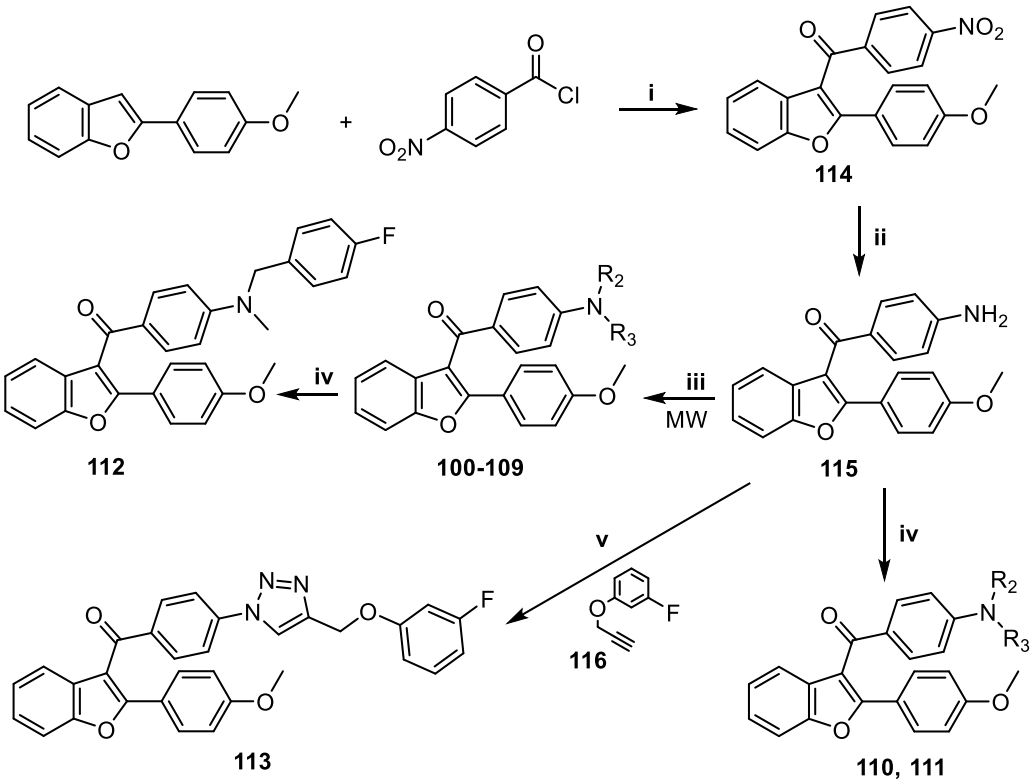
n= 5, 4, 3, 2,

Reagents and conditions: i) K_2CO_3, acetone, reflux; ii) KOH, ethanol, reflux; iii) $SOCl_2$, reflux

The reaction scheme starts with an alkylation of the hydroxyl group of the para-hydroxyethyl benzoate in basic conditions with 1-bromo-*ω*-chloroalkanes in order to obtain various analogues. The reaction is chemoselective on brominated position to achieve derivatives 92-95 from which thanks to a saponification with potassium hydroxide compounds 96-99 were formed. The acid was then transformed into the corresponding chloride by treatment with thionyl chloride.

	n
80	5
81	4
82	3
83	2

SCHEME 13

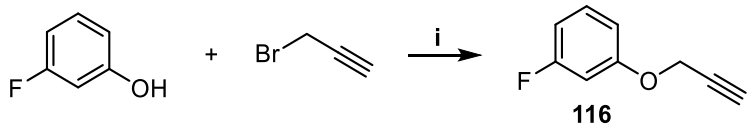


Reagents and conditions: i) $SnCl_4$, DCM, 0°C; ii) $SnCl_2$, ethanol, reflux; iii) KI, K_2CO_3, CH_3CN, 110°C, 150W, MW; iv) tetrabutylammonium hydrogensulfate, DCM/NaOH 50%, r.t.; v) tert-butyl nitrite, $TMSN_3$, CH_3CN, r.t.

The benzofuran ring was derivatized with Friedel Craft acylation using p-nitrobenzoylchloride in presence of $SnCl_4$. The nitro group was reduced to the corresponding amine refluxing with $SnCl_2$;

then with the aid of microwaves, in presence of KI and K_2CO_3, mono- and di-alkylated compounds (100-109) were obtained. Compound 102 was methylated in a biphasic mixture of DCM/NaOH 50% using CH_3I in presence of tetrabutylammonium hydrogensulfate; with the same procedure, using the appropriate bromo-alkane, we obtained alkylated compounds 110-111. Compound 113 was formed using a click reaction, combining 115 with the alkyne 116 in presence of tert-butyl nitrite and $TMSN_3$ in CH_3CN, forming in this way the triazole.

SCHEME 14

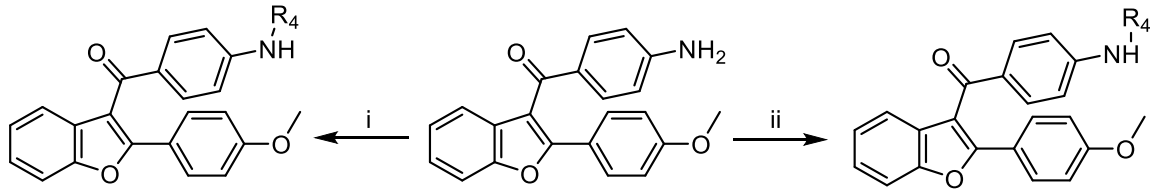


116

Reagents and conditions: i) K_2CO_3, acetone

The 3-fluorophenol reacted with propargyl bromide in presence of K_2CO_3 in acetone in order to achieve compound 116.

SCHEME 15

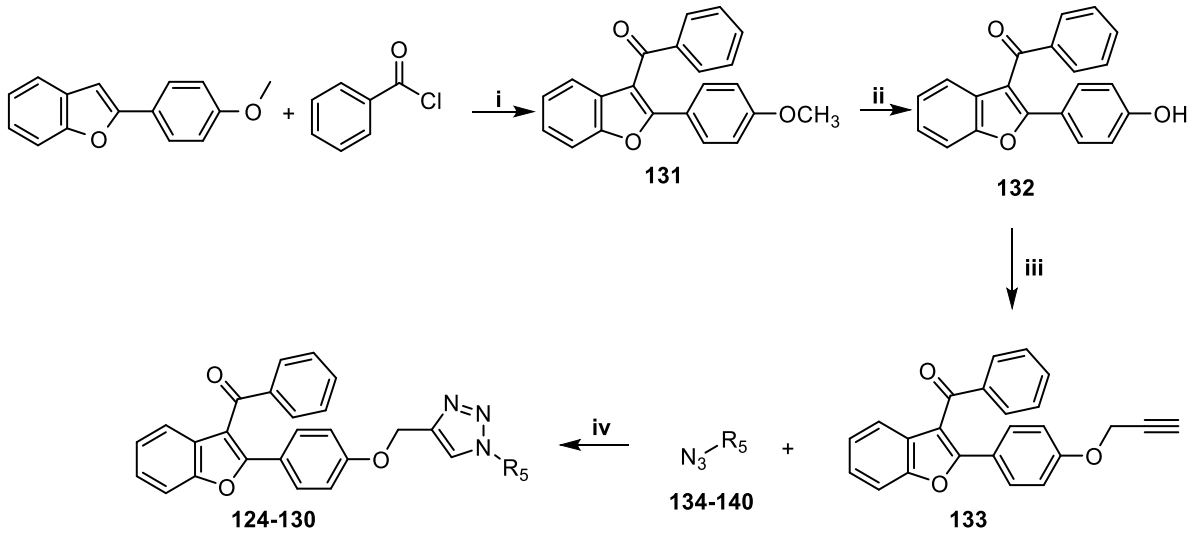


117-121 **114** **122, 123**

Reagents and conditions: i) K_2CO_3, toluene; ii) DCC, DCM, N_2 atmosphere, r.t.

Compounds 117-121 were obtained thanks to an amidation conducted in thermal conditions in presence of potassium carbonate in toluene. The amide of compound 122, 123 was formed in presence of DCC, in DCM, under nitrogen atmosphere.

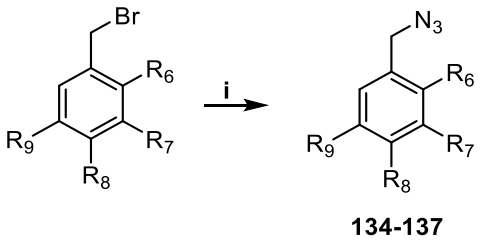
SCHEME 16



Reagents and conditions: i) $SnCl_4$, DCM, 0°C; ii) BBr_3, DCM, N_2 atmosphere; iii) propargyl bromide, K_2CO_3, toluene, reflux; iv) TEA, $CuSO_4$, sodium ascorbate, DMSO, r.t.

The benzofuran ring was derivatized with Friedel Craft acylation using benzoyl chloride in presence of $SnCl_4$. The methoxyl group was deprotecting using BBr_3, and then it was functionalized with propargyl bromide. Thank a click reaction conducted in presence of $CuSO_4$, sodium ascorbate and the various azides, the triazole rings were formed in order to reach compounds 124-130.

SCHEME 17

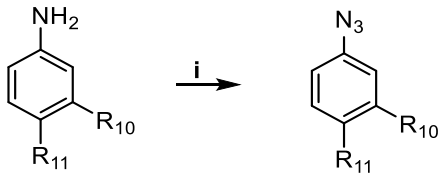


Reagents and conditions: i) NaN_3, DMF, 80°C; or NaN_3, DMSO, r.t.

Bromo-benzyl derivatives were converted in benzylazides using sodium azide in DMF at 80°C, or using sodium azide in DMSO at room temperature.

	R_6	R_7	R_8	R_9
134	H	H	F	H
135	H	F	H	F
136	F	H	F	H
137	H	H	OCH_3	H

SCHEME 18



138-140

Reagents and conditions: i) H_2O, HCl 37%, $NaNO_2$, NaN_3, r.t.

The functionalization of the aniline in azide was performed forming the diazonium salt with $NaNO_2$ and subsequently adding sodium azide.

	R_{10}	R_{11}
138	F	OCH_3
139	F	H
140	F	F

3.4 Biological results and discussion

Given the large number of targets for which benzofuran-inspired molecules was designed and the fact that the synthesis of the designed molecules was completed close to the end of my PhD, currently various biological studies are still ongoing, in particular we are waiting for BACE1 and β-aggregation biological activities.

Thus, in this thesis, only preliminary data will be presented and therefore complete biological framework is not available.

SERIES 1

	Ki on CB_1 μM m±ds	**CB_1% displacement at 10 μM**	**Ki on CB_2 μM m±ds**	**CB_2 %displacement at 10 μM**	**AChE % inhib**	**IC_{50} BuChE μM**
76	1.76 ± 0.36	65.34	>10	33.56	11.06	1.09 ± 0.07
77	0.86 ± 0.13	60.90	>10	35.05	n.a	219 ± 94.0
78	0.50 ± 0.05	70.23	0.12±0.02	85.07	n.a.	135 ± 6.o
79	0.43 ± 0.08	80.00	0.69 ± 0.00	73.92	n.a.	14.0 ± 0.9

Biological data showed that **76** and **77** (n = 5 and n = 4, respectively) had moderate affinity and good selectivity for CB1 receptors and **76** has also good activity on BuChE.

With the shortening of the chain the affinity for CBs was increased, but the compounds lost their selectivity towards CB1 receptors. Compound **78** (n= 3), in fact, had submicromolar affinity both for CB1 and for CB2, but it lost activity on BuChE. Instead, **79** recovers inhibitory activity of BuChE. **78** is the compound that proved to have the greatest affinity towards CB2. None of the compounds proved to be active on AChE.

SERIES 2

	Ki on CB_1 μM m±ds	CB_1 %displacement at 10 μM	Ki on CB_2 μM m±ds	CB_2 %displacement at 10 μM	% inhib BuChE	IC_{50} BuChE μM
100	>10	41.91	168±11.29	78.00		
101	>10	27.05	925.63±60.15	69.29		
102	0.78±0.34	68.64	0.02±0.00	75.57	60.8± 1.1	13.4±0.5
103	>10	29.14	0.44±0.04	66.30		
104	>10	22.07	0.30±0.06	62.39	<10	n. d.
105	>10	25.03	>10	26.56		
106	>10	49.90	1.34±0.38	68.48 (25μM)	<10	n. d.
107	>10	38.25	1.02±0.12	67.91 (25μM)		
108	2.60±0.05	92.27 (25μM)	0.025±0.001	79.89	<10	n. d.
109	0.4±0.17	85.11	0.108±0.015	87.17	23.5±0.6	n. d.
110	0.085±0.02	87.14	0.069±0.004	83.57		
111	1.67±0.66	69.39	0.401±0.76	74.42		
112	0.02±0.00	90.60	0.002±0.000	96.06	10.1±02	n.d.

Data listed in the table demonstrated that many compounds of series 2 had remarkable biological properties. First of all, regarding CB1 receptors, we noticed that **100**, which has the benzyl ring without substituents, does not show affinity at all. The introduction of a fluoro or cyano substituent in para position of the benzyl ring (**102** and **108**, respectively), appeared to be important for a significant affinity, while the introduction of a methoxyl (**107**) led to the loss of affinity. Positive was also the introduction of methylcyclopropane **(109)**.

The introduction of an alkyl chain (**110**-**111**) present in anandamide and THC too, provided interesting affinity for the compounds best being where the derivative was with a 5 carbon chain. The most potent compound in the series is derivative **112** with the tertiary nitrogen, but with a small substituent.

Although almost all the substituents demonstrated more or less affinity for CB2 receptors, the same SAR of CB1, relating to the para substituted benzyl, in particular with fluorine and cyano substituents, also applies for CB2, although showing affinity values much more noticeable.
In particular, compounds **102** and **108** have Ki of 20 and 25 nM, respectively; on the other hand, the introduction of a 5 units alkyl chain gives to **110** a Ki of 69 nM, demonstrating high selectivity and affinity.
Selectivity is more pronounced towards the CB2 receptors, for which Ki values were quite lower than those for CB1. Of particular interest appeared to be the central role of the tertiary amine; in fact compound **112** clearly showed that the affinities towards the CB1 and CB2 increased 39 times and 10 times respectively. However, of greater importance appears to be the small size of the substituent on the nitrogen, in fact a methyl group gives to compound **112** a nanomolar affinity. This is also demonstrated by the affinity of the disubstituted compounds **103** and **105** for CB2: the first one shows acceptable affinity while, the second one, with a bulkier substituent, totally lost the affinity.
Finally, only **102** had activity on BuChE and all compounds are inactive towards AChE; maybe it is due to the rigidity of the structure that hinders the molecules to enter in the gorges.

SERIES 3

	Ki on CB_1 μM m±ds	**CB_1 %displacement at 10 μM**	**Ki on CB_2 μM m±ds**	**CB_2 %displacement at 10 μM**	**% inhib BuChE**	**IC_{50} BuChE μM**
113	>10	29.18	>10	21.07	<10	n. d.

Compound 113 showed no activity and affinity towards the selected targets.

SERIES 4

	Ki on CB_1 μM m±ds	**CB_1 %displacement at 10 μM**	**Ki on CB_2 μM m±ds**	**CB_2 %displacement at 10 μM**
117	>10000	23.11%	>10000	11.54%
118	>10000	23.56.%	>10000	22.10%
119	>10000	14.69%	>10000	28.15%
120	>10000	36.95%	>10000	22.89%

121	597.90±69.13	75.52%	>10000	37.02%
122	>10000	33.18%	>10000	37.24%
123	>10000	34.48%	>10000	33.16%

Compounds of series 4 turned out to have no affinity for CBs. However, their amide group was inserted to obtain activity on BACE1; unfortunately biological results for the latter are not available yet.

SERIES 5

	Ki on CB_1 µM m±ds	**CB_1 %displacement at 10 µM**	**Ki on CB_2 µM m±ds**	**CB_2 %displacement at 10 µM**	**IC_{50} on FAAH (µM) n≥2**	**%inib BuChE**
124	>10	32.95	>10	34.61	n.a.	
125	0.45±0.19	79.65	>10	26.03	3.01±0.13	24.4±0.1
126	>10	47.58	>10	43.27	n.a.	
127	2.67±0.13	85.14	>10	45.02	n.a.	
128	>10	20.00	>10	23.46	n.a.	
129	>10	37.86	>10	35.87	n.a.	
130	>10	38.13	>10	32.86	n.a.	

Biological results of series 5 showed that only **125** and **127** had affinity for CB1 receptors. No compounds of this series shows affinity for CB2s and activity on BuChE. **125**, the most potent one, also showed a promising inhibitory action on FAAH.

3.5 Conclusions

In continuing our studies on the design of new multi-target compounds for the treatment of AD, several modifications of the promising lead, previously identified among a new series of hybrid molecules based on the frameworks of our AChE/ BuChE inhibitors and of SKF-64346, were performed. With the aim of further optimizing this scaffold and broadening the biological profile of the molecules by investigating their potential action on additional targets involved in AD, five small series of compound were designed and synthesized. The different modifications introduced in the structure of the lead compound led to an increase in activity towards one or more of the selected targets for various compounds.

Some compounds of series 1 showed interesting increased activity, in particular compound **79,** if compared with the lead molecule, improved the affinity for CB1 and gained affinity for CB2; moreover it enhanced the activity on BuChE. **78** acquired instead an interesting affinity for CB2 receptors. Structure activity relationships suggest that, with the shortening of the alkyl chain, the affinity for the CB tends to increase

Biological results of series 2 demonstrated that the insertion of the para-fluorine seems to be essential. In fact, fluorine atoms were also introduced in the amine backbone, in order to evaluate their possible role in activity. Indeed the fluorine atom, due to its small size and high electronegativity, has recently received increasing interest in medicinal chemistry, since its introduction into potential drugs or diagnostic could improve pharmacokinetic and physical-chemical properties, such as metabolic stability or membrane permeation. [49] Furthermore, a greater affinity of fluorinated compounds for the different target proteins has been documented. [50]

Moreove,r with compound **112** the importance of the fundamental role of the tertiary amine, if substituted with a not bulky group (methyl group) has been demonstrated.

In series 5 the first compound with interesting activity on FAAH (compound **125**), which also appears to have affinities with the CB1 was identified.

Finally, these studies showed that it is possible to obtain compounds able to modulate both classic and novel emerging targets involved in AD. These new compounds can be considered as a platform for further studies aimed at improving their multifaceted activities for the identification of new molecular entities.

In conclusion, these studies could demonstrate that drug discovery paradigm is shifting from one-drug–one-target strategy to one-drug-multiple-targets strategy. The diverse targets can be hit not only by multiple components but also by one compound or even by a single pharmacophore. Thanks to the advancement in high throughput drug screening and computer-aided drug design, there is less and less technical hurdle in finding more multipotent agents to fulfill the new strategy.

CHAPTER 2

4 INTRODUCTION

Ten months out of the three years of PhD were spent at the "Institute of Medical Sciences", Aberdeen, UK, under the supervision of Professor Matteo Zanda. During this period, I was involved in different projects. One of these was entitled: “Mapping the Brain with PET Radiolabeled Cannabinoid (CB1) Ligands PET brain”.

As explained before, Cannabinoid receptors are members of G-protein coupled receptors (GPCRs).[51, 52] Two types of cannabinoid receptor were discovered so far, CB1 and CB2, and both of them were widely studied. CB2 receptors are more expressed in peripheral nervous system (PNS) cells[53] while CB_1 receptors are mostly localized in the brain.[54] Some studies showed the presence of CB1 in the PNS[55] and of CB2 in the central nervous system, although in low density[56]. Moreover, CB1 receptors were associated with many disorders like depression,[57] anxiety,[58] Alzheimer disease, chronic pain[59] and obesity.[60] Consequently, several cannabinoid ligands were developed as drug candidates.

PROJECT 3

4.1 Aim of project 3

The work was based on the concept that the study of the role of the cannabinoid system in the brain is still largely unexplored; moreover, the relationship between CB_1 receptors functional modification, density and distribution and the onset of a pathological state is still not well understood.

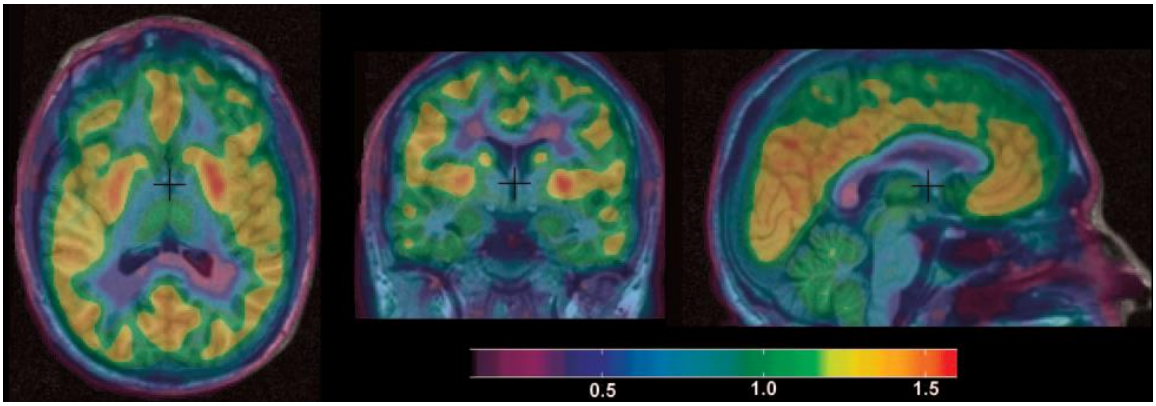
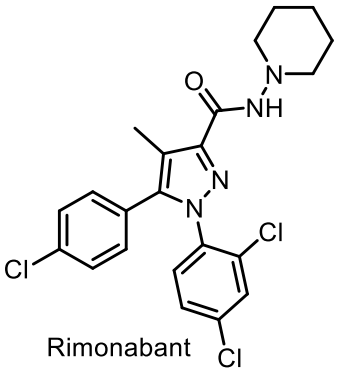


Figure 28: Uptake of [18F] MK-9470, inverse agonist (human IC50, 0.7 nM) for the cannabinoid CB1 receptor in human brain. Baseline images (120–180 min after tracer injection, day 1 of subject 1) show the regional distribution of tracer binding was consistent with labelling CB1R and with rhesus monkey images. Color scale corresponds to SUV units.[61]

Taking this into account, the development of radio-ligands suitable for in vivo PET (positron emission tomography) functional imaging of CB_1 receptors remains an important area of research in medicine and drug development and may lead to the development of new therapeutic strategies for the early stages of AD. We decided to synthesize radioligands for CB_1 receptors, which, through the PET (fig. 28), could provide reliable measurements of density and distribution of CB_1 receptors in the brain. PET would allow the *in vivo* determination of specific biomarkers; in this way, the accuracy of the diagnosis of AD would be improved.

To date, few radiotracers were synthesized based on the structure of SR141716 (Rimonabant), (fig. 29), a potent pyrazole-core inverse agonist of CB1 receptors discovered by Sanofi-Synthelabo (now Sanofi-Aventis) in 1994. This drug was marketed in Europe as an anti-obesity drug but subsequently withdrawn from the market owing to its side-effects, which included severe depression and suicidal thoughts. Until today, most of the derivatives that were synthesized and tested in vivo demonstrated unsatisfactory brain imaging results due to their poor ability to cross the blood-brain barrier.



Rimonabant

hCB1 CHO cells:	hCB2 CHO cells :
Ki (95% CL)= 18.7 (11.1–31.4) nM	Ki (95% CL)= 1.40 x 103 (500–3.70 103) nM
% Max displacement (95% CLb)= 90.2 (85.0–95.3	% Max displacement (95% CLb)= 92.4 (70.4–114)

Figure 29

Extensive theoretical and experimental structure-activity relationship studies were performed on Rimonabant analogues for identifying a general pharmacophore. The two aromatic rings in positions 1 and 5 of the pyrazole ring favourably interrelate with hydrophobic interactions with Trp279/Phe200/Trp356 and Tyr275/Trp255/Phe278 respectively (fig. 30) and, at the same, the aminopiperidine cyclohexyl could interact with the cavity constituted by Val196/Phe170/Leu387 and Met384.[62] In addition, the hydrogen bond between the ligand's amidic oxygen and the receptor residue Lys192 plays a key role in the binding, giving the inverse agonism of Rimonabant.

The aim of this work was the design and synthesis of analogues of Rimonabant that could be used as tracers for PET imaging and that might also be developed into a theranostic or multi-modal imaging tool by radioiodination.

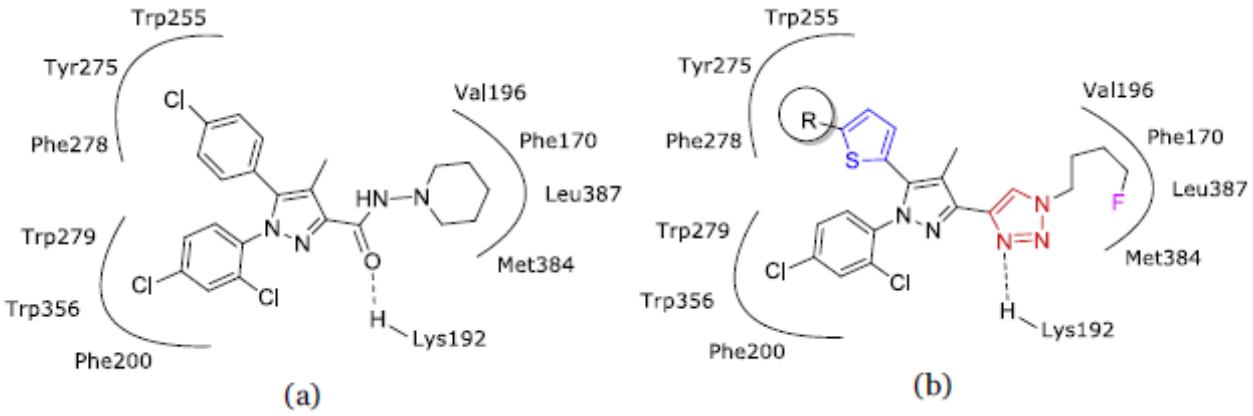
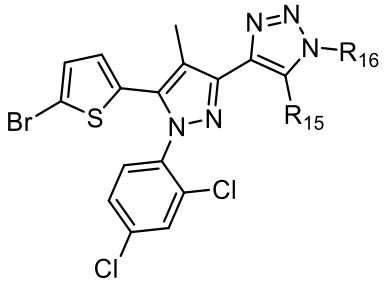
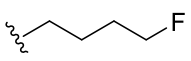
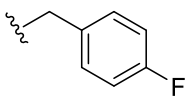


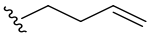
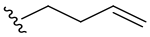
Figure 30: structure-activity relationship Rimonabant and Rimonabant analogues

Considering these information we decided to replace the carbonyl-aminopiperidine residue in position 3 with a 4-(1,2,3-triazolyl) function, since any of the triazolyl sp2 nitrogen atoms could act as hydrogen bond acceptor with Lys192. The 1,2,3-triazole would carry a N-(4-fluorobutyl) group, which should be used for [18F]radiofluorination and could be located in the lipophilic pocket. Many compounds had an iodine atom in position 5 of the triazole; this substitution could be useful for radioiodination. Finally, we planned to replace the 4-chlorophenyl group in 5-position with a 5-substituted 2-thiophenyl residue, which was previously shown to be an advantageous structural modification leading to high-affinity CB1 ligands. Four compounds, shown in table 8, were synthesized.[63]

TABLE 8

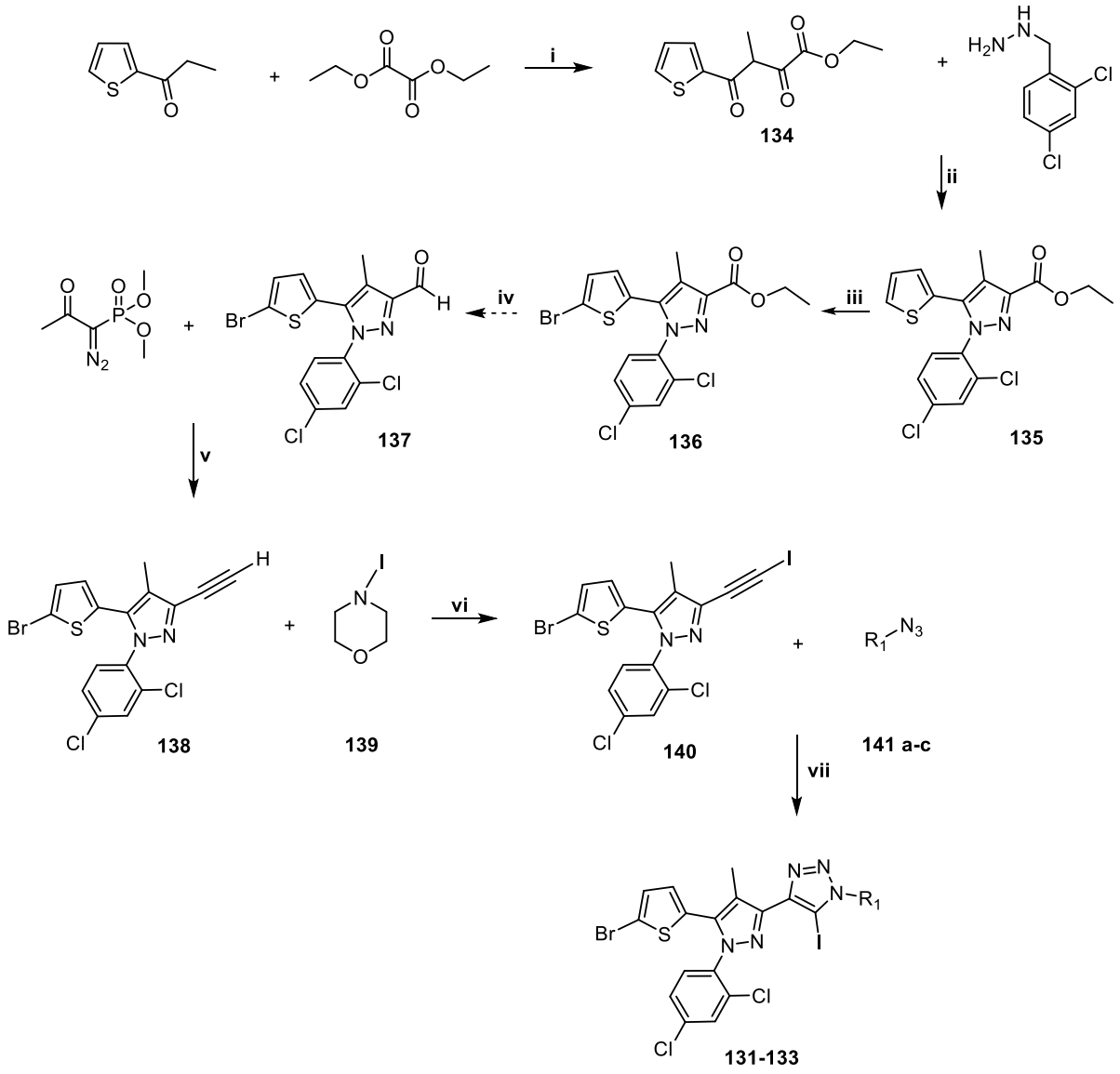


	R	R_1
131	I	
132	I	

133	I	
142	H	

4.2 Chemistry

SCHEME 19

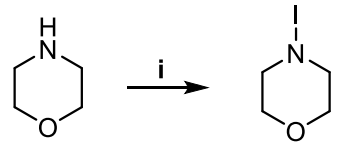


Reagents and conditions: i) EtONa/EtOH, r.t., overnight; ii) 2,4-dichlorophenylhydrazine hydrochloride, EtOH, reflux, overnight; iii) NBS, CH_3CN, from 0 8C to r.t., overnight; iv) DIBAL-H, DCM, -78 °C, 4 h; v) dimethyl 1-diazo-2-oxopropylphosphonate, K_2CO_3, MeOH, r.t., overnight; vi) 4-iodomorpholine, CuI, THF, r.t. 1 h; vii) azido-compound, CuI, TEA, THF, r.t, 72 h

The synthesis started from 1-(thiophen-2-yl) propan-1-one which was condensed with diethyl oxalate in presence of sodium ethylate to give, in 85% yield, the 1,3-diketoester (**135**)as a tautomeric mixture, predominantly containing the alkenylidene structure. Subsequently, tricarbonyl compound and 2,4-dichlorophenylhydrazine were heated in ethanol to afford the pyrazole **136** in rather modest

yield (32%). The latter was regioselectively brominated, employing NBS, to afford the corresponding bromothiophene **137** in good yield (83%). The following conversion was accomplished through a DIBAL-H hydride reduction, providing the aldehyde **138** which was homologated under Bestmann-Ohira alkynylation conditions to generate the alkyne **139** in a moderate yield (55%).
The intermediate **139** was iodinated in a good yield (74%) using 4-iodomorpholine as iodine source. Next, a copper-catalysed azide-iodoalkyne cycloaddition afforded the desired compound **131-134** in moderate yield.

SCHEME 20

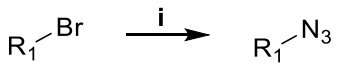


140

Reagents and conditions: i) iodine, CH_3OH, r.t.

Iodomorpholine was obtained treating morpholine with iodine in methanol.

