Design and Synthesis of Multi Target Compounds for the Treatment of Alzheimer’s Disease

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Esame finale anno 2009
“Dulce est desipere in loco”.....Orazio
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1. Introduction

1.1. Alzheimer’s disease

Alzheimer's disease (AD), also called Senile Dementia of the Alzheimer Type (SDAT), is the most common form of dementia. This neurodegenerative disease is named after the German psychiatrist Alois Alzheimer, who first described it in 1906. Generally it is diagnosed in people over 65 years of age\(^1\), but the less-prevalent early-onset Alzheimer's can occur much earlier. The disease course can be divided into four stages\(^2\) and although each sufferer experiences Alzheimer's in a unique way, there are many common symptoms with a progressive pattern of cognitive and functional impairment from pre-dementia, to early, moderate and advanced dementia.

1.1.1. Predementia

The first symptoms are often mistaken as related to ageing or stress and they can affect the most complex daily living activities. The most noticeable deficit is memory loss, which shows up as difficulty in remembering recently learned facts and inability to acquire new informations. Subtle problems with the executive functions of attentiveness, planning, flexibility, and abstract thinking, or impairments in semantic memory (memory of meanings, and concept relationships), can also be symptomatic of the early stages of AD. Apathy can be observed at this stage, and remains the most persistent neuropsychiatric symptom throughout the course of the disease.

1.1.2. Early dementia

In people with AD the increasing impairment of learning and memory eventually leads to a definitive diagnosis. In a small proportion of them, difficulties with language, executive functions, perception (agnosia), or execution of movements (apraxia) are more prominent than memory problems. AD does not affect all memory capacities equally. Older memories of the person's life (episodic memory), facts learned (semantic memory), and implicit memory (the memory of the body on how to do things) are affected to a lesser degree than new facts or memories. Language problems are mainly characterized by a shrinking vocabulary and decreased word fluency, which lead to a general impoverishment of oral and written language. In this stage, the person with
Alzheimer's is usually capable of adequately communicating basic ideas. While performing fine motor tasks such as writing, drawing or dressing, certain movement coordination and planning difficulties may be present, making sufferers appear clumsy. 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.

1.1.3. Moderate dementia

Progressive deterioration eventually hinders independence. Speech difficulties become evident due to an inability to recall vocabulary, which leads to frequent incorrect word substitutions (paraphasias). Reading and writing skills are also progressively lost. Complex motor sequences become less coordinated as time passes, reducing the ability to perform most normal daily living activities. During this phase, memory problems worsen, and the person may fail to recognise close relatives. Long-term memory, which was previously intact, becomes impaired, and behavioral changes become more prevalent. Common neuropsychiatric manifestations are wandering, irritability and labile affect, leading to crying, outbursts of unpremeditated aggression, or resistance to caregiving. Approximately 30% of patients also develop illusionary misidentifications and other delusional symptoms. Urinary incontinence can develop.

1.1.4. Advanced dementia

During this last stage of AD, the patient is completely dependent upon caregivers. Language is reduced to simple phrases or even single words, eventually leading to complete loss of speech. Despite the loss of verbal language abilities, patients can often understand and return emotional signals. Although aggressiveness can still be present, extreme apathy and exhaustion are much more common results. Patients will ultimately not be able to perform even the most simple tasks without assistance. Muscle mass and mobility deteriorate to the point where they are bedridden, and they lose the ability to feed themselves. Finally comes death, usually caused directly 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. Fewer than three percent of individuals live more than fourteen years after diagnosis.

1.1.5. Pathological hallmarks

Although the origin of AD is still unknown, three pathological hallmarks have been identified: amyloid-β plaques, neurofibrillary tangles (NFTs) and synaptic loss. At autopsy, the AD brain is
characterized by a number of important pathological changes, including a marked loss of neurons and synapses in many areas of the CNS, especially in regions involving higher order cognitive functions such as hippocampus and the association cortices. In addition, there is a global and dramatic reduction of neurotransmitters level, noradrenaline, dopamine, serotonin, glutamate, substance P and acetylcholine (ACh). Such depletion of neurotransmitters among which ACh is the most important one, is almost certainly the cause of the previously described clinical manifestations of AD.

1.1.6. Epidemiology and social costs

Two main measures are used in epidemiological studies: incidence and prevalence. Incidence is the number of new cases per thousand person–years; while prevalence is the total number of cases of the disease in the population at a given time. Regarding incidence, studies provide rates between 10–15 thousand person–years for all dementias and 5–8 for AD, which means that half of new dementia cases each year are AD. Advancing age is a primary risk factor for the disease and incidence rates are not equal for all ages: every five years after the age of 65, the risk of acquiring the disease approximately doubles, increasing from 3 to as much as 69 per thousand person years. There are also sex differences in the incidence rates, women having a higher risk of developing AD particularly in the population older than 85. The World Health Organization estimated that in 2005 0.379% of people worldwide (26 millions) had dementia, and that the prevalence would increase to 0.441% in 2015 and to 0.556% in 2030, likely tripling by the year 2050. Dementia, and specifically Alzheimer's disease, may be among the most costly diseases for society in developed states and despite numbers vary between studies, dementia costs worldwide have been calculated around $160 billion, of which about $100 billion each year only in the United States. In addition to the impact on healthcare budget, there is also the emotional as well as the physical stress brought to the family of the sufferers.

1.2. Ethiopathology

There are two main hypotheses trying to explain the onset of the disease: the first and oldest one, on which most currently available drug therapies are based, is the cholinergic hypothesis\(^3\), which
proposes that AD is caused by a severe impairment of the cholinergic system; the second one presented in 1991 is the *amyloid hypothesis*\(^{(4)}\), and postulates that amyloid beta (A\textsubscript{\textbeta}) deposits are the fundamental cause of the disease. Other processes seem to be involved, such as inflammatory injury, oxidative stress\(^{(5)}\) and intracellular deposition of neurofibrillary tangles\(^{(6)}\) made of hyperphosphorilated tau protein, pointing out the multi-factorial nature of AD and the urge for understanding how these processes are related in the cascade-events leading to its onset.

1.2.1. Cholinergic hypothesis

The cholinergic hypothesis\(^{(7)}\) was initially presented about 30 years ago and has become well established in the past decade almost to the point of dogma. In fact, starting from the mid-1970s, several groups, involved in the study of AD brains, reported loss of the main markers of the cholinergic activity such as significatively reduced choline acetyltransferase (CAT) activity in the cortex and hippocampus being CAT the enzyme responsible for the synthesis of acetylcholine from its precursors (acetyl coenzyme A and choline). Several other parameters of cholinergic function have been shown to be deranged in AD. High-affinity choline uptake, acetylcholine release and synthesis, together with cortical acetylcholine levels are in fact all reduced. The cholinergic hypothesis provided the first rational approach in the treatment of AD: six classes of compounds might be developed to contrast cholinergic deficit in AD patients.

1) Cholinesterase inhibitors (ChEIs), which block the enzyme acetylcholinesterase and thereby increase the synaptic availability of ACh by reducing its degradation.

2) Choline precursors, such as phosphatidylcholine, aimed at increasing the bioavailability of choline.

3) ACh releaser, which should facilitate the release of ACh from presynaptic end terminals.

4) M\textsubscript{1} and M\textsubscript{3} receptors agonists, which mimic ACh on postsynaptic end terminals.

5) M\textsubscript{2} and M\textsubscript{4} receptors antagonists, which regulate ACh release via negative feedback (auto receptors).

6) Nicotinic agonists, which should enhance ACh.

Acetylcholinesterase inhibitors seem to be the most effective strategy within the cholinergic approach in the treatment of AD.
1.2.2. *Acetylcholinesterase*

In 1914, Dale postulated an enzyme system responsible for terminating the physiological role of ACh at the cholinergic synapses. He differentiated between the muscarine- and nicotine-like actions of choline esters on different tissues and so he proposed: “it seems not improbable that an esterase contributes to the removal of acetylcholine from the circulation”. It was not until 1926 that Loewi and Navratil experimentally demonstrated acetylcholinesterase existence and in 1932 Stedman prepared a crude extract of an “ACh-splitting” enzyme from horse serum, which he called “choline esterase”. We now know that two enzymes efficiently hydrolyze ACh, acetylcholinesterase and butyrylcholinesterase (BuChE), also known as pseudocholinesterase or non specific cholinesterase. Those enzymes, which preferentially hydrolyze acetyl esters such as ACh, are called AChE and those which prefer other types of esters such as butyrylcholine (BuCh) are termed BuChE.

1.2.3. *Localization and function*

The principal biological role of AChE is the termination of impulse transmission at cholinergic synapses, by rapid hydrolysis of the neurotransmitter acetylcholine, within both the central and the peripheral nervous system; hence its location is related to cholinergic system. It has been reported that AChE is able to hydrolyze substance P and enkephalins too. Moreover, AChE shows peptidase and amidase activities. BuChE is found not only in association with neuronal cells in brain, but also with glial cells, in particular at the blood-brain barrier (BBB) level.

<table>
<thead>
<tr>
<th>Localization</th>
<th>AChE</th>
<th>BuChE</th>
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<tbody>
<tr>
<td>Brain</td>
<td>- Cholinergic Neuron</td>
<td>- Glial Cells</td>
</tr>
<tr>
<td>Blood-Brain Barrier</td>
<td>- Neuromuscular Junct.</td>
<td>- Heart</td>
</tr>
<tr>
<td>Periphery</td>
<td>- Heart</td>
<td>- Liver</td>
</tr>
</tbody>
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Table 1. Localization of AChE and BuChE

The main function of AChE is the rapid hydrolysis of the neurotransmitter ACh at the cholinergic synapses: its catalytic mechanism is among the most efficient known, rate approaches that of a diffusion-controlled reaction, the substrate turnover is 25000 molecules sec⁻¹, and each turnover lasts about 40 µs. The hydrolysis reaction proceeds by nucleophilic attack of the carbonyl carbon,
involving acylation of the enzyme and release of choline. This is followed by a rapid hydrolysis of the acylated enzyme yielding acetic acid, and restoring the esteratic site.

\[
\text{CH}_3\text{COOCH}_2\text{CH}_2\text{N}^+\text{(CH}_3)_3 + \text{AChE} \rightarrow \text{CH}_3\text{CO-}\text{AChE} + \text{HOCH}_2\text{CH}_2\text{N}^+\text{(CH}_3)_3 \rightarrow \text{AcOH} + \text{AChE}
\]

The function of BuChE remains to be fully elucidated in both the brain and the systemic circulation, to date any specific natural substrate is known though is able to hydrolyze ACh. BuChE is found in association with the dopaminergic system where it may have a modulator role and is found in high concentrations in liver, suggesting a role in lipid metabolism, as well as in the cardiac muscle. Its massive presence in serum suggests a critical role in degradation of drugs such as succinyl choline, heroin, cocaine and physostigmine and activation of certain others like bambuterol. Recently it has been found that BuChE is a co-regulator of ACh levels in the advanced stage of AD, suggesting that its inhibition may play an important role for the therapeutic strategies.

<table>
<thead>
<tr>
<th>AChE</th>
<th>Peripheral Organs</th>
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<tbody>
<tr>
<td>-Brain cholinergic</td>
<td>-Peripheral cholinergic neurotransmission</td>
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<tr>
<td>-Neurotransmission</td>
<td>-Cardiovascular regulation</td>
</tr>
<tr>
<td>-Memory</td>
<td>-Neuromuscular activity</td>
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<td></td>
<td>-Papillary control</td>
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<table>
<thead>
<tr>
<th>BuChE</th>
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<tbody>
<tr>
<td>-Modulation of dopamine neurotransmission</td>
<td>- Drug metabolism</td>
</tr>
<tr>
<td></td>
<td>- Antitoxin</td>
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</table>

Table 2. Functions of AChE and BuChE

1.2.4. Structure

Knowledge of the three dimensional (3D) structure of AChE is essential for understanding its remarkable catalytic efficacy, for rational drug design and for developing new therapeutic approaches. The various oligomeric forms of AChE in the electric organ of certain fish, *Electrophorus* and *Torpedo*, are structurally homologous to those in vertebrate muscle and nerve. Highly purified preparations from this abundant sources of AChE facilitated research on this enzyme. The traditional view of the active site of AChE was initially considered to consist of two
subsites: an electron rich “anionic” site, to which the positively charged quaternary nitrogen moiety binds, and an esteratic site containing the catalytic residues. A second “anionic” site, which is known as the peripheral “anionic” site (PAS) was later proposed on the basis of binding of bis-quaternary compounds.

![Fig. 2. Schematic representation of the binding sites of AChE: esteratic site, anionic binding site as well as the peripheral anionic binding site are shown](Image)

The nucleophile was assumed to be a serine residue, with a histidine residue enhancing its nucleophilicity. AChE was classified as a serine hydrolase, and therefore assumed to contain a catalytic triad of Glu-His-Ser at the esteratic site. A great leap forward in the understanding of catalytic mechanism, and mode of action of inhibitors, came in 1991 with the determination of the three dimensional structure of dimeric *Torpedo californica* AChE (*Tc*AChE). The structure was determined to a resolution of 2.8 Å, which has more recently been refined to 2.2 Å by Sussman himself. According to the agreement adopted at the Oholo Conference in 1992, residues are numbered from the first one of the mature protein. The enzyme monomer is an α/β protein (MW 65612) containing 537 amino acids. The molecule possesses an ellipsoidal shape with dimensions of about 45 Å by 60 Å by 65 Å. It consists of a 12-stranded central mixed β-sheet surrounded by 14 α-helices and bears a striking resemblance to several hydrolases. The position of the active residue serine in *Tc*AChE was established by irreversibly labeling it with [*3H*]isopropyl fluorophosphate, followed by tryptic digestion and analysis of the tryptic peptides localizing it to Ser^{200}. Both kinetic and chemical studies implicate a His residue in the active site. Mutagenesis studies identified the catalytic Histidine with His^{440}. The glutamic group of the catalytic triad was confirmed to be Glu^{327} by site-directed mutagenesis on the related *Torpedo marmorata* AChE. The active site was found to be located 20 Å from the enzyme surface at the bottom of a narrow gorge, lined with 14
aromatic residues which may be important in guiding the substrate to the active site. There is not discernable “anionic” site, the quaternary nitrogen of choline binds chiefly through interactions with the \( \pi \) electrons of the Trp\(^{84}\) residue. It is interesting to compare the structure of AChE and BuChE, since they possess 53% sequence homology, which has permitted the modeling of BuChE on the basis of 3D structure of \( TcAChE \). Six aromatic residues, which are conserved in AChE and line the gorge leading to the active site, are absent in BuChE. Computer modeling has shown that two of these residues, Phe\(^{288}\) and Phe\(^{290}\), which are replaced in BuChE by leucine and valine, respectively, may prevent bulky esters of choline from entering into the acyl binding pocket of AChE. Mutagenesis experiments have confirmed this model. In addition to the subsite of the catalytic centre, AChE possesses one more additional binding site for ACh and other quaternary ligands. Such peripheral anionic binding site is located at the lip of the gorge. In BuChE Trp\(^{279}\) is missing.

1.2.5. Mechanism of action

The catalytic triad has been termed “charge relay system”. In human AChE (\( huAChE \)), the triad includes Ser\(^{203}\), the imidazole ring of His\(^{447}\) and the carboxylic group of Glu\(^{334}\). During the binding of ACh to AChE, the charge relay system causes electron shifts yielding to the acylation of the enzyme. The tetrahedral intermediate collapses to the acylated enzyme by acid catalyzed expulsion of choline by His\(^{447}\). The acylated enzyme complex is finally rapidly hydrolyzed, regenerating active enzyme by releasing acetic acid.

Fig. 3. Schematic mechanism of ACh metabolism by AChE

The inhibition of AChE by excess substrate is one of the key features that distinguishes it from BuChE. It’s not known if the substrate inhibition has a biological role or it is just a consequence of AChE structure and mechanism. Various explanations have been proposed AChE could be allosterically regulated by the binding of ACh to the peripheral anionic site through conformational
changes at the active centre. The importance of the peripheral site in substrate inhibition has also been supported by Radic in 1991, based on studies of competition of the substrate with the peripheral site ligand propidium. However, studies on chicken AChE, which lacks the Tyr\textsuperscript{70} and Trp\textsuperscript{279} residues found in \textit{Torpedo californica} peripheral site, reveal substrate inhibition characteristics similar to those of \textit{Tc}AChE.