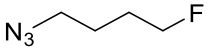
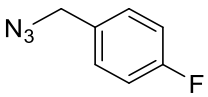
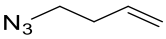
SCHEME 21



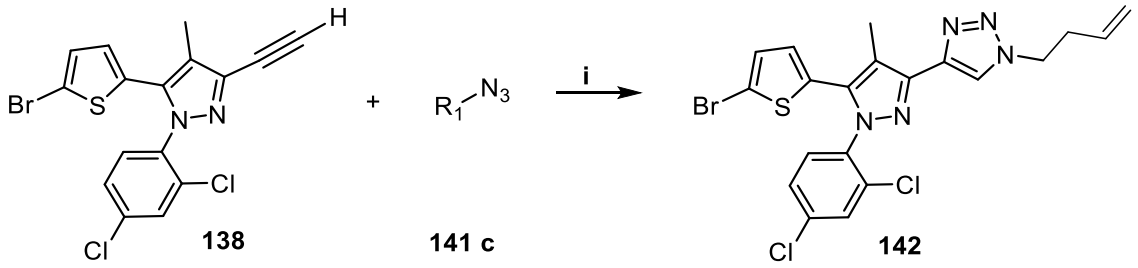
141 a-c

Reagents and conditions: i) NaN_3, H_2O/Acetone (1:4), r.t. or NaN_3, DMF, r.t.

Azido-compounds were formed combining the selected bromoderivative, dissolved in H_2O/Acetone or DMF, with sodium azide.

	R_1
141 a	
142 b	
143 c	

SCHEME 22



Reagents and conditions: i) 1-azido-4-fluorobutane, CuI, sodium ascorbate, tert-BuOH/H2O, r.t, overnight

The triazole in **142** was achieved by means of a copper-catalyzed azide-alkyne cycloaddition protocol, in presence of sodium ascorbate in tert-BuOH/H2O with an acceptable yield.

4.3 Biological results and discussion

At the moment only biological results about compound 131 are available. To present a clearer framework, in table 9 are shown also biological data of Rimonabant and of the non-iodinated analogue of **131** (fig. 31), previously synthesized.

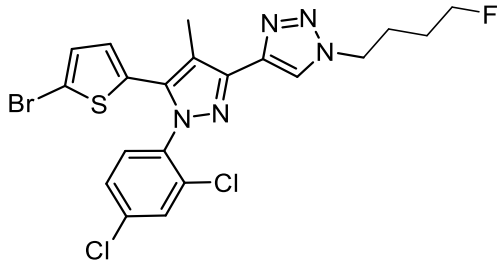


Figure 31: non-iodinated analogue of 131

TABLE 9

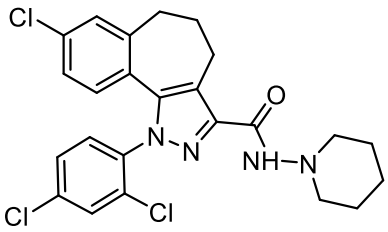
	hCB_1 CHO cells K_i (95% CL) nM	**hCB_1 CHO cells % Max displacement (95% CLb)=**	**hCB_2 CHO cells K_i (95% CL) nM**	**hCB_1 CHO cells % Max displacement (95% CLb)=**
131	422 (235–757) nM	71.8 (65.6–77.9)	n. a.	n. a.
non-iodinated analogue of 131	312 (113–862)	100 (81.0–120)	1.02×10^3 ($603–1.72 \times 10^3$)	92.0 (79.7–104)
Rimonabant	18.7 (11.1–31.4)	90.2 (85.0–95.3)	1.40×10^3 ($500–3.70 \times 10^3$)	92.4 (70.4–114)

Biological data show that the non-iodinated analogue of 131 is affine CB for the receptors although with less intensity than Rimonabant. On the other hand, compound **131** loses almost totally the affinity. This could explain that the polarized C_5-H bond of the triazole, that can act as a hydrogen bonding donor, deems to be essential for the affinity on CBs.

PROJECT 4

4.4 Aim of project 4

The aim of this project was the design and the synthesis of a series of novel 5-aryl-4-alkylpyrazole compounds, related to the potent and selective CB1 antagonist NESS-0327 (fig. 32),[64, 65,66] with the aim of obtaining different analogues, suitable as radioligands for PET imaging.



NESS-0327

Figure 32

The strategy was to modify NESS-0327in two different portions (fig. 33)

- the amidic portion (A)
- the aromatic substituent of the tricyclic moiety (B)

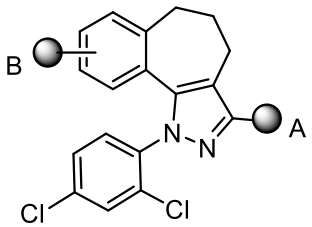
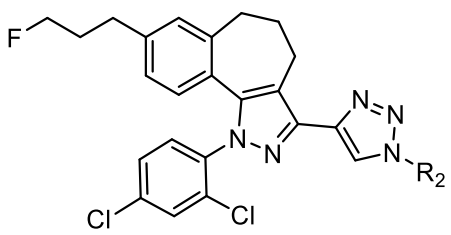
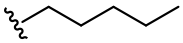
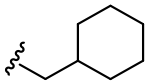


Figure 33

Various triazoles analogues (table 10) bearing a fluoro alkynyl chain, suitable for radiolabelling with ^{19}F radioisotope, replacing the bromine at position 8 of the tricyclic moiety, were designed; the amidic portion was replaced with an *N*-substituited triazole ring.

TABLE 10



	R_2
152	
153	
154	H
155	CH_3

As known, triazole group displays structural similarity with the amide bond, mimicking a *Z* or an *E* amide bond depending on its substitution pattern.[67]

The 1,4-disubstituted triazole moiety can be compared with the *Z*-amide bond. In fact, the lone pair of the 3-nitrogen mimics the one of the carbonyl oxygen of the amide bond, the polarized C_5-H bond can act as a hydrogen bonding donor, just like the amide N–H bond, and the electrophilic and polarized C_4 is electronically similar to the carbonyl carbon (fig. 34).

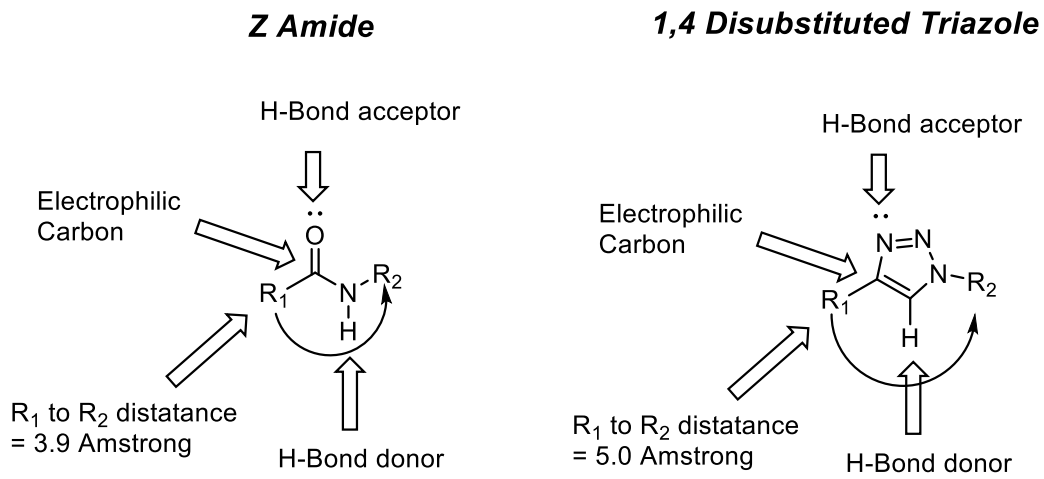


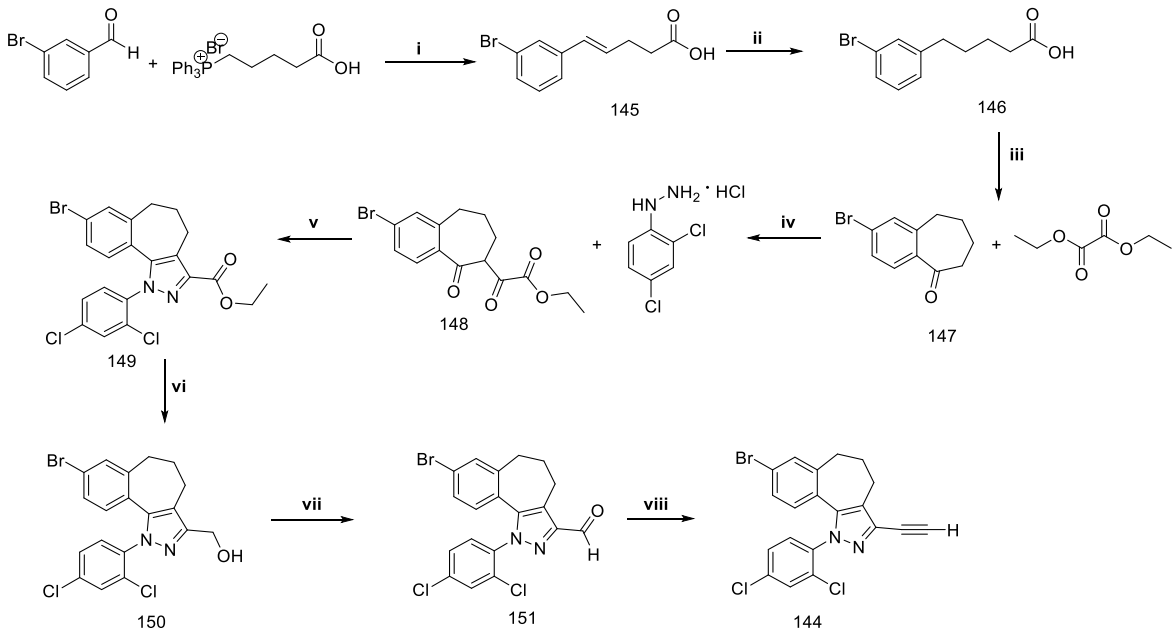
Figure 34

Moreover, the triazole was inserted considering the easiness of its synthesis: in general, the Sharpless click chemistry proves to be highly reproducible, quickly, cheap and simple to perform. The chlorine was changed with the bromine considering that in literature this substitution in NESS-0327 does not produce a large difference in terms of potency, activity and selectivity (CB1 *vs* CB2)[68], moreover bromine is more attractive from a synthetic point of view. In fact, a common intermediate for the syntheses of various analogues bearing the original chlorine was less useful in comparison to the one substituted with the bromine.

4.5 Chemistry

The key intermediate **144** was obtained with the following scheme of synthesis.

SCHEME 23

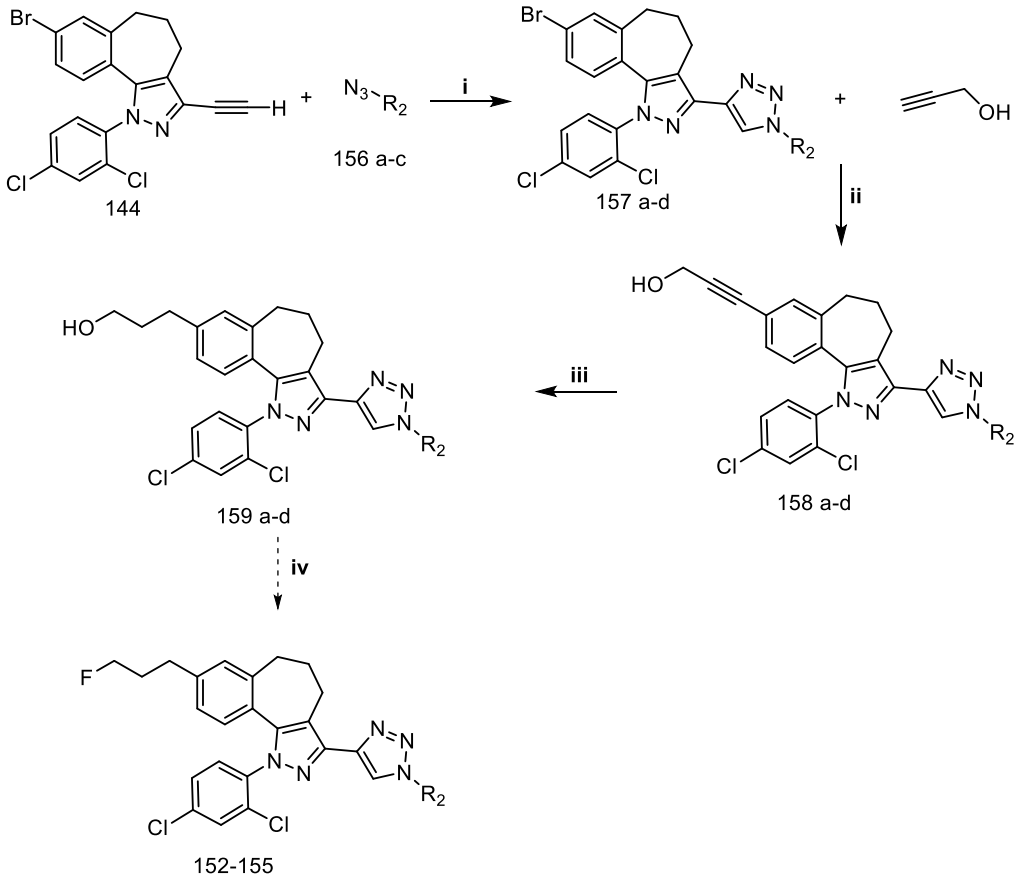


Reagents and conditions: i) tBuOK, DMSO, r.t.; ii) Pd/C, H_2, AcOEt, r.t.; iii) $COCl_2$, DCM, DMF (cat), 50°C, 2h; $AlCl_3$, r.t., overnight; iv) diethyloxalate, N_2, EtOH, r.t.; v) 2,4-dichloroaniline hydrochloride salt, EtOH, 80°C; vi) $LiAlH_4$, THF, 0°C to rt; vii) DMP, DCM, 0°C to r.t.; viii) Bestmann Ohira reagent, CH_3OH, K_2CO_3, r.t.

The synthesis of **144** started from the commercially available 3-bromobenzaldehyde, following the procedures previously published.[69] The aldehyde was submitted to a Wittig condensation with the phosphonium bromide by means of *t*-BuOK in DMSO to yield the pentenoic acid derivatives **145**. Then, the double bond was reduced using Pd/C, AcOEt, 1 atm of H_2, affording the desired product **146**. The subsequent transformation of **146** into the corresponding acyl chloride was carried out using thionyl chloride, while the ring closure was achieved via an intramolecular Friedel-Crafts acylation under standard conditions, affording the benzosuberone derivative **147**. After that, the reaction with diethyl oxalate NaOEt in EtOH provided diketoester **148** which was allowed to react with 2,4-dichlorophenylhydrazine hydrochloride to obtain compound **149**.

The aldehyde **151**,was reached with the total reduction of the ester of **149** using $LiAlH_4$ at 0ºC to give alcohol **150** and the following re-oxidation to aldehyde **151** using DMP at 0ºC. Finally, Bestmann-Ohira alkynylation was employed to obtain compound **144.**

SCHEME 24



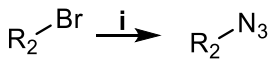
Reagents and conditions: i) azido-compound, $CuSO_4$, sodium ascorbate, $tBuOH/H_2O$; ii) propargyl alcohol, $Pd(PhP)_2Cl_2$, TBAF, 80°C; iii) Pd/C, EtOH, H_2 1 atm, r.t.; iv) DAST

Compound **144** was submitted to a click reaction using the properly azide in order to obtain analogue **157 a-d**. The copper/amine/solvent - free Sonogashira alkynylation[70] that was carried out using $Pd(PPh)_2Cl_2$, TBAF, 80ºC obtaining **158 a-d**. Then, the triple bond was hydrogenated before the deoxofluorination step, in order to avoid a side reaction that occurs using DAST on a similar substrate. In fact, previously, the formation of a side elimination product was observed. It was due to the creation of a propargyl cation intermediate[71] which reacts with toluene (employed as solvent) through a Friedel-Crafts propargylation, leading to an undesired product. Without the triple bond, no radical side process should take place.

As already published, the switch from a single bond to a triple bond, on similar substrate, does not affect the activity and the selectivity against the CB1 receptor.[72]

Unfortunately, during the hydrogenation the two chlorine atoms were lost and the last step of the synthesis was not performed.

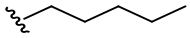
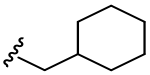
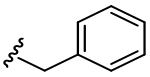
SCHEME 25



156 a-c

Reagents and conditions: i) NaN_3, acetone/H_2O, 60°C or NaN_3, DMSO, 80°C or NaN_3, DMF, 65°C

The synthesis of the azides starts from the corresponding bromide, using sodium azide in acetone/H_2O or in DMSO.

	R_2
156 a	
156 b	
156 c	

4.6 Conclusions

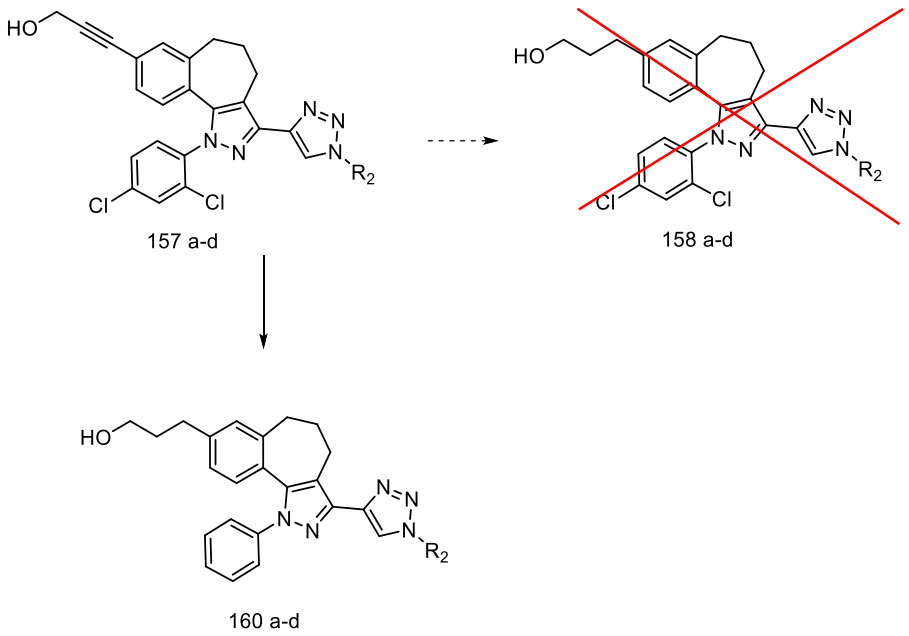


Figure 35

The hydrogenation of the triple bond had as a consequence the unexpected loss of the two chlorine atoms located on the aromatic ring (fig. 35). This event was confirmed by NMR and mass spectra. Unfortunately, considering the limited time spent at Institute of medical sciences, it was not possible to solve the problem and achieve the synthesis of the prefixed compounds. The work was after completed by Zanda's group.

CHAPTER 3

5 INTRODUCTION

5.1 Magnetic resonance imaging

NMR is the most powerful technique for molecule characterization. However, it suffers some weaknesses, such as low sensitivity due to Boltzmann distribution, according to which only 1 spin in 31,200 is NMR active at a magnetic field of 9.4 T (normal magnetic field for MRI). In order to enhance the signal, several hyperpolarization techniques, able to modify the Boltzmann distribution making the technique more sensitive, have been reported.[73]

Magnetic Resonance Imaging (MRI) thanks to imaging nuclei atoms inside the body provides to the visualization in detail of internal structures. MRI generally allows seeing the body structures rather than the pharmacokinetics or metabolism. This technique considers the magnetic properties of atomic nuclei, in particular the hydrogen (present in water molecules), the most abundant one in our bodies. Thanks to a different water concentration in each tissue, there is a dissimilar response to the applied magnetic field and, consequently, a different grey gradient in the final picture (fig. 36).

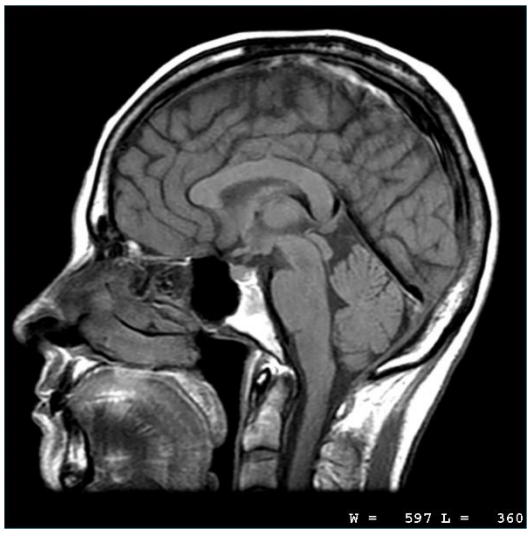


Figure 36

MRI scanner is a device in which the patient lies within a large, powerful magnet and the magnetic field is used to align the magnetization of some atomic nuclei in the body. Radio frequency magnetic fields are applied to systematically alter the alignment of this magnetization. Consequently, nuclei produce a rotating magnetic field that is detected by the scanner and the information is recorded in order to form an image of the desired area of the body.[74] Magnetic field gradients cause nuclei at different locations to precess at different speeds, which allows spatial information to be recovered using Fourier analysis of the measured signal. By using gradients in different directions, 2D images or 3D volumes can be obtained. MRI affords good contrast of different soft tissues of the body, which makes it useful in imaging brain, heart, muscles and cancers compared with other medical imaging techniques such as computed tomography (CT) or X-rays. The adverse side is the long scan times

to acquire a proper image. Some contrast agents have been used based on gadolinium, which changes the relaxation times of the molecules in the body, to enhance the signal and overcome the sensitivity problem. These agents alter the relaxation times of atoms within body tissues after oral or intravenous administration. In fact, MRI and NMR is quite an insensitive technique: for example, it is quite representative the difference in concentration in the preparation of a sample to be submitted to a normal ^{1}H-NMR experiment versus the amount of a compound submitted to an MS analysis. Another example is the time and concentration requested to run a normal ^{13}C-NMR experiment. Several scans are needed in order to achieve a good to optimal Signal-to-Noise: at least 4 for normal 1H-NMR, while 50 for 13C-NMR. That is due to the signal intensity that is related to relative populations of the magnetic levels probed during the experiment; this mechanism is called polarization. At thermal equilibrium the energy levels are close and there is little energy needed to promote the transition from one level to the other one. Moreover, the Boltzman distribution predicts that almost an equal number of nuclei will be in the two different energy levels, even in highest magnetic fields. An altered distribution of the nuclei population in the two levels would increase NMR signal because more nuclei are subjected to transition. This mechanism is called hyperpolarization.

5.2 Para Hydrogen Induced Polarisation

Para Hydrogen Induced Polarisation (PHIP) is a technique that induce polarisation into molecules. This method was used for the generation of PHIP enhanced contrast agents in Magnetic Resonance Imaging.[75] In the H_2 molecule the two hydrogen nuclei have spin = 1/2 and the molecule is NMR active. The two spins can couple and generate four spin isomers for the molecule as a triply degenerate and symmetric with respect to the exchange of nuclei state (orthohydrogen), finally, the remaining configuration corresponding at the parahydrogen, is antisymmetric with respect to exchange of nuclei and results in a singlet state. Even if these spin configurations are close in energy and basically equally at room temperature, p-H_2 is the most stable isomer and it is prevalent at low temperatures. The transformation from one isomer to the other is forbidden in fact, spins cannot simply flip from one state to another even if the thermodynamics are favorable. Thus is necessary a paramagnetic catalyst like charcoal, Fe_2O_3 or $Fe(OH)_3$.

When hydrogen reacts and its symmetry is broken in an oxidative addition reaction to a metal center, novel NMR effects can be seen thanks to the reaction that proceeds in a spin correlated manner.

In nature hydrogen is diffused in equal numbers of molecules in each of its four spin configurations, also the four possible configurations in the oxidative addition product of hydrogen on the metal center are equally populated.

Considering that the intensity of an NMR signal is proportional to the population difference among the energy levels (very low in this case), the number of nuclei able to undergoing the transition is very low and low intensity signals are detected (fig. 37). On the other hand, if it is possible to populate selectively only one of the spin configurations of the molecular hydrogen, the *αβ-βα* state or p-H_2, after the oxidative addition to the metal center only one of the spin states in the product would be selectively populated, with a resulting hyperpolarisation. In this case, the populations of each energy

level are very different to the usual Boltzmann distribution and the resultant NMR signals of the product show an enhancement.

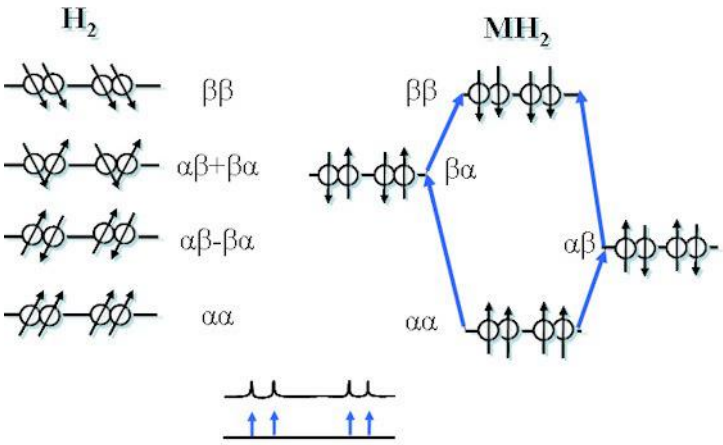


Figure 37: Boltzmann-derived distribution and resulting transitions and NMR signals

The enhancement worked if the resultant signals are antiphase: one set in absorption and the other in emission (fig. 38). After its embranchment, the sample relaxes back to thermal equilibrium.

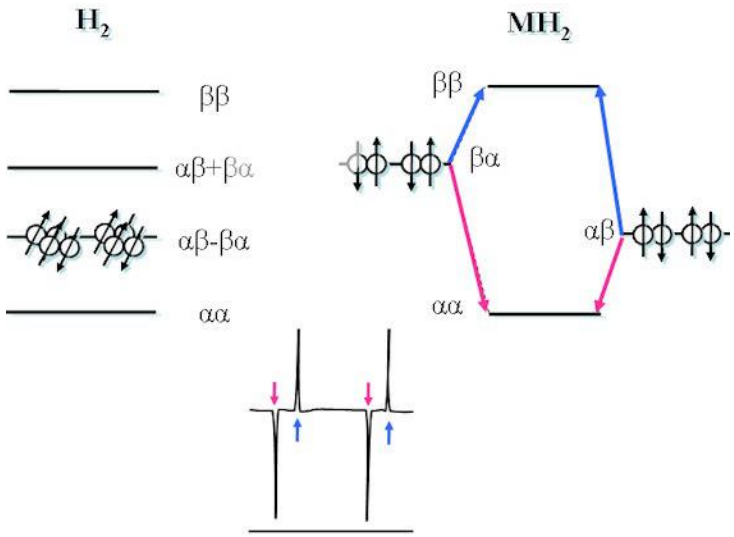


Figure 38: p-H_2 - distribution of population and resulting NMR signal

ALTADENA (Adiabatic Longitudinal Transport After Dissociation Engenders Nuclear Alignment) and PASADENA (Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment) are two old techniques used to obtain the p-H2 -induced polarization. Their fundamental requirement is that the molecule has to present a proton acceptor (double or triple bond).

SABRE (signal amplification by reversible exchange) a new method developed at the University of York, UK, in the group of Professor Simon Duckett, can achieve the PHIP without any chemical modification of the substrate. [76,77]

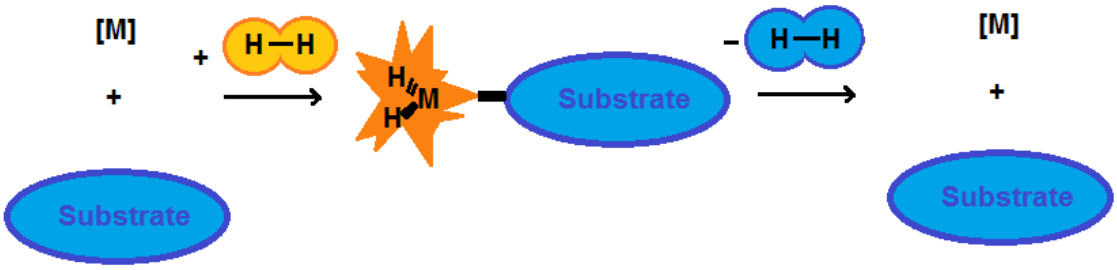


Figure: 39

More in detail, there is no chemical modification of the hyperpolarized material: it generates long lived spin states and then the polarization can be transferred to the heteronuclei through a suitable NMR sequence. SABRE is based on the transfer of the polarization present in the para-hydrogen to a pyridine or pyridyl moiety within a molecule through a catalyst center based on iridium metal (fig. 39). Probably the metal center has the function of a template and allows the p-H_2 and the substrate molecule to get close and participate in the magnetization exchange. All this process happens at low magnetic fields, and signal enhancement >100-fold could be observed.[78]

5.3 Nitric Oxide Synthases and arginine

Arginine, a natural amino acid among the 20 proteinogenic L-amino acids, is one of the most basic residues, with a pKa of 12.48. Arginine is the substrate for Nitric Oxide Synthases (NOS) enzyme, a class composed by three different isozyme forms (the Neuronal NOS, the Endothelial NOS and the Inducible NOS) with the function of the conversion of arginine to citrulline and nitric oxide (fig. 40).

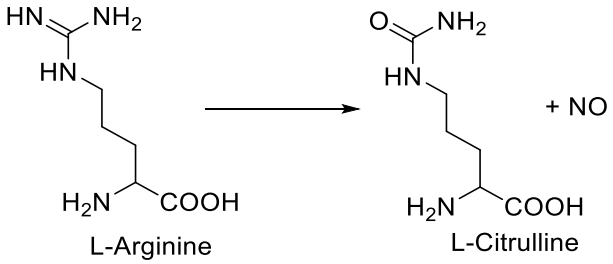


Figure 40

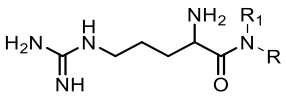
NO, produced in neuronal tissues with the function of intracellular second messenger for neurotransmission, is involved in the regulation of smooth muscle relaxation and blood pressure in the endothelial tissue; moreover, in macrophage cells, NO acts as an answer to inflammatory stimuli. NO has also an important role in cardiovascular diseases, such as hypertension.

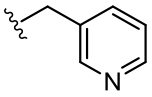
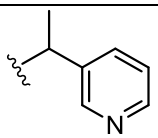
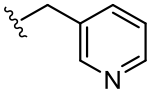
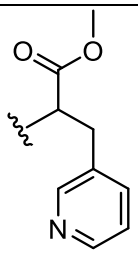
Many inhibitors are based on L-arginine; but a simple modification of the scaffold or capping an amine functionality could target only one of the different isoforms of the NOS enzyme.[79]

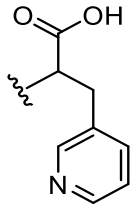
5.4 Aim of project 5

This project was developed in collaboration with the group of Professor Matteo Zanda at the University of Aberdeen, UK, the group of Professor Michael Frenneaux and doctor Dana Dawson at the University of Aberdeen and the group of Professor Simon B. Duckett at the University of York.
The aim was the synthesis of arginine analogues with a pyridyl/pycolyl moiety that could be potential substrate for eNOS enzyme and also could be suitable substrate for the transfer of polarization by p-H_2 molecules through SABRE technique.
In fact, considering that peculiar analogues and peptides involved in the catalytic process of the synthesis of the Nitric Oxide could be detected through Magnetic Resonance Imaging, synthesized compounds could be source of their NMR signal. For these reasons, the goal was to develop arginine analogues that could work as MRI contrasting agents which are target specific due to their exclusively location into NOS enzymes. This could result in the MRI technique as a more sensitive and faster technique.
For these reasons, five compounds were synthesized (table 11)

TABLE 11

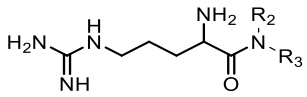


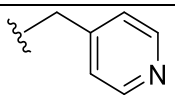
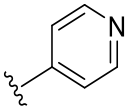
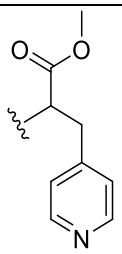
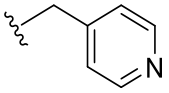
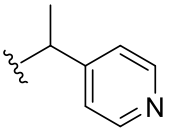
	Salt	R	R_1
160	·3HCl	H	
162	·3HCl	H	
164	·3HCl	CH_3	
166	·3HCl	H	

168	·3HCl	H	

Moreover, five compounds, already synthesized, were converted in hydrochloride salt (table 12).

TABLE 12

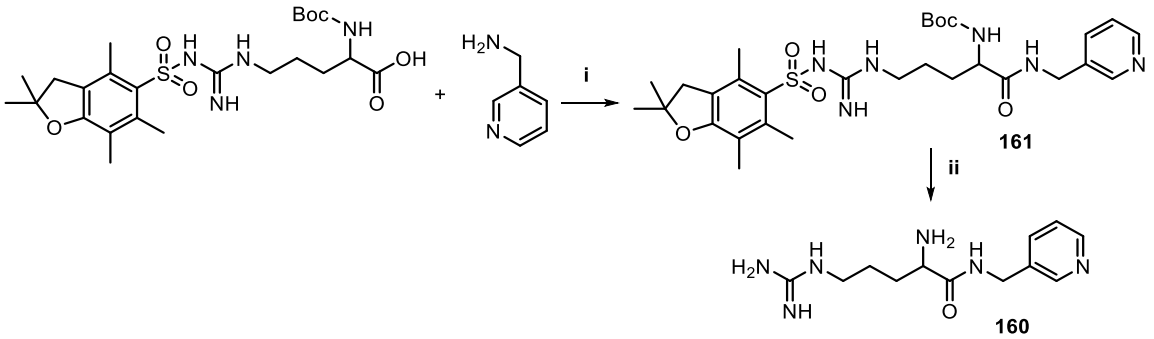


	Salt	**R_2**	**R_3**
171	·3HCl	H	
172	·3HCl	H	
173	·3HCl	H	
174	·3HCl	CH_3	
175	·3HCl	H	

5.5 Chemistry

Compounds showed in table 11 were synthesized following synthetic procedures explained in schemes 26-31.

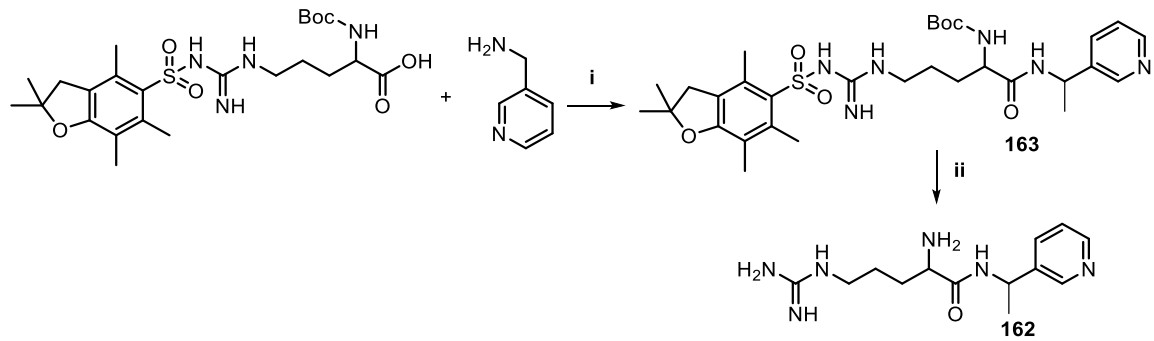
SCHEME 26



Reagents and conditions: i) HATU, TEA, DCM, r.t.; ii) CF_3COOH, r,t,; HCl 1N

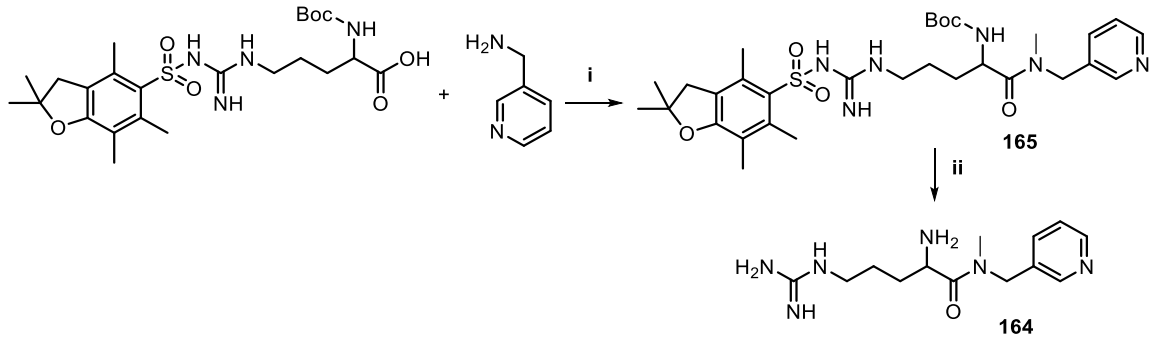
The small peptide 161 was obtained by simple coupling of the Boc-Pbf protected arginine with pyridin-3-ylmethanamine in presence of HATU and TEA; then the aminoacid derivative was deprotected using TFA. Finally, in order to reach the hydrochloride salt, compound was treated with HCl 1N and crystals of **160** were obtained thanks to freeze drying. Using the same procedure were obtained compounds **162, 164, 166** too.

SCHEME 27



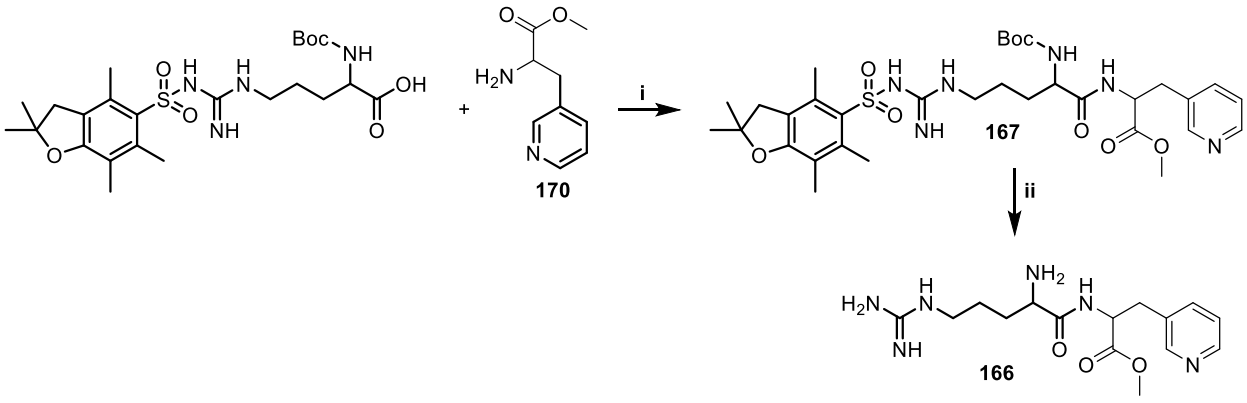
Reagents and conditions: i) HATU, TEA, DCM, r.t.; ii) CF_3COOH, r,t,; HCl 1N

SCHEME 28



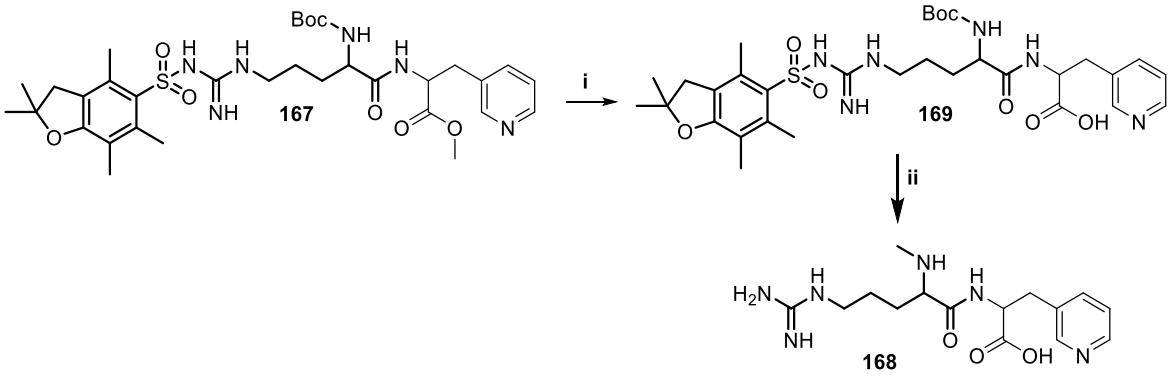
Reagents and conditions: i) HATU, TEA, DCM, r.t.; ii) CF_3COOH, r,t,; HCl 1N

SCHEME 29



Reagents and conditions: i) HATU, TEA, DCM, r.t.; ii) CF_3COOH, r,t,; HCl 1N

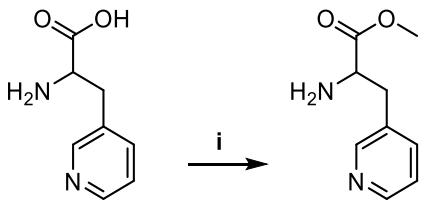
SCHEME 30



Reagents and conditions: i) HATU, TEA, DCM, r.t.; ii) LiOH, THF, H_2O; iii) CF_3COOH, r,t,; HCl 1N

Thanks to a saponification with LiOH the acid **169** was formed then, the aminoacid derivative was deprotected using TFA. Finally, in order to reach the hydrochloride salt, compound was treated with HCl 1N and crystals of **168** were obtained with the afford of the freeze drying.

SCHEME 31



170

Reagents and conditions: i) $SOCl_2$, CH_3OH, r.t.

2-amino-3-(pyridin-3-yl)propanoic acid was methylated using $SOCl_2$, in methanol, affording compound **165**.

5.6 Results and discussion

Compounds of **Table 11** and **Table 12** were tested with the eNOS enzyme according to a protocol based on a standard Nitrate-Nitrite colorimetric assay protocol by Dana Dawson's research group at the Institute of Medical Sciences, Aberdeen, UK.

Polarisation Tests

During a period as a visiting PhD student at The Centre for Hyperpolarisation in Magnetic Resonance (CHyM), York, UK, SABRE experiments of **160**, **164**, **171** and **174** were performed.
Considering previous studies, that demonstrated that the presence of the triflic acid as counterion interacted strongly with the catalyst causing an insignificant enhancement, all synthesized compounds were converted in hydrochloride salts. Unfortunately, HCl salts still interacted with the catalyst. For this reason why we had to remove it before reading out the results.

In order to analyze the enhancement of **171** we followed this procedure:
The reaction mixture was prepared dissolving approximately 10 to 20 mg of **171** in 0.6 mL of deuterated methanol, because its appearance is a very sticky oil, and it was impossible to weight out, adding 2 mg of the catalyst Ir(cod)(IMes)Cl. This solution was filtered through a home-made cartridge of K_2CO_3 to get rid of the HCl, which is known to deactivate the catalyst. Then the cartridge was washed out with 0.1 mL of the same solvent. Unfortunately, during the neutralization process, water was formed (fig. 41) causing the precipitation of an amount of catalyst.

$$2\ HCl + K_2CO_3 \rightarrow 2\ KCl + H_2O + CO_2$$

Figure 41

Even though, we were able to achieve the experiments listed below:

- ^1H thermal spectrum (fig. 42)
- ^1H Hyperpolarised spectrum, that showed 50.68-fold enhancement signal at 70 gauss (fig. 43)
- OPSY experiments at 0 gauss (fig. 44) and 70 gauss, that showed greater enhancement signal at 0 gauss; no enhancement for any proton in the arginine chain was observed but only on aromatic region.
- ^{13}C hyperpolarized spectrum at 0 gauss, that showed no enhancement.

NMR tube prepared was first degassed then, the *para*-hydrogen was introduced in the headspace, and the tube was shaken with the "shake-and-drop" technique close to the NMR instrument, where the magnetic field is approximately 70 G. The distance to the instrument where there is a 70 gauss magnetic field was calculated using a gauss meter.

In vitro, **171** indicated the highest activity among all ten tested compounds (about as twice active as native arginine). It also showed the best enhancement in the SABRE polarization tests.

The enhancement of **174** was analyzed following this procedure:

Even if **174** did not show an excellent activity in vitro, it was tested with SABRE method in order to observe the effect of the methyl substituent on the nitrogen.

The sample was prepared dissolving 2 mg of the catalyst Ir(cod)(IMes)Cl and 10.7 mg of **174** in 0,6 mL of deuterated methanol. Then, the solution was filtered through a K_2CO_3 home-made cartridge to get rid of the HCl part of the salt. Catalyst needed to be activated overnight.

Indeed, enhancement was lower than that of **171**, being only 17.25-fold. In the OPSY spectra no proton for the arginine chain was observed and only protons of the pyridyl moiety can be detected.

0 gauss gave better enhancement than 70 gauss, therefore ^{13}C spectrum was collected hyperpolarizing at that magnetic field, but no enhancement was observed.

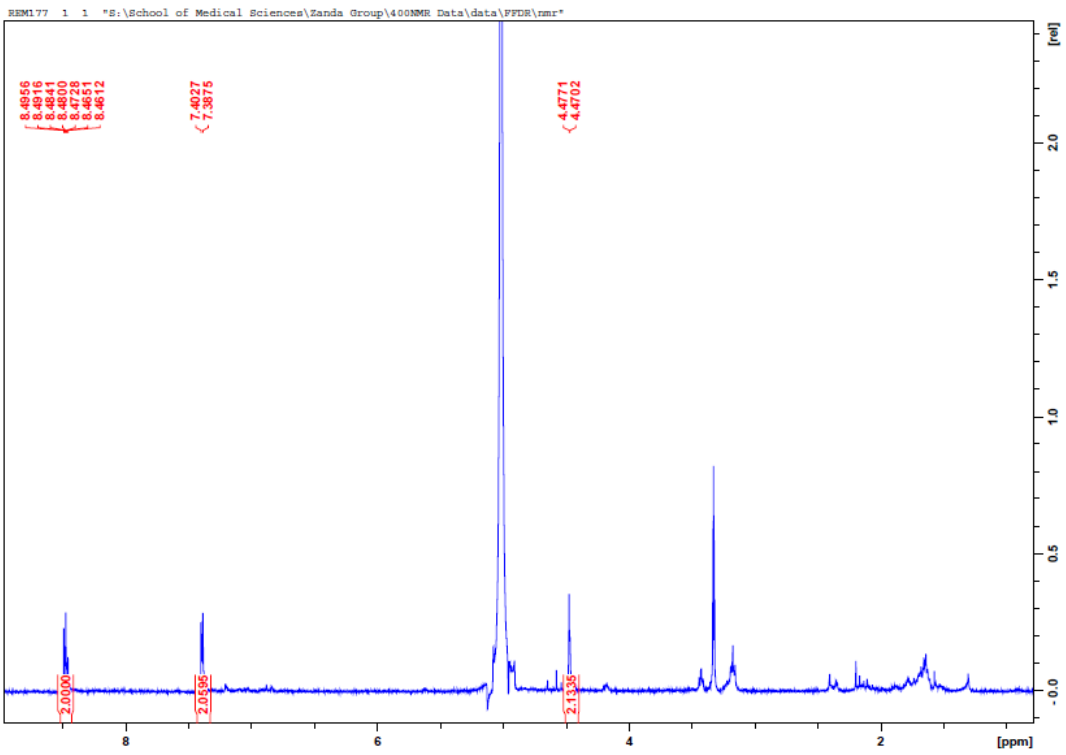


Figure 42

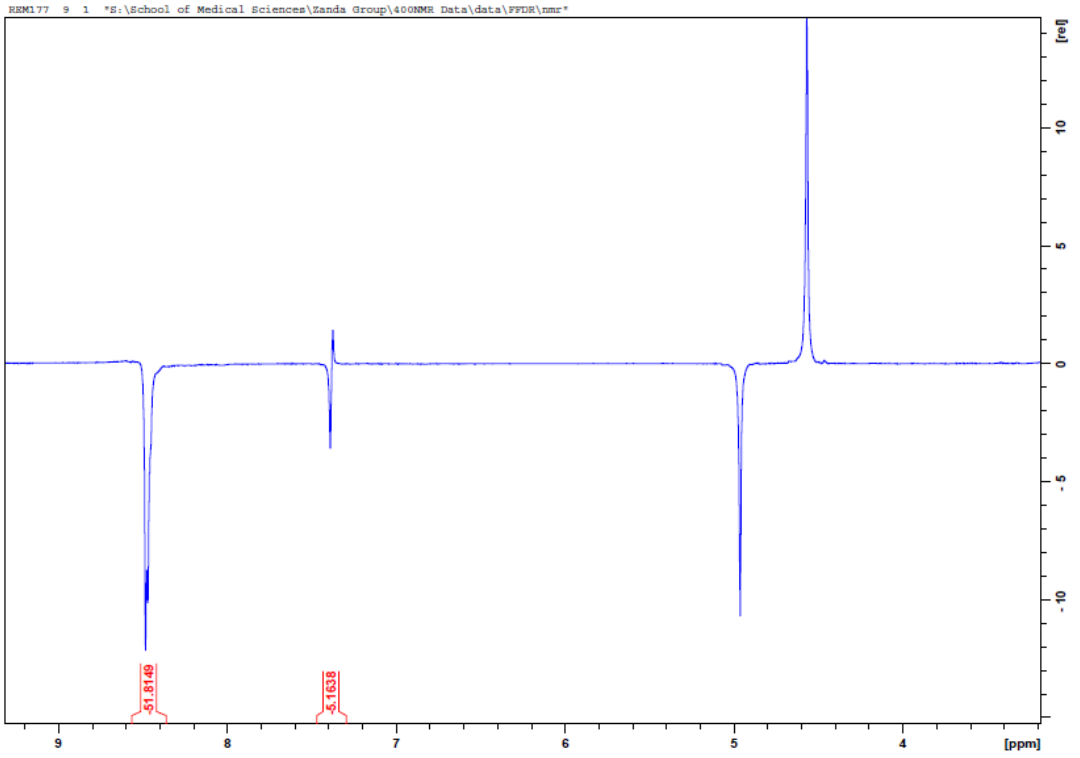


Figure 43

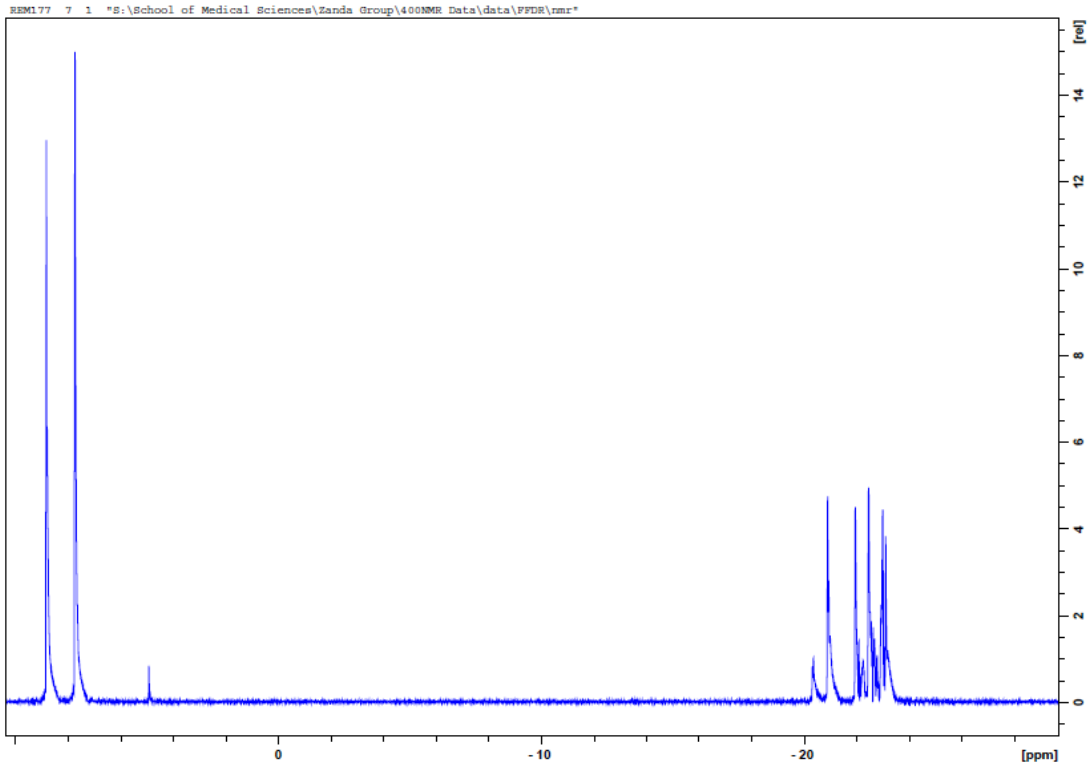


Figure 44

The enhancement of **160** was analyzed following this procedure:
10.1 mg of **160** were dissolved with 0.6 mL of deuterated methanol and 2 mg of the catalyst Ir(cod)(IMes)Cl. Then, it was filtered through the K_2CO_3 cartridge. Enhancement shown was of 39.47-fold, spread in the four protons of the pyridyl moiety (o: o: p: m are 12.62: 14.73: 9.17: 2.95). OPSY was found to be better at 0 gauss, but ^{13}C was not even read out, because it was supposed the enhancement of the protons was not enough to be transferred to the carbon.

The enhancement of **164** was analyzed following this procedure:
9.7 mg of the **164** were dissolved in a solution of 2 mg of the catalyst Ir(cod)(IMes)Cl in 0.6 mL of deuterated methanol. Some spectra were recorded like that, after degassing the tube, showing enhancement only on the first read out and only 0.4 on the ortho protons, and no signal for the hydrides was observed. The reason could be that this compound could form a very stable complex with the catalyst that did not dissociate, so catalyst is saturated, and therefore blocked. If this happens, the guanidine moiety of the arginine is the most likely part to be blocking the Ir center. Moreover it could be due to a too slow or quick exchange. To rule out that possibility, hyperpolarization was achieved at 9ºC and at 35ºC, but still the same spectrum could be observed. This indicates the temperature and the rapidity of the polarization transfer is most likely not the cause of the lack of polarization within the molecule.

5.7 Conclusions

Previous work showed that no data were available for unprotected compounds, with the triflic acid as counterion that interacted with the catalyst; therefore hyperpolarisation process could not be properly performed. In this project, with the use of the free amine, we obtained interesting results in particular for **171** that showed a 53-fold enhancement in the signal, also for both protons ortho and meta even if ^{13}C NMR did not show any enhancement.

The results obtained in York constitute a significant step forward showing an enhancement almost as strong as the picolyl moiety itself as previous studies demonstrated. This means that optimization can be now performed, with the goal of obtaining a much greater enhancement.

CHAPTER 4

6 EXPERIMENTAL SECTION

Chemistry

General Methods.

Melting points were measured in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. Direct infusion ES-MS spectra were recorded on a Waters Micromass ZQ 4000 apparatus. ^{1}H, ^{13}C, ^{19}F NMR experiments were recorded in $CDCl_3$, unless differently indicated, on Varian VXR 400 MHz instrument. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet) or m (multiplet). Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040-0.063 mm; Merck) by flash chromatography. Compounds were named following IUPAC rules as applied by Beilstein-Institut AutoNom (version 2.1), a PC integrated software package for systematic names in organic chemistry. HPLC-MS: Agilent Technologies HP1100 instrument, equipped with a ZOBRAX-Eclipse XDB-C8 Agilent Technologies column, mobile phase: H_2O/CH_3CN, 0.4 mL/min, gradient from 30 to 80% of CH_3CN in 8min, 80% of CH_3CN until 25min, coupled with an Agilent Technologies MSD1100 single-quadrupole mass spectrometer: full scan mode from m/z = 50 to 2600, scan time 0.1s in positive ion mode, ESI spray voltage 4500V, nitrogen gas 35psi, drying gas flow 11.5 mL/min, fragmentor voltage 20V.

PROJECT 1

3-(3-chloropropoxy)-6H-benzo[c]chromen-6-one (57)

To a suspension of 2.58g (11.37 mmol, 3eq) of DDQ (2,3-dichloro-5,6-dicyano-p-benzoquinone) in 30 mL of anhydrous dioxane, 1.1g (3.79 mmol, 1eq) of 3-(3-chloropropoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one were added and the mixture was stirred and refluxed for 24h. The solution was concentrated under vacuum, then water was added and the aqueous phase was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude was purified via flash chromatography: petroleum ether/ethyl acetate 3:2. 0.23g of **57** were obtained. Yield: 21.10% M.P.: 84-85° C; ^{1}H-NMR δ 8.35 (d, *J* = 8.8 Hz, 1H), 8.11 - 7.95 (m, 2H), 7.83 (t, J = 8.2 Hz, 1H), 7.55 (t, J = 6.4 Hz, 1H), 7.09- 6.81 (m, 2H), 4.19 (t, J = 11.2 Hz, 2H), 3.78 (t, J = 15.6 Hz, 2H), 2.35 – 2.26 (m, 2H)

7-hydroxy-3-phenyl-2H-chromen-2-one (58a)

0.17g (7.35 mmol) of Na were dissolved in methanol. The solution was added to 1.0g (7.35 mmol) of phenylacetic acid previously dissolved in methanol. The mixture was concentrated under vacuum, then ethanol was added and it concentrated again. 1.01g (7.350 mmol) of 2,4-

dihydroxybenzaldehyde and an excess of acetic anhydride were added to the reaction flask that was stirred and heated at 180 °C for 5h. The reaction was poured in a solution of sodium bicarbonate(10%), then it was filtered and allowed to dry. The solid was dissolved in DCM then was washed with NaOH 2N and water; the organic phase was concentrated under vacuum. The compound was further purified via flash chromatography: petroleum ether/ethyl acetate 7:3. Obtained compound was deacetylated with an excess of K_2CO_3 in acetone warming up to reflux. 0.65g of neat compound **58a** were obtained. Yield: 37.14% M.P.: 175.5-177° C; ^{1}H NMR δ 9.49 (s, 1H), 8.05 (s, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.48 - 7.54 (m, 3H), 6.91 (d, *J* = 10.8 Hz, 1H), 6.81 (s, 1H)

7-hydroxy-3-(4-methoxyphenyl)-2H-chromen-2-one (58b)

Compund **58b** was obtained following the same procedure described for compound **58a** using p-methoxy phenylacetic acid and 2,4-dihydroxybenzaldehyde. Yield: 8,7 %; M.P.:158.7-160.8° C; ^{1}H-NMR δ 9.49 (s, 1H), 8.05 (s, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 10.8 Hz, 1H), 6.81 (s, 1H), 3.92 (s, 3H)

6-hydroxy-3-(4-methoxyphenyl)-2H-chromen-2-one (58c)

Compund **58c** was obtained following the same procedure described for compound **58a** using p-methoxy phenylacetic acid and 2,5-dihydroxybenzaldehyde. Yield: 49.7% M.P.: 186.6-187.3° C. ^{1}H-NMR δ 9.49 (s, 1H), 8.11 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.48 - 7.32 (m, 3H), 7.08 - 6.91 (m, 2H), 3.68 (s, 3H)

3-(4-chlorophenyl)-7-hydroxy-2H-chromen-2-one (58d)

Compund **58d** was obtained following the same procedure described for compound **58a** using p-chlorophenylacetic acid and 2,4-dihydroxybenzaldehyde. Yield: 48.27% M.P.: >250° C; ^{1}H-NMR δ 9.49 (s, 1H), 8.11 (s, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.48 (dd, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 10.8 Hz, 1H), 6.81 (s, 1H)

7-(3-chloropropoxy)-4-methyl-2H-chromen-2-one (17)

To a solution of 1.26g (7.16 mmol) of 7-hydroxy-4-methyl-2H-chromen-2-one in acetone (100 mL), 2.47g (17.89 mmol, 2.5eq) of K_2CO_3 and 1.67g (10.738 mmol, 1.5eq, d=1.592) of 1-bromo-3-chloropropane were added. The reaction was refluxed for 24h, then it was filtered hot and concentrated under vacuum. The crude was crystallized with petroleum ether. 1.42g of neat compound 17. Yield: 79% M.P.: 79-80° C; ^{1}H NMR δ 7.58 (d, J = 8.2 Hz, 1H), 6.95 - 6.81 (m, 2H), 6.15 (s, 1H),.4.19 (t, J = 11.2 Hz, 2H), 3.78 (t, J = 15.6 Hz, 2H), 2.45 (s, 3H), 2.35 – 2.26 (m, 2H)

3-(3-chloropropoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (18)

Compound **18** was obtained following the same procedure described for compound **17** using 3-hydroxy-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one as starting material. Yield: 78% M.P.: 99.8-

101.0° C. ^{1}H NMR δ 7.44 (d, J = 8.8 Hz, 1H). 6.90 - 6.70 (m, 2H), 4.07 (t, J = 12.4 Hz, 2H), 3.85 - 3.70 (m, 2H), 2.75 (t, J = 11.6 Hz, 2H), 2.57-2.54 (m, 2H), 2.00 – 1.97 (m, 2H), 1.86 – 1.78 (m, 4H)

7-(3-chloropropoxy)-3-phenyl-2H-chromen-2-one (19)

Compound **19** was obtained following the same procedure described for compound **17** using compound **58a** as starting material. Yield: 48.78% M.P.: 108-110° C; ^{1}H NMR δ 7.75 (s, 1H), 7.68 (d, J = 8.8 Hz, 2H), 7.45 – 737 (m, 4H), 6.87 (d, J = 10 Hz, 2H), 4.19 (t, J = 11.6 Hz, 2H),3.76 (t, J = 12.8 Hz, 2H), 2.31 – 2.26 (m, 2H)

7-(3-chloropropoxy)-3-(4-methoxyphenyl)-2H-chromen-2-one (20)

Compound **20** was obtained following the same procedure described for compound **17** using compound **58b** as starting material. Yield: 51.97% M.P.: 108-114.5° C; ^{1}H NMR δ 7.81 (s, 1H), 7.51 - 7.25 (m, 4H), 7.01 - 6.87 (m, 3H), 4.19 (t, J = 11.2 Hz, 2H), 3.92 (s, 3H), 3.78 (t, J = 15.6 Hz, 2H), 2.35 – 2.26 (m, 2H)

6-(3-chloropropoxy)-3-(4-methoxyphenyl)-2H-chromen-2-one (21)

Compound **21** was obtained following the same procedure described for compound **17** using compound **58c** as starting material. Yield: 49.53% M.P.: 113.9-116.2° C; ^{1}H NMR 7.89 (s, 1H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.49 - 7.31 (m, 3H), 7.08 - 6.91 (m, 2H), 4.19 (t, J = 11.2 Hz, 2H), 3.95 (s, 3H), 3.78 (t, J = 15.6 Hz, 2H), 2.35 – 2.26 (m, 2H)

3-(4-chlorophenyl)-7-(3-chloropropoxy)-2H-chromen-2-one (22)

Compound **22** was obtained following the same procedure described for compound **17** using compound **58d** as starting material. Yield: 74.38% M.P.: 157° C; ^{1}H NMR δ 7.76 (s, 1H), 7.66 (dd, *J* = 8.4 Hz, 2H), 7.46-7.40 (m, 3H), 6.89-6.87 (m, 2H), 4.22-4.18 (m, 2H), 3.79-3.75 (m, 2H), 2.31-2.26 (m, 2H)

7-(3-chloropropoxy)-2H-chromen-2-one (23)

Compound **23** was obtained following the same procedure described for compound **17** using 7-hydroxycoumarin as starting material. Yield: 95.92% M.P.: 104.3° C; ^{1}H NMR δ 7.60 (d, *J* = 9.5 Hz, 1H), 7.33 (d, *J* = 8.6 Hz, 1H), 6.80 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.76 (d, *J* = 2.3 Hz, 1H), 6.20 (d, *J* = 9.5 Hz, 1H), 4.13 (t, *J* = 5.8 Hz, 2H), 3.71 (t, *J* = 6.3 Hz, 2H), 2.23 (p, *J* = 6.0 Hz, 2H)

8-(3-chloropropoxy)-5H-chromeno[2,3-b]pyridin-5-one (24)

Compound **24** was obtained following the same procedure described for compound **17** using 8-hydroxy-5H-chromeno[2,3-b]pyridin-5-one as starting material. Yield: 95.92% M.P.: 104.3° C; ^{1}H NMR δ 8.72 (d, *J* = 8 Hz, 2H), 8.252 (d, *J* = 8.8 Hz, 1H), 7.45 – 7.42 (m, 1H), 7.03 – 6.98 (m, 2H), 4.28 (t, *J* = 12 Hz, 2H), 3.79 (t, *J* = 12.4 Hz, 2H), 2.35 – 2.31 (m, 2H)

7-((5-chloropentyl)oxy)-2H-chromen-2-one (25)

Compound **25** was obtained following the same procedure described for compound **17** using 7-hydroxycoumarin and 1-Bromo-5-chloropentane as starting materials. Yield: 51.21% M.P.: 52-54° C; ^{1}H NMR δ 7.60 (d, *J* = 9.5 Hz, 1H), 7.33 (d, *J* = 8.6 Hz, 1H), 6.80 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.76 (d, *J* = 2.3 Hz, 1H), 6.20 (d, *J* = 9.5 Hz, 1H), 4.13 (t, *J* = 5.8 Hz, 2H), 3.71 (t, *J* = 6.3 Hz, 2H), 1.98-1.89 (m, 4H), 1.69-1.68 (m, 2H)

3-((5-chloropentyl)oxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (26)

Compound **26** was obtained following the same procedure described for compound **17** using 3-hydroxy-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one and 1-Bromo-5-chloropentane as starting materials. Yield: 96.15%; yellow oil; ^{1}H NMR δ 7.38 (d, *J* = 8.8 Hz, 1H), 6.76 (d, *J* = 8.8 Hz, 1H), 6.70 (s, 1H), 3.94 (t, *J* = 12.4 Hz, 2H), 3.50 (t, *J* = 12.8 Hz, 2H), 2.67 – 2.56 (m, 2H), 2.49 – 2.48 (m, 2H), 1.83 - 1.61 (m, 8H), 1.55 – 1.52 (m, 2H)

8-((5-chloropentyl)oxy)-5H-chromeno[2,3-b]pyridin-5-one (27)

Compound **27** was obtained following the same procedure described for compound **17** using 8-hydroxy-5H-chromeno[2,3-b]pyridin-5-one and 1-Bromo-5-chloropentane as starting materials. Yield: 67.86% M.P.: 109.8-110.5° C; ^{1}H NMR δ 8.72-8.69 (m, 2H), 8.24 (dd, *J* = 7.6 Hz, 1H), 7.45 (dd, *J* = 12.4 Hz, 1H), 6.99 (d, *J* = 10Hz, 2H), 4.12 (t, *J* = 12.8 Hz, 2H), 3.61 (t, *J* = 13.2 Hz, 2H), 1.92-1.87 (m, 4H), 1.69-1.68 (m, 2H)

7-(3-iodopropoxy)-4-methyl-2H-chromen-2-one (28)

A mixture of 1.42g (5.63 mmol) of **17** and 0.84g (5.63 mmol) di NaI in 75 mL of methyl ethyl ketone was refluxed for 3h, then it was concentrated under reduced pressure. The crude was dissolved in DCM and washed with water. The organic layer was collected and the solvent was evaporated under reduced pressure. Compound **28** was used for the next reaction without any further purification. 1.53g of neat compound.Yield: 79.27%

3-(3-iodopropoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (29)

Compound **29** was obtained following the same procedure described for compound **28** using compound **18** as starting material. Yield: 88.49% M.P.: 99.8-101.0° C. ^{1}H NMR: δ 1.5-2.0 (m,5H), 2.1-2.4 (m,2H), 2.5 (s,2H), 2.7 (s,2H), 3.3 (t,1H), 3.7 (t,1H), 4.0-4.4 (m,2H), 6.7-6.9 (m,2H), 7.1-7,4 (m,1H), 7.4 (d,1H).