1.2.6. \textit{Amyloid-}β\textit{peptide hypothesis}

Two of the major markers\textsuperscript{(8,9)} of AD are the extracellular deposition of fibrous protein aggregates called senile or amyloid plaques, and NFTs in brain regions responsible for high cognitive function, such as hippocampus and association cortices.

![Fig. 4. Neurofibrillary tangles (left) and Aβ plaques (right)](image)

The amyloid plaque as described by Alzheimer was isolated by Glenner and Wong in 1984. It is composed of an approximately 4 KDa peptide, that aggregates into a fibrillar, β-pleated structure. On the basis of its size and secondary structural characteristic, the peptide has been referred to by a variety of names including A4 peptide, β-peptide and βA4. According to accepted amyloid nomenclature, the peptide is now referred to as amyloid-β or Aβ. Even though a casual link between plaque formation and AD has not been firmly established, increasing evidences suggest that amyloid deposition may play a critical role in the neurodegenerative process. The plaque are primarily composed of aggregates of 39–43 amino acids (Aβ), of which the 42 amino acid peptide is the most prone to aggregation and thus, plaque formation. The “amyloid hypothesis” postulates that Aβ\textsubscript{42}, in particular, is casual in the disease process. This hypothesis is supported primarily by genetic evidence from individuals who greatly overproduce Aβ\textsubscript{42}. A growing effort is directed towards therapeutic strategies, which target the effects of Aβ as treatment for the cause of AD.
These strategies are complimentary to symptomatic cognition-improvement therapies based on AChE inhibitors and M1 agonists. The Aβ strategies take three forms:

1) Disruption of Aβ production from its precursor protein (APP)
2) Protection from neurotoxicity of the Aβ aggregates
3) Inhibition of the aggregation of Aβ monomer into the neurotoxic aggregates that constitute the plaques

1.2.7. Amyloid-β peptide formation

It is presently thought that Aβ is generated after sequential cleavage of the amyloid precursor protein, a transmembrane glycoprotein of undetermined function. APP can be processed by α-, β- and γ-secretases; Aβ protein is generated by successive action of the β (BACE) and γ secretases. The γ secretase, which produces the C-terminal end of the Aβ peptide, cleaves within the transmembrane region of APP and can generate a number of isoforms of 39-43 amino acid residues in length. The most common isoforms are Aβ40 and Aβ42; the shorter form is typically produced by cleavage that occurs in the endoplasmic reticulum, while the longer form is produced by cleavage in the trans-Golgi network. The Aβ40 form is the more common of the two, but Aβ42 is the more fibrillogenic and is thus associated with disease states. Mutations in APP associated with early-onset Alzheimer's have been noted to increase the relative production of Aβ42, and thus one suggested avenue of Alzheimer's therapy involves modulating the activity of β (BACE) and γ secretases to produce mainly Aβ40.

![Fig. 5](image)

Fig. 5 a) Schematic representation of a possible processing pathway of the amyloid precursor protein (APP) by α, β and γ secretases; b) representation of the amyloid–β theory.

Particularly, the proteolysis takes place just outside the membrane, releasing soluble APP (β-APPs) and leaving behind a 99 residue membrane-associated C-terminal fragment, C99. Alternatively, APP is cut by α-secretase, a membrane-bound metallo-protease, to produce α-APPs and an 83
residue membrane-associated C-terminal fragment, C83. Both C99 and C83 are substrates for γ-secretase, an enzyme that carries out an unusual proteolysis in the middle of the transmembrane domain of APP, resulting in formation of the 4 kDa Aβ from C99 and p3, a 3 kDa N-terminally truncated form of Aβ, from C83. AChE may also fulfill non-cholinergic roles. In AD brains, cortical AChE activity is associated predominantly with the amyloid core of mature senile plaques, pre-amyloid diffuse deposits and cerebral blood vessels. Previous studies have indicated that AChE binds to Aβ and induces Aβ fibril formation, forming macromolecular complexes with the growing fibrils. These studies suggested that a specific motif, located close to the rim of the active site gorge of the enzyme, may be involved in accelerating fibril formation. This motif, as elucidated by Inestrosa and co-workers(15,16), matches with the PAS since both PAS inhibitors and a monoclonal antibody directed toward the PAS, blocked the amyloidogenic effect of AChE.

1.2.8. BACE (beta amyloid cleaving enzyme)(9)

β-Secretase (BACE) is a proteolytic enzyme involved in the processing of an integral membrane protein known as amyloid precursor protein, or APP. The proteolysis of APP by BACE, followed by subsequent C-terminal cleavage(s) by γ-secretase, results in the formation of the amyloid β (Aβ) peptide. Aβ is a neurotoxic and highly aggregatory peptide segment of APP that is the principal component of the neuritic plaque found in the brains of Alzheimer’s disease (AD) patients. Because of the apparent causal relationship between Aβ and AD, the so-called “secretases” (especially BACE) that produce Aβ have been targeted.

1.2.9. BACE inhibitors(9)

Peptidomimetic BACE Inhibitors. Substrate based inhibitors of BACE were designed using the knowledge of the specificity and kinetics of BACE. Tang and co-workers at the University of Oklahoma have reported on the development of peptidic hydroxyethylene-based BACE inhibitor OM99-2, which has a BACE IC50 of 0.002 μM. (10) They have further reported on using X-ray structure-based modification of the lead 1 that led to the discovery of a series of potent and considerably low molecular weight peptidomimetic BACE inhibitors such as 2.
Nonpeptidomimetic BACE Inhibitors. The most detailed account of nonpeptidomimetic BACE inhibitors to date comes from Vertex, via a published patent application. In late 2002, Vertex disclosed several hundred compounds along with associated $K_i$ ranges of BACE inhibition; these compounds spanned multiple classes of heterocyclic templates. Among the more potent classes reported (BACE $K_i < 3 \mu M$) were the halogen-substituted biarylnaphthalenes represented by 3. From these results, Vertex proposed the first 3-D pharmacophore map of BACE to guide the design and optimization of inhibitors, wherein HB represents hydrogen-bonding moiety interactions with the active site and other key residues of BACE and HPB represents hydrophobic moiety interactions with BACE subsites.

**Figure 6.** Vertex inhibitor and 3-D BACE pharmacophore map.
1.2.10. *Tau protein theory*\(^{(6)}\)

Tau proteins are microtubule-associated proteins that are abundant in neurons in the central nervous system and are less common elsewhere. They were discovered in 1975 in Marc Kirschner's laboratory at Princeton University. Tau proteins interact with tubulin to stabilize microtubules and promote tubulin assembly into microtubules. Tau has two ways of controlling microtubule stability: isoforms and phosphorylation. Six tau isoforms exist in brain tissue, and they are distinguished by their number of binding domains. Three isoforms have three binding domains and the other three have four binding domains. The binding domains are located in the carboxy-terminus of the protein and are positively-charged (allowing it to bind to the negatively-charged microtubule). The isoforms with four binding domains are better at stabilizing microtubules than those with three binding domains. The isoforms are a result of alternative splicing in exons 2, 3, and 10 of the *tau* gene. Phosphorylation of tau is regulated by a host of kinases, for example, PKN, a serine/threonine kinase. When PKN is activated, it phosphorylates tau, resulting in disruption of microtubule organization. Hyperphosphorylation of the tau protein (tau inclusions), however, can result in the self-assembly of tangles of paired helical filaments and straight filaments, which are involved in the pathogenesis of Alzheimer's disease. In fact, the observation that deposition of amyloid plaques does not correlate well with neuron loss has supported the *tau hypothesis*. In this model, hyperphosphorylated tau begins to pair with other threads of tau. Eventually, they form neurofibrillary tangles inside nerve cell bodies. When this occurs, the microtubules disintegrate, leading to collapse of the neuron's transport system. This may first result in malfunctions in biochemical communication between neurons, and later in the death of the cells.

![Fig. 7. Intracellular neurofibrillary tangles made of hyperphosphorilated tau protein](image)
1.3. Acetylcholinesterase inhibitors

Inhibition can be either reversible, by competitively preventing the substrate from reaching the active site; pseudo-irreversible, by covalent reaction with the active site serine, inactivating the catalytic ability of the enzyme or irreversible. Competitive inhibitors act by blocking substrate at the active site, non competitive inhibitors by binding to the peripheral site.

Organophosphorus compounds

Organophosphorus compounds such as diisopropyl fluorophosphates (DFP) are very potent inhibitors of AChE and are used as agricultural insecticides or as nerve gases in chemical warfare. These compounds react with the active site serine, forming a very stable covalent phosphoryl-enzyme complex.

Metriphonate

Metriphonate is a natural AChE inhibitor, which irreversibly phosphorilates the enzyme. This drug is unique among ChEIs for AD treatment; since in aqueous solution it spontaneously transforms in the active metabolite 2,2-dichlorovinyl dimethyl phosphate. The reaction doesn’t require enzymatic activation; the inhibition is mediated by a competitive drug interaction at the catalytic site, followed by dimethylphosphonorylation of a serine residue located in the active site of the enzyme.

Propidium

Propidium is a reversible AChE inhibitor. Its bulky structure doesn’t allow the molecule to penetrate into the gorge. It is the reference compound for the determination of the inhibition at the PAS.
(-) *Huperzine A*

(-) Huperzine A (Hup A) is a natural origin alkaloid isolated from *Huperzia serrata*. This alkaloid inhibits the enzyme by forming reversible complexes with it and it is able to reduce neuronal cells death caused by glutamate. This double action and the very low toxicity indicate Hup A as a promising drug for the treatment of AD. The crystal structure of Hup A complexed with *TcAChE* reveals an unexpected orientation of the ligand within the active site, as well as an unusual protein-ligand interaction and a significant change in the main chain conformation of the protein. The orientation of Hup A within the active site gorge appears to be almost orthogonal to the ACh molecule. Binding of Hup A to the PAS, near to Trp^{279} was also predicted by docking studies, but no evidence of this was seen in the crystallography studies.

*Galantamine*

Alkaloid galantamine\(^{14}\) was primarily studied for botanic purposes and only in 1986 for the treatment of AD. It is a reversible inhibitor that shows CNS selectivity and despite its less potency compared with tacrine and E2020, it has excellent pharmacological and pharmacokinetic profiles exhibiting very low hepatotoxicity and side effects. Galantamine is also a representative member of a class of ligands acting as noncompetitive nicotinic receptor agonist. Its action is exerted via an allosteric binding site on nAChR, distinct from the site for acetylcholine, improving the nicotinic response induced by ACh and competitive agonists. The cost of obtaining galanthamine from natural sources is very high slowing down studies about this drug. Total synthesis procedures developed so far, still have to be improved for large-scale industrial preparation. Galantamine (Reminyl\(^\text{®}\)) has now been approved for AD treatment in many countries among which Italy.
Trifluoromethyl ketons

Of particular interest are inhibitors, which act as transition-state mimics, such as trifluoromethyl ketons.

Trimethyl-[3-(2,2,2-trifluoro-acetyl)-phenyl]-ammonium (TMTFA) is a substrate-like inhibitor of AChE of this class, and it is one of the most potent reversible AChE inhibitors reported in the literature. Sussman and co-workers confirmed the nature of the binding mode of TMTFA with TcAChE by X-ray analysis. The active site serine covalently binds to the activated carbonyl of the trifluoroacetyl group, while the quaternary ammonium group favorably interacts with the adjacent aromatic residues Trp$^{84}$ and Phe$^{330}$ among others.

Physostigmine

The longest known AChE inhibitor is the natural alkaloid physostigmine (PHY), which is extracted from the Calabar Beans. This carbamylates the active site serine residue, greatly slowing the acylenzyme hydrolysis reaction compared with the acetylated enzyme. The AChE IC$_{50}$ is 14.1 nM but it is not selective for AChE versus BuChE and its half life (t$_{1/2}$) is 15-30 minutes; for these reasons in November 1998 the U.S. Food and Drug Administration (FDA) issued a non approval letter for PHY’s Alzheimer’s indication based mainly on a lack of efficacy as shown from results in Clinical Phase II and Phase III studies.

Physostigmine analogues

Derivatives of the alkaloid physostigmine, as well as physostigmine itself, inhibit the enzyme by carbamoylating the serine residue of the catalytic triad in a pseudoirreversible manner. Sequestration of AChE in its carbamoylated form precludes any further hydrolysis of ACh for an extended period of time. To ameliorate farmacokinetic properties of PHY, the carbamic substituent has been modified increasing the duration of action and maintaining the acetylcholinesterase
activity. *Phenserine*, the phenyl carbamate of (-)-eseroline, was prepared in 1916 from natural physostigmine by Polonovsky.

![Phenserine](image)

It’s a long acting drug and it inhibits AChE selectively, being more than 50 times more active on AChE than BuChE. *Eptastigmine*, a more recent physostigmine analogue, is the heptyl carbamate of (-)-eseroline.

![Eptastigmine](image)

Compared to physostigmine this compound has a longer duration of action ($t_{1/2} = 10$ hours) and the drug inhibits red blood cells AChE in a dose-dependent manner. Although clinical phase III studies showed a positive effect of eptastigmine compared to placebo, in particular in the more severely impaired patients, they were subsequently dropped because of toxicity problems.

*Rivastigmine*

![Rivastigmine](image)

Rivastigmine (SDZ-ENA-713/Exelon®) is a miotine derivative. It is a carbamic AChE inhibitor with a short half-life and duration of action of about 10 hours. This drug inhibits AChE by carbamoylating the serine residue of the catalytic triad in a pseudoirreversible manner. Based on Phase III clinical studies conducted in the U.S. a regimen of increasing dosage seems to be necessary in order to achieve maximum clinical benefits with minimal side effects. Rivastigmine under the name of Exelon® has been launched worldwide: Europe, South America, Asia, Africa and more recently also in the United States.
Tacrine

Tacrine (THA, Cognex®) was launched in 1993 as the first drug for the symptomatic treatment of AD. THA (9-amino-1,2,3,4-tetrahydroacridine) is a centrally active reversible inhibitor of AChE that shows a moderately long duration of action. The rationale for its use was related to the elevation of the acetylcholine levels that can compensate for the cholinergic deficit associated to the brain lesions present in mild to moderate AD. In the Tacrine-AChE complex, the tacrine moiety is staked against Trp$_{84}$, with the nitrogen in the ring forming a hydrogen bond with the main chain carbonyl oxygen of His$_{440}$; its amino nitrogen binds to a water molecule. The Phe$_{330}$ ring rotates to lie parallel to tacrine, which is sandwiched between the Phe$_{330}$ and Trp$_{84}$ rings. Because of the positive effects observed with THA, this compound has been used as a reference drug in the clinical development of other AChE inhibitors for both clinical efficacy and side effects.

Tacrine analogues

Several THA analogues are under preclinical or clinical evaluation, among them are amiridine and velnacrine. Velnacrine is an hydroxyl metabolite of tacrine, with a shorter half-life.

Pharmacodinamics trials with single doses of this inhibitor demonstrated statistically significant cognitive improvements compared with placebo, however about 20-30% of patients developed hepatotoxicity. Amiridine is now under phase III clinical studies in Japan.

Beneficial effects are produced at both the initial and marked stages of the disease, although the most significant results are obtained in patients with unmarked dementia. Some recent studies are addressed to tacrine-huperzine A hybrids and other alkylene-linked tacrine dimers.