7-(3-iodopropoxy)-3-phenyl-2H-chromen-2-one (30)

Compound **30** was obtained following the same procedure described for compound **28** using compound **19** as starting material. Yield: 86.96%

7-(3-chloropropoxy)-3-(4-methoxyphenyl)-2H-chromen-2-one (31)

Compound **31** was obtained following the same procedure described for compound **28** using compound **20** as starting material. Yield: 87.79%

6-(3-iodopropoxy)-3-(4-methoxyphenyl)-2H-chromen-2-one (32)

Compound **32** was obtained following the same procedure described for compound **28** using compound **21** as starting material. Yield: 73.45%

3-(4-chlorophenyl)-7-(3-chloropropoxy)-2H-chromen-2-one (33)

Compound **33** was obtained following the same procedure described for compound **28** using compound **22** as starting material. Yield: 91.15%

7-(3-iodopropoxy)-2H-chromen-2-one (34)

Compound **34** was obtained following the same procedure described for compound **28** using compound **23** as starting material. Yield: 86.92%;

8-(3-iodopropoxy)-5H-chromeno[2,3-b]pyridin-5-one (35)

Compound **35** was obtained following the same procedure described for compound **28** using compound **24** as starting material. Yield: 94.93%

7-((5-iodopentyl)oxy)-2H-chromen-2-one (36)

Compound **36** was obtained following the same procedure described for compound **28** using compound **25** as starting material. Yield: 86.56%

3-((5-iodopentyl)oxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (37)

Compound **37** was obtained following the same procedure described for compound **28** using compound **26** as starting material. Yield: 86.49%; yellow oil

8-((5-iodopentyl)oxy)-5H-chromeno[2,3-b]pyridin-5-one (38)

Compound **38** was obtained following the same procedure described for compound **28** using **27** as starting material. Yield: 70.96% M.P.: 97.2-97.8° C

3-(3-iodopropoxy)-6H-benzo[c]chromen-6-one (39)

Compound **39** was obtained following the same procedure described for compound **28** using **57** as starting material. Yield: 70.96%

7-(3-((3-hydroxybenzyl)(methyl)amino)propoxy)-4-methyl-2H-chromen-2-one (40)

A mixture composed by 1.53g (4.461 mmol) of **28**, 0,61g (4.461 mmol) of 3-((methylamino)methyl)phenol (0,004 moli) and 0,404g (4.461 mmol) of TEA in 20 mL of toluene was refluxed for 48h. The organic phase was washed with water, then the organic layer was anhydrified

with anhydrous Na_2SO_4 and it was concentrated under reduced pressure. The crude was purified by flash chromatography: toluene/acetone 3:2. 0.44g of neat compound were obtained. Yield: 28.02% M. P.: 133-135° C; ^{1}H NMR δ 7.58 (d, J = 8.2 Hz, 1H), 7.15 (t, *J* = 15.6 Hz , 1H), 6.95 - 6.79 (m, 5H), 6,15 (s, 1H),.4.19 (t, J = 11.2 Hz, 2H), 3.50 (s, 2H), 2.63 – 2.58 (m, 2H), 2.45 (s, 3H), 2.25 (s, 3H), 2.15 – 1.89 (m, 2H)

3-(3-((3-hydroxybenzyl)(methyl)amino)propoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (41)

Compound **41** was obtained following the same procedure described for compound **40** using compound **29** as starting material. Yield: 41.17%; purple oil; ^{1}H NMR δ 7.44 (d, *J* = 8.8 Hz, 1H), 7.14 (t, *J* = 15.6 Hz, 1H), 6.91 – 6.79 (m, 4H), 6.75 (dd, *J* = 10.4 Hz, 1H), 4.07 (t, *J* = 12.4 Hz, 2H), 3.45 (s, 2H), 2.57 (t, *J* = 11.6 Hz, 2H), 2.57 – 2.52 (m, 4H), 2.23 (s, 3H), 2.00 (m, 2H), 1.86- 1.78 (m, 4H)

7-(3-((3-hydroxybenzyl)(methyl)amino)propoxy)-3-phenyl-2H-chromen-2-one (42)

Compound **42** was obtained following the same procedure described for compound **40** using compound **30** as starting material. Yield: 85.10%; oil; ^{1}H NMR δ 7.77 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.48-7.38 (m, 4H), 7.15 (t, *J* = 15.6 Hz, 1H), 6.92 (d, *J* = 8 Hz, 2H), 6.85 (d, *J* = 10.4 Hz, 2H), 6.74 (d, *J* = 10.4 Hz, 1H), 4.11 (t, *J* = 12.8 Hz, 2H), 3.47 (s, 2H), 2.55 (t, *J* = 13.6 Hz, 2H), 2.24 (s, 3H), 2.01 (t, *J* = 13.2 Hz, 2H)

7-(3-((3-hydroxybenzyl)(methyl)amino)propoxy)-3-(4-methoxyphenyl)-2H-chromen-2-one (43)

Compound **43** was obtained following the same procedure described for compound **40** using compound **31** as starting material. Yield: 39.21%; oil; ^{1}H NMR δ 7.82 -7.78 (m, 3H),. 7.42 (d, *J* = 9.2 Hz, 1H), 7.15 (m, 2H), 7.02 (d, , *J* = 8 Hz, 2H), 6.95 - 6.76 (m, 5H), 4.11 (t, *J* = 12.8 Hz, 2H), 3.89 (s, 3H), 3.47 (s, 2H), 2.55 (t, *J* = 13.6 Hz, 2H), 2.24 (s, 3H), 2.01 (t, *J* = 13.2 Hz, 2H)

6-(3-((3-hydroxybenzyl)(methyl)amino)propoxy)-3-(4-methoxyphenyl)-2H-chromen-2-one (44)

Compound **44** was obtained following the same procedure described for compound **40** using compound **32** as starting material. Yield: 37.07%; oil; ^{1}H NMR δ 7.75 (s, 1H), 7.43 - 7.05 (m, 5H), 6.98 - 6.65 (m, 6H), 4.14 (t, *J* = 12.8 Hz, 2H), 3.87 (s, 3H), 3.46 (s, 2H), 2.57 (t, *J* = 13.6 Hz, 2H), 2.22 (s, 3H), 2.07 (t, *J* = 13.2 Hz, 2H)

3-(4-chlorophenyl)-7-(3-((3-hydroxybenzyl)(methyl)amino)propoxy)-2H-chromen-2-one (45)

Compound **45** was obtained following the same procedure described for compound **40** using compound **33** as starting material. Yield: 38.09% M.P.: 144-145° C; ^{1}H NMR δ 7.79 (s,1H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.44-7.41 (m, 3H), 7.15 (d, *J* = 15.6 Hz, 1H), 6.94 (d, *J* = 14.4 Hz, 2H), 6.85-6.83 (m, 2H), 4.13 (t, *J* = 12.8 Hz, 2H), 3.47 (s, 2H), 2.56 (d, *J* = 12.8 Hz, 2H), 2.24 (s, 3H), 2.04-2.00 (m, 2H)

7-(3-((3-hydroxybenzyl)(methyl)amino)propoxy)-2H-chromen-2-one (46)

Compound **46** was obtained following the same procedure described for compound **40** using compound **34** as starting material. Yield: 31.05%; brown oil; ^{1}H NMR δ 7.65 (d, *J* = 9.6 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.26 (s, 1H), 7.13 (t, *J* = 15.6, 2.4 Hz, 1H), 6.86-6.79 (m, 3H), 6.74 (dd, *J* = 7.6 Hz, 1H), 6.26 (d, *J* = 9.6 Hz, 1H), 4.07 (t, *J* = 12.8 Hz, 2H), 3.46 (s, 2H), 2.55 (t, *J* = 13.6 Hz, 2H), 2.23 (p, *J* = 6.0 Hz, 2H), 2.24 (s, 3H), 2.01-1.98 (m, 2H)

88-(3-((3-hydroxybenzyl)(methyl)amino)propoxy)-5H-chromeno[2,3-b]pyridin-5-one (47)

Compound **47** was obtained following the same procedure described for compound **40** using compound **35** as starting material. Yield: 73.62%; oil; ^{1}H NMR δ 8.65 (d, *J* = 12 Hz, 2H), 8.13 (d, *J* = 8.8 Hz, 1H), 7.38 (dd, *J* = 12.4 Hz, 1H), 7.17 -7.07 (m, 3H), 6.88 (dd, *J* = 10.8 Hz, 1H), 6.75 (d, *J* = 7.2 Hz, 1H), 6.71 (d, *J* = 8 Hz, 1H), 4.16 (t, *J* = 12.4 Hz, 2H), 3.41 (s, 2H), 2.52 (t, *J* = 13.2 Hz, 2H), 2.18 (s, 3H), 1.99 – 1.96 (m, 2H)

77-((5-((3-hydroxybenzyl)(methyl)amino)pentyl)oxy)-2H-chromen-2-one (48)

Compound **48** was obtained following the same procedure described for compound40 using compound **36** as starting material. Yield: 17.99%; oil; ^{1}H NMR δ 7.60 (d, *J* = 9.5 Hz, 1H), 7.33 (d, *J* = 8.6 Hz, 1H), 7.25 - 7.11 (m, 1H), 6.68-6.79 (m, 5H) 6.20 (d, *J* = 9.5 Hz, 1H), 4.13 (t, *J* = 5.8 Hz, 2H), 3.44 (s, 2H), 3.71 (t, *J* = 6.3 Hz, 2H), 2.24 (s, 3H), 1.98-1.89 (m, 4H), 1.69-1.68 (m, 2H)

3-((5-((3-hydroxybenzyl)(methyl)amino)pentyl)oxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (49)

Compound **49** was obtained following the same procedure described for compound **40** using compound **37** as starting material. Yield: 36.80%; yellow oil; ^{1}H NMR δ 7.42 (d, *J* = 8.8 Hz, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 6.85 – 6.78 (m, 3H), 6.73 (dd, *J* = 10.2, 2.0 Hz, 2H), 3.96 (t, *J* = 6.5 Hz, 2H), 3.45 (s, 2H), 2.74 (t, *J* = 5.8 Hz, 2H), 2.55 (t, *J* = 5.7 Hz, 2H), 2.47 – 2.38 (m, 2H), 2.21 (s, 3H), 1.88 – 1.73 (m, 6H), 1.58 (dd, *J* = 14.8, 7.6 Hz, 2H), 1.48 (dd, *J* = 14.9, 7.9 Hz, 2H)

8-((5-((3-hydroxybenzyl)amino)pentyl)oxy)-5H-chromeno[2,3-b]pyridin-5-one (50)

Compound **50** was obtained following the same procedure described for compound **40** using **38** as starting material. Yield: 64.27%; yellow oil; ^{1}H NMR δ 8.72 (d, *J* = 6.4 Hz, 2H), 8.21 (d, *J* = 8.8 Hz, 1H), 7.45 (dd, *J* = 5.2 Hz, 1H), 7.26 (s, 1H), 7.16 (t, *J* = 15.6 Hz, 1H), 6.97-6.93 (m, 2H), 6.88 (s, 1H), 6.84 (d, *J* = 7.2 Hz, 1H), 6.78 (d, *J* = 9.6 Hz, 1H), 4.06 (t, *J* = 12.8 Hz, 2H), 3.49 (s, 2H), 2.45 (d, *J* = 14 Hz, 2H), 2.25 (s, 3H), 1.87-1.79 (m, 2H), 1.66 (m, 2H), 1.55-1.48 (m, 2H)

3-(3-((3-hydroxybenzyl)amino)propoxy)-6H-benzo[c]chromen-6-one (51)

Compound **51** was obtained following the same procedure described for compound **40** using **39** as starting material. Yield: 48.67%; yellow pale oil; ^{1}H NMR δ 8.35 (d, *J* = 8.8 Hz, 1H), 8.11 - 7.95 (m, 2H), 7.83 (t, J = 8.2 Hz, 1H), 7.55 (t, J = 6.4 Hz, 1H),7.22 -7.09 (m, 3H), 6.95 - 6.72 (m, 3H), 4.09 (t, J = 11.2 Hz, 2H), 3.45 (s,2H), 2.5 (m, 2H), 2.15 (s, 3H), 2.05 - 1.89 (m, 2H),

3-((methyl(3-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)propyl)amino)methyl)phenyl (5-phenylpentyl)carbamate (1)

A mixture of 0.12g (0.34 mmol) of **40** and a catalytic amount of NaH in anhydrous toluene (20 mL) was stirred for 10 minutes. 0.06g (0.339 mmol) of **53a** were added to the mixture and the reaction was stirred at r.t. for 48h; then it was quenched with water and extracted with DCM. Compound **1** was purified via flash chromatography: toluene/acetone 4:1. Yield: 43.32%; M.P.: 82° C; ^{1}H NMR δ 7.45 - 7.05 (d, *J* = 9.2 Hz 1H), 7.38 (m, 8H), 6.95 (d, *J* = 8 Hz,1H), 6.70-6.9 (m, 2H), 6.12 (s, 1H), 5.35 (broad, 1H), 4.05 (t, *J* = 13.2 Hz, 2H), 3.50 (s, 2H), 3.20-3.38 (m, 2H), 2.45-2.70 (m, 4H), 2.38 (s, 3H), 2.25 (s, 3H), 1.85-2.05 (m, 2H), 1.52-1.73 (m, 4H), 1.3-1.5 (m, 2H); ^{13}C NMR δ 162.26, 161.49, 155.29, 152.72, 151.22, 142.43, 141.52, 137.98, 128.89, 128.36, 128.25, 128.66, 126.33, 125.35, 121.96, 120.33, 113.32, 112.98, 111.67, 101.22, 66.89, 62.12, 53.03, 42.44, 40.52, 35.77, 31.04, 29.69, 23.91, 26.35, 18.65; ES-MS *m/z*: 543 (M + H^{+}), 565 (M + Na)

3-((methyl(3-((6-oxo-7,8,9,10-tetrahydro-6H-benzo[c]chromen-3-yl)oxy)propyl)amino)methyl) phenyl (5-phenylpentyl)carbamate (2)

Compound **2** was obtained following the same procedure described for compound **1** using **41** and **53a** as starting materials. Yield: 67.56% M.P.: 82° C; ^{1}H NMR δ 7.43 (d, *J* = 8.8 Hz, 1H), 7.28-7.22 (m, 3H), 7.18-7.13 (m, 4H), 7.10 (d, *J* = 7.2 Hz, 1H), 6.99 (d, *J* = 8Hz, 2H), 6.81-6.79 (m, 2H), 5.30 (broad, 1H), 4.3 (t, *J* = 12.8 Hz, 2H), 3.5 (s, 2H), 3.28 (dd, *J* = 6.8 Hz, 2H), 2.76-2.73 (m, 2H), 2.63-2.50 (m, 6H), 2.25 (s, 3H), 1.96 (t, *J* = 13.2 Hz, 2H), 1.85-1.79 (m, 4H), 1.68-1.61 (m, 4H), 1.43-1.38 (m, 2H); ^{13}C NMR δ 162.26, 161.49, 155.29, 152.72, 151.22, 142.43, 141.52, 137.98, 128.89, 128.36, 128.25, 128.66, 126.33, 125.35, 121.96, 120.33, 119.59, 113.32, 112.98, 101.22, 67.17, 63.93, 55.78, 43.22, 41.65, 36.78, 29.87, 29.51, 27.26, 27.23, 26.54, 24.65, 24.40, 24.27; ES-MS *m/z*: 583 (M + H^{+}), 605 (M + Na)

3-((methyl(3-((6-oxo-6H-benzo[c]chromen-3-yl)oxy)propyl)amino)methyl)phenyl (5-phenylpentyl)carbamate (3)

Compound **3** was obtained following the same procedure described for compound **1** using **51** and **53a** as starting materials. Yield: 27.03%; M.P.: 79° C; ^{1}H NMR δ 8.37 (d, *J* = 8 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.78 (t, *J* = 15.2 Hz, 1H), 7.50 (t, *J* = 15.2 Hz, 1H), 7.28-7.23 (m, 4H), 7.18-7.15 (m, 3H), 7.11 (d, *J* = 7.6 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 5.21 (broad, 1H), 4.07 (t, *J* = 12.8 Hz, 2H), 3.51 (s, 2H), 3.26-3.24 (m, 2H), 2.61 (t, *J* = 15.2 Hz, 2H), 2.53 (t, *J* = 13.2 Hz, 1H), 2.27 (s, 3H), 1.67-1.58 (m, 4H), 1.41-1.37 (m, 2H); ^{13}C NMR δ 163.49, 158.20, 152.56, 151.20, 142.43, 136.54, 135.31, 134.86, 130.51, 128.91, 128.36, 128.25, 127.62, 126.33, 125.67, 124.38, 123.63, 121.95, 121.05, 120.30, 119.88, 113.11, 110.87, 102.06, 66.72, 62.11, 53.13, 41.18, 40.50, 35.78, 31.06, 30.08, 29.67, 26.44; ES-MS *m/z*: 579 (M + H^{+}), 601 (M + Na)

3-((methyl(3-((2-oxo-3-phenyl-2H-chromen-7-yl)oxy)propyl)amino)methyl)phenyl (5-phenylpentyl) carbamate (4)

Compound **4** was obtained following the same procedure described for compound **1** using **42** and **53a** as starting materials. Yield: 27.52%; oil; ^{1}H NMR δ 7.76 (s, 1H), 7.69 (d, *J* = 7.1 Hz, 2H), 7.47 – 7.35 (m, 4H), 7.30 – 7.22 (m, 3H), 7.17 (t, *J* = 7.4 Hz, 4H), 7.09 (d, *J* = 7.3 Hz, 1H), 6.99 (d, *J* = 7.4 Hz, 1H), 6.83 (m, 2H), 5.24 (broad, 1H), 4.06 (t, *J* = 6.5 Hz, 2H), 3.51 (s, 2H), 3.26 (dd, *J* = 13.4, 6.7 Hz, 2H), 2.66 – 2.58 (m, 2H), 2.52 (t, *J* = 6.5 Hz, 2H), 2.27 (s, 3H), 2.03 – 1.93 (m, 2H), 1.70 – 1.57 (m, 4H), 1.45 – 1.38 (m, 2H); ^{13}C NMR δ 161.80, 160.94, 155.20, 154.50, 151.29, 142.37, 140.07, 134.96, 129.39, 128.81, 128.43, 128.41, 128.35, 128.25, 126.40, 125.66, 124.71, 122.78, 121.36, 119.87, 114.22, 113.34, 113.02, 101.04, 66.35, 54.84, 53.76, 52.85, 41.20, 35.75, 31.02, 29.24, 26.33, 14.10; ES-MS *m/z*: 605 (M + H+), 627 (M + Na)

3-(((3-((3-(4-methoxyphenyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)(methyl) amino)methyl) phenyl (5-phenylpentyl)carbamate (5)

Compound **5** was obtained following the same procedure described for compound **1** using **43** and **53a** as starting materials. Yield: 42.85 %; M.P.: 123-124° C; ^{1}H NMR δ 7.71 (s, 1H), 7.66 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.6 Hz, 1H), 7.27 (s, 3H), 7.18 (t, J = 7.2 Hz, 5H), 6.99 (t, J = 9.1 Hz, 3H), 6.87 – 6.80 (m, 2H), 5.26 (broad, 1H), 4.08 (t, J = 6.5 Hz, 2H), 3.86 (s, 3H), 3.52 (s, 2H), 3.34 – 3.19 (m, 2H), 2.67 – 2.58 (m, 2H), 2.58 – 2.49 (m, 2H), 2.28 (s, 3H), 2.08 – 1.95 (m, 2H), 1.63 (m, 4H), 1.46 – 1.39 (m, 3H); ^{13}C NMR δ 161.94, 161.25, 159.79, 155.06, 154.71, 151.21, 142.43, 140.87, 138.89, 136.38, 129.60, 128.90, 128.46, 128.35, 128.25, 127.43, 125.61, 124.12, 121.96, 120.30, 113.86, 113.38, 113.25, 100.77, 66.89, 62.13, 55.33, 53.06, 42.44, 41.18, 40.54, 35.77, 31.03, 26.93, 26.42; ES-MS m/z: 635 (M + H^{+}), 657 (M + Na)

3-(((3-((3-(4-methoxyphenyl)-2-oxo-2H-chromen-6-yl)oxy)propyl)(methyl) amino) methyl) phenyl (5-phenylpentyl) carbamate (6)

Compound **6** was obtained following the same procedure described for compound **1** using **44** and **53a** as starting materials. Yield: 29.58 %; M.P.: 83.2-84 ° C; ^{1}H NMR δ 7.77 (s, 1H), 7.44 – 7.33 (m, 2H), 7.29 – 7.24 (m, 6H), 7.21 – 7.13 (m, *J* = 7.3 Hz, 4H), 7.13 – 7.09 (m, 1H), 7.00 (d, *J* = 7.4 Hz, 1H), 6.94 (d, *J* = 7.8 Hz, 1H), 6.84 (d, *J* = 8.7 Hz, 2H), 5.25 (s, 1H), 4.07 (t, *J* = 6.7 Hz, 2H), 3.86 (s, 3H), 3.52 (s, 2H), 3.27 (dd, *J* = 6.0 Hz, 2H), 2.68 – 2.58 (m, 2H), 2.58 – 2.47 (m, 2H), 2.28 (s, 3H), 2.04 – 1.91 (m, 2H), 1.72 – 1.59 (m, 4H), 1.47 – 1.33 (m, 3H).; ^{13}C NMR δ 162.28, 160.91, 159.33, 155.33, 154.70, 151.21, 142.42, 140.87, 140.27, 136.39 ,129.40, 128.91, 128.72, 128.36, 128.25, 125.65, 124.29, 121.95, 120.76, 120.30, 114.07, 113.47, 113.04, 100.78, 66.93, 62.14, 55.32, 53.05, 42.45, 41.19, 35.77, 31.03, 29.69, 26.93, 26.35; ES-MS *m/z*: 635 (M + H^{+})

3-(((3-((3-(4-chlorophenyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)(methyl)amino)methyl)phenyl (5-phenylpentyl)carbamate (7)

Compound **7** was obtained following the same procedure described for compound **1** using **45** and **53a** as starting materials. Yield: 23.47 %; M.P.: 76-77° C; ^{1}H NMR δ 7.75 (s, 1H), 7.66 (d, *J* = 8 Hz,

2H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.14-7.16 (m, 4H), 7.22-7.24 (m, 3H), 7.10 (d, *J* = 7.7 Hz, 2H), 6.99 (d, *J* = 7.7 Hz, 2H), 6.85 (d, *J* = 8 Hz, 2H), 5.22 (broad, 1H), 4.06 (t, 2H), 3.27 (s, 2H), 3.27 (dd, *J* = 6.8 Hz, 2H), 2.61 (t, *J* = 15.2 Hz, 2H), 2.52 (t, *J* = 13.2 Hz, 2H), 2.27 (s, 3H), 1.98 (t, *J* = 13.6 Hz, 2H) 1.62-1.55 (m, 4H), 1.49-1.38 (m, 2H); ^{13}C NMR δ 162.46, 160.80, 155.39, 154.68, 151.20, 142.39, 140.82, 140.20, 134.38, 133.45, 129.64, 128.91, 128.76, 128.60, 128.35, 128.25, 125.67, 125.61, 123.24, 121.95, 120.29, 113.62, 112.92, 100.82, 66.95, 62.12, 53.04, 42.44, 41.17, 35.76, 31.02, 29.67, 26.91, 26.33; ES-MS *m/z*: 639 (M + H^+), 661 (M + Na)

3-((methyl(3-((2-oxo-2H-chromen-7-yl)oxy)propyl)amino)methyl)phenyl heptylcarbamate (8)

Compound **8** was obtained following the same procedure described for compound **1** using **46** and **53b** as starting materials. Yield: 80 %; M.P.: 84-85° C; ^{1}H NMR δ 7.61 (d, *J* = 9.4 Hz, 1H), 7.31 (d, *J* = 9.0 Hz, 1H), 7.22 (dd, *J* = 13.9, 6.2 Hz, 1H), 7.10 (s, 1H), 7.06 (d, *J* = 7.4 Hz, 1H), 6.96 (d, *J* = 7.8 Hz, 1H), 6.77 (d, *J* = 4.8 Hz, 2H), 6.21 (d, *J* = 9.4 Hz, 1H), 5.32 (broad, 1H), 4.01 (t, *J* = 6.3 Hz, 2H), 3.47 (s, 2H), 3.22 (dd, *J* = 13.2, 6.6 Hz, 2H), 2.49 (t, *J* = 6.5 Hz, 2H), 2.23 (s, 3H), 2.01 – 1.90 (m, 2H), 1.60 – 1.48 (m, 2H), 1.37 – 1.17 (m, 8H), 0.84 (t, *J* = 5.8 Hz, 3H); ^{13}C NMR δ 162.39, 161.42, 155.85, 154.72, 151.18, 143.58, 140.59, 128.90, 128.61, 125.62, 122.02, 120.32, 113.20, 112.72, 112.29, 101.21, 66.84, 62.05, 53.05, 42.36, 41.27, 31.71, 29.79, 28.91, 26.83, 26.76, 22.56, 14.05; ES-MS *m/z*: 481 (M + H^+), 503 (M + Na)

3-((methyl(3 -((6-oxo-7,8,9,10-tetrahydro-6H-benzo[c]chromen-3-yl)oxy)propyl) amino) methyl)phenyl heptylcarbamate (9)

Compound **9** was obtained following the same procedure described for compound **1** using **41** and **53b** as starting materials. Yield: 61.42 %; M.P.: 86-87° C ; ^{1}H NMR δ 7.43 (d, *J* = 9.2 Hz, 1H), 7.24 (t, *J* = 17.6 Hz, 1H), 7.13 (s, 1H), 7.09 (d, *J* = 7.2 Hz, 1H), 6.99 (d, *J* = 8 Hz, 1H), 6.81 (d, *J* = 6.4 Hz, 2H), 5.29 (broad, 1H), 4.03 (t, *J* = 12.8 Hz, 2H), 3.49 (s, 2H), 3.28-3.23 (dd, *J* = 20.4 Hz, 1H), 2.75 (m, 2H), 2.57-2.49 (m, 4H), 2.25 (s, 3H), 1.99-1.95 (m, 2H), 1.85-1.79 (m, 4H), 1.56 (t, 2H), 1.31-1.28(m, 8H), 0.88 (t, *J* = 13.2 Hz, 3H); ^{13}C NMR δ 162.27, 160.99, 154.72, 153.51, 151.23, 147.37, 140.83, 128.87, 125.54, 123.94, 121.94, 120.26, 119.88, 113.52, 112.57, 101.05, 66.71, 62.10, 53.19, 42.38, 41.27, 31.72, 29.81, 28.91, 26.97, 26.69, 25.20, 23.81, 22.55, 21.69, 21.39, 14.03; ES-MS *m/z*: 535 (M + H^+), 557 (M + Na)

3-((methyl(3-((5-oxo-5H-chromeno[2,3-b]pyridin-8-yl)oxy)propyl)amino)methyl)phenyl heptylcarbamate (10)

Compound **10** was obtained following the same procedure described for compound **1** using **47** and **53b** as starting materials. Yield: 74.07 %; M.P.: 68° C; ^{1}H NMR δ 8.76 – 8.67 (m, 2H), 8.22 (d, *J* = 8.9 Hz, 1H), 7.45 (dd, *J* = 7.4, 4.9 Hz, 1H), 7.33 – 7.21 (m, 1H), 7.16 – 7.05 (m, 2H), 7.05 – 6.87 (m, 3H), 5.34 (broad, 1H), 4.14 (t, *J* = 6.5 Hz, 2H), 3.52 (s, 2H), 3.26 (dd, *J* = 13.5, 6.8 Hz, 2H), 2.54 (t, *J* = 6.5 Hz, 2H), 2.29 (s, 3H), 2.10 – 1.91 (m, 2H), 1.58 – 1.49 (m, 2H), 1.39 – 1.24 (m, 8H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR δ 176.86, 165.69, 160.93, 157.87, 153.68, 151.56, 142.41, 137.59, 129.25, 128.41, 125.96, 122.35, 121.33, 120.64, 119.88, 117.33, 115.51, 114.86, 101.56, 67.43, 62.53,

53.27, 42.82, 41.64, 41.02, 32.07, 30.19, 29.30, 27.14, 22.91, 14.39; ES-MS *m/z*: 532 (M + H^+), 554 (M + Na)

3-((methyl(3-((2-oxo-2H-chromen-7-yl) oxy)propyl) amino)methyl)phenyl (4-hexylphenyl) carbamate (11)

Compound **11** was obtained following the same procedure described for compound **1** using **46** and **53c** as starting materials. Yield: 42.86 %; M.P.: 93-94° C; ^{1}H NMR δ 7.61 (d, *J* = 9.6 Hz, 1H), 7.39 (d, *J* = 8 Hz, 2H), 7.32 (d, *J* = 10.8 Hz, 1H), 7.26 (d, *J* = 8 Hz, 1H), 7.20 (s, 2H), 7.14-7.11 (m, 2H), 7.04 (dd, *J* = 8 Hz, 1H), 6.81 (d, *J* = 10 Hz, 1H), 6.24 (d, *J* = 9.2 Hz, 1H), 4.06 (t, *J* = 12.8 Hz, 2H), 3.51 (s, 2H), 2.58-2.51 (m, 4H), 2.26 (s, 3H), 1.98-1.95 (m, 2H), 1.60-1.57 (m, 2H), 1.33-1.29 (m, 6H), 0.88 (t, *J* = 13.6 Hz, 3H); ^{13}C NMR δ 162.46, 161.51, 155.89, 150.80, 143.54, 142.41, 141.09, 138.16, 135.12, 128.99, 128.92, 128.60, 125.96, 121.98, 120.30, 118.88, 118.68, 113.25, 112.75, 112.30, 101.28, 66.79, 62.11, 53.02, 42.44, 35.27, 31.69, 31.46, 28.88, 26.91, 22.58, 14.06; ES-MS *m/z*: 543 (M + H^+), 565 (M + Na)

3-((methyl(3-((2-oxo-2H-chromen-7-yl) oxy)propyl)amino) methyl)phenyl (6-phenylhexyl) carbamate (12)

Compound **12** was obtained following the same procedure described for compound **1** using **46** and **52** as starting materials. Yield: 34.84 %; oil; ^{1}H NMR δ 7.63 (d, *J* = 9.2 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.29-7.24 (m, 3H), 7.17-7.13 (m, 4H), 7.09 (d, *J* = 8 Hz, 1H), 6.97 (d, *J* = 8 Hz, 1H), 6.81 (d, *J* = 7.6 Hz, 2H), 6.25 (d, *J* = 9.6 Hz, 1H), 5.23 (broad, 1H), 4.04 (t, *J* = 13.2 Hz, 2H), 3.49 (s, 2H), 3.27-3.23 (m, 4H), 2.60 (t, *J* = 15.6 Hz, 2H), 2.51 (t, *J* = 13.2 Hz, 2H), 2.26 (s, 3H), 1.98 (dd, *J* = 13.2 Hz, 2H), 1.64-1.38 (m, 4H), 1.38-1.34 (m, 6H), 1.13 (t, *J* = 14.4 Hz, 2H); ^{13}C NMR δ 162.43, 155.91, 151.21, 143.54, 142.62, 128.91, 128.60, 128.35, 128.22, 128.20, 125.64, 125.60, 125.57, 122.01, 120.33, 119.81, 113.24, 112.76, 112.30, 101.23, 66.88, 62.07, 53.02, 42.39, 41.22, 35.83, 31.37, 29.75, 28.88, 26.84, 13.85; ES-MS *m/z*: 543 (M + H^+), 565 (M + Na)

3-((methyl(5-((2-oxo-2H-chromen-7-yl) oxy)pentyl) amino)methyl)phenyl (5-phenylpentyl) carbamate (13)

Compound **13** was obtained following the same procedure described for compound **1** using **48** and **53a** as starting materials. Yield: 35.02 %; oil; ^{1}H NMR δ 7.63 (d, *J* = 9.2 Hz, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.28-7.24 (m, 3H), 7.19-7.10 (m, 5H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.84-6.80 (m, 2H), 6.25 (d, *J* = 9.6 Hz, 1H), 5.01 (broad, 1H), 3.99 (t, *J* = 12.8 Hz, 2H), 3.47 (s, 2H), 3.27 (dd, *J* = 20.4 Hz, 2H), 2.62 (t, *J* = 15.2 Hz, 2H), 2.41 (t, *J* = 14 Hz, 2H), 2.19 (s, 3H), 1.81 (t, *J* = 14.4 Hz, 2H), 1.68-1.47 (m, 8H), 1.41-1.38(m, 2H); ^{13}C NMR δ 162.38, 161.27, 155.88, 154.62, 151.08, 143.44, 142.35, 140.80, 128.90, 128.66, 128.35, 128.26, 125.68, 122.01, 120.10, 119.81, 112.94, 112.85, 112.34, 101.33, 68.51, 61.95, 57.14, 42.27, 41.15, 35.75, 31.01, 29.68, 28.79, 27.02, 26.32, 23.70

3-((methyl(5-((2-oxo-2H-chromen-7-yl)oxy)pentyl)amino)methyl)phenyl heptylcarbamate (14)

Compound **14** was obtained following the same procedure described for compound **1** using **48** and **53b** as starting materials. Yield: 50.60%; oil; ^{1}H NMR δ 7.64 (d, *J* = 9.2 Hz, 1H), 7.36 (d, *J* = 8 Hz, 1H), 7.27 (t, *J* = 15.6 Hz, 1H), 7.14-7.10 (m, 2H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.83-6.79 (m, 2H), 5.17 (broad, 1H), 3.99 (t, *J* = 12.8 Hz, 2H), 3.47 (s, 2H), 3.26-3.21 (dd, *J* = 7.0 Hz, 2H), 2.39 (t, *J*= 13.6 Hz, 2H), 2.19 (s, 3H), 1.84-1.77 (m, 2H), 1.57-1.46 (m, 6H), 1.32-1.28 (m, 8H), 0.88 (t, *J*= 13.2 Hz, 3H); ^{13}C NMR δ 162.38, 161.31, 155.86, 154.66, 151.09, 143.50, 128.89, 128.69, 125.66, 122.02, 120.11, 119.83, 112.93, 112.83, 112.33, 101.30, 68.51, 61.96, 57.15, 42.28, 41.25, 31.71, 29.81, 28.91, 28.80, 27.04, 26.69, 23.70, 22.56, 14.06; ES-MS *m/z*: 509 (M + H^{+}), 531 (M + Na), 547 (M + K)

3-((methyl(5-((5-oxo-5H-chromeno[2,3-b]pyridin-8-yl)oxy)pentyl)amino)methyl)phenyl heptylcarbamate (15)

Compound **15** was obtained following the same procedure described for compound **1** using **49** and **53b** as starting materials. Yield: 74.91%; M.P.: 73° C; ^{1}H NMR δ 7.43 (d, *J* = 8.7 Hz, 1H), 7.31 – 7.27 (m, 1H), 7.16 – 7.08 (m, 2H), 7.00 (d, *J* = 7.3 Hz, 1H), 6.85 – 6.76 (m, 2H), 5.05 (broad, 1H), 3.98 (t, *J* = 6.4 Hz, 2H), 3.48 (s, 2H), 3.24 (dd, *J* = 13.2, 6.6 Hz, 2H), 2.74 (t, *J* = 5.6 Hz, 2H), 2.56 (t, *J* = 5.6 Hz, 2H), 2.39 (t, *J* = 6.9 Hz, 2H), 2.18 (s, 3H), 1.92 – 1.71 (m, 6H), 1.61 – 1.42 (m, 6H), 1.37 – 1.24 (m, 8H), 0.88 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR δ 162.12, 160.87, 153.41, 151.00, 147.20, 140.84, 128.80, 125.57, 123.92, 121.89, 120.26, 119.97, 119.81, 113.48, 112.23, 101.04, 68.27, 61.89, 57.11, 42.21, 41.17, 31.63, 29.73, 28.82, 27.00, 26.61, 25.12, 23.73, 23.65, 22.48, 21.62, 21.31, 17.79, 13.96; ES-MS *m/z*: 563 (M + H^{+}), 585 (M + Na)

3-(((5-((5-oxo-5H-chromeno[2,3-b]pyridin-8-yl)oxy)pentyl)amino)methyl)phenyl heptylcarbamate (16)

Compound **16** was obtained following the same procedure described for compound **1** using **50** and **53b** as starting materials. Yield: 56.23%; oil; ^{1}H NMR δ 8.71 (d, *J* = 6.8 Hz, 2H), 8.23 (d, *J* = 8.8 Hz, 1H), 7.44-7.41 (dd, *J* = 5.2 Hz, 1H), 7.28 (m, 1H), 7.14-7.11 (m, 2H), 7.01-6.96 (m, 3H), 5.03 (broad, 1H), 4.09 (t, *J*= 13.2 Hz, 2H), 3.48 (s, 2H), 3.27-3.22 (dd, *J* = 6.8 Hz, 2H), 2.40 (t, *J*= 13.6 Hz, 2H), 2.20 (s, 3H), 1.88-1.82 (m, 2H), 1.57-1.53 (m, 6H), 1.31-1.28 (m, 8H), 0.88 (t, *J*= 13.2 Hz, 3H); ^{13}C NMR δ 176.49, 165.30, 160.49, 157.72, 154.59, 153.42 ,151.12, 137.18, 128.93, 128.16, 125.73, 122.06, 120.95, 120.17, 119.81, 116.94, 115.20, 114.28, 101.26, 68.76, 61.91, 57.05, 42.21, 41.26, 31.69, 29.81, 28.88, 28.73, 26.94, 26.68, 23.69, 22.53, 14.01; ES-MS *m/z*: 560 (M + H^{+}), 582 (M + Na)

(6-chlorohexyl)benzene (54)

To a cold solution of 1.00g (5.62 mmol, d=0.953) of 6-phenyl-1-hexanol and 0.57g (5.62 mmol, d=0.726) of TEA in 30 mL of DCM, 0.67g (5.62 mmol, d=1.631) of $SOCl_2$ were added. The solution was stirred at 0° C for 1h, then it was stirred at r.t. for 48h. The solvent was removed under reduced pressure, then the solution was alkalinized with Na_2CO_3 and **54** was extracted with DCM. 0.90g of

neat compound were obtained. Yield: 81.81%; oil; ^{1}H NMR δ 7.25 – 7.21 (m, 2H), 7.14 – 7.12 (m, 3H), 3.44 (t, *J* = 13.2 Hz, 2H), 2.59 (t, *J* = 15.2 Hz, 2H), 1.72 - 1.68 (m, 2H), 1.61 – 1.57 (m, 2H), 1.43 – 1.39 (m, 2H), 1.34 – 1.28 (m, 2H)

2-(6-phenylhexyl)isoindoline-1,3-dione (55)

0.91g (4.65 mmol, 1.1eq) of **54** were dissolved in 2.5 mL of DMF and 0.78g (4.23 mmol) of K-phthalimide were added to the solution. The reaction was refluxed for 2h; then it was poured in water and the suspension was filtered. 1.15g of neat compound were obtained. Yield: 88.60% M.P.: 40-41° C; ^{1}H NMR δ 7.85 – 7.83 (m, 1H), 7.71 – 7.69 (m, 1H), 7.27 – 7.26 (m, 4H), 7.17 – 7.14 (m, 3H), 3.67 (t, *J* = 14.8 Hz, 2H), 2.59 (t, *J* = 15.6 Hz, 2H), 1.67 – 1.59 (m, 4H), 1.38 – 1.36 (m, 4H)

6-phenylhexan-1-amine (56)

1.15g (3.75 mmol) of **55** were dissolved in ethanol, then 0.37g (11.61 mmol, d=1.029, 3.1eq) of hydrazine monohydrate were added. The reaction was refluxed for 5h, then it was allowed to cool. 1.3 mL of HCl 37% were added to the mixture and the solution was refluxed for 30 minutes, then it was concentrated under reduced pressure. The mixture was washed with water and **56** was extracted with DCM. 0.69g of neat compound. Yield: 96.53%; oil; ^{1}H NMR δ 7.27 – 7.25 (m, 2H), 7.17 – 7.14 (m, 4H), 2.71 (t, *J* = 14 Hz, 2H), 2.58 (t, *J* = 15.2 Hz, 2H), 1.62 – 1.58 (m, 2H), 1.48 – 1.43 (m, 2H), 1.37 – 1.33 (m, 4H)

(6-isocyanatohexyl)benzene (52)

0.57g (5.84 mmol, 1.5eq) of phosgene solution 20% in toluene were added dropwise to a solution of 0.69g (3.89 mmol, 1eq) of **56** and 2.83 mL of TEA in toluene at 0° C. The solution was stirred for 2h at r.t.; 0.57g (5.84 mmol, 1.5eq) of phosgene solution 20% in toluene were added again and the solution was stirred for 90 minutes. The reaction was bubbled with N_2, then it was washed with water. The solvent was removed. Compound **52** was used crude for next step of reaction.

(5-isocyanatopentyl)benzene (53a)

A mixture of 1g (5.21 mmol) of 6-phenylhexsanoic acid and 10 mL of $SOCl_2$ was refluxed for 5h, then $SOCl_2$ was removed. To an ice-cold mixture of 0.42g (7.28 mmol, 1.4eq) of NaN_3 in 2 mL of water, a solution of 6-phenylhexanoyl chloride in 2 mL of acetone was added, keeping the temperature below 10° C. The mixture was stirred for 1h, then the layers were separated and the lower acqueous layer was discarded; the upper layer was slowly and dropwise added to 30 mL of benzene heated to 60° C and the mixture was kept at 60-70° C until gas production ceased; then it was filtered and the solvent was removed. Compound **54a** was used crude, without any further purification, for next step of reaction; oil

1-isocyanatoheptane (53b)

Compound **53b** was obtained following the same procedure described for compound **53a** using octanoic acid as starting material; oil

1-hexyl-4-isocyanatobenzene (53c)

Compound **53c** was obtained following the same procedure described for compound **53a** using 4-hexylbenzoic acid as starting material; oil

7-(prop-2-yn-1-yloxy)-2H-chromen-2-one (63)

To a solution of 1.00g (6.17 mmol) of 7-hydroxycoumarin in 40 mL of acetone, 0.88g (7.40 mmol, 1.2 eq, d=1.335) of propargyl bromide and 1.00g of anhydrous K_2CO_3 were added. The mixture was stirred and refluxed for 8h, then it was filtered hot and it was concentrated under reduced pressure. The crude was crystallized with petroleum ether. 1.22g of neat compound **63** were obtained. Yield: 97%; M.P.: 120-121° C; ^{1}H NMR δ 7.26 (d, *J* = 9.5 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 6.87 (s, 1H), 6.56 (d, *J* = 2.3 Hz, 1H), 6.52 (dd, *J* = 8.5, 2.4 Hz, 1H), 5.89 (d, *J* = 9.5 Hz, 1H), 4.37 (d, *J* = 2.4 Hz, 2H), 2.19 (t, *J* = 2.4 Hz, 1H).

7-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (65a)

To a solution of 0.50g (2.497 mmol, 1 eq) of **63** in 15 mL of DMSO, 0.53g (3.247 mmol, 1.3eq) of **64a** and 0.02g (0.250 mmol, 1/10eq, d=0.726) of TEA were added. 0.04g (0.250 mmol, 1/10eq) of $CuSO_4$ and 0.25g (1.249 mmol, ½eq) of sodium ascorbate were dissolved in water and were stirred for 5 min; the solution was added to the reaction flask and it was stirred at r.t. for 24h. A saturated solution of NH_4Cl was added to the reaction and the organic compound was extracted with ethyl acetate. The mixture was purified via flash chromatography: toluene/acetone 4:1; 0.89g of neat compound **65a** were obtained. Yield: 98%; M.P.: 134-136° C; ^{1}H NMR (200 MHz, $CDCl_3$,) δ 7.63 (d, *J* = 9.4 Hz, 1H), 7.54 (s, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 6.91 (m, 4H), 6.26 (d, *J* = 9.4 Hz, 1H) , 5.48 (s, 2H), 5.22 (s, 2H), 3.81 (s, 3H)

7-((1-(3-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (65b)

Compound **65b** was obtained following the same procedure described for compound **65a** using **63** and **64b** as starting materials. Yield: 94.81%; M.P.: 101-102° C; ^{1}H NMR (400 MHz, $CDCl_3$,) δ 7.59 (m, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.25 (t, J= 15.6 Hz, 1H), 6.88-6.76 (m, 4H), 6.20 (d, J= 9.6 Hz, 1H), 5.47 (s, 2H), 5.18 (s, 2H), 3.73 (s, 3H)

7-((1-(4-hydroxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (66a)

0.89g (2.449 mmol) of **65a** were dissolved in anhydrous DCM under nitrogen atmosphere in a two neck flask. The temperature was allowed to 0° C. 4.0 mL of BBr_3 were added dropwise to the reaction that was stirred at r.t. for 48h. The reaction was quenched with water and it was washed with brine. The organic layer was concentrated under reduced pressure. The crude was purified via flash chromatography: toluene/acetone 4:1; 0.32g neat of compound **66a** were obtained. Yield: 37%; yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$,) δ 7.49 (d, *J* = 9.4 Hz, 1H), 7.54 (s, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 2H), 6.88 (m, 4H), 6.26 (d, *J* = 9.4 Hz, 1H), 5.98 (s, 1H), 5.46 (s, 2H), 5.21 (s, 2H)

7-((1-(3-hydroxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (66b)

Compound **66b** was obtained following the same procedure described for compound **66a** using **65b** as starting material. Yield: 20.83%; M.P.: 65-66° C; ^{1}H NMR (401 MHz, cdcl$_3$) δ 7.61 (m, 2H), 7.34 (d, *J* = 11.2 Hz, 1H), 7.24 (t, 1H), 6.85 (m, 4H), 6.24 (d, *J* = 9.4 Hz, 1H), 5.47 (s, 2H), 5.18 (s, 2H)

4-((4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)phenyl (5-phenylpentyl)carbamate (59)

To a reaction flask with 0.13g (0.372 mmol)of **66a** dissolved in anhydrous toluene a catalytic amount of NaH was added and the reaction was stirred for 10 min. 0.07g (0.372 mmol) compound **53a** were added. The solution was stirred at r.t. for 60h, then it was poured in water and extracted with DCM. Compound **59** was purified via flash chromatography: toluene/acetone 4:1; then it was crystalized at cold conditions with methanol. 0.04g of neat compound **59** were obtained. Yield: 20%; M.P.: 129.8-130.1° C; ^{1}H NMR δ 7.61 (d, *J* = 9.5 Hz, 1H), 7.54 (s, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.26 (m, 4H), 7.19 – 7.07 (m, 4H), 6.95 – 6.82 (m, 2H), 6.24 (d, *J* = 9.5 Hz, 1H), 5.50 (s, 2H), 5.20 (s, 2H), 5.04 (br, *J* = 5.4 Hz, 1H), 3.23 (dd, *J* = 13.3, 6.8 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H), 1.72 – 1.49 (m, 4H), 1.38 (m, 2H); ^{13}C NMR δ 161.27, 161.06, 155.68, 154.16, 151.46, 143.29, 142.29, 131.05, 129.23, 128.87, 128.35, 128.28, 125.72, 122.86, 122.37, 113.44, 112.97, 112.73, 102.10, 62.30, 53.73, 41.20, 35.74, 30.99, 29.64, 26.28

3-((4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)phenyl (5-phenylpentyl)carbamate (60)

Compound **60** was obtained following the same procedure described for compound **59** using **66b** and **53a** as starting materials. Yield: 21.73%; oil; ^{1}H NMR δ 7.61 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.33 (t, *J* = 8.2 Hz, 2H), 7.25 (dd, *J* = 9.6, 5.4 Hz, 2H), 7.16 (3H), 7.13 – 7.00 (m, 3H), 6.89 (m, 2H), 6.23 (d, *J* = 9.5 Hz, 1H), 5.51 (s, 2H), 5.20 (s, 2H), 5.13 (broad, 1H), 3.23 (dd, *J* = 13.3, 6.8 Hz, 2H), 2.61 (t, *J* = 7.7 Hz, 2H), 1.62 (m, 4H), 1.39 (t, 2H); ^{13}C NMR δ 161.31, 161.13, 155.71, 154.24, 151.60, 143.36, 142.35, 135.59, 130.10, 128.92, 128.40, 128.33, 125.76, 124.81, 123.08, 122.24, 121.42, 113.47, 113.01, 112.86, 102.12, 62.36, 53.90, 41.26, 35.79, 31.04, 29.67, 26.35

4-((4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)phenyl heptylcarbamate (61)

Compound **61** was obtained following the same procedure described for compound **59** using **66a** and **53b** as starting materials. Yield: 22.22%; M.P.:152.2-152.6° C; ^{1}H NMR δ 7.61 (d, *J* = 9.5 Hz, 1H), 7.54 (s, 1H), 7.35 (d, *J* = 8.7 Hz, 1H), 7.30 – 7.20 (m, 3H), 7.13 (d, *J* = 8.3 Hz, 2H), 6.90 (d, *J* = 7.6 Hz, 2H), 6.24 (d, *J* = 9.5 Hz, 1H), 5.51 (s, 2H), 5.21 (s, 2H), 5.02 (broad, 1H), 3.24 (dd, *J* = 13.4, 6.7 Hz, 2H), 1.28 (m, 8H), 0.87 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR δ 161.26, 161.06, 155.66, 154.16, 153.37, 152.26, 151.47, 143.30, 131.01, 129.22, 128.8, 122.3, 113.41, 112.95, 112.73, 102.07, 62.25, 53.80, 41.30, 31.69, 29.74, 28.88, 26.66, 22.55, 14.04; ES-MS *m/z*: 491 (M + H^{+}), 513 (M + Na)

3-((4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)phenyl heptylcarbamate (62)

Compound **62** was obtained following the same procedure described for compound **59** using **66b** and **53b** as starting materials. Yield: 32.46%; M.P.:>250° C; ^{1}H NMR δ 7.62 (d, *J* = 9.5 Hz, 1H), 7.58 (s, 1H), 7.35 (t, *J* = 9.4 Hz, 2H), 7.15 – 7.04 (m, 3H), 6.94 – 6.86 (m, 2H), 6.25 (d, *J* = 9.5 Hz, 1H), 5.52 (s, 2H), 5.22 (s, 2H), 5.14 (broad, 1H), 3.24 (dd, *J* = 13.4, 6.7 Hz, 2H), 1.40 – 1.20 (m, 8H), 0.88 (t, *J* = 6.2 Hz, 3H); ^{13}C NMR δ 161.23, 161.05, 155.64, 154.16, 151.55, 143.38, 143.28, 135.50, 130.02, 128.84, 124.72, 122.95, 122.16, 121.36, 113.40, 112.93, 112.77, 102.04, 62.29, 53.82, 41.28, 31.67, 29.72, 28.86, 26.65, 22.53, 14.02; ES-MS *m/z*: 491 (M + H^{+}), 513 (M + Na), 529 (M + K)

1-(azidomethyl)-4-methoxybenzene (64a)

To a mixture of 0.548g (3.500 mmol, 1eq, d=1.155) of 4-methoxybenzylchloride dissolved in 15 ml of anhydrous DMF, 0.46g (7.000 mmol, 2eq) of NaN_3 were added. The mixture was stirred at 80° C for 6h. The reaction was quenched with water and the crude was extracted with diethyl ether. The compound was used without any further purification for the next step; 0.53g of compound **64a** were obtained. Yield: 93%; colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$,) δ 7.23 (d, *J* = 7.2 Hz, 2H), 6.88 (d, *J* = 7.2 Hz, 2H), 4.24 (s, 2H), 3.79 (s, 3H)

1-(azidomethyl)-3-methoxybenzene (64b)

Compound **64b** was obtained following the same procedure described for compound **64a** using 3-methoxybenzylchloride as starting material. Yield: 92.73%; oil; ^{1}H NMR (400 MHz, $CDCl_3$,) δ 6.99 (t, *J* = 8 Hz, 1H), 6.60-6.56 (m, 3H), 4.01 (s, 2H), 3.50 (s, 3H)

7-((1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (72a)

To a solution of 0.43g (2.161 mmol, 1 eq) of **63** in 15 mL of DMSO, 0.70g (2.809 mmol, 1.3eq) of **71a** and 0.02g (0.216 mmol, 1/10eq, d=0.726) of TEA were added. 0.04g (0.216 mmol, 1/10eq) of $CuSO_4$ and 0.25g (1.081 mmol, ½eq) of sodium ascorbate were dissolved in water and were stirred for 5 min; the solution was added to the reaction flask and it was stirred at r.t. for 24h. A saturated solution of NH_4Cl was added to the reaction and the organic compound was extracted with ethyl acetate. The nmr of the crude product showed that the reaction had occurred. The mixture was purified via flash chromatography: toluene/acetone 4:1; During purification the molecule was deprotected, therefore peaks corresponding to the TBDMS disappeared from the NMR spectrum. 0.18g of neat compound **72a** were obtained. Yield: 25%; M.P.: 239° C; ^{1}H NMR (DMSO) δ 9.97 (s, 1H), 8.81 (s, 1H), 8.00 (d, *J* = 9.5 Hz, 1H), 7.66 (dd, *J* = 8.8, 2.6 Hz, 2H), 7.19 (s, 1H), 7.06 (d, *J* = 8.7 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.31 (d, *J* = 9.6 Hz, 1H), 5.33 (s, 2H)

7-((1-(3-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one (72b)

Compound **72b** was obtained following the same procedure described for compound **72a** using **63** and **71b** as starting materials. Yield: 62.90%; M.P.: 218° C; ^{1}H NMR (400 MHz, DMSO) δ 10.07 (s, 1H), 8.94 (s, 1H), 8.01 (d, *J* = 9.5 Hz, 1H), 7.66 (d, *J* = 8.6 Hz, 1H), 7.38 (t, *J* = 8.3 Hz, 1H), 7.33 – 7.28 (m, 2H), 7.20 (d, *J* = 1.8 Hz, 1H), 7.06 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.89 (d, *J* = 8.1 Hz, 1H), 6.32 (d, *J* = 9.5 Hz, 1H), 5.34 (s, 2H)

4-(4-(((2-oxo-2H-chromen-7-yl) oxy)methyl)- 1H-1,2,3-triazol-1-yl) phenyl (5-phenylpentyl) carbamate (67)

To a mixture of 0.10g (0.298 mmol) of **72a** in anhydrous toluene, a catalytic amount of NaH was added and the reaction was stirred for 10 min. 0.06g (0.298 mmol) compound **53a** were added, then the solution was stirred at r.t. for 60h; it was poured in water and extracted with DCM. Compound **67** was crystalized with methanol. 0.08g of neat compound **67** were obtained. Yield: 50%; M.P.: 168.4-168.6° C; ^{1}H NMR δ 8.06 (s, 1H), 7.73 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 9.5 Hz, 1H), 7.45 – 7.37 (m, 1H), 7.31 (m, 4H), 7.20 (t, *J* = 6.8 Hz, 3H), 6.98 (d, *J* = 5.5, 2.3 Hz, 2H), 6.29 (d, *J* = 9.5 Hz, 1H), 5.35 (s, 2H), 5.09 (broad, *J* = 5.7 Hz, 1H), 3.29 (dd, *J* = 13.3, 6.8 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H), 1.66 (m, 4H), 1.51 – 1.37 (m, 2H); ^{13}C NMR δ 161.18, 160.99, 155.71, 153.89, 151.34, 143.71, 143.24, 142.26, 133.79, 128.93, 128.34, 128.28, 125.72, 122.89, 121.64, 121.24, 113.54, 113.07, 112.69, 102.14, 62.23, 41.24, 35.73, 30.97, 29.63, 26.27

3-(4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl (5-phenylpentyl)carbamate (68)

Compound **68** was obtained following the same procedure described for compound **67** using **72b** and **53a** as starting materials. Yield: 51.28%; M.P.:132.1-132.3° C**;** ^{1}H NMR δ 8.09 (s, 1H), 7.65 (d, *J* = 9.5 Hz, 1H), 7.60 (d, *J* = 6.8 Hz, 2H), 7.51 (t, *J* = 8.2 Hz, 1H), 7.41 (d, 1H), 7.32 – 7.15 (m, 6H), 6.97 (d, *J* = 6.4 Hz, 2H), 6.28 (d, *J* = 9.5 Hz, 1H), 5.35 (s, 2H), 5.11 (broad, 1H), 3.28 (dd, *J* = 13.3, 6.8 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H), 1.73 – 1.61 (m, 4H), 1.43 (dd, *J* = 15.2, 8.1 Hz, 2H); ^{13}C NMR δ 161.15, 160.99, 155.72, 153.73, 151.91, 143.84, 143.24, 137.35, 130.44, 128.95, 128.35, 128.29, 125.73, 122.10, 121.21, 117.12, 114.34, 113.56, 113.09, 112.67, 102.19, 62.24, 41.26, 35.73, 30.97, 29.62, 26.27; ES-MS *m/z*: 525 (M + 1)

4-(4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl heptylcarbamate (69)

Compound **69** was obtained following the same procedure described for compound **67** using **72a** and **53b** as starting materials. Yield: 64.29%; M.P.:180-181° C; ^{1}H NMR δ 8.05 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.64 (d, *J* = 9.5 Hz, 1H), 7.43 – 7.36 (m, 1H), 7.31 (d, *J* = 8.8 Hz, 2H), 6.97 (dd, *J* = 4.4, 2.2 Hz, 2H), 6.27 (d, *J* = 9.5 Hz, 1H), 5.33 (s, 2H), 5.11 (broad, 1H), 3.28 (dd, *J* = 13.4, 6.7 Hz, 2H), 1.58 (m, 2H), 1.31 (m, 8H), 0.89 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR δ 161.18, 161.02, 155.69, 153.92, 151.36, 143.68, 143.28, 133.72, 128.94, 122.90, 121.62, 121.35, 113.51, 113.05, 112.70, 102.10, 62.21, 41.35, 31.69, 29.73, 28.88, 26.67, 22.55, 14.03; ES-MS *m/z*: 499 (M + Na)

3-(4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl heptylcarbamate (70)

Compound **70** was obtained following the same procedure described for compound **67** using **72b** and **53b** as starting materials. Yield: 41.17%; M.P.:127°; ^{1}H NMR δ 8.09 (s, 1H), 7.63 (d, *J* = 9.5 Hz, 1H), 7.57 (m, 2H), 7.48 (t, *J* = 8.1 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 6.94 (m, 2H), 6.26 (d, *J* = 9.4 Hz, 1H), 5.32 (s, 2H), 5.21 (broad, 1H), 3.26 (d, *J* = 6.3 Hz, 2H), 1.58 (m, 3H), 1.29 (m, 8H), 0.88 (s, 3H); ^{13}C NMR δ 161.15, 161.03, 155.68, 153.83, 151.94, 143.75, 143.28, 137.34, 130.42, 128.95, 122.10, 121.27, 117.04, 114.31, 113.50, 113.06, 112.67, 102.13, 62.20, 41.36, 31.69, 29.72, 28.88, 26.66, 22.55, 14.04; ES-MS *m/z*: 477 (M + H^{+}), 499 (M + Na)

4-((tert-butyldimethylsilyl)oxy)aniline (73a)

To a solution of 0.50g (4.582 mmol, 1eq) of p-aminophenol in 10 mL of DMF, 0.94g (13.746 mmol, 3eq) of imidazole and 0.83g (5.498 mmol, 1.2eq) of TBDMS-Cl were added. The mixture was stirred at r.t. for 12h, then the solution was poured in water and it was extracted with ether. Compound **73a** was purified via flash chromatography: petroleum ether/ethyl acetate 4:1; 0.82g of neat compound were obtained. Yield: 58.42%; dark red oil; ^{1}H NMR δ 6.64 (d, *J* = 8.5 Hz, 2H), 6.56 (d, *J* = 8.5 Hz, 2H), 0.93 (s, 9H), 0.13 (s, 6H).

3-((tert-butyldimethylsilyl)oxy)aniline (73b)

Compound **73b** was obtained following the same procedure described for compound **73a** using 3-aminophenol as starting material. Yield: 79.92%; brow oil**;** ^{1}H NMR δ 7.01 (t, *J* = 16 Hz, 1H), 6.32 – 6.26 (m, 2H), 6.21 (s, 1H), 3.61 (s, 1H), 0.99 (s, 9H), 0.21 (s, 6H).

(4-azidophenoxy)(tert-butyl)dimethylsilane (71a)

0.82g (3.661 mmol, 1eq) of **73a**, 9.5 mL of water and 2.85 mL of HCl 37% were added to a round-bottom flask that was cooled at 0° C in a ice bath. A solution of 0.25g (3.661 mmol, 1eq) of $NaNO_2$, in H_2O, was added dorpwise to the reaction flask. The mixture was stirred for 10'. 0.29g (4.392 mmol, 1.2eq) of NaN_3 were added in portions and the reaction was stirred for 1h. The reaction was quenched with water and compound **71a** was extracted with ethyl acetate. 0.06g of neat compound were obtained. Yield: 58.83%; black oil; ^{1}H NMR δ 6.89 – 6.76 (m, 4H), 0.90 (s, 9H), 0.09 (s, 6H).

(3-azidophenoxy)(tert-butyl)dimethylsilane (71b)

Compound **71b** was obtained following the same procedure described for compound **71a** using **73b** as starting material. Yield: 65.93%; oil; ^{1}H NMR δ 7.18 (t, *J* = 6.8 Hz, 1H), 6.60 (d, *J* = 7.8 Hz, 2H), 6.49 (d, *J* = 8.9 Hz, 1H), 0.90 (s, 9H), 0.09 (s, 6H)

N-cyclopentyl-2-(3- ((methyl(3- ((2-oxo-2H-chromen-7-yl)oxy) propyl) amino) methyl) phenoxy)acetamide (74)

To a solution of 0.35g (1.032 mmol, 1eq) of **46** in 20 mL of acetone, 0.25g (1.366 mmol, 1.3eq) of 2-chloro-N-cyclopentylacetamide and 0.70g of K_2CO_3 were added and the mixture was stirred and refluxed for 12h, then the solution was concentrated under reduced pressure. Compound **74** was

purified via flash chromatography: toluene/acetone 3:2; 0,00g of neat compound were obtained. Yield: oil ^{1}H NMR δ 7.60 (d, *J* = 9.5 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.87 (s, 1H), 6.79 – 6.72 (m, 3H), 6.40 (d, *J* = 7.9 Hz, 1H), 6.20 (d, *J* = 9.5 Hz, 1H), 4.47 (s, 2H), 4.13 – 4.03 (m, 2H), 3.50 (s, 2H), 2.97 – 2.83 (m, 1H), 2.55 (dd, *J* = 13.0, 6.6 Hz, 2H), 2.16 (s, 3H), 2.07 – 1.95 (m, 2H), 1.73 – 1.58 (m, 4H), 1.51 – 1.36 (m, 4H); ^{13}C NMR δ 167.07, 162.20, 161.14, 157.23, 155.78, 143.35, 141.26, 129.41, 128.63, 122.48, 115.08, 113.06, 112.88, 112.68, 112.36, 101.40, 67.79, 67.14, 63.89, 55.92, 54.32, 43.01, 35.65, 27.25, 24.64

N-cyclohexyl-2- (3- ((methyl(3- ((2-oxo-2H-chromen-7-yl) oxy) propyl) amino) methyl) phenoxy) acetamide (75)

Compound **75** was obtained following the same procedure described for compound **74** using 2-chloro-N-cyclohexylacetamide as starting material. Yield: 65.93%; oil**;** ^{1}H NMR δ 7.60 (d, *J* = 9.5 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.87 (s, 1H), 6.79 – 6.72 (m, 3H), 6.40 (d, *J* = 7.9 Hz, 1H), 6.20 (d, *J* = 9.5 Hz, 1H), 4.38 (s, 2H), 4.04 (dd, *J* = 7.9, 4.6 Hz, 2H), 3.89 – 3.74 (m, 1H), 3.45 (s, 2H), 2.52 (t, *J* = 6.9 Hz, 2H), 2.20 (s, 3H), 2.01 – 1.93 (m, 2H), 1.93 – 1.83 (m, 2H), 1.72 – 1.62 (m, 2H), 1.62 – 1.53 (m, *J* = 9.2, 3.8 Hz, 1H), 1.40 – 1.26 (m, 2H), 1.20 – 1.07 (m, 2H); ^{13}C NMR δ 167.07, 162.20, 161.14, 157.23, 155.78, 143.35, 141.26, 129.41, 128.63, 122.48, 115.08, 113.06, 112.88, 112.68, 112.36, 101.40, 67.29, 66.53, 62.21, 53.47, 47.74, 42.21, 32.90, 26.90, 25.36, 24.71

PROJECT 2

ethyl 4-((5-chloropentyl)oxy)benzoate (92)

To a mixture of 1.00g (6.024 mmol, 1eq) of 4-hydroxybenzoate dissolved in acetone, 1.67g (9.036 mmol, 1.5eq) of 1-Bromo-5-chloropenthane and 2.13g of K_2CO_3 were added. The reaction was refluxed and stirred for 10h, then it was filtered hot and concentrated under vacuum. Compound **92** was purified via flash chromatography thanks to a mobile phase of toluene. 1.60g of neat compound were obtained. Colorless oil. Yield: 98.40%; ^{1}H NMR δ 7.99 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 9.2 Hz, 2H), 4.37-4.32 (m, 2H), 4.02 (t, *J* = 12.4 Hz, 2H), 3.56 (t, *J* = 13.2 Hz, 2H), 1.89-1.80 (m, 4H), 1.67-1.61 (m, 2H), 1.38 (t, *J* = 14.4 Hz, 3H)

ethyl 4-(4-chlorobutoxy)benzoate (93)

Compound **93** was obtained following the same procedure described for compound **92** using 1-Bromo-4-chlorobutane as starting material. Yield: 91.44%; oil; ^{1}H NMR δ 8.01 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 4.38-4.33 (m, 2H), 4.08 (t, *J* = 12.4 Hz, 2H), 3.64 (t, *J* = 11.2 Hz, 2H), 2.00-1.98 (m, 4H), 1.39 (t, *J* = 14 Hz, 3H)

ethyl 4-(3-chloropropoxy)benzoate (94)

Compound **94** was obtained following the same procedure described for compound **92** using 1-Bromo-3-chloropropane as starting material. Yield: 96.09%; colorless oil**;** ^{1}H NMR δ 8.02 (d, *J* = 9.2

Hz, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 4.38-4.33 (m, 2H), 4.19 (t, *J* = 11.6 Hz, 2H), 3.76 (t, *J* = 12.8 Hz, 2H), 2.30-2.56 (m, 2H), 1.39 (t, *J* = 14.4 Hz, 3H)

ethyl 4-(2-chloroethoxy)benzoate (95)

Compound **95** was obtained following the same procedure described for compound **92** using 1-Bromo-2-chloroethane as starting material. Yield: 85.79%; oil; ^{1}H NMR δ 8.02 (d, *J* = 9.2 Hz, 2H), 6.95 (d, *J* = 9.2 Hz, 2H), 4.39-4.33 (m, 2H), 4.29 (t, *J* = 11.6 Hz, 2H), 3.85 (t, *J* = 11.6 Hz, 2H), 1.39 (t, *J* = 14.4 Hz, 3H)

4-((5-chloropentyl)oxy)benzoic acid (96)

To a solution of 1.58g (5.85 mmol, 1eq) of **92** in 40 mL of ethanol, a solution of 0.49g (8.78 mmol, 1.5eq) of KOH in 2 mL of H_2O was added and the mixture was stirred and refluxed for 3h. The ethanol was evaporated under vacuum then, 20 mL of HCl 1:1 were added to the reaction flask. A white solid precipitate was formed and it was filtrated. 1.37g of neat compound **96** were obtained. Yield: 96.75%; M.P.: 103.8° C; ^{1}H NMR δ 8.06 (d, *J* = 9.2 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 4.06-4.02 (m, 2H), 3.58 (t, *J* = 13.2 Hz, 2H), 1.90-1.82 (m, 4H), 1.68-1.61 (m, 2H)

4-(4-chlorobutoxy)benzoic acid (97)

Compound **97** was obtained following the same procedure described for compound **92** using **93** as starting material. Yield: 92.43%; M.P.: 113° C; ^{1}H NMR δ 8.05 (d, *J* = 8.8 Hz, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 4.09-4.06 (m, 2H), 3.63 (t, *J* = 12 Hz, 2H), 2.00-1.98 (m, 4H)

4-(3-chloropropoxy)benzoic acid (98)

Compound **98** was obtained following the same procedure described for compound **92** using **94** as starting material. Yield: 87.14%; M.P.: 102° C; ^{1}H NMR δ 8.04 (d, *J* = 9.2 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 4.17 (t, *J* = 11.6 Hz, 2H), 3.74 (t, *J* = 12.4 Hz, 2H), 2.25 (t, *J* = 12 Hz, 2H),

4-(2-chloroethoxy)benzoic acid (99)

Compound **99** was obtained following the same procedure described for compound **92** using **95** as starting material. Yield: 91.77%; M.P.: 176-178° C; ^{1}H NMR 8.03 (d, *J* = 9.2 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.35 (t, *J* = 11.6 Hz, 2H), 3.91 (t, *J* = 12.4 Hz, 2H)

4-((5-chloropentyl)oxy)benzoyl chloride (80)

1.36g (5.62 mmol) of **96** were dissolved in 18 mL of $SOCl_2$; the reaction flask was stirred and refluxed for 5h, then the solvent was removed with a trap. 1.40g of compound **80** were obtained and it was used for the next reaction without any further purification. Yield: 95.89%; yellow oil.