**Tacrine dimers**

Since bis(7)-tacrine, a heptamethylene-linked dimer of the first marketed anti-Alzheimer drug tacrine, was developed 1 decade ago,\(^{15-18}\) the search for inhibitors of acetylcholinesterase able to simultaneously bind to its catalytic and peripheral binding sites has become an area of very active research. Several classes of dual binding site AChE inhibitors have been developed by connecting through a suitable linker the two interacting units, which are generally derived from known AChE inhibitors either commercialized or under development.\(^{19-21}\) The success of the dual binding site strategy is evidenced by the large increase in AChE inhibitory potency of these dimers or hybrids relative to the parent compounds from which they have been designed. Further interest comes from the fact that some of these dual binding site AChE inhibitors have been shown to inhibit the aggregation of β-amyloid peptide (Aβ),\(^{9-18}\) which is a key event in the neurotoxic cascade of Alzheimer’s disease (AD).\(^{19,20}\) This effect, which has been related to the blockade of the AChE peripheral site\(^ {16}\) by dual binding site AChE inhibitors, makes these compounds very promising disease-modifying anti-Alzheimer drug candidates. The general structure of tacrine dimers can be represented as follows:

![Structure of tacrine dimer]

Two 9-amino-1,2,3,4-tetrahydroacridine moieties are linked one to another through an opportune spacer that most of the times happens to be an heptyl chain. There have been also heterodimers linking THA to different kinds of chemical motifs (mainly trimethoxy substituted benzenes, lipoic acid, melatonin, NO donor) sometimes through spacers other than alkyl-chains such as hydrazide-, amide- and triazole-based linkers. In these structures the 9-amino-1,2,3,4-tetrahydroacridine moiety is sometimes found to carry a chlorine substituent at C 6 position.
**Donepezil**

![Donepezil molecule](image)

Donepezil hydrochloride (E2020, Aricept®) is the second drug approved by the U.S. FDA for the treatment of mild to moderate AD. This class of $N$-benzylpiperidines emerges from Eisai Company; through a random screening they found a $N$-benzylpiperazine derivative showing a promising AChE inhibitory activity. When $N$-benzylpiperazine was replaced with $N$-benzylpiperidines a dramatic increase in anti-acetylcholinesterase activity was detected. After various modifications they obtained Donepezil. They studied the possible interaction of the new compound with the enzyme finding multiple modes of binding, but only few years later Sussman and co-workers reported the crystal structure of a complex of donepezil with $Tc$AChE. The X-ray structure shows that the elongated donepezil molecule spans the entire length of the active-site gorge of the enzyme. It thus interact with both the anionic subsite at the bottom of the gorge, and with the peripheral anionic site, near its entrance, via aromatic staking interactions with conserved aromatic residues. E2020 doesn’t directly interact with either the catalytic triad or with the “oxyanion hole”.

**Fig. 8.** E2020 (in green) binds along the active site and interacts with the PAS at the top and with the AS at the bottom of the gorge
Donepezil shows superior efficacy, minimal side effects, and high brain selectivity compared to THA and PHY. E2020 and related compounds are shown to reversibly and specifically inhibit AChE by forming a complex in which the $N$-benzylpiperidine group presumably interacts with the anionic site, which recognizes the quaternary ammonium group of ACh. Clinical trials have shown that the inhibition of BuChE may be linked with side effects. $N$-benzylpiperidine compounds are highly selective for AChE over BuChE demonstrating an exceptional safety profile ($IC_{50}$ for AChE is 5.7 nM while for BuChE is about 7000 nM).

1.4. Therapeutic Approaches

Four drugs are currently approved by regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) to treat the cognitive manifestations of AD: three are acetylcholinesterase inhibitors and the other one is memantine, an NMDA receptor antagonist. No drug has an indication for delaying or halting the progression of the disease. Since reduction in the activity of the cholinergic neurons is a well-known feature of Alzheimer's disease, acetylcholinesterase inhibitors are employed to reduce the rate at which acetylcholine (ACh) is broken down, thereby increasing the concentration of ACh in the brain and combating the loss of ACh caused by the death of cholinergic neurons. As of 2008, the cholinesterase inhibitors approved for the management of AD symptoms are donepezil (brand name Aricept®), galantamine (Reminyl®), and rivastigmine (branded as Exelon® and Exelon Patch®). There is evidence for the efficacy of these medications in mild to moderate Alzheimer's disease, and some evidence for their use in the advanced stage. Only donepezil is approved for treatment of advanced AD dementia. The use of these drugs in mild cognitive impairment has not shown any effect in a delay of the onset of AD. The most common side effects are nausea and vomiting, both of which are linked to cholinergic excess. These side effects arise in approximately ten to twenty percent of users and are mild to moderate in severity. Less common secondary effects include muscle cramps, decreased heart rate (bradycardia), decreased appetite and weight, and increased gastric acid production. Glutamate is a useful excitatory neurotransmitter of the nervous system, although excessive amounts in the brain can lead to cell death through a process called excitotoxicity, which consists of the overstimulation of glutamate receptors. Excitotoxicity was shown to occur in Alzheimer's disease, but also in other neurological diseases such as Parkinson's disease and multiple sclerosis. Memantine (brand names Akatinol®, Axura®, EbixaAbixa®, Memox® and Namenda®), is a noncompetitive NMDA receptor antagonist, first used as an anti-influenza agent. It acts on the glutamatergic system by blocking NMDA receptors and inhibiting their overstimulation by glutamate. Memantine has been shown to be moderately efficacious in the
treatment of moderate to severe Alzheimer’s disease. Its effects in the initial stages of AD are unknown. Reported adverse events with memantine are infrequent and mild, including hallucinations, confusion, dizziness, headache and fatigue. The combination of memantine and donepezil has been shown to be "of statistically significant, but clinically marginal effectiveness". Antipsychotic drugs are modestly useful in reducing aggression and psychosis in Alzheimer's patients with behavioural problems, but are associated with serious adverse effects, such as cerebrovascular events, movement difficulties or cognitive decline, that do not permit their routine use. When used in the long-term, they have been shown to associate with increased mortality.

1.5. New research’s directions

As of 2008, the safety and efficacy of more than 400 pharmaceutical treatments are being investigated in clinical trials worldwide, and approximately one-fourth of these compounds are in Phase III trials, which is the last step prior to review by regulatory agencies. One area of clinical research is focused on treating the underlying disease pathology. Reduction of amyloid beta levels is a common target of compounds under investigation. Immunotherapy or vaccination for the amyloid protein is one treatment modality under study. Unlike preventative vaccination, the putative therapy would be used to treat people already diagnosed. It is based upon the concept of training the immune system to recognise, attack, and reverse deposition of amyloid, thereby altering the course of the disease. An example of such a vaccine under investigation was ACC-001, although the trials were suspended in 2008. Another similar agent is bapineuzumab, an antibody designed as identical to the naturally-induced anti-amyloid antibody. Other approaches are neuroprotective agents, such as AL-108, and metal-protein interaction attenuation agents, such as PBT2. A TNFα receptor fusion protein, etanercept has showed encouraging results. In 2008, two separate clinical trials showed positive results in modifying the course of disease in mild to moderate AD with methylthioninium chloride (trade name rember), a drug that inhibits tau aggregation, and dimebon, an antihistamine.

1.6. The Multi Target Directed Ligands (MTDL) paradigm

For much of the past century, drug discovery largely has relied on the use of animal models of disease as the first-line screen for testing the compounds produced by medicinal chemists. This in vivo pharmacology approach had the benefit of highlighting compounds that exhibited both desirable pharmacokinetic and pharmacodynamic profiles. A major disadvantage of this approach was that an animal model was essentially a “black box”. When compounds were inactive, it was
unclear whether this was because they no longer interacted with a molecular target or simply because they had failed to reach the site of action. In many cases, the molecular target(s) driving the desired pharmacological effect had not been identified, and inevitably many older generation drugs cross-reacted with targets that caused detrimental side effects. Inexorably, the drug discovery paradigm shifted toward a reductionist “one-target, one-disease” approach that continues to dominate the pharmaceutical industry today. Many successful drugs have emerged from this strategy, and it will no doubt remain dominant for many years to come. However, despite the best efforts of drug discoverers, many diseases remain inadequately treated. There is an increasing readiness to challenge the current paradigm and to consider developing agents that modulate multiple targets simultaneously (polypharmacology), with the aim of enhancing efficacy or improving safety relative to drugs that address only a single target. There are three possible approaches to polypharmacology (Figure 9) \(^{(48,49)}\).

![Figure 9. Three main clinical scenarios for multitarget therapy](image)

Traditionally, clinicians have treated unresponsive patients by combining therapeutic mechanisms with cocktails of drugs. Most frequently, the cocktail is administered in the form of two or more individual tablets (scenario A). However, the benefits of this approach are often compromised by poor patient compliance, particularly for treating asymptomatic diseases such as hypertension. Recently, there has been a move toward multicomponent drugs whereby two or more agents are coformulated in a single tablet to make dosing regimes simpler and thereby to improve patient compliance (scenario B). An alternative strategy is to develop a single chemical entity that is able to modulate multiple targets simultaneously (scenario C). Across the pharmaceutical industry, scenario B is increasingly providing an attractive opportunity for enhancing R&D output. Several multicomponent drugs have recently been launched, such as Caduet (amlodipine/atorvastatin) and Vytorin (ezetimibe/simvastatin) that were approved in 2004 for the treatment of cardiovascular disease. However, there are significant risks involved in the development of multicomponent drugs. There is the commercial uncertainty arising from the risk that clinicians might still prefer
prescribing combinations of existing monotherapies that may offer greater dose flexibility and lower cost treatment, particularly in the case of generic drugs. Differences in the relative rates of metabolism between patients can produce highly complex pharmacokinetic (PK)/pharmacodynamic (PD) relationships for multicomponent drugs, leading to unpredictable variability between patients and calling for extensive and expensive clinical studies. Compared to multicomponent drugs, the multielelgand approach (scenario C) has a profoundly different risk-benefit profile. A downside is that it is significantly more difficult to adjust the ratio of activities at the different targets. However, this increased complexity in the design and optimization of such ligands is shifted toward the earlier and therefore less expensive stages of the drug discovery process. The clinical development of multiple ligands, in terms of the risks and costs involved, is in principle no different from the development of any other single entity. Another advantage is a lower risk of drug-drug interactions compared to cocktails or multicomponent drugs. While many currently marketed drugs are in essence multiple ligands, very few were rationally designed to be so.

1.6.1. Strategies for designing multiple ligands

Conceptually, there are two quite different methods of generating chemical matter with which to commence a DML project: knowledge-based approaches and screening approaches. Knowledge-based approaches rely on existing biological data from old drugs or other historical compounds, from either literature or proprietary company sources. Serendipitous approaches involve the screening of either diverse or focused compound libraries. Typically, diversity-based screening involves the high-throughput screening (HTS) of large, diverse compound collections at one target, and any actives are then triaged on the basis of activity at the second target. In focused screening, compound classes that are already known to provide robust activity at one of the targets of interest, A, are screened for signs of activity at a new target, B. Even if only weak activity is observed for target B, this can provide a useful baseline for increasing that activity by incorporating structural elements from the more potent selective ligands for target B. For both the screening or knowledge-based approaches, the identification of a lead compound with appropriate activity at both targets A and B is unlikely. In reality, a lead generation, or “hit-to-lead” phase, will be required. In one scenario, two compounds that bind with very high selectivity to their respective targets are used as the starting points. To incorporate activity at both targets into a single molecule (“designing in”), structural elements from the two selective ligands are combined. Incorporating a second activity into a compound that has no measurable affinity for that target, while retaining affinity for the original target, is not an easy task. However, many literature examples testify to the fact that it can often be achieved. Perhaps a more tractable scenario is to first identify a compound that has at least
minimal activity at both targets. In this case, the activity at both targets must be modulated to achieve an optimal ratio. In a third scenario, a compound is identified that possesses activity at both targets A and B but also possesses undesirable activity at another target C. The optimization strategy must then focus on “designing out” this cross-reactivity. In some cases, a compound may possess more than one undesired activity, and this will inevitably increase the complexity of the task. By far the most common trajectory for generating leads is to convert a ligand with a single activity into a dual ligand. The conversion of single ligands into triple ligands is more challenging and is much rarer. As the number of targets in a profile increases, the “designing in” philosophy will become less attractive compared to the “designing out” approach starting from a nonselective ligand. The lead candidate will usually lack the optimal ratio of in vitro activities. In lead optimization, the ratio is adjusted so that both targets are modulated to an appropriate degree in vivo at similar plasma or brain concentrations. In most examples, the aim has been to obtain in vitro activities within an order of magnitude of each other, with the assumption that this will lead to similar levels of receptor occupancy in vivo. However, this may not necessarily be the case, and assuming a validated animal model is available, the testing of a lead candidate in vivo may help to clarify the required ratio of in vitro activities. Ultimately, feedback from clinical studies will be required to identify the optimal ratio that can be used to drive the design of “follow-up” compounds. In addition to adjusting the ratio of activities, optimizing wider selectivity against a broad panel of targets is often required. This will be particularly intricate for targets for which a large number of subtypes or isozymes exist. Many publications do not discuss the key issue of global selectivity, so it is frequently difficult to judge whether real selectivity for the disease-relevant targets has been achieved. Again, animal models and subsequent clinical studies will provide essential feedback on the level of cross-reactivity that can be tolerated. A particular challenge in lead optimization is to optimize the PK profile and to obtain physicochemical properties that are consistent with good oral absorption.
Figure 10. Different strategies for DML projects. In the lead generation phase, knowledge-based or screening approaches are used to provide starting compounds that may be highly selective (little or no activity at a second target), moderately selective, or nonselective (with undesired activity). The subsequent strategy involves “designing in”, “balancing”, or “designing out” activities, respectively. As well as balancing the activity ratio, lead optimization provides other major challenges, in particular adsorption, distribution, metabolism, excretion, and toxicity (ADME-T) optimization. In this schematic, activity at targets A and B is desired and activity at target C is undesired. The size of the target letter illustrates the affinity for that target.

1.6.2 Classification of designed multiple ligands

“Conjugates” are DMLs in which the molecular frameworks, which contain the underlying pharmacophore elements for each target, are well separated by a distinct linker group that is not found in either of the selective ligands (Figure 11). Most conjugates contain a metabolically stable linker. “Cleavable conjugates” employ a linker that is designed to be metabolized to release two ligands that interact independently with each target.

As the size of the linker decreases, a point is reached where the frameworks are essentially touching, and these DMLs can be regarded as “fused”. In the most common type of DML, the frameworks are “merged” by taking advantage of commonalities in the structures of the starting compounds. In reality, the degree of merger of the frameworks forms a continuum, with high molecular weight conjugates with lengthy linker groups representing one extreme. At the other extreme are examples where the frameworks, and underlying pharmacophores, are highly merged, giving rise to smaller and simpler molecules.
2. Aim of the thesis

The multi target directed ligand theory (MTDL) is becoming an interesting approach in the field of medicinal chemistry, being particularly used to combat multi-factorial diseases such as Alzheimer’s. Our research group\(^\text{(51-54)}\) has been involved in this topic for several years designing and synthesizing dual binding compounds addressed against this terrible neurodegenerative disease. With the aim of continuing this work, we have developed a series of “oxa” and “aza” heterocycles able to simultaneously improve the cholinergic system through the inhibition of AChE and BuChE and to slow down the self and the AChE-induced Aβ aggregation. Additionally, compounds with β-secretase (BACE) inhibitory activity have been developed with the aim of blocking Aβ formation. The thesis cab be divided in two parts: part A, dealing with compounds having a benzofuran related motif, and part B, with THA related compounds.