4-(4-chlorobutoxy)benzoyl chloride (81)

Compound **81** was obtained following the same procedure described for compound **80** using **97** as starting material. Yield: 96.00%; oil

4-(3-chloropropoxy)benzoyl chloride (82)

Compound **82** was obtained following the same procedure described for compound **80** using **98** as starting material. Yield: 98.33%; yellow oil.

4-(2-chloroethoxy)benzoyl chloride (83)

Compound **83** was obtained following the same procedure described for compound **80** using **99** as starting material. Yield: 91.77%; oil.

(4-((5-chloropentyl)oxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (84)

A mixture of 1.00g (4.49 mmol, 1eq) of 2-(4-methoxyphenyl)benzofuran and 1.40g (5.38 mmol, 1.2eq) of **80**, previously dissolved in anhydrous DCM under nitrogen atmosphere, were cooled to 0° C. 1.40g (5.38 mmol, 1.2eq) of $SnCl_4$ were added dropwise to the reaction flask then, the mixture was stirred at r.t. overnight. The reaction was quenched with ice and the organic compound was extracted with DCM. Compound **84** was purified via flash chromatography: petroleum ether/ethyl acetate 3:2. 1.42g of neat compound **84** were obtained. Yield: 73.27%; yellow oil; ^{1}H NMR δ 7.86 (d, *J* = 9.2 Hz, 2H), 7.69 (d, *J* = 9.2 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.29 (t, *J* = 15.6 Hz, 1H), 7.21 (t, *J* = 15.6 Hz, 1H), 6.84 - 6.78 (m, 4H), 3.95 (t, *J* = 12.8 Hz, 2H), 3.76 (s, 3H), 3.52 (t, *J* = 13.2 Hz, 2H), 1.84 – 1.74 (m, 4H), 1.61 – 1.55 (m, 2H)

(4-(4-chlorobutoxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (85)

Compound **85** was obtained following the same procedure described for compound **84** using **81** as starting material. Yield: 70.90%; oil; ^{1}H NMR δ 7.86 (d, *J* = 9.2 Hz, 2H), 7.69 (d, *J* = 9.2 Hz, 2H), 7.55 (d, *J* = 8 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.30 (t, *J* = 15.2 Hz, 1H), 7.23 (t, *J* = 14.8 Hz, 1H), 6.84 - 6.79 (m, 4H), 3.99 (t, *J* = 10.8 Hz, 2H), 3.78 (s, 3H), 3.59 (t, *J* = 12.4 Hz, 2H), 1.96 – 1.92 (m, 4H)

(4-(3-chloropropoxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (86)

Compound **86** was obtained following the same procedure described for compound **84** using **82** as starting material. Yield: 40.00%; yellow pale oil; ^{1}H NMR δ 7.88 (d, *J* = 9.2 Hz, 2H), 7.71 (d, *J* = 9.2 Hz, 2H), 7.55 (d, *J* = 8 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.30 (t, *J* = 15.2 Hz, 1H), 7.23 (t, *J* = 14.8 Hz, 1H), 6.87 (dd, *J* = 11.6 Hz, 4H), 4.15 (t, *J* = 10.8 Hz, 2H), 3.81 (s, 3H), 3.74 (t, *J* = 12.8 Hz, 2H), 2.31 – 2.20 (m, 2H)

(4-(2-chloroethoxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (87)

Compound **87** was obtained following the same procedure described for compound **84** using **83** as starting material. Yield: 61.11%; oil; ^{1}H NMR δ 7.88 (d, *J* = 8.4 Hz, 2H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.49 (d, *J* = 8 Hz, 1H), 7.33 (t, *J* = 15.2 Hz, 1H), 7.23 (t, *J* = 14.8 Hz, 1H), 6.86 (dd, *J* = 8.4 Hz, 4H), 4.24 (t, *J* = 11.2 Hz, 2H), 3.79 (s, 3H), 1.26 (t, *J* = 15.2 Hz, 2H)

(4-((5-iodopentyl)oxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (88)

A mixture of 2.50g (5.58 mmol, 1eq) of **84** and 0.84g (5.58 mmol, 1.2eq) of NaI in 70 mL of methyl ethyl ketone was stirred and refluxed for 5h. The solvent was removed under reduced pressure, then the organic compound was dissolved in DCM and it was washed trice with water. Collected organic layers were concentrate under vacuum. 2.38g of compound **88** were obtained and it was used for the next reaction without any further purification. Yield: 79.07%; oil.

(4-(4-iodobutoxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (89)

Compound **89** was obtained following the same procedure described for compound **88** using **85** as starting material. Yield: 93.37%; oil.

(4-(3-iodopropoxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (90)

Compound **90** was obtained following the same procedure described for compound **88** using **86** as starting material. Yield: 89.23%; oil.

(4-(2-iodoethoxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (91)

Compound **91** was obtained following the same procedure described for compound **88** using **87** as starting material. Yield: 90.77%; oil.

(4-((5-(benzyl(methyl) amino) pentyl)oxy) phenyl)(2-(4-methoxyphenyl) benzofuran-3-yl)methanone (76)

A mixture of 0.84g (1.56 mmol, 1eq) of **88**mand 0.38g (3.12 mmol, 2eq) of N-benzylmethylamine in 28 mL of toluene was stirred and refluxed for 60h; then the reaction mixture was washed thrice with water and the organic layers were concentrated under vacuum. Compound **76** was purified via flash chromatography: toluene/acetone 4:1; 0.48g of neat compound were obtained. Yield: 57.83%; dark yellow oil; ^{1}H NMR δ 7.86 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.32-7.19 (m, 7H), 6.84-6-78 (dd, 4H), 3.96 (t, *J* = 12.8 Hz, 2H), 3.78 (s, 3H), 3.47 (s, 2H), 2.37 (t, *J* = 14.4 Hz, 2H), 2.18 (s, 3H), 1.79-1.59 (m, 2H), 1.57-1.53 (m, 2H), 1.51-1.45 (m, 2H); ^{13}C NMR δ 190.97, 163.34, 160.63, 156.66, 153.51, 139.22, 132.28, 130.50, 129.66, 128.98, 128.91, 128.16, 126.87, 124.75, 123.49, 122.17, 121.12, 115.06, 114.16, 113.96, 110.99, 68.14, 62.38, 57.22, 55.29, 42.25, 28.96, 27.13, 23.78; ES-MS *m/z*: 534 (M + H^{+}), 556 (M + Na)

(4- (4- (benzyl (methyl) amino) butoxy)phenyl) (2-(4-methoxyphenyl) benzofuran- 3-yl) methanone (77)

Compound **77** was obtained following the same procedure described for compound **76** using **89** as starting material. Yield: 68.34%; yellow oil; ^{1}H NMR δ 7.85 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 9.2 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.30-7.13 (m, 7H), 6.82-6-76 (dd, 4H), 3.92 (t, *J* = 13.2 Hz, 2H), 3.74 (s, 3H), 3.46 (s, 2H), 2.38 (t, *J* = 14.4 Hz, 2H), 2.17 (s, 3H), 1.82-1.75 (m, 2H), 1.67-1.61 (m, 2H); ^{13}C NMR δ 190.95, 163.35, 160.67, 156.67, 153.55, 139.24, 132.29, 130.53

, 129.69, 129.00, 128.22, 126.93, 125.31, 124.79, 123.54, 122.19, 121.15, 115.09, 114.21, 113.99, 111.03, 68.00, 62.39, 56.73, 55.28, 42.19, 26.84, 23.70; ES-MS *m/z*: 520 (M + H^+), 542 (M + Na)

(4-(3-(benzyl(methyl)amino)propoxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (78)

Compound **78** was obtained following the same procedure described for compound **76** using **90** as starting material. Yield: 58.34%; yellow oil; ^{1}H NMR δ 7.86 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8 Hz, 1H), 7.47 (d, *J* = 7.2 Hz, 1H), 7.32-7.15 (m, 7H), 6.84-6-78 (dd, 4H), 4.03 (t, *J* = 12.4 Hz, 2H), 3.76 (s, 3H), 3.49 (s, 2H), 2.51 (t, *J* = 13.6 Hz, 2H), 2.14 (s, 3H), 1.94 (m, 2H); ^{13}C NMR δ 190.98, 163.29, 160.63, 156.65, 153.52, 139.02, 132.26, 130.49, 129.66, 128.91, 128.87 , 128.19, 126.94, 124.76, 123.50, 122.16, 121.11, 115.04, 114.19, 113.96, 111.00, 66.27, 62.43, 55.28, 53.45, 42.25, 27.04; ES-MS *m/z*: 506 (M + H^+), 528 (M + Na)

(4-(2-(benzyl(methyl)amino)ethoxy)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (79)

Compound **79** was obtained following the same procedure described for compound **76** using **91** as starting material. Yield: 28.38%; oil; ^{1}H NMR δ 7.84 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.13-7.28 (m, 4H), 7.25-7.21 (m, 3H), 6.81 (t, *J* = 17.6 Hz, 4H), 4.09 (t, *J* = 11.6 Hz, 2H), 3.77 (s, 3H), 3.61 (s, 2H), 2.82 (t, *J* = 12 Hz, 2H), 2.33 (s, 3H); ^{13}C NMR 190.91, 162.87, 160.57, 156.73, 153.45, 138.30, 132.18, 130.62, 129.63, 128.99, 128.79, 128.24, 127.13, 124.71, 123.46, 122.04, 121.05, 114.92, 114.15, 113.88, 110.94, 66.43, 62.62, 55.30, 42.88, 30.84; ES-MS *m/z*: 492 (M + H^+), 514 (M + Na)

(2- (4-methoxyphenyl) benzofuran-3-yl) (4-nitrophenyl) methanone (114)

A mixture of 1.10g (4.95 mmol, 1eq) of 2-(4-methoxyphenyl)benzofuran, and 1.10g (5.85 mmol, 1.2 eq) of 4-nitrobenzoyl chloride in anhydrous DCM under nitrogen atmosphere was cooled at 0° C, then 1.55g (5.95 mmol, 1.2 eq) of $SnCl_4$ were added dropwise to the flask. The reaction was stirred at r.t. for 10h, then it was quenched with ice and the organic compound was extracted with DCM. 1.44g of neat compound **114** were obtained. Yield: 78.26%; M.P.: 146° C; ^{1}H NMR δ 8.12 (d, *J* = 8.2 Hz, 2H), 7.89 (d, *J* = 8.2 Hz, 2H), 7.64 (d, *J* = 7.7 Hz, 1H), 7.59 – 7.48 (m, 3H), 7.37 (t, *J* = 7.7 Hz, 1H), 7.29 (t, *J* = 7.5 Hz, 1H), 6.77 (d, *J* = 8.3 Hz, 2H), 3.77 (s, 3H)

(4-aminophenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (115)

To a solution of 1.44g (3.86 mmol) of **114** in 100 mL of ethanol, 10.85g (48.25 mmol, 12.5 eq) of $SnCl_2$ were added and the reaction was refluxed for 2h; then it was cooled to r.t. and 40 mL of NaOH were added. The organic compound was exctracted with ethyl acetate. Compound **115** was purified via flash chromatography: petroleum ether/ethyl acetate 4:1; 1.18g of neat compound were obtained. Yield: 89.39%; M.P.: 183° C; 7.77 (d, *J* = 8.6 Hz, 2H), 7.73 (d, *J* = 8.9 Hz, 2H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.46 (d, *J* = 7.7 Hz, 1H), 7.31 (t, *J* = 8.3 Hz, 1H), 7.22 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.9 Hz, 2H), 6.57 (d, *J* = 8.7 Hz, 2H), 4.13 (s, 2H), 3.82 (s, 3H)

(4-(benzylamino)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (100) and (4-(dibenzylamino)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (101)

To a solution of 0.10g (0.29 mmol, 1eq) of **115** in 3 mL of CH_3CN, 0.05g (0.29 mmol, 1eq) of benzylbromide, 0.02g (0.10 mmol, 1/3 eq) of KI and 0.02g (0.10 mmol, 1/3 eq) of K_2CO_3 were added. The reaction was carried out with the microwave at 110° C, 150W, for 10 minutes; then it was poured in water and organic compounds were extracted in DCM. Compound **100** and **101** were separated and purified via flash chromatography: petroleum ether/ethyl acetate 7:3; 0.04g of **100** and 0.10g of **101** were obtained. compound **100:** yield: 31.85%; yellow oil; ^{1}H NMR δ 7.77 (d, *J* = 8.6 Hz, 2H), 7.71 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.43 (d, *J* = 7.7 Hz, 1H), 7.38 – 7.23 (m, 6H), 7.18 (t, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.50 (d, *J* = 8.6 Hz, 2H), 4.63 (s, 1H), 4.35 (d, *J* = 3.8 Hz, 2H), 3.79 (s, 3H); ^{13}C NMR δ 190.38, 160.37, 155.43, 153.40, 152.33, 138.00, 132.64, 129.29, 129.15, 128.75, 127.56, 127.36, 127.04, 124.51, 123.27, 122.36, 121.00, 115.31, 113.91, 111.62, 110.87, 55.26, 47.51; compound **101:** yield: 65.93%; yellow oil; ^{1}H NMR δ 7.77 (d, *J* = 9.0 Hz, 2H), 7.71 (d, *J* = 5.8 Hz, 2H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.47 (d, *J* = 7.7 Hz, 1H), 7.33 (m, 4H), 7.27 (m, 3H), 7.19 (m, *J* = 14.6, 7.3 Hz, 5H), 6.86 (d, *J* = 8.9 Hz, 2H), 6.64 (d, *J* = 9.0 Hz, 2H), 4.67 (s, 4H), 3.81 (s, 3H).; ^{13}C NMR δ 190.19, 160.30, 155.38, 153.34, 153.06, 136.97, 132.48, 129.27, 129.10, 128.74, 127.22, 126.34, 126.26, 124.46, 123.21, 122.37, 120.99, 115.35, 113.83, 111.20, 110.82, 55.21, 53.84

(4-((4-fluorobenzyl)amino)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (102) and (4-(bis(4-fluorobenzyl)amino)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (103)

Compound **102** and **103** were obtained following the same procedure described for compound **100** using **115** and 4-fluorobenzyl bromide as starting materials. Yield: 57.80%; Compound **102**: yield: 38.05%; yellow oil; ^{1}H NMR δ 7.71 (d, *J* = 8.8 Hz, 2H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.38 (d, *J* 7.6 Hz, 1H), 7.24-7.18 (m, 3H), 7.13 (t, *J* = 15.2 Hz, 1H), 6.95 (t, *J* = 17.2 Hz, 2H), 6.79 (d, *J* = 8.4 Hz, 2H), 6.44 (d, *J* = 8.8 Hz, 2H), 4.75 (broad,1H), 4.27 (d, *J* = 5.6 Hz, 2H), 3.74 (s, 3H); ^{13}C NMR δ 190.30, 163.27, 160.81, 160.39, 153.45, 152.65, 132.54, 129.43, 129.08, 128.06, 127.98, 126.74, 124.57, 123.31, 122.34, 121.04, 115.82, 115.61, 113.85, 111.29, 110.90, 55.27, 53.21; ES-MS *m/z*: 452 (M + H$^+$), 474 (M + Na); Compound **102**: yield: 24.68%; yellow oil; ^{1}H NMR δ 7.70 (d, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.45-7.39 (dd, *J* = 8 Hz, 4H), 7.22 (t, *J* = 14.4 Hz, 1H), 7.13 (t, *J* = 15.2 Hz, 1H), 7.06-7.03 (m, 4H), 6.94 (t, *J* = 17.2 Hz, 4H), 6.79 (d, *J* = 8.8 Hz, 2H), 6.56 (d, *J* = 9.2 Hz, 2H), 4.54 (s, 4H), 3.75 (s, 3H); ^{13}C NMR δ 190.36, 163.19, 160.39, 153.44, 152.65, 132.54, 129.43, 129.04, 128.06, 127.98, 126.74, 124.57, 123.31, 122.41, 121.05, 115.82, 115.61, 113.85, 112.01, 111.29, 110.90, 55.27, 53.22; ES-MS *m/z*: 560 (M + H$^+$), 582 (M + Na)

(4-((3,5-difluorobenzyl)amino)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (104) and (4-(bis(3,5-difluorobenzyl)amino)phenyl)-(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (105)

Compound **104** and **105** were obtained following the same procedure described for compound **100** using–**115** and 3,5-difluorobenzyl bromide as starting materials. Yield: 57.18%; Compound **104**:

yield: 73.02%; yellow oil; ^{1}H NMR δ 7.76 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8 Hz, 1H), 7.42 (d, *J* = 7.6 Hz, 1H), 7.28 (t, *J* = 15.6 Hz, 1H), 7.20 (t, *J* = 14.8 Hz, 1H), 6.84-6.79 (m, 4H), 6.67 (t, *J* = 17.6 Hz, 1H), 6.46 (d, *J* = 8.8 Hz, 2H), 4.77 (broad, 1H), 4.35 (d, *J* = 6 Hz, 2H), 3.78 (s, 3H); ^{13}C NMR δ 190.30, 164.65, 162.30, 160.52, 155.91, 153.42, 151.84, 141.21, 132.59, 129.44, 127.57, 124.61, 123.37, 122.25, 121.04, 113.90, 111.39, 110.91, 109.23, 108.98, 103.00, 55.27, 46.70; ES-MS *m/z*: 470 (M + H^+); Compound **105**: yield: 23.02%; yellow oil; ^{1}H NMR δ 7.82 (d, *J* = 9.2 Hz, 2H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 1H), 7.33-7.21 (m, 3H), 6.88 (d, *J* = 8.8 Hz, 2H), 6.75-6.66 (m, 5H), 6.60 (d, *J* = 9.2 Hz, 2H), 4.65(s, 4H), 3.82 (s, 3H); ^{13}C NMR δ 164.58, 162.10, 160.47, 155.69, 153.43, 151.75, 142.52, 132.61, 129.36, 129.08, 127.61, 124.59, 123.34, 122.29, 121.02, 115.21, 113.94, 111.79, 110.92, 109.76, 102.83, 55.27, 53.66; ES-MS *m/z*: 596 (M + H^+), 618 (M + Na)

(2-(4-methoxyphenyl)benzofuran-3-yl)(4-((pyridin-2-ylmethyl)amino)phenyl) methanone (106)
Compound **106** was obtained following the same procedure described for compound **100** using **115** and 2-(Bromomethyl)pyridine as starting materials. Yield: 31.56%; M.P.: 56° C; ^{1}H NMR δ 8.59 (d, *J* = 4.6 Hz, 1H), 7.80 (d, *J* = 8.5 Hz, 2H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.67 (t, *J* = 7.7 Hz, 1H), 7.53 (d, *J* = 8.2 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.32 – 7.26 (m, 2H), 7.21 (dd, *J* = 14.3, 7.0 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 6.57 (d, *J* = 8.5 Hz, 2H), 5.60 (broad, 1H), 4.49 (d, *J* = 5.0 Hz, 2H), 3.80 (s, 3H); ^{13}C NMR δ 190.43, 178.07, 160.34, 156.87, 155.42, 153.39, 152.22, 149.03, 136.99, 132.65, 129.29, 129.16, 126.81, 124.51, 123.27, 122.53, 121.84, 121.02, 115.30, 113.91, 111.73, 110.86, 55.26, 47.96; ES-MS *m/z*: 435(M + H^+), 457 (M + Na)

(4-((4-methoxybenzyl)amino)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl) methanone (107)
Compound **107** was obtained following the same procedure described for compound **100** using-**115** and 4-methoxybenzylchloride as starting materials. Yield: 29.46%; yellow oil; ^{1}H NMR δ 7.80 (d, *J* = 8.8 Hz, 2H), 7.74 (d, 2H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.31 (t, 1H), 7.21 (t, 1H), 6.88 (dd, 4H), 6.52 (d, *J* = 8.8 Hz, 2H), 4.30 (s, 2H), 3.82 (s, 3H), 3.81 (s, 3H); ^{13}C NMR δ 192.79, 162.80, 161.52, 157.82, 155.83, 154.75, 135.08, 132.36, 131.66, 131.18, 130.10, 129.43, 126.93, 125.68, 124.82, 123.44, 117.77, 116.58, 116.35, 114.01, 113.30, 57.70, 49.47

4-(((4-(2-(4-methoxyphenyl)benzofuran-3-carbonyl)phenyl)amino)methyl)benzonitrile (108)
Compound **108** was obtained following the same procedure described for compound **100** using **115** and 4-(Bromomethyl)benzonitrile as starting materials. Yield: 36.28%; yellow oil; ^{1}H NMR δ 7.76 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 8.9 Hz, 2H), 7.61 (d, *J* = 8.2 Hz, 2H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.34 – 7.16 (m, 2H), 6.85 (d, *J* = 8.9 Hz, 2H), 6.46 (d, *J* = 8.8 Hz, 2H), 4.87 (broad, 1H), 4.45 (d, *J* = 5.9 Hz, 2H), 3.82 (s, 3H); ^{13}C NMR δ 190.45, 160.44, 155.73, 153.42, 151.70 , 143.87, 132.61, 132.52 , 129.39 , 129.01, 128.20, 127.52, 125.27, 124.64, 123.36, 122.27, 120.97, 118.64, 113.91, 111.78, 111.27, 110.95, 55.31, 46.94; ES-MS *m/z*: 459 (M + H^+)

(4-((cyclopropylmethyl)amino)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (109)

Compound **109** was obtained following the same procedure described for compound **100** using **115** and chloromethylcyclopropane as starting materials. Yield: 25.88%; yellow oil**;** ^{1}H NMR δ 7.77 (d, *J* = 8.6 Hz, 2H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.28 (t, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.46 (d, *J* = 8.6 Hz, 2H), 4.37 (s, 1H), 3.82 (s, 3H), 2.99 (d, *J* = 7.1 Hz, 2H), 1.12 – 0.96 (m, 1H), 0.56 (d, *J* = 7.5 Hz, 2H), 0.24 (d, *J* = 5.0 Hz, 2H); ^{13}C NMR δ 190.24, 160.35, 155.24, 152.61, 132.71, 132.60, 129.45, 129.25, 126.59, 124.49, 123.24, 122.40, 121.02, 115.33, 113.93, 111.32, 110.87, 55.27, 48.18, 10.56, 3.50; ES-MS *m/z*: 398 (M + H^+), 420 (M + Na)

(2-(4-methoxyphenyl)benzofuran-3-yl)(4-(pentylamino)phenyl)methanone (110)

A mixture of 0.10g (0.29 mmol, 1eq) of **115** and 0.09g (0.58 mmol, 2eq) of 1-Bromopentane were dissolved in a biphasic mixture of DCM/NaOH 50%, 1,5:1 (10 mL/6,5 mL); then 0.20g (0.58 mmol, 2eq) of tetrabutylammonium hydrogensulfate were added and the reaction was stirred at r.t. for 4h. The organic layer was collected and it was washed with water. Compound **110** was purified via flash chromatography: petroleum ether/ethyl acetate 7:3. 0.01 g of neat compound **110** were obtained. Yield: 41.66%; oil; ^{1}H NMR δ 7.77 (d, *J* = 8.7 Hz, 2H), 7.72 (d, *J* = 8.9 Hz, 2H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.42 (d, *J* = 7.7 Hz, 1H), 7.28 (t, *J* = 11.3, 4.1 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 8.9 Hz, 2H), 6.45 (d, *J* = 8.8 Hz, 2H), 4.20 (s, 1H), 3.79 (s, 3H), 3.13 (t, *J* = 7.1 Hz, 2H), 1.59 (m, 2H), 1.36 (m, 4H), 0.90 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR δ 190.30, 160.35, 155.22, 153.41, 152.75, 132.71, 129.24, 128.24, 126.49, 124.48, 123.24, 122.44, 121.02, 115.49, 113.93, 111.28, 110.86, 55.27, 43.22, 29.14, 28.94, 22.38, 13.95

(4-(heptylamino)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (111)

Compound **111** was obtained following the same procedure described for compound **110** using **115** and 1-Bromoheptane as starting materials. Yield: 31.06%; oil; ^{1}H NMR δ 7.75 (dd, *J* = 17.3, 8.8 Hz, 4H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.27 (t, *J* = 14.5, 6.3 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.45 (d, *J* = 8.7 Hz, 2H), 4.23 (t, 1H), 3.79 (s, 3H), 3.12 (dd, *J* = 12.1, 6.7 Hz, 2H), 1.65 – 1.52 (m, 2H), 1.42 – 1.13 (m, 8H), 0.87 (t, *J* = 6.1 Hz, 3H); ^{13}C NMR δ 190.29, 160.35, 155.23, 153.41, 152.76, 132.71, 131.51, 129.24, 126.48, 124.48, 123.23, 122.44, 121.02, 115.42, 113.84, 111.28, 110.86, 55.27, 43.25, 31.71, 29.26, 28.98, 26.97, 22.55, 14.03

(4-((4-fluorobenzyl)(methyl)amino)phenyl)(2-(4-methoxyphenyl)benzofuran-3-yl)methanone (112)

Compound **112** was obtained following the same procedure described for compound **110** using **102** and CH_3I as starting materials. Yield: 13.87%; yellow oil; ^{1}H NMR δ 7.82 (d, *J* = 9.0 Hz, 2H), 7.74 (d, *J* = 8.9 Hz, 2H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 7.2 Hz, 1H), 7.22 (t, *J* = 7.4 Hz, 1H), 7.12 (dd, *J* = 8.4, 5.4 Hz, 2H), 7.01 (t, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 6.62 (d, *J* = 9.0 Hz, 2H), 4.58 (s, 2H), 3.82 (s, 3H), 3.09 (s, 3H); ^{13}C NMR δ 160.35, 155.37, 153.47,

153.06, 133.03, 132.95, 132.50, 129.29, 129.14, 128.00, 127.92, 125.98, 124.51, 123.25, 122.43, 121.01, 115.72, 115.51, 115.39, 113.90, 110.87, 55.27, 55.21, 38.55; ^{19}F NMR δ -115.36 – -115.51 (m); ES-MS *m/z*: 466 (M + H^+), 488 (M + Na), 504 (M + K)

1-fluoro-3-(prop-2-yn-1-yloxy)benzene (116)

To a solution of 1.00g (8.92 mmol, 1eq) of 3-fluorophenol in 100 mL of acetone, 1.30g (10.70 mmol, 1.2eq) of propargyl bromide and 1.00g of K_2CO_3 were added. The reaction was stirred and refluxed for 8h then it was filtered hot and it was concentrated under vacuum. Compound **116** was purified via flash chromatography: petroleum ether/ethyl acetate 9:1; 1.03g of neat compound **116** were obtained. Yield: 76.98%; oil; ^{1}H NMR (200 MHz) δ 7.35-7.28 (m, 1H), 6.98-6.64 (m, 3H), 4.63 (d, *J* = 7.2 Hz, 2H), 2.58-2.48 (m, 1H)

(4-(4-((3-fluorophenoxy) methyl)-1H-1,2,3-triazol-1-yl) phenyl)-(2-(4-methoxyphenyl) benzofuran-3-yl) methanone (113)

To a mixture cooled to 0° C, in a double-neck flask, of 0.38g (1.11 mmol, 1eq) of **115** in 14 mL of CH_3CN, 0.17g (1.66 mmol, 1.5eq) of *tert*-Butyl nitrite and 0.15g (1.33 mmol, 1.2eq) of $TMSN_3$ were added and the reaction was stirred at r.t. for 2h. 0.25g (1.66 mmol, 1.5eq) of **116** and a solution of 0.02g (0.11 mmol, 1/10eq) of $CuSO_4$ and 0.11g (0.55 mmol, ½eq) of sodium ascorbate in 2.05 mL of water were added dropwise to the reaction flask, that then was stirred for 10h. The reaction was quenched with water and the organic compound was extracted with ethyl acetate. Compound **113** was purified via flash chromatography: petroleum ether/ethyl acetate 3:1; 0.10g of neat compound **113** were achieved. Yield: 17.39%; oil; ^{1}H NMR δ 8.07 (s, 1H), 7.97 (d, *J* = 8.6 Hz, 2H), 7.72 (d, *J* = 8.6 Hz, 2H), 7.59 (m, 4H), 7.35 (t, 1H), 7.24 (m, 2H), 6.79 (m, 3H), 6.71 (m, 2H), 5.26 (s, 2H), 3.76 (s, 3H); ^{13}C NMR δ 190.59, 161.05, 158.89, 153.69, 144.91, 139.60, 138.05, 131.52, 130.45, 130.29, 128.24, 125.22, 123.95, 121.66, 121.23, 120.70, 119.86, 114.45, 113.99, 111.17, 110.31, 108.43, 108.22, 102.79, 102.55, 62.07, 55.32; ^{19}F NMR δ -111.11 – -111.23 (m); ES-MS *m/z*: 520 (M + H^+), 542 (M + Na)

3-hexyl-N-(4-(2-(4-methoxyphenyl)benzofuran-3-carbonyl)phenyl)benzamide (117)

A mixture of 0.15g (0.44 mmol, 1eq) of **115**, 0.15g of K_2CO_3 and 0.10g (0.437 mmol, 1eq) of 4-hexylbenzoyl chloride in toluene was stirred and refluxed for 24h. The solvent was removed under reduced pressure and compound **117** was purified via flash chromatography: petroleum ether/ethyl acetate 4:1; 0.09g of neat compound were obtained. Yield: 38.71%; yellow oil; ^{1}H NMR δ 7.95 (s, 1H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.69 (t, *J* = 8.4 Hz, 4H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.31 (m, 4H), 6.85 (d, *J* = 8.8 Hz, 2H), 3.79 (s, 3H), 2.66 (t, *J* = 15.6 Hz, 2H), 1.63 (t, *J* = 14 Hz, 2H), 1.34-1.25 (m, 6H), 0.88 (t, *J* = 13.6 Hz, 3H); ^{13}C NMR δ 191.14 , 165.79, 160.73, 157.41, 153.53, 147.80, 142.71, 133.39, 131.70, 131.38, 129.83, 128.81, 128.66, 127.10, 124.86, 123.62, 121.95, 121.13, 119.05, 114.80, 113.96, 111.03, 55.28, 35.82, 31.62, 31.07, 28.86, 22.55, 14.05

N-(4-(2-(4-methoxyphenyl)benzofuran-3-carbonyl)phenyl)-6-phenylhexanamide (118)

Compound **118** was obtained following the same procedure described for compound **117** using **115** and 6-phenylhexanoyl chloride as starting materials. Yield: 39.83%; yellow oil; ^{1}H NMR δ 7.84 (d, *J* = 8.6 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.51 (td, *J* = 15.6, 8.0 Hz, 4H), 7.35 – 7.13 (m,7), 6.83 (d, *J* = 8.8 Hz, 2H), 3.79 (s, 3H), 2.61 (t, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.74 (dt, *J* = 15.3, 7.6 Hz, 2H), 1.65 (dt, *J* = 15.4, 7.7 Hz, 2H), 1.46 – 1.34 (m, *J* = 15.3, 7.7 Hz, 2H); ^{13}C NMR δ 191.05, 171.36, 160.72, 157.35, 153.54, 142.38, 133.27, 131.36, 129.82, 128.66, 128.34, 128.25, 125.69, 124.86, 123.61, 121.99, 121.12, 118.60, 114.84, 113.95, 111.03, 106.17, 55.29, 37.71, 35.64, 31.07, 28.74, 25.16

4-fluoro-N-(4-(2-(4-methoxyphenyl)benzofuran-3-carbonyl)phenyl)benzamide (119)

Compound **119** was obtained following the same procedure described for compound **117** using **115** and 4-fluorobenzoyl chloride as starting materials. Yield: 52.45%; yellow oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.92-7.84 (m, 4H), 7.69-7.63 (m, 4H), 7.58-7.48 (m, 2H), 7.34 (t, *J* = 9.2 Hz, 1H), 7.26-7.11 (m, 3H), 6.86 (d, *J* = 9.2 Hz, 2H), 3.79 (s, 3H); ^{13}C NMR 191.04, 164.58, 163.83, 160.78, 157.55, 153.58, 142.24, 133.78, 131.41, 130.61, 129.90, 129.44, 128.63, 124.92, 123.66, 121.98, 121.14, 119.11, 116.10, 115.88, 113.98, 111.06, 55.29; ES-MS *m/z*: 466 (M + H^+), 488 (M + Na)

(E)-3-(3,5-difluorophenyl)- N-(4-(2- (4-methoxyphenyl) benzofuran-3-carbonyl)phenyl) acrylamide (120)

Compound **120** was obtained following the same procedure described for compound **117** using **115** and 3,5-difluoricinnamic acid, previously refluxed with 3 mL of $SOCl_2$ for 5h, as starting materials. Yield: 17.98%; yellow oil; ^{1}H NMR δ 7.88 (d, *J* = 8.6 Hz, 2H), 7.73 – 7.59 (m, *J* = 17.9, 7.3 Hz, 5H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 1H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.22 (t, *J* = 7.4 Hz, 1H), 7.02 (d, *J* = 5.9 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 3H), 6.53 (d, *J* = 15.4 Hz, 1H), 3.78 (s, 3H); ^{13}C NMR δ 191.07, 164.61, 163.00, 162.04, 161.85, 160.78, 157.57, 153.58, 142.22, 133.73, 131.41, 129.90, 128.63, 124.93, 123.67, 122.90, 121.97, 121.14, 118.98, 114.78, 113.97, 111.07, 110.76, 110.50, 55.31

N-(4-(2-(4-methoxyphenyl)benzofuran-3-carbonyl)phenyl)octanamide (121)

Compound **121** was obtained following the same procedure described for compound **117** using **115** and heptanoyl chloride as starting materials. Yield: 30.11%; oil; ^{1}H NMR δ 7.86 (d, *J* = 8.8 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.53 (m, 4H), 7.32-7.22 (m, 2H), 6.85(d, *J* = 8.8 Hz, 2H), 3.80 (s, 3H), 2.36 (t, *J* = 15.2 Hz, 2H), 1.34-1.29 (m, 6H), 0.88 (t, *J* = 13.6 Hz, 3H); ^{13}C NMR δ 191.01, 171.48, 160.72, 157.33, 153.54, 142.38, 133.35, 131.36, 129.83, 128.66, 124.84, 123.59, 122.01, 121.13, 118.58, 114.82, 113.95, 111.01, 55.29, 37.89, 31.48, 28.85, 25.31, 22.43, 13.98

2-(3,5-difluorophenyl)-N-(4-(2-(4-methoxyphenyl)benzofuran-3-carbonyl)phenyl)acetamide (122)

0.17g (0.82 mmol, 1.4eq) of DCC were added to a solution of 0.13g (0.76 mmol, 1.3 eq) of 2-(3,5-difluorophenyl)acetic acid in 10 mL of anhydrous DCM, under nitrogen atmosphere. The reaction

was cooled to 0° C, then 0.20g (0.58 mmol, 1eq) of **115** were added to the flask and the reaction was stirred at r.t. for 4h. The DCU was then filtrated and the resulting solution was concentrated under reduced pressure. The organic compound was purified via flash chromatography: petroleum ether/ethyl acetate 7:3. 0.10g of neat compound **122** were obtained. Yield: 34.64; yellow oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8 Hz, 1H), 7.48 (d, *J* = 8 Hz, 3H), 7.34-7.20 (m, 3H), 6.78-6.76 (m, 4H), 3.79 (s, 3H), 3.69 (s, 2H); ^{13}C NMR 190.98, 170.69, 160.78, 157.59, 153.57, 141.69 ,131.32, 129.91, 128.59, 124.92, 123.66, 121.95, 121.12, 118.81, 114.72, 113.96, 112.55, 112.36, 112.29, 111.06, 103.60, 103.35, 55.29, 44.22; ES-MS *m/z*: 498(M + H^+), 520 (M + Na)

3-(3,4-difluorophenyl)-N-(4- (2-(4-methoxyphenyl) benzofuran-3-carbonyl)phenyl) propanamide (123)

Compound **123** was obtained following the same procedure described for compound **122** using **115** and 3-(3,4-difluorophenyl)propanoic acid as starting materials. Yield: 20.24%; oil; ^{1}H NMR δ 7.84 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 9.2 Hz, 2H), 7.56 (d, *J* = 8 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.34-7.31 (m, 2H), 7.26-7.22 (m, 2H), 7.06-7.01 (m, 2H), 6.84 (d, *J* = 9.2 Hz, 2H), 3.79 (s, 3H), 2.99 (t, *J* = 14.8 Hz, 2H), 2.63 (d, *J* = 14.8 Hz, 2H); ^{13}C NMR 191.03, 160.75, 157.46, 155.24, 155.10, 153.48, 141.60, 131.34, 129.86, 128.62, 124.88, 124.24, 124.19, 123.62, 121.99, 121.11, 118.68, 117.34, 117.23, 117.18, 117.06, 114.74, 113.94, 111.03, 55.28, 30.19, 29.66; ES-MS *m/z*: 534 (M + Na)

(2-(4-methoxyphenyl)benzofuran-3-yl)(phenyl)methanone (131)

Under nitrogen atmosphere, a solution of 0.50g (2.23 mmol, 1eq) of 2-(4-methoxyphenyl)benzofuran in 10 mL of anhydrous DCM and 0.38g (2.68 mmol, 1.2eq) of benzoylchloride was cooled to 0° C; 0.70g (2.68 mmol, 1.2eq) of $SnCl_4$ were added dropwise to the flask and the reaction was stirred for 12h. The reaction was quenched with ice; the organic layer was collected and it was washed with water. Compound **131** was purified with flash chromatography: petroleum ether/ethyl acetate 4:1. 0.65g of neat compound were obtained. Yield: 88.79%; oil; ^{1}H NMR δ 7.84 (d, *J* = 6.8 Hz, 2H), 7.65 (d, *J* = 9.2 Hz, 2H), 7.56-7.46 (m, 3H), 7.33 (t, *J* = 15.6 Hz, 3H), 7.23 (t, *J* = 12.8 Hz, 1H), 6.81 (t, *J* = 8.4 Hz, 2H), 3.77 (s, 3H)

(2-(4-hydroxyphenyl)benzofuran-3-yl)-(phenyl)methanone (132)

In a two-neck flask, under nitrogen atmosphere, 0.61g (1.86 mmol, 1eq) of **131** were dissolved in 20 mL of anhydrous DCM and the tempereature was allowed to 0° C. 3.75g (2.56 mL, solution 1M in DCM) of BBr_3 were added dropwise and the reaction was stirred at r.t. overnight; then it was quenched with ice and the organic layer was washed with water. 0.58g of neat compound **132** were obtained and it was used without any further purification for next reactions. Yield: 99.32%; oil; ^{1}H NMR δ 7.84 (d, *J* = 7.1 Hz, 2H), 7.61 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.35 (t, *J* = 7.7 Hz, 3H), 7.26 – 7-24 (m, 1H), 6.76 (d, *J* = 8.8 Hz, 2H)

Phenyl(2-(4-(prop-2-yn-1-yloxy)phenyl)benzofuran-3-yl)methanone (133)

To a solution of 0.57g (1.81 mmol, 1eq) of **132** in 30 mL of acetone, 0.26g (2.18 mmol, 1.2eq) of propargyl bromide and 0.57g of anhydrous K_2CO_3 were added and the reaction was refluxed for 8h. The solvent was removed and the organic compound was cristallized with petroleum ether. 0.62g of **133** were obtained. The crude was used for the next reactions without any further purification. Yield: 97.04%; yellow oil; ^{1}H NMR δ 7.85 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.68 (d, *J* = 8.9 Hz, 2H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.51 (t, *J* = 7.3 Hz, 2H), 7.35 (t, *J* = 7.8 Hz, 3H), 7.26 – 7.22 (m, *J* = 7.3 Hz, 1H), 6.91 (d, *J* = 8.9 Hz, 2H), 4.69 (d, *J* = 2.4 Hz, 2H), 2.53 (t, *J* = 2.4 Hz, 1H)

(2-(4-((1-(4-fluorobenzyl)- 1H-1,2,3-triazol-4-yl)methoxy) phenyl)benzofuran-3-yl)(phenyl) methanone (124)

A solution of 0.01g (0.04 mmol, 1/10eq) of $CuSO_4$ and 0.04g (0.21 mmol, ½ eq) of sodium ascorbate in 1 mL of water was stirred for 5min, then it was added dropwise to a mixture of 0.15g (0.43 mmol, 1eq) of **133**, 0.08g (0.55 mmol, 1.3eq) of **134** and 0.01g (0.04 mmol, 1/10eq) of TEA in 5 mL of DMSO. The reaction was stirred at r.t for 12 h, then it was queched with 10 mL of a saturated solution of NH_4Cl and the organic compound was extracted with ethyl acetate. Compound **124** was purified via flash chromatography: petroleum ether/ethyl acetate 7:3. 0.12g of neat compound were obtained. Yield: 56.00%; M.P.: 183.6° C; ^{1}H NMR δ 7.81 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 8.7 Hz, 2H), 7.54 (d, *J* = 10.4 Hz, 2H), 7.51 – 7.44 (m, 2H), 7.32 (t, *J* = 7.7 Hz, 3H), 7.24 (m, 3H), 7.05 (t, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 5.49 (s, 2H), 5.15 (s, 2H); ^{13}C NMR δ 192.23, 163.93, 162.25, 161.47, 159.14, 157.70, 153.41, 137.74, 132.94, 129.88, 129.80, 129.60, 128.34, 128.25, 124.86, 123.57, 122.40, 121.16, 116.10, 115.88, 114.93, 114.48, 110.91, 61.80, 53.34; ^{19}F NMR δ -112.45 – -112.65 (m).

(2-(4-((1-(3,5-difluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)benzofuran-3-yl)(phenyl)methanone (125)

Compound **125** was obtained following the same procedure described for compound **124** using **133** and **135** as starting materials. Yield: 35.69%; yellow oil; ^{1}H NMR δ 7.84 (d, *J* = 7.2 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.56 (m, 2H), 7.49 (m, 2H), 7.34 (t, *J* = 15.6 Hz, 3H), 7.24 (t, 1H), 6.90 (d, *J* = 9.2 Hz, 2H), 6.78 (m, 3H), 5.50 (s, 2H), 5.19 (s, 2H); ^{13}C NMR δ 192.36, 164,60, 162,11, 159.26, 157.81, 153.59, 144.49, 138.08, 137.94, 133.09, 130.06, 129.78, 129.72, 128.41, 125.02, 123.73, 122.81, 121.34, 115.13, 114.63, 111.07, 11.91, 104.36, 61.96, 53.13; ES-MS *m/z*: 544 (M + Na)

(2-(4-((1-(2,4-difluorobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)phenyl)benzofuran-3-yl)(phenyl) methanone (126)

Compound **126** was obtained following the same procedure described for compound **124** using **133** and **136** as starting materials. Yield: 58.57%; M.P.: 152-153° C; ^{1}H NMR δ 7.81 (d, *J* = 7.3 Hz, 2H), 7.65 – 7.57 (m, 3H), 7.53 (d, *J* = 8.2 Hz, 1H), 7.47 (t, *J* = 8.2 Hz, 2H), 7.31 (t, *J* = 7.7 Hz, 3H), 7.28 – 7.18 (m, 2H), 6.91 – 6.78 (m, 4H), 5.51 (s, 2H), 5.13 (s, 2H); ^{13}C NMR δ 192.29, 159.25, 157.81, 153.52, 144.05, 137.86, 133.02, 131.57, 130.00, 129.71, 128.47, 128.34, 124.96, 123.66, 122.74,

122.51, 121.28, 115.04, 114.57, 112.26, 112.01, 111.00, 104.61, 104.36, 104.11, 61.87, 47.16; ^{19}F NMR δ -107.46 – -107.68 (m), -113.57 (q, *J* = 8.7 Hz)

(2-(4-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)benzofuran-3-yl)(phenyl) methanone (127)

Compound **127** was obtained following the same procedure described for compound **124** using **133** and **137** as starting materials. Yield: 22.79%; oil; ^{1}H NMR δ 7.82 (d, *J* = 7.3 Hz, 2H), 7.63 (d, *J* = 8.9 Hz, 2H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.47 (t, *J* = 3.6 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 3H), 7.27 – 7.19 (m, *J* = 12.2, 5.8 Hz, 3H), 6.88 (t, *J* = 8.2 Hz, 4H), 5.45 (s, 2H), 5.14 (s, 2H), 3.79 (s, 3H); ^{13}C NMR δ 192.34, 181.40, 159.34, 157.91, 156.55, 153.56, 143.87, 137.90, 133.02, 130.03, 129.74, 129.69, 128.51, 128.36, 126.27, 124.96, 123.69, 122.49, 122.37, 121.32, 115.05, 114.61, 114.48, 111.02, 62.01, 55.29, 53.79; ES-MS *m/z*: 516 (M + H^+)

(2-(4-((1-(3-fluoro-4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)benzofuran-3-yl) (phenyl) methanone (128)

Compound **128** was obtained following the same procedure described for compound **124** using **133** and **138** as starting materials. Yield: 49.75%; M.P.: 150-151° C; ^{1}H NMR δ 7.96 (s, 1H), 7.84 (d, 2H), 7.68 (d, *J* = 8.9 Hz, 2H), 7.59 – 7.42 (m, 5H), 7.35 (t, *J* = 7.7 Hz, 3H), 7.26 (t, 1H), 7.08 (t, *J* = 8.8 Hz, 1H), 6.94 (d, *J* = 8.9 Hz, 2H), 5.27 (s, 2H), 3.95 (s, 3H); ^{13}C NMR δ 192.35, 159.22, 157.72, 153.58, 153.50, 137.92, 133.06, 130.10, 129.77, 128.51, 128.39, 125.02, 123.72, 122.68, 121.34, 120.99, 116.41, 116.37, 115.14, 114.64, 113.72, 113.68, 111.06, 109.80, 109.57, 61.89, 56.50; ^{19}F NMR δ -131.24 (dd, *J* = 15.0, 5.7 Hz)

(2-(4-((1-(3-fluorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)benzofuran-3-yl)(phenyl)methanone (129)

Compound **129** was obtained following the same procedure described for compound **124** using **133** and **139** as starting materials. Yield: 57.55%; M.P.: 151-152° C; ^{1}H NMR δ 8.03 (s, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.56 – 7.41 (m, 6H), 7.31 (t, *J* = 7.6 Hz, 3H), 7.22 (dd, *J* = 13.6, 6.1 Hz, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 2H), 5.23 (s, 2H); ^{13}C NMR δ 192.35, 164.26, 161.79, 159.19, 157.80, 153.57, 144.63, 137.90, 133.08, 131.27, 131.18, 130.10, 129.77, 128.49, 128.41, 125.04, 123.73, 122.68, 121.33, 120.93, 115.82, 114.63, 111.07, 108.43, 108.17, 61.81; ^{19}F NMR δ -109.52 (td, *J* = 8.5, 5.6 Hz); ES-MS *m/z*: 512 (M + Na)

(2-(4-((1-(3,4-difluorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)benzofuran-3-yl)(phenyl)methanone (130)

Compound **130** was obtained following the same procedure described for compound **124** using **133** and **140** as starting materials. Yield: 63.97%; M.P.: 183.6° C; ^{1}H NMR δ 7.98 (s, 1H), 7.83 (d, *J* = 7.2 Hz, 2H), 7.71 – 7.61 (m, 3H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.51 – 7.43 (m, *J* = 8.1 Hz, 3H), 7.32 (dd, *J* = 16.6, 8.7 Hz, 4H), 7.23 – 7.18 (m, *J* = 7.6 Hz, 1H), 6.92 (d, *J* = 8.9 Hz, 2H), 5.27 (s, 2H); ^{13}C NMR δ 192.34, 159.13, 157.71, 153.57, 137.92, 133.09, 130.10, 129.78, 128.48, 128.39, 125.03,

123.72, 122.79, 121.34, 118.51, 118.34, 116.54, 116.50, 116.47, 116.43, 115.19, 114.63, 111.06, 110.84, 110.63, 61.81; ^{19}F NMR δ -133.08 – -133.26 (m), -135.80 – -136.02 (m)

1-(azidomethyl)-4-fluorobenzene (134)

To a solution of 0.14g (0.72 mmol, 1eq) of 4-fluorobenzyl bromide in 3.8 mL of anhydrous DMF, 0.09g (1.44 mmol, 2eq) of NaN_3 were added and the reaction was stirred at 80° C for 4h. The reaction was quenched with water and the orgnaic compound was extracted with ether. 0.10g of neat compound **134** were obtained and it was used crude for the next reaction. Yield: 91.98%; oil.

1-(azidomethyl)-3,5-difluorobenzene (135)

A solution of 0.36g (1.74 mmol) of 3,5-difluorobenzyl bromide and 0.13g (1.91 mmol, 1.1eq) of NaN_3 in 5 mL of DMSO was stirred at r.t. for 72h. The reaction was quenched with water and the organic compound was extrated with ethyl acetate. 0.24g of neat compound **135** were obtained and it was used crude for the next reaction. Yield: 81.71%; oil; ^{1}H NMR δ 6.86 (d, *J* = 12 Hz, 2H), 6.78(t, *J* = 11.2 Hz, 1H), 4.35 (s, 2H)

1-(azidomethyl)-2,4-difluorobenzene (136

Compound **136** was obtained following the same procedure described for compound **134** using 2,4-difluorobenzyl bromide as starting material. Yield: 90.40%; oil; ^{1}H NMR δ 6.95 (t, *J* = 8.8 Hz, 1H), 6.83 – 6.75 (m, 2H), 3.89 (s, 2H); ^{19}F NMR δ -131.72 – -131.89 (m)

1-(azidomethyl)-4-methoxybenzene (137

Compound **137** was obtained following the same procedure described for compound **134** using 4-methoxybenzyl bromide as starting material. Yield: 59.64%; oil;

4-azido-2-fluoro-1-methoxybenzene (138)

A mixture of 0.11g (0.77 mmol) of 3-fluoro-4-methoxy aniline in 2 mL of water and 0.6 mL of HCl 37% was cooled to 0° C, then a solution of 0.05g (0.77 mmol) of $NaNO_2$ in 0.33 mL of water was added dropwise to the reaction. The suspension was stirred for 10 min, then 0.05g (0.93 mmol) of NaN3 were added in portions to the reaction flask that was finally stirred for 90min. The organic compound was extracted with ethyl acetate and washed with brine. 0.08g of neat compound **136** were obtained and it was used crude for the next reaction. Yield: 63.03%; oil; ^{1}H NMR δ 7.28 – 7.18 (m, 1H), 6.84 – 6.72 (m, 2H), 4.27 (s, 3H); ^{19}F NMR δ 109.26 (dt, *J* = 15.9, 8.1 Hz), -113.64 (q, *J* = 8.8 Hz)

1-azido-3-fluorobenzene (139)

Compound **139** was obtained following the same procedure described for compound **138** using 3-fluoroaniline as starting material. Yield: 76.13%; oil; ^{1}H NMR δ 7.33-7.26 (m, 1H), 6.88-6.72 (m, 3H)

4-azido-1,2-difluorobenzene (140)

Compound **140** was obtained following the same procedure described for compound **138** using 3,4-difluoroaniline as starting material. Yield: 67.29%; oil;

PROJECT 3

Ethyl-3-methyl-2,4-dioxo-4-(thiophen-2-yl)butanoate (134)

Under a nitrogen atmosphere, 0.86g (37.50 mmol) of sodium were added in small portions to 25mL of dry ethanol and stirred at room temperature until all the sodium was dissolved. 7.6 mL (56.30 mmol) of diethyl oxalate were then added, followed by dropwise addition of a solution of 2.63 g (18.65 mmol) in dry ethanol (26 mL) commercially available of 1-(thiophen-2-yl) propan-1-one 2. The mixture was stirred at room temperature for 18 h, than slowly poured into a mixture of ice and aqueous 1 N HCl. The resulting mixture was extracted with Et_2O, the organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography: hexane/EtOAc 8:2, to give compound 3.8g **134** as a white solid. Yield: 85% Rf 0.57 (Hexane/EtOAc 8:2); ^{1}H NMR δ: 7.51 (d, 1H, J = 8.4 Hz), 7.37 (d, 1H, J = 8.4 Hz), 7.37 (t, 1H, J = 8.4 Hz), 4.70 (q, 1H, J = 7.0 Hz), 4.25 (q, 2H, J = 7.1 Hz), 1.45 (d, 3H, J = 7.0 Hz), 1.24 (t, 3H, J = 7.1 Hz); ^{13}C NMR δ: 190.1, 187.5, 160.4, 142.4, 135.3, 133.5, 128.6, 63.1, 52.6, 14.0, 13.4; MS (ESI): 241.0 (M + H^+), 263.0 (M+Na)

ethyl 1-(2,4-dichlorophenyl)-4-methyl-5-(thiophen-2-yl)-1H-pyrazole-3-carboxylate(135)

4.63g (20.51 mmol) of the α,γ-diketoester **134** were dissolved in 36mL of absolute EtOH; 4.38g (20.51 mmol) of 2,4-dichlorophenylhydrazine hydro-chloride were added in one portion, then the mixture was refluxed overnight. The solvent was removed under reduced pressured and the crude product was purified by flash chromatography: hexane/EtOAc 8:2. A final recrystallization (Hexane/EtOAc 7:3) gave 2.01g of compound **135** as a white solid. Yield: 32%. Rf 0.30 (Hexane/EtOAc 8:2); ^{1}H NMR δ: 7.46 (d, 1H, J = 2.2 Hz), 7.40 (d, 1H, J = 8.5 Hz), 7.38 (dd, 1H, J = 1.2, 5.1 Hz), 7.33 (dd, 1H, J = 2.2, 8.5 Hz), 7.02 (dd, 1H, J = 3.6, 5.1 Hz), 6.92 (dd, 1H, J = 1.2, 3.6 Hz), 4.47 (q, 2H, J = 7.1 Hz), 2.46 (s, 3H), 1.45 (t, 3H, J = 7.1 Hz); ^{13}C NMR δ: 162.7, 142.9, 137.8, 136.3, 136.0, 133.9, 131.0, 130.0, 128.9, 128.6, 127.8, 127.7, 127.2, 120.0, 61.0, 14.5, 9.9; MS (ESI): 381.0 (M + H^+)

ethyl 5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylate (136)

2.21g (1.31 mmol) of compound **135** were dissolved in 4.5mL of acetonitrile and the solution was cooled to 0° C. 0.39g (2.23 mmol) of NBS were added in small portions, than the mixture was stirred overnight at r.t. A saturated solution of $Na_2S_2O_3$ (5 mL) was added and the solvent was removed under reduced pressure. The resulting mixture was extracted with EtOAc, the organic layers were washed with water, brine, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography: hexane/EtOAc 8:2 to give 0.51g of compound 5 as a pale yellow solid. Yield: 83%; Rf 0.38 (Hexane/EtOAc 8:2); ^{1}H NMR δ: 7.45 (dd,

1H, J = 0.4, 2.0 Hz), 7.35 (d, 1H, J = 0.4 Hz), 7.33 (d, 1H, J = 2.0 Hz), 6.94 (d, 1H, J = 3.9 Hz), 6.63 (d, 1H, J = 3.9 Hz), 4.43 (q, 2H, J = 7.1 Hz), 2.41 (s, 3H), 1.40 (t, 3H, J = 7.1 Hz); ^{13}C NMR δ: 162.6, 142.8, 137.0, 136.7, 135.8, 134.1, 133.9, 131.0, 130.3, 130.3, 129.4, 128.0, 120.5, 115.1, 61.2, 14.6, 10.1; MS (ESI): 458.9 ($M + H^+$)

5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carbaldehyde (137)

Ester **5** (0.2 g, 0.43 mmol) was dissolved in anhydrous dichloromethane (2 mL), and the mixture was cooled to –78°C. DIBAL-H (0.5 mL) was added drop wise over 45 min and the mixture was stirred for 4h at –78°C. MeOH (0.5 mL) was added and the solvent was removed under reduced pressure. The resulting mixture was extracted with EtOAc, the organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/ EtOAc 5:5) to give aldehyde **6** (0.45 g, 55%) as a white solid. R*f* 0.50 (Hexane/EtOAc 5:5); ^{1}H NMR δ: 10.0 (s, 1H), 7.45 (d, 1H, *J* = 2.0), 7.31 (d, 1H, *J* = 2.0), 7.28-7.30 (m, 1H), 6,90 (d, 1H, *J* =3.9), , 6.58 (d, 1H, *J* = 3.5), 2.36 (s, 1H); MS (ESI): 414 ($M + H^+$)

5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-3-ethynyl-4-methyl-1H-pyrazole (138)

To an ice cold solution of 0.45g (1.09 mmol) of aldehyde **137** in 0.5 mL of MeO, 0.11g (0.86 mmol) of K_2CO_3 and 0.09g (0.52 mmol) of dimethyl 1-diazo-2-oxopropylphosphonate were added. After 5 minutes the ice bath was removed and the reaction was allowed to warm to r.t. and stirred for additional 12 hours. Rochelle salt and 2 mL of Et_2O were added. The organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography: hexane/ EtOAc 7:3 to give 0.15 g of the alkyne **138** as a withe solid. Yield: 55%; R*f* 0.82 (Hexane/EtOAc 7:3); ^{1}H NMR δ 7.45 (d, 1H, *J* = 2.0Hz), 7.35 (d, 1H, *J* = 8.4 Hz), 7.32 (dd, 1H, *J* = 2.0, 8.4 Hz), 6.94 (d, 1H, *J* = 3.9 Hz), 6.63 (d, 1H, *J* = 3.9 Hz), 3.21 (s, 1H), 2.18(s, 3H); ^{13}C NMR δ: 136.4, 135.3, 135.8, 133.7, 130.9, 130.7, 130.2, 130.1, 128.4, 127.9, 120.1, 114.5, 81.4, 75.0, 9.4; MS (ESI): 410 ($M + H^+$)

5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-3-(iodoethynyl)-4-methyl-1H-pyrazole (140)

To a solution of 0.59g (1.432 mmol, 1 eq) of alkyne **138** in 4 mL THF, 0.02g (0.072 mmol, 0.05 eq) of CuI and 0.34g (1.575 mmol, 1.1 eq) of 4-iodomorpholine (**139**)were added. The reaction mixture was stirred at room temperature for 1 hour. The mixture was filtered on a neutral alumina pad and the solvent was evaporated under reduced pressure. 0.57g of the iodinated derivative **140** was obtained as a yellow oil and was used without further purification. Yield: 74%; R*f* 0.84 (Hexane/EtOAc 7:3); ^{1}H NMR δ 7.38 (dd, 1H, *J* = 1.7, 0.8 Hz), 7.23-7.25 (m, 1H), 6.88 (s, 1H), 6.83 (d, 1H, *J* = 3.9 Hz), 6.51 (d, 1H, *J* = 3.9 Hz), 2.16 (s, 3H); MS (ESI): 536 ($M + H^+$)

4-(5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1-(4-fluorobutyl)- 5-iodo- 1H-1,2,3-triazole (131)

To a solution of 0.20g (0.37 mmol) propargyl iodide **140** in 5 mL THF, 0.40g (0.37 mmol) of 1-azido-4-fluorobutane (**141 a**), 0.04g(0.19 mmol) of CuI and 10 mL (0.74 mmol) of TEA were added. The reaction was stirred at r.t. for 72 hours, then quenched with 10 mL of a 10% aqueous solution of NH_4OH and the solvent was removed under reduced pressure. The crude product was diluted with Et_2O, washed with water, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/ EtOAc 4:1) to give 0.10g of the triazole **131** as a white solid. Yield: 42%; R*f* 0.52 (Hexane/EtOAc 4:1); ^{1}H NMR δ 7.50 (d, 1H, *J* = 1.9 Hz), 7.30-7.38 (m, 2H), 6.96 (d, 1H, *J* = 3.9 Hz), 6.66 (d, 1H, *J* = 3.9 Hz), 4.55 (m, 3H), 4.44 (t, 1H, *J* = 5.8 Hz), 2.41 (s, 3H), 2.05-2.19 (m, 2H), 1.79-1.87 (m, 1H), 1.70-1.79 (m, 1H); ^{13}C NMR δ: 145.1, 143.1, 136.0, 133.9, 131.2, 130.28, 130.31, 128.7, 127.9, 116.9, 114.4, 84.0, 82.3, 50.3, 27.4, 27.2, 26.0, 10.1; ^{19}F NMR: -219.3 (s, 1F); MS (ESI): 656 (M + H^+)

4-(5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1-(4-fluorobenzyl)-5-iodo-1H-1,2,3-triazole (132)

Compound **132** was obtained following the same procedure described for compound **131** using **141 b** as starting material. Yield: 46.86%; ^{1}H NMR δ 7.53 – 7.49 (m, 1H), 7.39 – 7.31 (m, 4H), 7.08 – 7.02 (m, 2H), 6.98 (d, *J* = 3.9 Hz, 1H), 6.68 (d, *J* = 3.9 Hz, 1H), 5.67 (s, 2H), 2.44 (s, 3H); ^{13}C NMR δ: 163.94, 161.48, 145.55, 143.02, 136.13, 136.00, 133.85, 131.17, 130.85, 130.26, 130.13, 129.91, 129.83, 128.68, 127.86, 116.87, 115.98, 115.76, 114.42, 53.63, 10.12; ^{19}F NMR δ: -113.05 (s); MS (ESI): 690 (M + H^+)

4-(5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1-(but-3-en-1-yl)-5-iodo-1H-1,2,3-triazole (133)

Compound **133** was obtained following the same procedure described for compound **131** using **141 c** as starting material. Yield: 50.70%; ^{1}H NMR δ 7.43 (d, *J* = 2.1 Hz, 1H), 7.32 – 7.23 (m, 2H), 6.88 (d, *J* = 3.9 Hz, 1H), 6.58 (d, *J* = 3.9 Hz, 1H), 5.75 (dd, *J* = 17.1, 10.2 Hz, 1H), 5.11 – 4.98 (m, 2H), 4.50 – 4.38 (m, 2H), 2.65 (dd, *J* = 14.6, 7.0 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR δ: 144.94, 143.20, 136.19, 135.96, 135.90, 133.87, 132.91, 131.26, 130.90, 130.27, 130.12, 128.71, 128.64, 127.85, 118.45, 116.88, 114.37, 50.16, 34.09, 10.10; MS (ESI): 636 (M + H^+)

4-(5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1-(but-3-en-1-yl)-1H-1,2,3-triazole (134)

Compound **134** was obtained following the same procedure described for compound **131** using **141 c** as starting material. Yield: 52.82%; ^{1}H NMR δ 7.86 (s, 1H), 7.41 (d, *J* = 2.0 Hz, 1H), 7.32 – 7.24 (m, 2H), 6.88 (d, *J* = 3.9 Hz, 1H), 6.59 (d, *J* = 3.9 Hz, 1H), 5.78 – 5.63 (m, 1H), 5.07 – 4.99 (m, 2H), 4.41 (t, *J* = 7.1 Hz, 2H), 2.63 (q, *J* = 7.0 Hz, 2H), 2.46 (s, 3H); ^{13}C NMR δ: 144.01, 142.08, 136.33,

136.12, 133.96, 133.08, 131.11, 131.05, 130.23, 130.10, 128.73, 128.69, 127.93, 121.22, 118.54, 115.73, 114.35, 49.74, 34.36, 10.11; MS (ESI): 508 ($M + H^+$)

4-iodomorpholine (139)

To a solution of 4g (31.5 mmol) of iodine in 63 mL of MeOH, 2.75 mL (31.5 mmol) morpholine were added dropwise and the mixture was stirred for 1 hour. The precipitate formed was collected by filtration, dried under vacuum and used crude, without further purification. R*f* 0.32 (DCM/MeOH 95:5); ^{1}H NMR δ 3.69-3.77 (m, 2H), 2.89-3.00 (m, 2H).

1-azido-4-fluorobutane (141 a)

To a stirred solution of 0.50g (2.61 mmol) of 1-bromo-4-fluorobutane in 40mL of water/acetone (1:4), 0.25g (3.96 mmol) of NaN_3 were added. The resulting suspension was stirred at r.t. for 24h. The mixture was extracted with DCM, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. 0.30g of **141 a** were obtained as a yellow oil and was used without further purification. Yield: 76% ^{1}H NMR δ 4.22 (s, 2H), 6.95-7.01 (m, 2H), 7.19-7.23 (m, 2H)

1-(azidomethyl)-4-fluorobenzene (141 b)

Compound **141 b** was obtained following the same procedure described for compound **141 a** using 4-fluoro benzylbromide as starting material.**141 b** was used without further purification.