PART A

The benzofuran moiety is abundant both in natural and synthetic molecules possessing potent biological activities. Compounds carrying the mentioned motif are known for having a good binding affinity for Aβ\(_{1-42}\), being an isosteric analogue of Thioflavin-T (the dye used to visualize plaques composed of Aβ found in the brains of Alzheimer's disease patients). A series of benzofuran derivatives\(^\text{(55)}\) was synthesized by scientists of Smith Kline Beecham Pharmaceuticals that are able to block Aβ self-aggregation at μM concentration. According to the MTDL approach, we decided to synthesize a series of hybrid compounds (see chart 1A) carrying the benzofuran motif of SKF-64346 linked through an appropriate heptyloxy spacer to a N-methylbenzylamine moiety, that in our previous works\(^\text{(52,54,56-57)}\) resulted good at contacting the anionic binding site within the AChE gorge, inhibiting ACh breakdown by means of cation-π interactions with Trp\(^\text{86}\). Afterwards, we performed SAR studies on our lead compound 4c modifying both the furan ring and the alkoxy chain. In the first case a series of Friedel & Craft acylations at C\(_3\) of the benzofuran ring led to derivatives able to modulate Aβ self-aggregation measured on the Aβ\(_{25-35}\) fragment (11-28, 35-37, 39-40). A methoxy substituent was introduced at C\(_6\) position to evaluate the effect on the anti-aggregatory activity (62) Furthermore, a methylene insertion at C\(_2\) has been made to evaluate whether the coplanarity between the two aromatic rings plays a role towards the biological activity or not (66). In the second case, we have tried to optimize the cholinesterase activity by shortening and lengthening the alkoxy spacer chain (4a-b, 4d-e and 6-10), and moving this latter from the
para to the meta position of the phenyl ring searching for the optimal orientation within the gorge of AChE (4f, 21-28). A carbamic derivative (30) has been synthesized to evaluate a different inhibitory mechanism involving the catalytic triad.

**Chart 1A**

![Chart of compounds](image)

**Fig. 12.** Representation of the rationale underlying part A. Linking two different moieties, each one addressing to a different target, results in the realization of hybrid compounds with combined activity.

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**Tab. 3.** Summary of compounds synthesized within part A with the exception of 66 previously shown in chart 1A

**PART B**

Despite several reports about modifications of the tetrahydroaminoacridine nucleus (THA) and the structure-activity relationships studies (SAR) of this class of compounds, the study of tacrine analogues is still of interest to medicinal chemists dealing with AD research worldwide. In particular, following the MTDL paradigm, heterodimeric structures have led to very interesting compounds designed with the aim of conferring, two or even more biological activities, implicated in the selected pathology to the same molecule. Here follows our contribute to the topic. Part of the work was aimed at connecting through an alkyl tether of a variable length (6-10 carbons) the 1,2,3,4-tetrahydroacridine moiety (known for firmly being stacked against the aromatic rings of Trp⁸⁶ and Tyr³³⁷ at the catalytic site of human AChE) to a morpholinic ring, that could be able to interact with Trp²⁸⁶ at the PAS, inhibiting the AChE-induced Aβ aggregation. Morpholine has been chosen among other aliphatic bases because of its more favorable pharmacokinetic properties (45a-
We kept then the heptyl chain as the optimal spacer and replaced morpholine by different heterocyclic scaffolds previously reported by our research group (azaxanthone\textsuperscript{(58)}, indenoquinoline\textsuperscript{(59-61)} and benzofuran\textsuperscript{(62)} cores) obtaining compounds \textbf{52, 49a, 53a-b}. With the aim of improving the cholinesterase activity, a chlorine atom was introduced in position 6 of the THA ring on the tacrine-indenoquinoline hybrid compound \textbf{49b}, furthermore to selectively address the 6-Cl-THA moiety towards the catalytic site, an analogue of \textbf{49b} was synthesized carrying a further chlorine atom in position 7 of the indenoquinoline ring so that, because of the steric hindrance, this latter shouldn’t penetrate within the gorge of the enzyme (\textbf{49c}). An indenoquinolinic homodimer (\textbf{49d}) was synthesized as well.

\textbf{Fig. 13.} Representation of the rationale underlying part B. \textbf{Tab. 4.} Summary of compounds synthesized within part B.
3. Chemistry

PART A

The reported benzofuran derivatives were prepared by conventional synthetic methods. In scheme 1A, the synthesis of compounds 6-28 and 30 is illustrated. The first step is a modified intramolecular Wittig reaction leading to the formation of the benzofuran rings in moderate-good yields (1a-b). Demethylation with BBr₃ proved to be very effective on this substrate affording, in almost quantitative yield, the corresponding phenols (2a-b), that subsequently underwent alkylation with di-alo-alkyl-chains of various length using K₂CO₃ as the base and affording alo-alkyloxy-derivatives in good to moderate yields (3a-f). Condensation with N-benzylmethylamine by refluxing in toluene afforded compounds 4a-f, in low to moderate yields. A series of Friedel & Craft acylations, with selected acylchlorides, occurring at position 3 of the furanic ring, afforded compounds 6-20, in generally poor yields. Performing F&C acylations prior than condensation with N-benzylmethylamine proved to be a better strategy affording compounds 21-28 in higher overall yields. The synthesis of 30 required condensation of {2-[4-(7-Bromoheptyloxy)phenyl] benzofuran-3-yl}-p-tolylmethanone with 3-Methyl amino methylphenol followed by carbamoylation using NaH and Methyl isocianate in DCM. Scheme 2A illustrates the synthesis of compounds 35-37 and 39-40. In these cases 4c represents the key intermediate that underwent diversification by means of opportune F&C acylations, followed by condensations with selected amines, such as Et₃N and Morpholine. Scheme 3A illustrates the synthesis of compound 62. In particular, it is necessary to first prepare the non commercial (2-Hydroxy-5-methoxy-benzyl)-triphenylphosphonium bromide (55) by reducing 2-Hydroxy-5-methoxy benzaldehyde to the corresponding benzylic alcohol (54) with NaBH₄, and then treating 54 with Triphenylphosphonium bromide. 55 undergoes intramolecular Wittig reaction with 4-Benzoxycybenzoyle chloride (58). Removal of the protective group is conducted by hydrogenolysis leaving the phenolic function (60) available for the further sequence of reactions similar to those already described for the synthesis of 4c. Scheme 4A illustrates the synthesis of 66 which follows the same route for preparing 4c with the only difference of using 4-Methoxyphenylacetyl chloride as the starting material.
Reagents and conditions: a) Et$_3$N, Toluene, reflux; b) BBr$_3$, DCM, r.t.; c) X(CH$_2$)$_n$X, K$_2$CO$_3$, Acetone, reflux; d) N-benzylmethylamine, Toluene, reflux; e) selected acylchloride, SnCl$_4$, DCM, r.t.; f) 2-[4-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl]-p-tolylmethanone, 3-Methylamino methyl phenol, Toluene, reflux; g) Methylisocianate, NaH, DCM, r.t.
Reagents and conditions: a) Br(CH₂)₂Cl, K₂CO₃, Acetone, reflux; b) NaOH, EtOH, reflux; c) SOCl₂, reflux; d) selected acylchloride, SnCl₄, DCM, r.t.; e) selected amine, Toluene, reflux;
Reagents and conditions: a) NaBH₄, EtOH, r.t.; b) PPh₃-HBr, acetonitrile, reflux; c) Et₃N, Toluene, reflux; d) H₂, Pd/C, THF, r.t.; e) 1,7-Dibromoheptane, K₂CO₃, Acetone, reflux; f) N-methylbenzylamine, Toluene, reflux. i) BnBr, K₂CO₃, Acetone, reflux; ii) NaOH, EtOH, reflux; iii) SOCl₂, reflux.
Reagents and conditions: a) Et₃N, Toluene, reflux; b) BBr₃, DCM, r.t.; c) Br(CH₂)₇Br, K₂CO₃, Acetone, reflux; d) N-benzylmethylamine, Toluene, reflux.
The reported tacrine derivatives were prepared by conventional synthetic methods. In scheme 1B, dibromoalkyl-chains of various length (6 to 10 carbons) were turned to primary amines using Gabriel’s procedure. In particular, after creating the phtalic end, 41a-e bromoalkyl derivatives were reacted with Morpholine, affording 42a-e subsequently converted in 43a-e using hydrazine. Aromatic substitution of 43a-e with 44, previously obtained by reacting anthranilic acid with cyclohexanone in POCl₃, afforded compounds 45a-e. In scheme 2B, the synthesis of compounds 49a-d started by building intermediates 46a-b and 47a-b, prepared by condensation of the selected anthranilonitrile with cyclohexanone or indanone, respectively in the presence of ZnCl₂. These latter were reacted with 1,7-dibromoheptane in DMSO made basic by KOH affording 48a-b, subsequently condensed with 46a-b and 47a to give the desired compounds 49a-d. Synthesis of 52 was accomplished starting from azaxanthone 50 which undergoes alkylation with 1,7-dibromoheptane giving the bromoheptyloxy-derivative (51), subsequently condensed with 46a using a phase transfer catalyst in an heterogeneous medium made of DCM and 50% NaOH solution. Finally, compounds 53a-b were obtained by reacting intermediates 3c and 3f with 46b at room temperature in DMSO, using KOH as the base.
Reagents and conditions: a) DMF, reflux; b) Morpholine, Toluene, reflux; c) Hydrazine monohydrate, EtOH, reflux, then HCl; d) PhOH, 130 °C; e) POCl₃, reflux.
Reagents and conditions: a) ZnCl$_2$, 130 °C; b) Br(CH$_2$)$_7$Br, KOH, DMSO, r.t.; c) 46a-b or 47a KOH, DMSO, r.t.; d) Br(CH$_2$)$_7$Br, K$_2$CO$_3$, Acetone, reflux; e) 46a, tetra-$n$-butyl ammonium hydrogen sulphate, NaOH/DCM 50%, r.t.; f) 46b, KOH, DMSO, r.t.
4. Results and discussion

In this section, available biological data are shown and discussed. Parts A and B deal respectively with benzofuran and tacrine derivatives. A detailed description of analysis procedures used for testing the activity of our compounds is reported at the end of the experimental section.

PART A

The inhibitory activities of the newly synthesized compounds against both cholinesterases were studied using the method of Ellman\(^{(33)}\) to determine the rate of acetylthiocholine or butyrylthiocholine hydrolysis in the presence of the inhibitor and are reported in Table 5, expressed as IC\(_{50}\) values. The inhibitory activity of A\(_\beta\) fibril formation was studied with an original in vitro assay that uses UV-vis measurements and electron microscopy\(^{(34)}\) (Figure 14). The A\(_\beta\)\(_{25-35}\) amino acid peptide, which preserves the properties of neurotoxicity and aggregation, was used.\(^{(35,36)}\) For the compounds exhibiting an inhibitory activity at least equal to that of curcumin (compound reported to have antiamyloidogenic properties\(^{(37)}\)), IC\(_{50}\) values were calculated as reported in Table 5. The neuroprotective effects of the most interesting A\(_\beta\) antiaggregating compounds were also determined against the A\(_\beta\)\(_{25-35}\) peptide induced toxicity in human neuronal line SH-SY5Y cells using a colorimetric MTT assay \(^{(38,39)}\) (Table 6 and Figure 15).

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Tab. 5. Available data within the benzofuran derivatives series

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Rivastigmine  
Curcumin

Fig. 14. Aβ$_{25-35}$ fibril inhibition compared to that of curcumin. The mean ± SD values from three independent experiments are shown.
The anticholinesterase activity of the new molecules proved to be generally better toward BuChE than AChE. In detail, 11 and 14 showed the best AChE inhibitory activity of the series (17.4 and 10.5 μM, respectively), still keeping a fairly good BuChE inhibition (1.83 and 1.82 μM, respectively), probably as a consequence of a higher affinity. There are differences in terms of topology between AChE and BuChE, since in this latter enzyme Lys\textsuperscript{286} and Val\textsuperscript{288} line the gorge, compared to the large Phe of the corresponding residues of AChE, and this could allow bulky compounds to better fit inside the gorge of BuChE and to stabilize its occupancy probably by means of hydrophobic interactions. In particular 4c, the lead compound, was found to possess a good BuChE inhibitory activity (0.28 μM), about 100-fold higher than its activity toward AChE. Several lines of evidence indicate that BuChE might be a co-regulator of the activity of the neurotransmitter ACh\textsuperscript{40} and that it might be important to inhibit this enzyme in the treatment of AD. Several drugs that proved to be selective BuChE inhibitors have been evaluated; for example, rivastigmine showed clinical efficacy without remarkable side effects.\textsuperscript{41} It is intriguing that specific BuChE inhibitors not only improve cognition, presumably through an increase in acetylcholine concentration, but also reduce levels of APP, which is the source of Aβ peptide, the main component of plaques in AD. The effect of these compounds on APP seems to be independent of their ability to inhibit BuChE enzymatic activity, and it has been suggested that it involves interactions with interleukin-1, a proinflammatory molecule that has also been implicated in the pathogenesis of AD.\textsuperscript{42} Compounds bearing a second amine moiety, such as 35-37 and 39-40, were not able to improve AChE inhibition. Compound 30 bearing a methylcarbamic group (acting as a pseudo-irreversible inhibitor), showed an higher activity (IC\textsubscript{50} = 0.34 ± 0.029 μM) compared to the [Image] **Fig.15.** Compound 12 protects human neuronal SH-SY5Y cells Aβ\textsubscript{25-35} peptide induced toxicity. The neuronal viability was determined by the MTT assay (as described in Supporting Information) after 3 h of incubation with 10 μM Aβ\textsubscript{25-35} peptide in the presence or absence of various concentrations of 3. The results are expressed as percentage of control cells. **Tab.6.** Neuroprotective activity of 12 on human neuronal cells SH-SY5Y
reference rivastigmine (IC$_{50}$ = 3.03 ± 0.21 μM) toward AChE (Data for BuChE not available). Compared to 4c, compounds with shorter or longer spacer-chains such as 4a-b and 4d-e showed no activity toward AChE (IC$_{50}$ > 10 μM), while 4b and 4d kept a fairly good activity toward BuChE (IC$_{50}$ = 6.60±0.23 μM and 7.47±0.34 respectively). Moving the heptyloxy chain from para to meta position of the phenyl ring (4f) lowered the activity toward BuChE, loosing AChE inhibition compared to our lead compound. Regarding Aβ fibril inhibition, 4c showed a higher potency compared to that of the standard curcumin (IC$_{50}$ = 7 and 10 μM, respectively). Acylation of 4c with acetylchloride led to 11, whose activity was surprisingly proaggregatory, whereas acylation of 4c with benzoyl chloride retained the activity (12, IC$_{50}$ = 12.5 μM), suggesting that the introduction of an aryl moiety in position 3 of the benzofuran ring was tolerated. Modifications of 12 by the introduction of a methyl group in the meta or para position on the aryl group led respectively to 13 and 14 and resulted in a lower activity for 13, whereas 14 retained the potency of the unsubstituted 12. In the opposite direction, the introduction of a methoxy group in the meta position (15) resulted in a slightly lower but still remarkable activity, though moving the methoxy group from the meta to the para position (16) was considerably detrimental for the activity. Moreover, the acylation of 4c with 3,4-dimethoxybenzoyl chloride led to 16, which surprisingly proved to have remarkable proaggregating activity. Compounds 30, 35-37 and 39-40 proved to weakly inhibit amyloid aggregation. The chain length turned out to play a role too, since the propyloxy derivative 4a had a poor inhibition, while moving through the homologus series the activity rose. Pentoxy (4b) and octyloxy (4d) derivatives showed their selves being equactive with the standard curcumine, while nonyloxy derivative was more active (4e, IC$_{50}$ = 5.5 μM). The most potent inhibitor of the series (4f, IC$_{50}$ = 3.9 μM ) was obtained by moving the heptyloxy chain to the meta position. The assembly of Aβ aggregates, derived from Aβ oligomers, into fibrils is toxic to neurons. The formation of Aβ is related to protein misfolding, since the conformational transition to β-sheet leads to a faster formation of aggregates.\(^{43}\) Thus, compounds that are able to slow or block the amyloid polymerization process could be considered potential drugs for inhibition of AD progression. During incubation of Aβ in the presence of 4c, only small bulk aggregates were visible and no characteristic Aβ fibrils were observed in the electron micrographs (Figure 16). Interestingly, 12 also showed a marked neuroprotective effect against Aβ25-35 peptide induced neurotoxicity (Tab. 6). As reported in Figure 15,
treatment of SH-SY5Y cells with 12 at 10 and 30 μM significantly reduced the neuronal viability loss evoked by Aβ<sub>25-35</sub> peptide, in a dose-dependent manner, with a maximum of inhibition of 63%. Since the same trend was not observed with 4c, these results prove that the modification of 4c by introduction of an aryl moiety in the benzofuran ring is crucial for the observed neuroprotective effects. Aβ<sub>25-35</sub> peptide contains a number of hydrophobic residues (i.e., Ile<sup>31</sup>, Ile<sup>32</sup>, and Met<sup>35</sup>) that are critical for neurotoxicity and aggregation processes.<sup>44,45</sup> In this regard, recent studies have suggested that unaggregated Aβ<sub>25-35</sub> and Aβ<sub>31-35</sub> peptides could initiate a cascade of events leading to neurotoxicity solely after their internalization within the neuronal cells.<sup>46</sup> Neuroprotective effects of 12 against Aβ<sub>25-35</sub> toxicity could be ascribed to its hydrophobic properties (compare 12 with 4c: 8.00 vs 6.80 log <i>P</i>) and its ability to block the interaction of Aβ<sub>25-35</sub> with the lipid bilayer of the neuronal plasma membrane. It is therefore reasonable to predict that there are adequate opportunities for functionally important hydrophobic interactions between 12 and the Aβ<sub>25-35</sub> peptide. Thus, these preliminary results could encourage further studies to elucidate the neuroprotective mechanisms of 12.