4-azidobut-1-ene (141 c)

To a solution of 0.10g (0.741 mmol, 1eq) of 4-bromo-1-butene dissolved in 0.5 mL of DMF, 0.07g (1.111 mmol, 1.5eq) of NaN_3 were added. The resulting suspension was stirred at r.t. for 24h. The reaction was quenched with water, then the organic compound was extracted with Et_2O. **141 c** was used without any further purification and the solvent was not removed; the solution was directly added to next reactions.

PROJECT 4

(E)-5-(3-bromophenyl)pent-4-enoic acid (145)

20.03g (47.19 mmol, 1.1eq) of (4-carboxybutyl) triphenylphosponium bromide were dissolved in 20 mL of dry DMSO. 10.11g (90.1 mmol, 2.1eq) of tBuOK were added to the solution and it was stirred for 30min. Only when all was dissolved, 7.94 mmol (42.9 mmol, 1eq) of 3-bromobenzaldehyde were added then the solution was stirred overnight at r.t. The reaction was quenched with water and extracted with DMC. The aqueous phase was acidified with conc. HCl and extracted with DCM. The organic layer was washed with water, dried and concentrated. **145** was purified via flash chromatography hexane/ethyl acetate 9:1. 4.95g of neat compound **145** were obtained. Compound was obtained in a mixture of E/Z (72% of E and 28% Z); Yield: 45.43% Compare with spectra previously reported in literature.

5-(3-bromophenyl)pentanoic acid (146)

To a solution of 1.20g (4.725 mmol) of **145** dissolved in 20 mL of ethyl acetate, 0.10g (0.945 mmol) were added. The suspension was stirred for 24h at r.t. then it was filtered and concentrated. 1.20g of neat compound **146** were obtained. Yield: 98%; ^{1}H NMR δ: 7.46 (m, 2H), 7.18 – 7.05 (m, 2H), 2.57 (dd, *J* = 22.0, 14.9 Hz, 2H), 2.36 (t, *J* = 6.9 Hz, 2H), 1.66 (dt, *J* = 7.3, 3.6 Hz, 4H)

2-bromo-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (147)

A solution of 6.49g (25.46 mmol, 1eq) of **146** dissolved in 36mL of anhydrous DCM was cooled at 0° C then, 2.18mL (25.46 mmol) of $COCl_2$ in 0.5mL of DMF were added. The reaction was stirred at r.t. for 2h, then 15,65g (25.46 mmol) of $AlCl_3$ in 5mL of DCM were added to the solution. The reaction was stirred overnight then it was extracted with DCM. The compound was purified via flash chromatography: hexane/ ethyl acetate 9:1. 3.65g of neat compound **147** were obtained. Yield: 60.24%; ^{1}H NMR δ: 7.59 (d, *J* = 8.3 Hz, 1H), 7.44 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.38 (d, *J* = 1.9 Hz, 1H), 2.94 – 2.83 (m, 2H), 2.72 (dd, *J* = 6.9, 5.1 Hz, 2H), 1.93 – 1.75 (m, 4H)

ethyl 2-(2-bromo-5-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulen-6-yl)-2-oxoacetate (148)

0.69g (30.20 mmol, 2eq) of Na were dissolved in 70 mL of EtOH under nitrogen atmosphere then 2.20g (15.126 mmol, 1 eq) of diethyloxalate were added, subsequently 3.60g (15.126, 1 eq) of **147** were added dropwise. The reaction was stirred overnight and then was quenched with HCl 1N. The organic compound was extracted with DCM and the solvent was removed under reduced pressure. 4.66g of compound **148** were obtained and it was used crude for next reaction. Yield: 91.19%

ethyl 8-bromo-1-(2,4-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxylate (149)

4.66g (13.786 mmol, 1eq) of **148** were dissolved in 86mL of ethanol and 2.94g (13.786 mmol, 1 eq) of 2,4-dichloroaniline hydrochloride salt were added then, the solution was stirred at 80° C for 4h then the solvent was removed. The organic compound was purified via flash chromatography: hexane/ethyl acetate 4:1. 4.92g of neat compound **149** were obtained. Yield: 74.77%; ^{1}H NMR δ: 7.53 (d, *J* = 8.1 Hz, 1H), 7.47 (d, *J* = 1.9 Hz, 1H), 7.40 (dd, *J* = 10.6, 2.3 Hz, 2H), 7.17 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.54 (d, *J* = 8.2 Hz, 1H), 4.45 (q, *J* = 7.1 Hz, 2H), 3.22 (s, 2H), 2.66 (t, *J* = 6.5 Hz, 2H), 2.26 (s, 2H), 1.43 (t, *J* = 7.1 Hz, 3H)

(8-bromo-1-(2,4-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazol-3-yl)methanol (150)

To a solution of 0.41g (10.816 mmol, 1.1eq) of $LiAlH_4$ in 20mL of anhydrous THF, 4.90g (9.833 mmol, 1eq) of **149** in 30mL of THF were added dropwise at 0° C. The reaction was warmed gradually to r.t.and it was stirred until 149 disappeared. The solution was allowed to 0° C again and the reaction was quenched with H_2O and NaOH, then it was stirred for 30 min. The organic compound was extracted with ethyl acetate and was purified via flash chromatography: hexane/ethyl acetate 7:3. 3.44g of neat compound **150** were obtained. Yield: 80.00%; ^{1}H NMR δ: 7.46 – 7.40 (m, 3H),

7.39 – 7.34 (m, 1H), 7.15 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.53 (d, *J* = 8.3 Hz, 1H), 4.79 (s, 2H), 2.76 – 2.63 (m, 2H), 2.22 (t, *J* = 19.9 Hz, 2H), 2.00 (s, 2H).

8-bromo-1-(2,4-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carbaldehyde (151)

3.44g (7.854 mmol) of **150** were dissolved in 45 mL of DCM and the solution was allow to 0° C; then 3.99g (9.425 mmol, 1.2eq) of DMP were added. The solution was warmed to r.t and stirred for 4h; then 155mL of saturated solution of N_2HCO_3 and 8.95g (56.54 mmol, 7.2 eq) of $Na_2S_2O_3$ were added. The organic compound was extracted with Et_2O. 3.10g of neat compound **151** were obtained and it was used crude for the next step of reaction. Yield: 90.52%; ^{1}H NMR δ 10.13 (s, 1H), 7.53 – 7.40 (m, 4H), 7.18 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.55 (t, *J* = 6.0 Hz, 1H), 3.23 (d, *J* = 32.9 Hz, 2H), 2.68 (dd, *J* = 12.8, 6.4 Hz, 2H), 2.29 (d, *J* = 32.1 Hz, 2H).

8-bromo-1-(2,4-dichlorophenyl)-3-ethynyl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole (144)

To a solution of 3.74g (8.578 mmol, 1eq) of **151** dissolved in 50mL of MeOH**,** 3.90g (28.307 mmol, 3.3 eq) of K_2CO_3 and 4.36g (22.695 mmol, 2.6eq) of Bestmann-Ohira reagent were added and the solution was stirred overnight at r.t.; then it was quenched with 30mL of aqueous $NaHCO_3$ 5% and it was extracted with Et_2O. The organic compound was purified via flash chromatography: Et_2O/Hexane 1:9. 1.2g of neat compound **144** were obtained. Yield: 32.43%. ^{1}H NMR δ; 7.50 – 7.35 (m, 4H), 7.16 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.52 (d, *J* = 8.3 Hz, 1H), 3.27 (s 1H), 2.74 – 2.63 (m, 2H), 2.58 (s, 2H), 2.24 (s, 2H).

1-azidopentane (156 a)

To a solution of 0.21g (1.400 mmol, 1eq) of 1-bromopentane in 5.4mL of H_2O/acetone 1:4, 0.13g (2.100 mmol, 1.5eq) of NaN_3 were added and the suspension was stirred at r.t. for 24h. Then, the organic compound was extracted with DCM, the solvent was removed and compound **156 a** was used crude for the next reaction.

(azidomethyl)cyclohexane (156 b)

To a solution of 0.09g (1.489 mmol, 1.1eq) of NaN_3 in 10mL of DMSO, 0.24g (1.354mmol, 1eq) of (Bromomethyl)cyclohexane were added and the solution was stirred at 80° C overnight. Then, the cool reaction was quenched with water and extracted with ethyl acetate. Compound **156 b** was used crude for the next step of reaction.

(azidomethyl)benzene (156 c)

To a solution of 0.07g (0.0463 mmol) of benzylbromide in 2mL of DMF, 0.06g (0.926 mmol) of NaN_3 were added and the solution was stirred for 3h at 65 C. The reaction was quenched with water and the organic compound was extracted with ether. Compound **156 c** was used crude for the next reaction.

8-bromo-1- (2,4-dichlorophenyl)- 3- (1-pentyl-1H-1,2,3-triazol-4-yl)- 1, 4, 5, 6- tetrahydrobenzo [6,7] cyclohepta [1,2-c]pyrazole (157 a)

To a solution of 0.40g (0.926mmol, 1eq) of **144** dissolved in 11mL of tBuOH and 5mL of H_2O, 0.11g (0.926mmol) of **156 a**, 0.005g (0.037mmol, 0.04eq) of $CuSO_4$ and 0.04g (0.185mmol, 0.2eq) of sodium ascorbate were added. The reaction was stirred at r.t. overnight then it was quenched with a saturated solution of NH_4Cl and it was extracted with ethyl acetate. The organic compound was purified via flash chromatography: hexane/ethyl acetate 7:3. 0.48g of neat compound **157 a** were obtained. Yield: 96%; ^{1}H NMR δ: 7.96 (s, 1H), 7.52 – 7.36 (m, 4H), 7.17 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.57 (d, *J* = 8.3 Hz, 1H), 4.41 (t, *J* = 7.2 Hz, 2H), 3.27 (s, 2H), 2.72 (d, *J* = 6.2 Hz, 2H), 2.31 (s, 2H), 2.00 – 1.91 (m, 2H), 1.42 – 1.30 (m, 4H), 0.90 (t, *J* = 7.0 Hz, 3H).

8-bromo-3- (1- (cyclohexylmethyl)- 1H-1,2,3-triazol-4-yl)-1- (2,4-dichlorophenyl)- 1, 4, 5, 6-tetrahydrobenzo [6,7]cyclohepta[1,2-c]pyrazole (157 b)

Compound **157 b** was obtained following the same procedure described for compound **157 a** using **144** and **156 b** as starting materials. Yield: 50.60%;^{1}H NMR δ: 7.94 (s, 1H), 7.54 – 7.34 (m, 4H), 7.17 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.57 (d, *J* = 8.2 Hz, 1H), 4.24 (d, *J* = 7.1 Hz, 2H), 3.09 (m, 2H), 2.71 (m, 2H), 2.31 (s, 2H), 1.93 (m, 1H), 1.80 – 1.55 (m, 6H), 1.31 – 1.08 (m, 2H), 1.00 (m, 2H).

3- (1-benzyl-1H-1,2,3-triazol-4-yl)- 8-bromo-1- (2,4-dichlorophenyl)- 1, 4, 5, 6- tetrahydrobenzo [6,7]cyclohepta[1,2-c]pyrazole (157 c)

Compound **157 c** was obtained following the same procedure described for compound **157 a** using **144** and **156 c** as starting materials. Yield: 48.62%; ^{1}H NMR δ 7.80 (s, 1H), 7.43 – 7.22 (m, 9H), 7.09 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.48 (d, *J* = 8.3 Hz, 1H), 5.51 (s, 2H), 3.21 (m, 2H), 2.63 (m, 2H), 2.23 (s, 2H

8-bromo- 1- (2,4-dichlorophenyl)- 3- (1-methyl-1H-1,2,3-triazol-4-yl)- 1, 4, 5, 6-tetrahydrobenzo [6,7]cyclohepta[1,2-c]pyrazole (157 d)

Compound **157 d** was obtained following the same procedure described for compound **157 a** using **144** and CH_3I as starting materials. Yield: 30.29%; ^{1}H NMR δ 7.88 (s, 1H), 7.48 – 7.27 (m, 4H), 7.10 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.56 – 6.46 (m, 1H), 4.08 (s, 3H), 2.65 (t, *J* = 6.3 Hz, 2H), 2.23 (m, 2H), 1.64 (d, *J* = 4.3 Hz, 2H).

3-(1-(2,4-dichlorophenyl)-3-(1-pentyl-1H-1,2,3-triazol-4-yl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazol-8-yl)prop-2-yn-1-ol (158 a)

To 0.08g (0.146mmol, 1eq) of **157 a**, 0.003g (0.00438mmol, 3%eq) of $PdCl_2(PPh_3)_2$, 0.12mL (0.438 mmol, 3eq) of TBAF and 0.01g (0.1606mmol, 1.1eq) of propargyl alcohol were added. The reaction was stirred under nitrogen atmosphere at 80° C overnight then, it was quenched with water and extracted with ethyl acetate. Compound 158a was purified via flash chromatography: hexane/ethyl acetate 7:3. 0.03g of neat compound were obtained. Yield: 39.63%; ^{1}H NMR δ: 8.01 (d, *J* = 5.8 Hz,

1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.46 – 7.37 (m, 3H), 7.12 (dd, *J* = 8.0, 1.7 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 4.52 (d, *J* = 7.5 Hz, 2H), 4.43 (t, *J* = 7.2 Hz, 2H), 2.76 (t, *J* = 6.3 Hz, 2H), 2.32 (s, 2H), 2.01 – 1.92 (m, 2H), 1.43 – 1.34 (m, 4H), 1.31 – 1.23 (m, 2H), 0.92 (t, *J* = 7.0 Hz, 3H)

3-(3-(1-(cyclohexylmethyl)-1H-1,2,3-triazol-4-yl)-1-(2,4-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazol-8-yl)prop-2-yn-1-ol (158 b)

Compound **158 b** was obtained following the same procedure described for compound **158 a** using **157 b** as starting material. Yield: 29.49%;^{1}H NMR δ: 7.87 (s, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.37 – 7.27 (m, 3H), 7.02 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.57 (d, *J* = 8.0 Hz, 1H), 4.40 (s, 2H), 4.16 (d, *J* = 6.2 Hz, 2H), 2.66 (t, *J* = 6.2 Hz, 2H), 2.23 (s, 2H), 1.93 – 1.78 (m, 2H), 1.72 – 1.53 (m, *J* = 23.7, 11.7 Hz, 6H), 1.17 – 1.06 (m, 3H), 1.01 – 0.89 (m, 2H).

3-(3-(1-benzyl-1H-1,2,3-triazol-4-yl)-1-(2,4-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazol-8-yl)prop-2-yn-1-ol (158 c)

Compound **158 c** was obtained following the same procedure described for compound **158 a** using **157 c** as starting material. Yield: 58.00%; ^{1}H NMR δ 7.81 (s, 1H), 7.33 – 7.23 (m, 9H), 7.02 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.56 (d, *J* = 8.0 Hz, 1H), 5.51 (s, 2H), 4.41 (d, *J* = 5.9 Hz, 2H), 3.21 (m, 2H), 2.63 (m, 2H), 2.23 (s, 2H)

3-(1-(2,4-dichlorophenyl)-3-(1-methyl-1H-1,2,3-triazol-4-yl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazol-8-yl)prop-2-yn-1-ol (158 d)

Compound **158 d** was obtained following the same procedure described for compound **158 a** using **157 d** as starting material. Yield: 37.65%; ^{1}H NMR δ 7.88 (s, 1H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.36 – 7.26 (m, 3H), 7.03 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.58 (d, *J* = 8.0 Hz, 1H), 4.41 (d, *J* = 5.6 Hz, 2H), 4.09 (s, 3H), 2.66 (t, *J* = 6.3 Hz, 2H), 2.21 (s, 2H), 1.66 (t, *J* = 5.9 Hz, 2H).

3-(3-(1-pentyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazol-8-yl)propan-1-ol (159 a)

To a solution of 0.03g (0.058 mmol) of **158 a** dissolved in 3mL of dry ethanol, a catalytic amount of Pd/C (10%) was added then, the solution was stirred under nitrogen atmosphere for 3 days. The reaction was filtered through a celite pad. The organic compound was purified via flash chromatography: hexane/ethyl acetate 7:3. 0.02g of neat compound **159 a** were obtained. Yield: 75.78%; ^{1}H NMR δ 8.01 (s, 1H), 7.38 (m, 4H), 7.34 (m, 1H), 7.20 (d, *J* = 1.5 Hz, 1H), 6.90 (dd, *J* = 7.9, 1.8 Hz, 1H), 6.74 (d, *J* = 7.9 Hz, 1H), 4.44 (t, *J* = 7.2 Hz, 2H), 3.72 (t, *J* = 6.4 Hz, 2H), 3.03 (t, *J* = 7.3 Hz, 2H), 2.79 (t, *J* = 6.6 Hz, 2H), 2.75 – 2.62 (m, 2H), 2.40 – 2.27 (m, 2H), 2.02 – 1.87 (m, 4H), 1.42 – 1.34 (m, *J* = 8.5, 4.7 Hz, 2H), 1.32 – 1.23 (m, 2H), 0.99 – 0.80 (m, 3H); ^{13}C NMR δ 149.74, 142.81, 142.10, 141.68, 140.64, 140.05, 129.67, 128.97, 128.69, 127.82, 127.30, 125.89, 124.94, 120.84, 119.73, 62.36, 50.33, 33.97, 32.74, 31.88, 31.59, 29.99, 28.60, 22.12, 20.58, 13.84; MS (ESI): 456 (M + H^{+}), 478 (M + Na)

3-(3-(1-(cyclohexylmethyl)-1H-1,2,3-triazol-4-yl)-1-phenyl-1,4,5,6-tetrahydrobenzo[6,7] cyclohepta[1,2-c]pyrazol-8-yl)propan-1-ol (159 b)

Compound **159 b** was obtained following the same procedure described for compound **159 a** using **158 b** as starting material. Yield: 97.15%;^{1}H NMR δ: 8.00 (s, 1H), 7.40- 7.36 (m, 4H), 7.34 (m, 1H), 7.19 (d, *J* = 1.5 Hz, 1H), 6.88 (dd, *J* = 7.9, 1.8 Hz, 1H), 6.73 (d, *J* = 7.9 Hz, 1H), 4.26 (dd, *J* = 7.1, 3.5 Hz, 2H), 3.77 – 3.62 (m, 2H), 3.01 (dd, *J* = 14.3, 7.2 Hz, 1H), 2.83 – 2.62 (m, 4H), 2.31 (dd, *J* = 8.0, 5.0 Hz, 2H), 1.99 – 1.87 (m, 2H), 1.72 (dd, *J* = 22.4, 11.3 Hz, 6H), 1.21 (t, *J* = 14.0 Hz, 4H), 1.11 – 0.97 (m, 2H);); ^{13}C NMR δ 149.74, 142.80, 142.08, 141.67, 140.62, 140.01, 129.65, 128.96, 128.69, 127.83, 127.31, 125.87, 124.91, 120.82, 119.73, 62.27, 56.51, 53.81, 38.77, 33.94, 32.79, 31.83, 30.51, 29.27, 26.08, 25.53; MS (ESI): 482 (M + H^{+}).

3-(1-phenyl-3-(1H-1,2,3-triazol-4-yl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazol-8-yl)propan-1-ol (159 c)

Compound **159 c** was obtained following the same procedure described for compound **159 a** using **158 c** as starting material. Yield: 76.18%;^{1}H NMR δ: 8.05 (s, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.38 – 7.24 (m, 3H), 7.11 (d, *J* = 7.0 Hz, 1H), 7.06 (d, *J* = 11.3 Hz, 1H), 6.80 (dd, *J* = 7.9, 1.7 Hz, 1H), 6.57 (dd, *J* = 7.7, 3.9 Hz, 1H), 3.68 – 3.51 (m, 2H), 2.70 – 2.54 (m, 4H), 2.25 – 2.17 (m, 2H), 1.88 – 1.75 (m, 2H), 1.62 – 1.48 (m, 2H); ^{13}C NMR δ 142.87, 142.21, 141.79, 136.62, 135.39, 132.77, 130.60, 130.36, 129.88, 127.88, 127.08, 126.93, 126.14, 122.70, 118.70, 62.27, 33.85, 32.71, 31.68, 31.49, 21.08; MS (ESI): 386 (M + H^{+})

3-(3-(1-methyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazol-8-yl)propan-1-ol (159 d)

Compound **159 d** was obtained following the same procedure described for compound **159 a** using **158 d** as starting material. Yield: 69.76%;^{1}H NMR δ: 8.01 (s, 1H), 7.44 – 7.31 (m, 5H), 7.23 – 7.17 (m, 1H), 6.94 – 6.87 (m, 1H), 6.74 (d, *J* = 7.9 Hz, 1H), 4.21 – 4.15 (m, 3H), 3.75 – 3.66 (m, 2H), 3.00 (t, *J* = 7.4 Hz, 2H), 2.79 (t, *J* = 6.6 Hz, 2H), 2.76 – 2.67 (m, 2H), 2.36 – 2.27 (m, 2H), 1.94 (dt, *J* = 15.7, 6.4 Hz, 2H); ^{13}C NMR δ 142.87, 142.21, 141.79, 136.62, 135.39, 132.77, 130.60, 130.36, 129.22, 128.95, 127.85, 125.76, 124.90, 122.66, 118.70, 62.37, 36.83, 33.75, 31.86, 30.73, 25.41, 20.71; MS (ESI): 400 (M + H^{+}), 422 (M + Na)

PROJECT 5

tert-butyl (1-oxo-5- (3- ((2,2,4,6,7- pentamethyl- 2, 3- dihydrobenzofuran- 5-yl) sulfonyl) guanidino)-1-((pyridin-3-ylmethyl)amino)pentan-2-yl)carbamate (161)

To a solution of 0.50g (0.949 mmol, 1eq) of the protected aminoacid Boh-Arg(pbf)-OH dissolved in 10mL of DCM, 0.505g (1.328 mmol, 1.4eq) of coupling agent HATU were added followed by 0.28g (2.752 mmol, 2.9eq) of TEA and, finally, by 0.12 (1.140 mmol, 1.2eq) of pyridin-3-ylmethanamine. The resulting lightly yellow mixture was stirred at r.t. for 6h then, the solvent was removed. The reaction was worked up by washing with HCl 1N and a saturated solution of sodium bicarbonate

and extracted with ethyl acetate. The crude was purified via flash chromatography: DCM/Methanol 9.5:0.5; 0.38g of neat compound **167** were obtained. Yield: 82.12%; ^{1}H NMR δ 8.46 (s, 1H), 8.41 (dd, *J* = 4.8, 1.5 Hz, 1H), 7.95 (s, 1H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.17 (dd, *J* = 7.7, 4.9 Hz, 1H), 6.41 (s, 2H), 5.83 (d, *J* = 7.8 Hz, 1H), 4.37 (qd, *J* = 15.3, 6.0 Hz, 2H), 4.21 (s, 1H), 3.22 (d, *J* = 36.0 Hz, 2H), 2.94 (s, 2H), 2.54 (s, 3H), 2.46 (s, 3H), 2.17 (s, 2H), 2.09 (m, 3H), 1.79 (d, *J* = 7.1 Hz, 1H), 1.58 (dt, *J* = 19.0, 10.5 Hz, 2H), 1.45 (s, 6H), 1.37 (s, 9H).

tert-butyl (1-oxo-5- (3- ((2,2,4,6,7- pentamethyl- 2,3- dihydrobenzofuran- 5-yl) sulfonyl) guanidino)-1- ((1- (pyridin-3-yl) ethyl) amino) pentan-2-yl) carbamate (163)

Compound **163** was obtained following the same procedure described for compound **161** using 1-(pyridin-3-yl)ethan-1-amine as starting material. Yield: 68.58%; ^{1}H NMR δ 8.51 (s, 1H), 8.41 – 8.36 (m, 1H), 7.97 – 7.77 (m, 1H), 7.62 (s, 1H), 7.16 (ddd, *J* = 12.5, 7.8, 4.9 Hz, 1H), 6.42-6.39 (m, 3H), 6.01 – 5.75 (m, 1H), 5.05 – 4.91 (m, 1H), 4.28 – 4.01 (m, 1H), 3.09 (d, *J* = 55.2 Hz, 2H), 2.95 (d, *J* = 20.1 Hz, 2H), 2.57 (s, 3H), 2.47 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H), 1.74 (dd, *J* = 13.2, 5.6 Hz, 1H), 1.63-1.58 (m, 2H), 1.43 (s, 6H), 1.38 – 1.30 (m, 9H)

tert-butyl (1-(methyl(pyridin-3-ylmethyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)carbamate (165)

Compound **165** was obtained following the same procedure described for compound **161** using N-methyl-1-(pyridin-3-yl)methanamineas starting material. Yield: 93.68%; ^{1}H NMR δ ^{1}H NMR δ 8.47 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.44 (s, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.24 (dd, *J* = 7.6, 4.7 Hz, 1H), 6.39 – 6.29 (m, *J* = 19.5 Hz, 2H), 5.72 (d, *J* = 8.2 Hz, 1H), 4.55 (t, *J* = 12.3 Hz, 2H), 3.40 (s, 2H), 3.19-3.17 (m, 2H), 2.96 (s, 2H), 2.84 (dd, *J* = 15.1, 4.8 Hz, 2H), 2.78 (s, 3H), 2.54 (s, 3H), 2.47 (s, 3H), 1.73 -1.54 (m, 2H), 1.42 (s, 6H), 1.40 (s, 9H)

methyl 2-(2- ((tert-butoxycarbonyl) amino)- 5- (3- ((2,2,4,6,7- pentamethyl- 2,3-dihydrobenzofuran-5-yl) sulfonyl) guanidino) pentanamido)- 3- (pyridin- 3-yl) propanoate (167)

Compound **167** was obtained following the same procedure described for compound **161** using N-methyl-1-(pyridin-3-yl)methanamineas starting material. Yield: 93.68%; ^{1}H NMR δ 8.39 (dd, *J* = 4.7, 1.6 Hz, 2H), 7.65 (d, *J* = 6.3 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.18 (dd, *J* = 7.7, 4.9 Hz, 1H), 6.42 (s, 2H), 6.26 (s, 1H), 5.78 (d, *J* = 7.6 Hz, 1H), 4.78 (td, *J* = 8.2, 5.5 Hz, 1H), 3.69 (s, 3H), 3.17 (dd, *J* = 14.1, 5.1 Hz, 3H), 3.01 (dd, *J* = 14.0, 8.4 Hz, 1H), 2.94 (s, 2H), 2.58 (s, 2H), 2.51 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.77 – 1.54 (m, 3H), 1.50 (s, 6H), 1.38 (s, 9H)

2-amino-5-guanidino-N-(pyridin-3-ylmethyl)pentanamide (160)

0.55g (0.893 mmol) of 161 were dissolved in 10mL of TFA then the reaction was stirred at room temperature for 4.5h. The solvent was removed with N_2 and, finally, with rotavapor. The precipitate was washed with diethyl ether. Compound 160 was dissolved in 5mL of HCl and 5mL of water and it was evaporated with freeze drying. This process was repeated three times in order to obtain **160**

as hydrochloride salt. ^{1}H NMR (D_2O) δ 8.70 (s, 1H), 8.67 (d, *J* = 5.8 Hz, 1H), 8.49 (d, *J* = 8.2 Hz, 1H), 8.00 (dd, *J* = 8.1, 5.9 Hz, 1H), 4.63 – 4.54 (m, 2H), 4.03 (t, *J* = 6.6 Hz, 1H), 3.15 (t, *J* = 6.9 Hz, 2H), 1.87 (td, *J* = 8.7, 2.3 Hz, 2H), 1.61 – 1.48 (m, 2H)

2-amino-5-guanidino-N-(1-(pyridin-3-yl)ethyl)pentanamide (162)

Compound **162** was obtained following the same procedure described for compound **160** using **163** as starting material. ^{1}H NMR (D_2O) δ 8.74 (d, *J* = 7.8 Hz, 1H), 8.67 (dd, *J* = 11.1, 5.7 Hz, 1H), 8.53 (d, *J* = 6.5 Hz, 1H), 8.01 (dt, *J* = 8.3, 5.2 Hz, 1H), 5.13 (q, *J* = 7.3 Hz, 1H), 3.99 (dt, *J* = 9.9, 5.0 Hz, 1H), 3.19 (t, *J* = 6.8 Hz, 1H), 3.09 (t, *J* = 6.9 Hz, 1H), 1.95 – 1.76 (m, 2H), 1.67 – 1.58 (m, 1H), 1.53 (dd, *J* = 7.1, 3.9 Hz, 3H), 1.46 – 1.34 (m, 1H)

2-amino-5-guanidino-N-methyl-N-(pyridin-3-ylmethyl)pentanamide (164)

Compound **164** was obtained following the same procedure described for compound **160** using **165** starting material. ^{1}H NMR (D_2O) δ 8.69 (s, 2H), 8.50 – 8.43 (m, *J* = 8.0 Hz, 1H), 8.01 (dd, *J* = 8.1, 6.1 Hz, 1H), 4.93 – 4.82 (m, *J* = 12.6 Hz, 1H), 4.68 – 4.62 (m, *J* = 8.0 Hz, 1H), 4.55 (dd, *J* = 7.0, 5.1 Hz, 1H), 3.18 (dd, *J* = 14.4, 7.7 Hz, 2H), 3.13 (s, 3H), 1.94-1.89 (m, 2H), 1.68 – 1.55 (m, 2H)

Methyl 2-(2-amino-5-guanidinopentanamido)-3-(pyridin-3-yl)propanoate (166)

Compound **166** was obtained following the same procedure described for compound **160** using **167** starting material. ^{1}H NMR (D_2O) δ 8.68 (s, 1H), 8.64 (d, *J* = 5.8 Hz, 1H), 8.49 (d, *J* = 8.1 Hz, 1H), 7.97 (dd, *J* = 8.0, 6.0 Hz, 1H), 4.90 (dd, *J* = 8.8, 5.9 Hz, 1H), 3.96 (dd, *J* = 8.0, 4.8 Hz, 1H), 3.70 (s, 3H), 3.53 – 3.39 (m, 1H), 3.35 – 3.20 (m, 1H), 3.15 (dd, *J* = 12.8, 6.1 Hz, 2H), 1.86 (dd, *J* = 15.1, 8.3 Hz, 2H), 1.59 (td, *J* = 13.7, 6.6 Hz, 2H)

2-(2-((tert-butoxycarbonyl)amino)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamido)-3-(pyridin-3-yl)propanoic acid (169)

To a solution of 0.43g (0.6278 mmol, 1eq) of **167** dissolved in 26mL of THF and 9mL of water, 0.05g (1.2556 mmol, 2eq) of LiOH were added and it was stirred at r.t. for 2h. The reaction was quenched with HCl 1N and the THF was evaporated. Then, the organic compound was extracted with ethyl acetate. 0.16g of neat compound **169** were obtained. Yield: 37.82%; ^{1}H NMR δ 9.01 (s, 1H), 8.39 (dd, *J* = 4.7, 1.6 Hz, 2H), 7.65 (d, *J* = 6.3 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.18 (dd, *J* = 7.7, 4.9 Hz, 1H), 6.42 (s, 2H), 6.26 (s, 1H), 5.78 (d, *J* = 7.6 Hz, 1H), 4.78 (td, *J* = 8.2, 5.5 Hz, 1H), 3.17 (dd, *J* = 14.1, 5.1 Hz, 3H), 3.01 (dd, *J* = 14.0, 8.4 Hz, 1H), 2.94 (s, 2H), 2.58 (s, 2H), 2.51 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.77 – 1.54 (m, 3H), 1.50 (s, 6H), 1.38 (s, 9H)

2-(2-amino-5-guanidinopentanamido)-3-(pyridin-3-yl)propanoic acid (169)

Compound **168** was obtained following the same procedure described for compound **160** using **169** starting material. ^{1}H NMR (D_2O) δ 8.66 – 8.61 (m, 2H), 8.47 (d, *J* = 8.3 Hz, 1H), 7.96 (dd, *J* = 8.0, 5.8 Hz, 1H), 4.68 – 4.59 (m, 1H), 3.97 (t, *J* = 6.3 Hz, 1H), 3.37 (dd, *J* = 14.4, 6.2 Hz, 1H), 3.28 – 3.19 (m, 1H), 3.15 (t, *J* = 6.8 Hz, 2H), 1.87 (dd, *J* = 14.9, 8.1 Hz, 2H), 1.67 – 1.50 (m, 2H).

methyl 2-amino-3-(pyridin-3-yl)propanoate (170)

To a cooled (0° C) solution of 0.25g (1.506 mmol) of 3-(3-pyridyl)-L-alanine dissolved in 5mL of anhydrous methanol, 0.4mL of $SOCl_2$ were added dropwise and the reaction was stirred at r.t. for 24H. The solvet was removed under reduced pressure and 0.26g of neat compound **170** were obtained. Yield: 95.91%; ^{1}H NMR (D_2O) δ 8.74 – 8.68 (m, 2H), 8.49 (d, *J* = 8.2 Hz, 1H), 8.00 (dd, *J* = 8.1, 5.8 Hz, 1H), 4.52 (dd, *J* = 7.7, 6.6 Hz, 1H), 3.74 (s, 3H), 3.52 (dd, *J* = 14.9, 7.8 Hz, 1H), 3.44 (dd, *J* = 14.8, 6.

PROCEDURE 1

0.500 mmol of aginine derivative was dissolved in 5mL of HCl and 5mL of water. Then the solvent was evaporated with the afford of the freeze drying. The process was repeated three times until the total conversion of the salt.

Compounds 171-175 were converted from trifluoroacetic salt into hydrochloride salt following procedure 1.

7 REFERENCES

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