**PART B**

The inhibitory activities of the newly synthesized compounds against both cholinesterases were studied using the method of Ellman<sup>33</sup> to determine the rate of acetylthiocholine or butyrylthiocholine hydrolysis in the presence of the inhibitor and are reported in Table 7, expressed as IC<sub>50</sub> values together with data from BACE 1 inhibition obtained by spectrofluorometric analyses. Docking simulations were carried out by means of the GOLD software 6 (v.3.0.1) and using the crystal structure of human AChE (figure 17).

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<th>BACE IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
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Tab. 7. Available data for the tacrine derivatives series

Fig. 17. Docking simulation of compound 49c within the human AChE gorge.

With regard to the AChE inhibition, all the new molecules (with the exception of 52) proved to be more active compared to the standard tacrine. Compounds 49b and 49c were the most active of the series at AChE inhibition (IC$_{50}$ = 1.22±0.13 μM and 4.59±0.87 μM respectively). In light of this increased activity, a docking study of 49c was performed, (figure 17) pointing out the existence of favorable interactions within the hAChE gorge. Particularly, the indenoquinolinic moiety is suggested to establish interactions with Trp$^{286}$ and Tyr$^{72}$ in a “sandwich-like” manner. The tacrine motif, addressed toward the central anionic site (CAS), is shown to interact with Trp$^{86}$ by means of
a π–π interaction. The chlorine atom at position 6 is meant to fill a lipophilic pocket, close to the CAS (central anionic site), via hydrophobic interactions. All the compounds showed a good inhibition of BuChE with generally poor selectivity with regard to BuChE/AChE ratio. Rivastigmine also inhibits BuChE, providing dual AChE and BuChE inhibition. It has been suggested that dual inhibition may afford several advantages, including greater and broader symptomatic effects, particular behavioural benefits and the absence of AChE upregulation. These benefits are likely to increase with time, because, as AD progresses, AChE activity decreases by up to 45%, while BuChE activity increases by 40-90%. The gradual shift in the enzyme responsible for degrading ACh from AChE to BuChE during AD progression could be responsible for the inefficacy of AChE inhibitors, that do not achieve dual inhibition. Consequently, there is a rationale for switching from a selective AChE inhibitor to an inhibitor of both AChE and BuChE\(^{50}\). Furthermore compounds 49a and 49b were tested for the inhibition of BACE1 and 49b showed a remarkable activity (78.16% of inhibition at 4.75 μM with \(IC_{50} = 0.97 \mu M\)).

### 5. Conclusions

In conclusion, because of the multifactorial nature of AD, molecules that modulate the activity of a single protein target are unable to significantly modify the progression of the disease. With regard to part A of the thesis, we have reported a new series of hybrid molecules based on the frame works of our AChE/BuChE inhibitors and of SKF-64346. Promising hits proved to be 4c, with very good inhibitory activity of BuChE and Aβ aggregation, and 12, which turned out to inhibit AChE/ BuChE enzymes and showed remarkable inhibition of Aβ aggregation and Aβ neurotoxicity. Compounds 4e and 4f were able to potently inhibit Aβ aggregation while 17-20 are expected to possess a good inhibition of Aβ neurotoxicity because of their bigger lipophilicity which probably makes these compounds more prone to penetrate through cellular barriers. With regard to part B a series of heterodimers structurally related to tacrine has been developed which possesses a very good activity at cholinesterase inhibition. Compounds 49b and 49c turned out to be very potent reversible inhibitors of AChE. Particularly, 49c showed a good inhibition of BACE1 and a mode of docking within AChE gorge which is going to probably give the compound the ability of blocking AChE-induced β-amyloid aggregation by interactions with the PAS. These evidences make 49c a very promising lead compound for the development of new potent MTDL’s for the treatment of AD.
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 and $^{13}$C NMR experiments were recorded in CDCl$_3$, unless differently indicated, on Varian VXR 300 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), m (multiplet) or br (broad). Wherever analyses are only indicated with elements symbols, analytical results obtained for those elements are within 0.4 % of the theoretical values. 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.

**2-(4-Methoxyphenyl)benzofuran (1a).** A stirred suspension of 4-Metoxybenzoylchloride (5 g, 29 mmol), 2-Hydroxybenzyltriphenyl phosphonium bromide (12 g, 27 mmol) and Et$_3$N (11.1 mL, 80 mmol) in toluene (125 mL) was refluxed for 10 hours. The reaction mixture was filtered under vacuum and evaporated to dryness. EtOH (150 mL) was added to the oily residue and the flask kept in the freezer overnight. A yellowish solid formed which was filtered off and purified by flash chromatography on silica gel (Petroleum Ether/Ethyl Acetate 95:5), affording 1a as a white solid (3.58 g, 55%). mp 153 °C. $^1$H NMR (CDCl$_3$) $\delta$: 3.85 (s, 3H), 6.86 (s, 1H), 6.92-7.01 (m, 2H), 7.18-7.28 (m, 2H), 7.32-7.60 (m, 2H), 7.75-7.85 (m, 2H).

**2-(3-Methoxyphenyl)benzofuran (1b).** A stirred suspension of 3-Metoxybenzoylchloride (5 g, 29 mmol), 2-Hydroxybenzyltriphenyl phosphonium bromide (12 g, 27 mmol) and Et$_3$N (11.1 mL, 80 mmol) in Toluene (125 mL), was refluxed for 10 hours. The reaction mixture was filtered under vacuum and evaporated to dryness. EtOH (150 mL) was added to the oily residue and the flask kept in the freezer overnight. A yellowish solid formed which was filtered off and purified by flash chromatography on silica gel (Petroleum Ether/Ethyl Acetate 96:4), affording 1b as a colorless oil (3.00 g, 50%). $^1$H NMR (CDCl$_3$) $\delta$: 3.87 (s, 3H), 6.82-6.95 (m, 1H), 6.92-7.01 (m, 2H), 7.18-7.28 (m, 2H), 7.32-7.60 (m, 2H), 7.75-7.85 (m, 2H).

**4-Benzofuran-2-yl-phenol (2a).** To a cold (0 °C) solution of 1a (3.50 g, 16 mmol) in DCM anhydrous (300 mL), BBr$_3$ (22 mL of 1M solution in DCM) was added slowly. The resulting
mixture was stirred overnight at room temperature. The reaction was quenched with ice/water and stirred for 30 minutes. The organic layer was washed with water (3 x 50 mL), then with brine (3 x 25 mL) and dried over Na$_2$SO$_4$. The solvent was removed affording 2a (3.10 g, 93%) as a white solid. mp 142 °C. $^1$H NMR (CDCl$_3$) $\delta$: 4.87 (br, 1H), 6.86-6.94 (m, 3H), 7.21-7.25 (m, 2H), 7.42-7.56 (m, 2H), 7.69-7.84 (m, 2H). ES-MS m/z: 209 (M – 1).

3-Benzofuran-2-yl-phenol (2b). To a cold (0 °C) solution of 1b (3 g, 13 mmol) in DCM anhydrous (250 mL), BBr$_3$ (18 mL of 1M solution in DCM) was added slowly. The resulting mixture was stirred overnight at room temperature. The reaction was quenched with ice/water and stirred for 30 minutes. The organic layer was washed with water (3 x 50 mL), then with brine (3 x 25 mL) and dried over Na$_2$SO$_4$. The solvent was removed by rotary evaporation affording 2b (2.65 g, 97%) as a white solid. m.p. 123 °C. $^1$H NMR (CDCl$_3$) $\delta$: 4.92 (br, 1H), 6.83-6.97 (m, 3H), 7.18-7.28 (m, 2H), 7.43-7.58 (m, 2H), 7.70-7.79 (m, 2H). ES-MS m/z: 209 (M – 1).

2-[4-(3-Chloropropoxy)phenyl]benzofuran (3a). A stirred mixture of 2a (1 g, 4.7 mmol), 1-Bromo-3-Chloropropane (0.94 mL, 9.5 mmol) and K$_2$CO$_3$ (1.2 g) was refluxed in acetone (100 mL) for 20 hours. The suspension was hot filtered and the solvent was removed. After adding Petroleum Ether, the residue was kept in the freezer overnight and the white solid that formed was filtered off, affording 3a (0.93 g, 69 %). mp 137-138 °C. $^1$H NMR (CDCl$_3$) $\delta$: 2.23-2.31 (quintet, J = 6.0 Hz, 2H), 3.77-3.80 (m, 2H), 4.17-4.22 (t, J = 4.5 Hz, 2H), 6.87 (s, 1H), 6.94-6.98 (d, J = 12 Hz, 2H), 7.18-7.32 (m, 2H), 7.47-7.58 (m, 2H), 7.76-7.80 (d, J = 12 Hz, 2H).

2-[4-(5-Chloropentyloxy)phenyl]benzofuran (3b). Using the previous procedure and starting from 2a (0.34 g, 1.6 mmol) and 1-Bromo-5-Chloropentane (0.42 mL, 3.2 mmol), 3b (0.35 g, 69 %) was obtained as a white solid. mp 115 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.60-1.71 (m, 2H), 1.83-1.90 (m, 4H), 3.56-3.61 (t, $J$ = 6.3 Hz, 2H), 4.01-4.05 (t, $J$ = 6.0 Hz, 2H), 6.88 (s, 1H), 6.95-6.98 (d, $J$ = 8.4 Hz, 2H), 7.21-7.26 (m, 2H), 7.48-7.56 (m, 2H), 7.77-7.80 (d, $J$ = 12 Hz, 2H).

2-[4-(7-Bromoheptyloxy)phenyl]benzofuran (3c). Using the previous procedure and starting from 2a (0.35 g, 1.6 mmol) and 1,7-Dibromoheptane (0.57 mL, 3.3 mmol), 3c (0.34 g, 58 %) was obtained as a white solid. mp 92 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.25-1.60 (m, 6H), 1.71-1.98 (m, 4H), 3.39-3.44 (t, $J$ = 5.0 Hz, 2H), 3.96-4.05 (t, $J$ = 9.0 Hz, 2H), 6.87 (s, 1H), 6.90-6.93 (d, $J$ = 8.4 Hz, 2H), 7.23-7.29 (m, 2H), 7.49-7.58 (m, 2H), 7.75-7.78 (d, $J$ = 12 Hz, 2H).

2-[4-(8-Bromooctyloxy)phenyl]benzofuran (3d). Using the previous procedure and starting from 2a (0.45 g, 2.1 mmol) and 1,8-Dibromoocctane (0.79 mL, 4.3 mmol), 3d (0.51 g, 61 %) was
obtained as a white solid. mp 112 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.37-1.58 (m, 8H), 1.76-1.92 (m, 4H), 3.39-3.44 (t, $J = 5.0$ Hz, 2H), 3.98-4.02 (t, $J = 6.6$ Hz, 2H), 6.88 (s, 1H), 6.95-6.97 (d, $J = 6.9$ Hz, 2H), 7.20-7.25 (m, 2H), 7.48-7.56 (m, 2H), 7.71-7.80 (dd, $J_1 = 2.1$ Hz; $J_2 = 6.9$ Hz, 2H).

2-[4-(9-Bromonyloxy)phenyl]benzofuran (3e). Using the previous procedure and starting from 2a (0.57 g, 2.7 mmol) and 1,9-Dibromononane (1.1 mL, 5.4 mmol), 3e (0.82 g, 73 %) was obtained as a white solid. mp 108 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.34-1.56 (m, 10H), 1.76-1.95 (m, 4H), 3.39-3.44 (t, $J = 5.0$ Hz, 2H), 3.98-4.02 (t, $J = 6.6$ Hz, 2H), 6.88 (s, 1H), 6.95-6.97 (d, $J = 6.9$ Hz, 2H), 7.20-7.25 (m, 2H), 7.48-7.56 (m, 2H), 7.71-7.80 (dd, $J_1 = 2.1$ Hz; $J_2 = 6.9$ Hz, 2H).

2-[3-(7-Bromoheptyloxy)phenyl]benzofuran (3f). Using the previous procedure and starting from 2b (2.65 g, 13 mmol) and 1,7-Dibromoheptane (4.3 mL, 25 mmol), 3f (4 g, 81%) was obtained as a white solid. mp 145 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.23-1.58 (m, 6H), 1.69-1.98 (m, 4H), 3.37-3.42 (t, $J = 5.0$ Hz, 2H), 3.97-4.06 (t, $J = 9.0$ Hz, 2H), 6.89 (s, 1H), 6.92-6.95 (d, $J = 8.4$ Hz, 2H), 7.24-7.31 (m, 2H), 7.50-7.56 (m, 2H), 7.78-7.81 (d, $J = 12$ Hz, 2H).

[3-(4-Benzofuran-2-yl-phenoxy)propyl]benzylmethylamine (4a). A stirred solution of 3a (0.9 g, 3.1 mmol) and N-Benzylmethylamine (0.80 mL, 6.2 mmol) in toluene (100 mL) was refluxed in the presence of catalytic amount of NaI for 20 hours. The mixture was washed with water (3 x 25 mL) and the organic layer was dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (Toluene/Acetone 96:4), affording 4a as a yellowish solid (1.1 g, 95%). mp 84 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.96-2.04 (quintet, $J = 6.0$ Hz, 2H), 2.23 (s, 3H), 2.52-2.60 (t, $J = 12.0$ Hz, 2H), 3.51 (s, 2H), 4.05-4.13 (t, $J = 12.0$ Hz, 2H), 6.84 (s, 1H), 6.95-6.97 (d, $J = 6.9$ Hz, 2H), 7.15-7.30 (m, 7H), 7.45-7.55 (m, 2H), 7.75-7.80 (m, 2H). ES-MS m/z: 372 (M + 1).

[5-(4-Benzofuran-2-yl-phenoxy)pentyl]benzylmethylamine (4b). Using the previous procedure and starting from 3b (0.34 g, 1.6 mmol), 4b was obtained as a yellowish solid (0.26 g, 40%). mp 57 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.47-1.59 (m, 4H), 1.75-1.81 (m, 2H), 2.18 (s, 3H), 2.34-2.41 (t, $J = 7.4$ Hz, 2H), 3.47 (s, 2H), 3.92-3.99 (t, $J = 6.2$ Hz, 2H), 6.84 (s, 1H), 6.90-6.95 (d, $J = 9.2$ Hz, 2H), 7.18-7.31 (m, 7H), 7.46-7.51 (m, 2H), 7.73-7.78 (d, $J = 8.8$ Hz, 2H). ES-MS m/z: 400 (M + 1).

[7-(4-Benzofuran-2-yl-phenoxy)heptyl]benzylmethylamine (4c). Using the previous procedure and starting from 3c (0.49 g, 1.3 mmol), 4c was obtained as a yellowish solid (0.51 g, 92%). mp 57 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.27-1.59 (m, 8H), 1.75-1.85 (m, 2H), 2.19 (s, 3H), 2.32-2.39 (t, $J = 7.4$ Hz, 2H). ES-MS m/z: 428 (M + 1).
Hz, 2H), 3.42 (s, 2H), 3.94-4.01 (t, J = 6.2 Hz, 2H), 6.84 (s, 1H), 6.91-6.96 (d, J = 9.2 Hz, 2H), 7.18-7.31 (m, 7H), 7.46-7.51 (m, 2H), 7.73-7.78 (d, J = 8.8 Hz, 2H). ES-MS m/z: 428 (M + 1).

[8-(4-Benzofuran-2-yl-phenoxy)octyl]benzylmethylamine (4d). Using the previous procedure and starting from 3d (0.51 g, 1.3 mmol), 4d was obtained as a white solid (0.2 g, 36%). mp 69-70 °C. 1H NMR (CDCl3) δ: 1.32-1.58 (m, 10H), 1.75-1.85 (m, 2H), 2.18 (s, 3H), 2.32-2.39 (t, J = 7.4 Hz, 2H), 3.47 (s, 2H), 3.97-4.03 (t, J = 6.2 Hz, 2H), 6.84 (s, 1H), 6.94-6.99 (d, J = 9.2 Hz, 2H), 7.19-7.32 (m, 7H), 7.47-7.54 (m, 2H), 7.76-7.81 (d, J = 8.8 Hz, 2H). ES-MS m/z: 442 (M + 1).

[9-(4-Benzofuran-2-yl-phenoxy)nonyl]benzylmethylamine (4e). Using the previous procedure and starting from 3e (0.82 g, 2.0 mmol), 4e was obtained as a white solid (0.53 g, 58%). mp 79 °C. 1H NMR (CDCl3) δ: 1.34-1.53 (m, 12H), 1.75-1.86 (m, 2H), 2.22 (s, 3H), 2.37-2.42 (t, J = 7.8 Hz, 2H), 3.51 (s, 2H), 4.02-4.06 (t, J = 6.6 Hz, 2H), 6.91 (s, 1H), 6.94-6.99 (d, J = 9.2 Hz, 2H), 7.24-7.36 (m, 7H), 7.52-7.58 (m, 2H), 7.80-7.84 (dd, J1 = 2.4 Hz; J2 = 6.9 Hz, 2H). ES-MS m/z: 456 (M + 1).

[7-(3-Benzofuran-2-yl-phenoxy)heptyl]benzylmethylamine (4f). Using the previous procedure and starting from 3f (4 g, 10 mmol), 4f was obtained as a yellowish oil (0.84 g, 20%). 1H NMR (CDCl3) δ: 1.20-1.59 (m, 8H), 1.69-1.87 (m, 2H), 2.17 (s, 3H), 2.32-2.39 (t, J = 7.4 Hz, 2H), 3.42 (s, 2H), 3.94-4.01 (t, J = 6.2 Hz, 2H), 6.84 (s, 1H), 6.91-6.96 (d, J = 9.2 Hz, 2H), 7.18-7.31 (m, 7H), 7.46-7.51 (m, 2H), 7.73-7.78 (d, J = 8.8 Hz, 2H). ES-MS m/z: 428 (M + 1).

Compounds 5 g-r were obtained according to the following general procedure:

SnCl4 (1.2 eq.) was added dropwise to a mixture of 3f (1 eq.) and the selected acylchloride (1.2 eq.) in dry dichloromethane and the resulting solution was stirred at room temperature overnight. The reaction was quenched with ice/water and stirred for 1 h. The organic layer was separated and the aqueous one was extracted with dichloromethane. The combined organic extracts were dried (Na2SO4), filtered and concentrated under reduced pressure to afford the desired 3-acylated benzofurans (5 g-r), generally as honey colored oils purified by flash chromatography.

{2-[3-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl}phenylmethanone (5g). (0.09 g, 28%). (Petroleum ether/Ethyl acetate 98:2). 1H NMR (CDCl3) δ: 1.25-1.58 (m, 6H), 1.62-1.95 (m, 4H), 3.35-3.42 (t, J = 6.6 Hz, 2H), 3.80-3.86 (t, J = 6.2 Hz, 2H), 6.83-6.87 (dt, J1 = 1.0 Hz, J2 = 7.6 Hz, 1H), 7.11-7.40 (m, 7H), 7.47-7.58 (m, 2H), 7.76-7.80 (d, J = 8.0 Hz, 2H).
{2-[3-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl-p-tolymethanone (5h). (0.09 g, 27%). (Petroleum ether/Ethyl acetate 98:2). $^1$H NMR (CDCl$_3$) $\delta$: 1.25-1.58 (m, 6H), 1.62-1.95 (m, 4H), 2.36 (s, 3H), 3.37-3.44 (t, $J = 6.6$ Hz, 2H), 3.79-3.85 (t, $J = 6.2$ Hz, 2H), 6.82-6.86 (dt, $J_1 = 1.0$ Hz, $J_2 = 7.6$ Hz, 1H), 7.12-7.38 (m, 7H), 7.48-7.59 (m, 2H), 7.75-7.79 (d, $J = 8.0$ Hz, 2H).

{2-[3-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl-m-tolymethanone (5i). (0.06 g, 18%). (Petroleum ether/Ethyl acetate 98:2). $^1$H NMR (CDCl$_3$): $\delta$: 1.41-1.59 (m, 6H), 1.70-1.95 (m, 4H), 2.28 (s, 3H), 3.38-3.45 (t, $J = 6.6$ Hz, 2H), 3.78-3.84 (t, $J = 6.6$ Hz, 2H), 6.81-6.87 (m, 1H), 7.14-7.40 (m, 7H), 7.56-7.67 (m, 4H).

{2-[3-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl-3-methoxyphenylmethanone (5j). (0.07 g, 21%). (Petroleum ether/Ethyl acetate 98:2). $^1$H NMR (CDCl$_3$): $\delta$: 1.40-1.58 (m, 6H), 1.60-1.95 (m, 4H), 3.38-3.45 (t, $J = 6.6$ Hz, 2H), 3.73 (s, 3H), 3.78-3.84 (t, $J = 6.2$ Hz, 2H), 6.82-6.88 (m, 1H), 7.01-7.06 (m, 1H), 7.15-7.41 (m, 8H), 7.56-7.62 (m, 2H).

{2-[3-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl-2-methoxyphenylmethanone (5k). (0.2 g, 59%). (Petroleum ether/Ethyl acetate 94:6). $^1$H NMR (CDCl$_3$) $\delta$: 1.42-1.58 (m, 6H), 1.62-1.98 (m, 4H), 3.38-3.45 (t, $J = 6.6$ Hz, 2H), 3.55 (s, 3H), 3.81-3.87 (t, $J = 6.2$ Hz, 2H), 6.67-6.71 (d, $J = 8.4$ Hz, 1H), 6.84-6.92 (m, 2H), 7.12-7.38 (m, 6H), 7.45-7.56 (m, 2H), 7.73-7.78 (m, 1H).

{2-[3-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl-3,5-dimethoxyphenylmethanone (5l). (0.07 g, 16%). (Petroleum ether/Ethyl acetate 90:10). $^1$H NMR (CDCl$_3$) $\delta$: 1.41-1.58 (m, 6H), 1.62-1.95 (m, 4H), 3.38-3.45 (t, $J = 6.6$ Hz, 2H), 3.68 (s, 6H), 3.78-3.84 (t, $J = 6.2$ Hz, 2H), 6.56-6.58 (t, $J = 2.2$ Hz, 1H), 6.83-6.90 (m, 1H), 6.98 (s, 1H), 6.99 (s, 1H), 7.13-7.41 (m, 5H), 7.56-7.66 (m, 2H).

{2-[3-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl-3,4-dimethoxyphenylmethanone (5m). (0.13 g, 36%). (Petroleum ether/Ethyl acetate 90:10). $^1$H NMR (CDCl$_3$): $\delta$: 1.30-1.56 (m, 6H), 1.60-1.98 (m, 4H), 3.37-3.44 (t, $J = 6.6$ Hz, 2H), 3.80-3.90 (m, 8H), 6.72-6.88 (m, 2H), 7.16-7.59 (m, 9H).

{2-[3-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl-2,3-dimethoxyphenylmethanone (5n). (0.17 g, 47%). (Petroleum ether/Ethyl acetate 90:10). $^1$H NMR (CDCl$_3$) $\delta$: 1.42-1.51 (m, 6H), 1.72-1.92 (m, 4H), 3.37-3.44 (t, $J = 6.6$ Hz, 2H), 3.74 (s, 3H), 3.82 (s, 3H), 3.87-3.94 (t, $J = 6.6$ Hz, 2H), 6.81-6.93 (m, 4H), 7.13-7.40 (m, 5H), 7.51-7.65 (m, 2H).

{2-[4-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl]naphthalen-1-yl-methanone (5o). (0.06 g, 16%). (Petroleum ether/Ethyl acetate 98:02). $^1$H NMR (CDCl$_3$) $\delta$: 1.25-1.58 (m, 6H), 1.62-1.95 (m,
4H), 3.37-3.44 (t, J = 6.6 Hz, 2H), 3.83-3.89 (t, J = 6.6 Hz, 2H), 6.60-6.64 (m, 2H), 7.20-7.38 (m, 4H), 7.50-7.65 (m, 7H), 7.84-7.90 (d, 2H), 8.55-8.60 (m, 1H).

(2-[4-(7-Bromoheptyloxy)phenyl]benzofuran-3-yl)naphthalen-2-yl-methanone (5p). (0.16 g, 47%). (Petroleum ether/Ethyl acetate 98:02). $^1$H NMR (CDCl$_3$) $\delta$: 1.56 (m, 6H), 1.64-1.92 (m, 4H), 3.37-3.44 (t, J = 6.6 Hz, 2H), 3.83-3.89 (t, J = 6.6 Hz, 2H), 6.73-6.78 (m, 2H), 7.21-7.38 (m, 3H), 7.42-7.63 (m, 4H), 7.64-7.90 (m, 4H), 7.95-8.01 (m, 1H), 8.34 (s, 1H).

Biphenyl-4-yl-[2-[4-(7-bromoheptyloxy)phenyl]benzofuran-3-yl]methanone (5q). (0.22 g, 58%). (Petroleum ether/Ethyl acetate 98:02). $^1$H NMR (CDCl$_3$) $\delta$: 1.58 (m, 6H), 1.62-1.95 (m, 4H), 3.36-3.43 (t, J = 6.6 Hz, 2H), 3.87-3.94 (t, J = 6.6 Hz, 2H), 6.78-6.82 (dd, J$_1$ = 2.2 Hz, J$_2$ = 7.0 Hz, 2H), 7.24-7.50 (m, 5H), 7.51-7.71 (m, 8H), 7.90-7.93 (m, 2H).

Anthracen-9-yl-[2-[4-(7-bromoheptyloxy)phenyl]benzofuran-3-yl]methanone (5r). (0.08 g, 18%). (Petroleum ether/Ethyl acetate 98:02). $^1$H NMR (CDCl$_3$) $\delta$: 1.61 (m, 6H), 1.62-1.95 (m, 4H), 3.40-3.47 (t, J = 6.6 Hz, 2H), 3.77-3.83 (t, J = 6.2 Hz, 2H), 6.29 (br, 2H), 6.72-7.82 (m, 10H), 7.84-8.12 (m, 4H), 8.34 (s, 1H).

2-[4-[3-(Benzylmethylamino)propoxy]phenyl]benzofuran-3-yl-(4-methoxyphenyl)methanone (6). To a cooled solution (0 °C) of 4a (0.54 g, 1.4 mmol) and 4-Methoxybenzoylchloride (0.3 g, 1.7 mmol) in DCM anhydrous (25 mL), SnCl$_4$ (0.44 g, 1.7 mmol) was added dropwise with stirring. The mixture was allowed to reach room temperature then stirred overnight. The reaction was quenched with ice/water and stirred for 30 minutes. The organic layer was washed with water (3 x 10 mL) and brine (3 x 10 mL), then dried over Na$_2$SO$_4$ anhydrous and evaporated to dryness. The crude was purified by flash chromatography (Toluene/Acetone 96:4) affording 6 as a pale yellow oil (0.04 g, 6%). $^1$H NMR (CDCl$_3$) $\delta$: 1.94-2.02 (quintet, J = 6.9 Hz, 2H), 2.23 (s, 3H), 2.52-2.57 (t, J = 6.9 Hz, 2H), 3.53 (s, 2H), 3.82 (s, 3H), 3.98-4.03 (t, J = 6.3 Hz, 2H), 6.79-6.85 (m, 4H), 7.22-7.32 (m, 7H), 7.45-7.53 (m, 2H), 7.65-7.68 (dd, J$_1$ = 2.1 Hz; J$_2$ = 6.9 Hz, 2H), 7.86-7.89 (dd, J$_1$ = 2.1 Hz; J$_2$ = 6.9 Hz, 2H). ES-MS m/z: 528 (M + 1).

2-[4-[5-(Benzylmethylamino)pentyloxy]phenyl]benzofuran-3-yl-(4-methoxyphenyl)methanone (7). Following the previous procedure and starting from 4b (0.13 g, 0.32 mmol), 7 was obtained as a colorless oil (0.03 g, 17%) after flash chromatography (from Toluene/Acetone 90:10 to Toluene/Acetone 20:80). $^1$H NMR (CDCl$_3$) $\delta$: 1.39-1.65 (m, 4H), 1.68-1.85 (m, 2H), 2.18 (s, 3H), 2.34-2.41 (t, J = 7.4 Hz, 2H), 3.47 (s, 2H), 3.82 (s, 3H), 3.92-3.99 (t, J = 6.2 Hz, 2H), 6.79-6.85 (m,
4H), 7.22-7.32 (m, 7H), 7.45-7.53 (m, 2H), 7.65-7.68 (dd, \( J_1 = 2.1 \) Hz; \( J_2 = 6.9 \) Hz, 2H), 7.86-7.89 (dd, \( J_1 = 2.1 \) Hz; \( J_2 = 6.9 \) Hz, 2H). ES-MS \( m/z \): 534 (M + 1).

2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl-(4-methoxyphenyl)methanone (8). Following the previous procedure and starting from 4c (0.18 g, 0.41 mmol), 8 was obtained as a colorless oil (0.09 g, 39%) after flash chromatography (from Toluene 100 to Toluene/Acetone 94:6). \(^1\)H NMR (CDCl\(_3\)) \( \delta \): 1.21-1.45 (m, 6H), 1.68-1.81 (m, 4H), 2.18 (s, 3H), 2.42 (t, 2H), 3.60 (s, 2H), 3.82 (s, 3H), 3.95 (2H, 6.78-6.97 (m, 4H, Ar), 7.10-7.60 (m, 9H, Ar), 7.68 (d, 2H, Ar), 7.87 (d, 2H, Ar). ES-MS \( m/z \): 562 (M+1). Anal. C\(_{37}\)H\(_{39}\)NO\(_4\) (C, H, N).

2-{4-[8-(Benzylmethylamino)octyloxy]phenyl}benzofuran-3-yl-(4-methoxyphenyl)methanone (9). Following the previous procedure and starting from 4d (0.18 g, 0.41 mmol), 9 was obtained as a colorless oil (0.05 g, 21%) after flash chromatography (from Toluene 100 to Toluene/Acetone 94:6). \(^1\)H NMR (CDCl\(_3\)) \( \delta \): 1.30-1.61 (m, 10H), 1.68-1.83 (m, 2H), 2.18 (s, 3H), 2.34-2.41 (t, \( J = 7.4 \) Hz, 2H), 3.47 (s, 2H), 3.82 (s, 3H), 3.92-3.99 (2H, J = 6.2 Hz, 2H), 6.79-6.85 (m, 4H), 7.22-7.32 (m, 7H), 7.45-7.53 (m, 2H), 7.65-7.68 (dd, \( J_1 = 2.1 \) Hz; \( J_2 = 6.9 \) Hz, 2H), 7.86-7.89 (dd, \( J_1 = 2.1 \) Hz; \( J_2 = 6.9 \) Hz, 2H). ES-MS \( m/z \): 576 (M+1).

2-{4-[9-(Benzylmethylamino)nonyloxy]phenyl}benzofuran-3-yl-(4-methoxyphenyl)methanone (10). Following the previous procedure and starting from 4e (0.48 g, 1.0 mmol), 10 was obtained as a pale yellow oil (0.03 g, 5%) after flash chromatography (Toluene/Acetone 96:4). \(^1\)H NMR (CDCl\(_3\)) \( \delta \): 1.30-1.61 (m, 12H), 1.68-1.83 (m, 2H), 2.18 (s, 3H), 2.34-2.41 (t, \( J = 7.4 \) Hz, 2H), 3.47 (s, 2H), 3.82 (s, 3H), 3.92-3.99 (2H, J = 6.2 Hz, 2H), 6.79-6.85 (m, 4H), 7.22-7.32 (m, 7H), 7.45-7.53 (m, 2H), 7.65-7.68 (dd, \( J_1 = 2.1 \) Hz; \( J_2 = 6.9 \) Hz, 2H), 7.86-7.89 (dd, \( J_1 = 2.1 \) Hz; \( J_2 = 6.9 \) Hz, 2H). ES-MS \( m/z \): 590 (M+1).

Compounds 11-16 were obtained according to the following general procedure:

SnCl\(_4\) (1.2 eq.) was added dropwise to a mixture of 4c (1 eq.) and the selected acylchloride (1.2 eq.) in dry DCM and the resulting solution was stirred at room temperature overnight. The reaction was quenched with ice/water and stirred for 1 h. The organic layer was separated and the aqueous one was extracted with DCM. The combined organic extracts were dried (Na\(_2\)SO\(_4\)), filtered and concentrated under reduced pressure to afford the desired 3-acylated benzofurans (11-16), generally as honey colored oils purified by flash chromatography. (Toluene/Acetone 90:10)

1-(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)ethanone (11). 15 % yield.

\(^1\)H NMR (CDCl\(_3\)) \( \delta \): 1.37-1.58 (m, 6H), 1.78-1.85 (m, 4H), 2.20 (s, 3H), 2.38 (t, 2H), 2.42 (s, 3H),
3.48 (s, 2H), 4.05 (t, 2H), 7.02 (d, 2H, Ar), 7.20-7.40 (m, 7H, Ar), 7.43-7.52 (m, 1H, Ar), 7.68 (d, 2H, Ar), 8.05-8.15 (m, 1H, Ar). ES-MS m/z: 470 (M+1). Anal. C_{31}H_{35}NO_{3} (C, H, N).

(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)phenylmethanone (12). 55 % yield. \(^{1}\)H NMR (CDCl\(_3\)) \(\delta\): 1.25-1.48 (m, 6H), 1.68-1.82 (m, 4H), 2.20 (s, 3H), 2.38 (t, 2H), 3.47 (s, 2H), 3.98 (t, 2H), 6.78 (d, 2H, Ar), 7.18-7.58 (m, 12H, Ar), 7.62 (d, 2H, Ar), 7.82 (d, 2H, Ar). ES-MS m/z: 532 (M+1). Anal. C_{36}H_{37}NO_{3} (C, H, N).

(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)-m-tolylmethanone (13). 38 % yield. \(^{1}\)H NMR (CDCl\(_3\)) \(\delta\): 1.25-1.45 (m, 6H), 1.62-1.82 (m, 4H), 2.18 (s, 3H), 2.25-2.42 (m, 5H), 3.48 (s, 2H), 3.98 (t, 2H), 6.80 (m, 2H, Ar), 7.16-7.58 (m, 11H, Ar), 7.62-7.80 (m, 4H, Ar). ES-MS m/z: 546 (M+1). Anal. C_{37}H_{39}NO_{3} (C, H, N).

(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)-p-tolylmethanone (14). 41 % yield. \(^{1}\)H NMR (CDCl\(_3\)) \(\delta\): 1.23-1.58 (m, 6H), 1.61-1.81 (m, 4H), 2.18 (s, 3H), 2.39 (t, 2H), 3.48 (s, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 3.98 (t, 2H), 6.78-7.75 (m, 16H, Ar). ES-MS m/z: 592 (M+1). Anal. C_{38}H_{41}NO_{5} (C, H, N).

Compounds 17-28 were obtained according to the following general procedure:

A stirred solution of the opportune 5 g-r (1 eq.) and N-Benzylmethylamine (2 eq.) in toluene was refluxed for 48 hours. The mixture was washed with water. The organic layer was dried over Na\(_2\)SO\(_4\) and the solvent was removed. The residue was purified by flash chromatography (Toluene/Acetone 98:2) to afford 17-28 generally as a yellowish oil.

(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)naphthalen-1yl-methanone (17). (0.04 g, 62%). \(^{1}\)H NMR (CDCl\(_3\)) \(\delta\): 1.21-1.62 (m, 8H), 1.63-1.79 (m, 2H), 2.18 (s, 3H), 2.39 (t, 2H), 3.48 (s, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 3.98 (t, 2H), 6.78-7.75 (m, 16H, Ar). ES-MS m/z: 592 (M+1). Anal. C_{38}H_{41}NO_{5} (C, H, N).
(m, 8H), 7.52-7.63 (m, 7H), 7.83-7.88 (d, J = 8.2 Hz, 2H), 8.55-8.59 (d, J = 7.8 Hz, 1H). ES-MS m/z: 582 (M + 1).

(2-{4-[7-(Benzylmethy lamino)heptyloxy]phenyl}benzofuran-3-yl)naphthalen-2yl-methanone (18). (0.17 g, 99%). $^1$H NMR (CDCl$_3$) $\delta$: 1.25-1.60 (m, 8H), 1.64-1.80 (m, 2H), 2.18 (s, 3H), 2.31-2.39 (t, J = 7.4 Hz, 2H), 3.48 (s, 2H), 3.81-3.88 (t, J = 6.6 Hz, 2H), 6.73-6.77 (d, J = 8.8 Hz, 2H), 7.18-7.38 (m, 8H), 7.41-7.85 (m, 8H), 7.96-8.02 (m, 1H), 8.33 (s, 1H). $^{13}$C NMR (CDCl$_3$) $\delta$: 25.86, 27.19, 27.24, 28.95, 29.18, 42.15, 57.35, 62.24, 67.93, 111.04, 114.42, 114.93, 121.21, 121.73, 123.65, 124.84, 125.10, 126.55, 126.87, 127.67, 128.14, 128.31, 128.39, 128.73, 129.03, 129.52, 129.85, 132.04, 132.39, 135.21, 135.56, 138.97, 150.90, 153.56, 158.04, 160.31, 192.28, 197.95. ES-MS m/z: 582 (M + 1).

(2-{4-[7-(Benzylmethy lamino)heptyloxy]phenyl}benzofuran-3-yl)biphenyl-4-yl-methanone (19). (0.18 g, 73%). $^1$H NMR (CDCl$_3$) $\delta$: 1.21-1.62 (m, 8H), 1.64-1.80 (m, 2H), 2.20 (s, 3H), 2.37-2.44 (t, J = 7.4 Hz, 2H), 3.50 (s, 2H), 3.87-3.93 (t, J = 6.6 Hz, 2H), 6.78-6.83 (d, J = 8.8 Hz, 2H), 7.19-7.45 (m, 11H), 7.54-7.68 (m, 7H), 7.89-7.93 (d, J = 8.2 Hz, 2H). ES-MS m/z: 608 (M + 1).

Anthracen-9-yl-(2-{4-[7-(benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)methanone (20). (0.06 g, 70%). $^1$H NMR (CDCl$_3$) $\delta$: 1.25-1.62 (m, 8H), 1.64-1.80 (m, 2H), 2.22 (s, 3H), 2.37-2.44 (t, J = 7.4 Hz, 2H), 3.52 (s, 2H), 3.76-3.83 (t, J = 6.6 Hz, 2H), 6.18-6.40 (m, 2H), 7.25-7.52 (m, 15H), 7.89-7.93 (dd, J$_1$ = 2.2 Hz, J$_2$ = 6.6 Hz, 4H), 8.34 (s, 1H). $^{13}$C NMR (CDCl$_3$) $\delta$: 25.94, 27.20, 27.32, 28.96, 29.24, 42.13, 57.35, 62.22, 67.88, 110.97, 113.14, 118.73, 121.05, 122.55, 124.38, 124.90, 125.19, 125.25, 126.54, 126.66, 127.00, 128.20, 128.37, 128.49, 128.71, 129.12, 130.00, 130.99, 135.12, 153.82, 158.54, 160.18, 182.87, 194.44, 197.98. ES-MS m/z: 632 (M + 1).

(2-{3-[7-(Benzylmethy lamino)heptyloxy]phenyl}benzofuran-3-yl)phenylmethanone (21). (0.09 g, 65%). $^1$H NMR (CDCl$_3$) $\delta$: 1.21-1.62 (m, 8H), 1.63-1.79 (m, 2H), 2.18 (s, 3H), 2.32-2.39 (t, J = 7.2 Hz, 2H), 3.47 (s, 2H), 3.81-3.88 (t, J = 6.6 Hz, 2H), 6.89-6.94 (m, 1H), 7.16-7.62 (m, 15H), 7.80-7.93 (m, 2H). $^{13}$C NMR (CDCl$_3$) $\delta$: 25.98, 27.33, 29.09, 29.27, 42.25, 56.88, 57.47, 62.33, 67.99, 111.20, 113.87, 116.13, 116.74, 120.73, 121.46, 123.81, 125.36, 126.85, 128.15, 128.41, 128.51, 129.05, 129.41, 129.79, 130.49, 133.14, 137.82, 139.18, 150.95, 153.76, 157.35, 158.97, 192.30. ES-MS m/z: 532 (M + 1).

(2-{3-[7-(Benzylmethy lamino)heptyloxy]phenyl}benzofuran-3-yl)-p-tolylmethanone (22). (0.08 g, 82.3%). $^1$H NMR (CDCl$_3$) $\delta$: 1.25–1.62 (m, 8H), 1.64-1.80 (m, 2H), 2.19 (s, 3H), 2.35-2.40 (m,
5H), 3.48 (s, 2H), 3.77-3.84 (t, J = 6.6 Hz, 2H), 6.82-6.86 (m, 1H), 7.11-7.42 (m, 12H), 7.50-7.58 (t, J = 9.2 Hz, 2H), 7.75-7.79 (d, J = 7.6 Hz, 2H). ES-MS m/z: 546 (M + 1).

(2-[3-[7-(Benzylmethylamino)heptyloxy]phenyl]benzofuran-3-yl)-m-tolylmethanone  (23). (0.05 g, 77%). $^1$H NMR (CDCl$_3$) $\delta$: 1.22-1.62 (m, 8H), 1.64-1.80 (m, 2H), 2.20 (s, 3H), 2.28 (s, 3H), 2.34-2.41 (t, J = 7.6 Hz, 2H), 3.50 (s, 2H), 3.77-3.83 (t, J = 6.6 Hz, 2H), 6.82-6.86 (d, J = 7.8 Hz, 1H), 7.16-7.42 (m, 12H), 7.50-7.58 (t, J = 9.2 Hz, 2H), 7.75-7.79 (d, J = 7.6 Hz, 2H). ES-MS m/z: 546 (M + 1).

(2-[3-[7-(Benzylmethylamino)heptyloxy]phenyl]benzofuran-3-yl)-3-methoxyphenylmethanone (24). (0.05 g, 77%). $^1$H NMR (CDCl$_3$) $\delta$: 1.22-1.62 (m, 8H), 1.64-1.80 (m, 2H), 2.20 (s, 3H), 2.28 (s, 3H), 2.34-2.41 (t, J = 7.6 Hz, 2H), 3.50 (s, 2H), 3.77-3.83 (t, J = 6.6 Hz, 2H), 6.82-6.86 (d, J = 7.8 Hz, 1H), 7.16-7.42 (m, 12H), 7.50-7.58 (t, J = 9.2 Hz, 2H), 7.75-7.79 (d, J = 7.6 Hz, 2H). ES-MS m/z: 546 (M + 1).

(2-[3-[7-(Benzylmethylamino)heptyloxy]phenyl]benzofuran-3-yl)-2-methoxyphenylmethanone (25). (0.15 g, 70%). $^1$H NMR (CDCl$_3$) $\delta$: 1.28-1.62 (m, 8H), 1.65-1.82 (m, 2H), 2.19 (s, 3H), 2.33-2.40 (t, J = 7.6 Hz, 2H), 3.48 (s, 2H), 3.53 (s, 3H), 3.79-3.86 (t, J = 6.6 Hz, 2H), 6.83-6.87 (m, 1H), 6.98-7.05 (m, 1H), 7.16-7.20 (m, 2H), 7.22-7.32 (m, 8H), 7.37-7.41 (m, 3H), 7.56-7.63 (m, 2H). ES-MS m/z: 562 (M + 1).

(2-[3-[7-(Benzylmethylamino)heptyloxy]phenyl]benzofuran-3-yl)-3,4-dimethoxyphenyl
methanone (26). (0.09 g, 65%). $^1$H NMR (CDCl$_3$) $\delta$: 1.25-1.62 (m, 8H), 1.65-1.82 (m, 2H), 2.20 (s, 3H), 2.34-2.41 (t, J = 7.6 Hz, 2H), 3.49 (s, 2H), 3.73 (s, 3H), 3.77-3.84 (t, J = 6.2 Hz, 2H), 6.83-6.87 (m, 1H), 6.98-7.05 (m, 1H), 7.16-7.20 (m, 2H), 7.22-7.32 (m, 8H), 7.37-7.41 (m, 3H), 7.56-7.63 (m, 2H). ES-MS m/z: 562 (M + 1).

(2-[3-[7-(Benzylmethylamino)heptyloxy]phenyl]benzofuran-3-yl)-2,3-dimethoxyphenyl
methanone (27). (0.14 g, 77%). $^1$H NMR (CDCl$_3$) $\delta$: 1.27-1.61 (m, 8H), 1.65-1.83 (m, 2H), 2.18 (s, 3H), 2.33-2.40 (t, J = 7.8 Hz, 2H), 3.48 (s, 2H), 3.73 (s, 3H), 3.79-3.89 (t, J = 6.6 Hz, 2H), 6.83-6.88 (m, 1H), 7.23-7.39 (m, 10H), 7.43-7.60 (m, 4H). ES-MS m/z: 592 (M + 1).

(2-[3-[7-(Benzylmethylamino)heptyloxy]phenyl]benzofuran-3-yl)-3,5-dimethoxyphenyl
methanone (28). (0.05 g, 67%). $^1$H NMR (CDCl$_3$) $\delta$: 1.25-1.61 (m, 8H), 1.65-1.83 (m, 2H), 2.18 (s, 3H), 2.33-2.40 (t, J = 7.8 Hz, 2H), 3.48 (s, 2H), 3.73 (s, 3H), 3.79-3.93 (t, J = 6.6 Hz, 2H), 6.80-6.95 (m, 4H), 7.12-7.34 (m, 10H), 7.51-7.55 (m, 1H), 7.63-7.68 (m, 1H). ES-MS m/z: 592 (M + 1).
(s, 1H), 6.84-6.88 (m, 1H), 6.98-6.99 (d, J = 2.2 Hz, 2H), 7.14-7.41 (m, 10H), 7.56-7.67 (m, 2H). ES-MS m/z: 592 (M + 1).

[2-(4-{7-[(3-Hydroxybenzyl)methylamino]heptyloxy}phenyl)benzofuran-3-yl]-p-tolylmethanone (29). A stirred solution of {2-[4-{(7-Bromoheptyloxy)phenyl]benzofuran-3-yl}]-p-tolylmethanone (0.7 g, 1.38 mmol) and 3-Methyaminomethylphenol (0.38 g, 2.77 mmol) in toluene (120 mL) was refluxed for 20 hours. The mixture was washed with water, the organic layer was dried over Na₂SO₄ and the solvent was removed. The residue was purified by flash chromatography (Toluene/Acetone 90:10), to afford 29 as a yellowish oil (0.16 g, 21%). ¹H NMR (CDCl₃) δ: 1.20-1.60 (m, 8H), 1.62-1.83 (m, 2H), 2.19 (s, 3H), 2.23-2.41 (m, 5H), 3.42 (s, 2H), 3.83-3.97 (t, 2H), 6.63-6.87 (m, 4H), 7.11-7.38 (m, 6H), 7.39-7.58 (m, 2H), 7.62-7.81 (m, 4H).

Methyl carbamic acid 3-{[methyl-{(7-[4-{3-methylbenzoyl}benzofuran-2-yl]phenoxy}heptyl)-amino]methyl}phenyl ester (30). To a solution of 29 (0.1 g, 0.18 mmol) in DCM, NaH (0.004 g, 0.18 mmol) and methyl isocyanate (0.010 g, 0.18 mmol) were added. The mixture was stirred for 24 h then quenched with ice/water and extracted with DCM. The organic layer was dried and evaporated. The crude was purified by flash chromatography (Toluene/Acetone 60:40), affording 30 as a clear oil (0.06 g, 54%). ¹H NMR (CDCl₃) δ: 1.21-1.59 (m, 8H), 1.63-1.82 (m, 2H), 2.18 (s, 3H), 2.23-2.41 (m, 5H), 2.82-2.91 (d, 3H), 3.45 (s, 2H), 3.83-3.97 (t, 2H), 4.91-5.02 (br, 1H), 6.62-6.85 (m, 4H), 7.09-7.41 (m, 6H), 7.47-7.59 (m, 2H), 7.61-7.84 (m, 4H). ES-MS m/z: 620 (M + 1).

4-(2-Chloroethoxy)benzoic acid ethyl ester (31a). A stirred mixture of Ethyl-4-hydroxybenzoate (3 g, 18 mmol), 1-Bromo-2-chloroethane (3 mL, 36 mmol) and K₂CO₃ (4.5 g) in acetone (150 mL) was refluxed for 20 hours and then hot filtered. The solvent was removed and the residue treated with Petroleum Ether (150 mL) and kept in the freezer overnight. The white solid that formed was filtered off affording 31a (1.64 g, 40%). mp 73 °C. ¹H NMR (CDCl₃) δ: 1.25-1.70 (t, J = 7.8 Hz, 3H), 3.80-4.00 (t, J = 6.2 Hz, 2H), 4.22-4.60 (m, 4H), 6.95-7.15 (d, J = 8.4 Hz, 2H), 7.99-8.27 (d, J = 8.4 Hz, 2H).

3-(2-Chloroethoxy)benzoic acid ethyl ester (31b). A stirred mixture of Ethyl-3-hydroxybenzoate (3 g, 18 mmol), 1-Bromo-2-chloroethane (3 mL, 36 mmol) and K₂CO₃ (4.5 g) in acetone (150 mL) was refluxed for 20 hours and then hot filtered. The solvent was removed and the residue was purified by flash chromatography (Toluene/Acetone 90:10), affording 31b as a colorless oil (1.22 g, 30%). ¹H NMR (CDCl₃) δ: 1.34-1.38 (t, J = 6.00 Hz, 3H), 3.75-3.82 (t, J = 6.21 Hz, 2H), 4.18-4.23 (t, J = 6.42 Hz, 2H), 4.32-4.38 (q, J = 6.54 Hz, 2H), 7.05-7.17 (d, J = 7.4 Hz, 1H), 7.29-7.47 (t, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.62-7.71 (d, J = 7.2 Hz, 1H).
4-(2-Chloroethoxy)benzoic acid (32a). To a stirred solution of 31a (1.64 g, 7.2 mmol) in EtOH (50 mL), KOH (0.6 g, 10.8 mmol), previously solubilized in the minimum amount of water, was added dropwise. The reaction mixture was refluxed for 3 hours. The solvent was removed and the residue diluted with water and cooled to 0 °C. The solution was made acid by adding HCl 6N dropwise with stirring. The white solid that formed was filtered off affording 32a (1.14 g, 79%). mp 177 °C.

1H NMR (CDCl3) δ: 3.79-3.99 (t, J = 6.2 Hz, 2H), 4.23-4.59 (m, 4H), 6.92-7.12 (d, J = 8.4 Hz, 2H), 8.05-8.32 (d, J = 8.4 Hz, 2H).

3-(2-Chloroethoxy)benzoic acid (32b). Using the previous procedure and starting from 31b (1.22 g, 5.3 mmol), 32b (0.93 g, 80%) was obtained as a white solid. mp 153 °C.

1H NMR (CDCl3) δ: 3.75-3.82 (t, J = 6.21 Hz, 2H), 4.18-4.23 (t, J = 6.42 Hz, 2H), 7.05-7.17 (d, J = 7.4 Hz, 1H), 7.29-7.47 (t, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.62-7.71 (d, J = 7.2 Hz, 1H).

4-(2-Chloroethoxy)benzoyl chloride (33a). 32a (1.14 g, 5.7 mmol) was suspended in SOCl2 (7 mL, 98 mmol), and refluxed with stirring for 12 hours. The excess of SOCl2 was removed under vacuum and collected using a trap affording 33a which was used for the next step without further purification. The yield was considered as quantitative.

3-(2-Chloroethoxy)benzoyl chloride (33b). Using the previous procedure and starting from 32b (0.93 g, 4.6 mmol), 33b was obtained and used for the next step without further purification. The yield was considered as quantitative.

(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)-[4-(2-chloroethoxy)phenyl]-methanone (34a). To a cooled solution (0 °C) of 4c (1.2 g, 2.8 mmol) and 33a (0.73 g, 3.4 mmol) in DCM anhydrous (50 mL), SnCl4 (0.87 g, 3.4 mmol) was added dropwise with stirring. The mixture was allowed to reach room temperature then stirred overnight. The reaction was quenched with ice/water and stirred during 30 minutes. The organic layer was washed with water (3 x 10 mL) and brine (3 x 10 mL), then dried over Na2SO4 anhydrous and the solvent removed. The crude was purified by flash chromatography (Toluene/Acetone 20:80), affording 34a as a dark yellow oil (0.92 g, 54%). 1H NMR (CDCl3) δ: 1.17-1.59 (m, 8H), 1.60-1.85 (m, 2H), 2.19 (s, 3H), 2.30-2.37 (t, J = 7.4 Hz, 2H), 3.45 (s, 2H), 3.70-3.85 (t, J = 6.2 Hz, 2H), 3.86-3.97 (t, J = 8.4 Hz, 2H), 4.15-4.26 (t, J = 8.4 Hz, 2H), 6.74-6.83 (m, 4H), 7.00-7.96 (m, 13H).

(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)-[3-(2-chloroethoxy)phenyl]-methanone (34b). Using the previous procedure and starting from 4c (0.62 g, 1.45 mmol) and 33b (0.38 g, 1.7 mmol), 34b was obtained as a dark yellow oil (0.53 g, 60%). 1H NMR (CDCl3)
δ: 1.17-1.59 (m, 8H), 1.56-1.79 (m, 2H), 2.15 (s, 3H), 2.32-2.39 (t, J = 7.4 Hz, 2H), 3.47 (s, 2H), 3.73-3.88 (t, J = 6.2 Hz, 2H), 3.84-3.95 (t, J = 8.4 Hz, 2H), 4.16-4.27 (t, J = 8.4 Hz, 2H), 6.72-6.87 (m, 4H), 7.05-8.02 (m, 13H).

(2-[4-(Benzylmethylamino)heptyloxy]phenyl)benzofuran-3-yl-[4-(2-diethylaminoethoxy)phenyl)methanone (35). A stirred solution of 34a (0.46 g, 0.75 mmol) and diethylamine (0.32 mL, 3.0 mmol) was refluxed in toluene (100 mL) for 20 hours in the presence of a catalytic amount of NaI. The mixture was washed with water (3 x 25 mL), then with brine (3 x 25 mL). The organic layer was collected and dried over Na₂SO₄ anhydrous. The solvent was removed and the residue was purified by flash chromatography (Toluene/Acetone 20:80), affording 35 as a dark yellow oil (0.04 g, 9%). ¹H NMR (CDCl₃) δ: 1.05-1.15 (t, J = 8.4 Hz, 6H), 1.18-1.62 (m, 8H), 1.63-1.85 (m, 2H), 2.19 (s, 3H), 2.32-2.43 (t, J = 7.4 Hz, 2H), 2.58-2.71 (q, J = 8.2 Hz, 4H), 2.82-2.94 (t, J = 8.4 Hz, 2H), 3.55 (s, 2H), 3.87-3.99 (t, J = 6.2 Hz, 2H), 4.05-4.12 (t, J = 8.4 Hz, 2H), 6.74-6.83 (m, 4H), 7.00-7.96 (m, 13H). ES-MS m/z: 647 (M + 1).

(2-[4-(Benzylmethylamino)heptyloxy]phenyl)benzofuran-3-yl-[3-(2-diethylaminoethoxy)phenyl)methanone (36). Using the previous procedure and starting from 34b (0.46 g, 0.75 mmol), 36 was obtained as a dark yellow oil (0.03 g, 8%). ¹H NMR (CDCl₃) δ: 1.02-1.12 (t, J = 8.4 Hz, 6H), 1.17-1.61 (m, 8H), 1.62-1.86 (m, 2H), 2.21 (s, 3H), 2.35-2.46 (t, J = 7.4 Hz, 2H), 2.60-2.73 (q, J = 8.2 Hz, 4H), 2.80-2.92 (t, J = 8.2 Hz, 2H), 3.55 (s, 2H), 3.87-3.99 (t, J = 6.2 Hz, 2H), 4.02-4.09 (t, J = 8.4 Hz, 2H), 6.75-6.86 (m, 4H), 7.00-7.94 (m, 13H). ES-MS m/z: 647 (M + 1).

(2-[4-(Benzylmethylamino)heptyloxy]phenyl)benzofuran-3-yl-[4-(2-morpholinoethoxy)phenyl)methanone (37). Using the previous procedure and starting from 34a (0.4 g, 0.57 mmol) and morpholine (0.1 g, 1.14 mmol), 37 was obtained as a dark yellow oil (0.08 g, 21%). ¹H NMR (CDCl₃) δ: 1.21-1.58 (m, 8H), 1.62-1.83 (m, 2H), 2.20 (s, 3H), 2.30-2.42 (t, J = 6.23 Hz, 2H), 2.50-2.62 (t, J = 7.3 Hz, 4H), 2.73-3.83 (t, J = 6.4 Hz, 2H), 3.43 (s, 2H), 3.65-3.80 (t, J = 8.2 Hz, 4H), 3.87-3.98 (t, J = 6.3 Hz, 2H), 4.08-4.19 (t, J = 6.2 Hz, 2H), 6.78-6.84 (d, J = 6.2 Hz, 2H), 7.12-7.41 (m, 10H), 7.50-7.62 (m, 4H), 7.83-7.95 (m, 2H). ES-MS m/z: 661 (M + 1).

(2-[4-(Benzylmethylamino)heptyloxy]phenyl)benzofuran-3-yl-[4-(chloromethyl)phenyl)methanone (38a). To a cool solution (0 °C) of 4c (0.43 g, 1.0 mmol) and 4-chloromethylbenzoylchloride (0.24 g, 1.25 mmol) in DCM anhydrous (50 mL), SnCl₄ (0.32 g, 1.25 mmol) was added dropwise with stirring. The mixture was allowed to reach room temperature then stirred overnight. The reaction was quenched with ice/water and stirred during 30 minutes. The organic layer was washed with water (3 x 10 mL) and with brine (3 x 10 mL), then dried over
Na$_2$SO$_4$ anhydrous and the solvent removed. The crude was purified by flash chromatography (Toluene/Acetone 60:40), affording 38a as a dark yellow oil (0.26 g, 43%). $^1$H NMR (CDCl$_3$) $\delta$: 1.02-1.60 (m, 8H), 1.62-1.88 (m, 2H), 2.20 (s, 3H), 2.33-2.44 (t, $J$ = 7.4 Hz, 2H), 3.46 (s, 2H), 3.85-3.97 (t, $J$ = 8.2 Hz, 2H), 4.58 (s, 2H), 6.77-6.83 (d, $J$ = 6.2 Hz, 2H), 7.05-7.38 (m, 11H), 7.50-7.63 (m, 4H), 7.79-7.92 (m, 2H).

(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)[3-(chloromethyl)phenyl]-methanone (38b). Using the previous procedure and starting from 4c (0.43 g, 1.0 mmol) and 3-Chloromethylbenzoyl chloride (0.24 g, 1.25 mmol), 38b was obtained after flash chromatography (Toluene/Acetone 60:40), as a dark yellow oil (0.27 g, 45%). $^1$H NMR (CDCl$_3$) $\delta$: 1.00-1.58 (m, 8H), 1.64-1.90 (m, 2H), 2.19 (s, 3H), 2.35-2.46 (t, $J$ = 7.4 Hz, 2H), 3.45 (s, 2H), 3.80-3.92 (t, $J$ = 8.2 Hz, 2H), 4.59 (s, 2H), 6.76-6.82 (d, $J$ = 6.2 Hz, 2H), 7.05-7.38 (m, 11H), 7.49-7.61 (m, 4H), 7.81-7.93 (m, 2H).

(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)[4-(diethylaminomethyl)phenyl]methanone (39). A stirred solution of 38a (0.26 g, 0.45 mmol) and diethylamine (0.1 mL, 0.90 mmol) was refluxed in toluene (100 mL) for 20 hours in the presence of a catalytic amount of NaI. The mixture was washed with water (3 x 25 mL), then with brine (3 x 25 mL). The organic layer was collected and dried over Na$_2$SO$_4$ anhydrous. The solvent was removed and the residue purified by flash chromatography (Toluene/Acetone 60:40), affording 39 as a dark yellow oil (0.05 g, 18%). $^1$H NMR (CDCl$_3$) $\delta$: 0.98-1.05 (t, $J$ = 8.2 Hz, 6H), 1.22-1.58 (m, 8H), 1.62-1.82 (m, 2H), 2.20 (s, 3H), 2.35-2.46 (m, 6H), 3.45 (s, 2H), 3.58 (s, 2H), 3.83-3.95 (t, $J$ = 8.2 Hz, 2H), 6.78-6.84 (d, $J$ = 6.2 Hz, 2H), 7.10-7.38 (m, 10H), 7.49-7.61 (m, 4H), 7.81-7.93 (m, 2H). ES-MS m/z: 617 (M + 1).

(2-{4-[7-(Benzylmethylamino)heptyloxy]phenyl}benzofuran-3-yl)[3-(diethylaminomethyl)phenyl]methanone (40). Using the previous procedure and starting from 38b (0.27 g, 0.47 mmol), 40 (0.06 g, 20%) was obtained as a dark yellow oil. $^1$H NMR (CDCl$_3$) $\delta$: 1.01-1.08 (t, $J$ = 8.2 Hz, 6H), 1.21-1.57 (m, 8H), 1.59-1.79 (m, 2H), 2.19 (s, 3H), 2.37-2.48 (m, 6H), 3.47 (s, 2H), 3.56 (s, 2H), 3.82-3.94 (t, $J$ = 8.2 Hz, 2H), 6.79-6.85 (d, $J$ = 6.2 Hz, 2H), 7.11-7.39 (m, 10H), 7.50-7.62 (m, 4H), 7.83-7.95 (m, 2H). ES-MS m/z: 617 (M + 1).

2-(6-Bromohexyl)isooindole-1,3-dione (41a). A suspension of Phtalimide potassium salt (5.3 g, 28.6 mmol) and 1,6-Dibromohexane (13.98 g, 57.3 mmol) in DMF (16 mL) was refluxed for 2 hours. The reaction mixture was poured in ice/water and the formed solid was filtered under
vacuum, obtaining 41a as a solid (5.37 g, 60%). mp 42-44 °C. \(^1\)H NMR (CDCl\(_3\)) \(\delta\) : 1.4 (m, 4H), 1.7 (m, 2H), 1.85 (m, 2H), 3.4 (m, 2H), 3.7 (t, 2H), 7.75 (m, 2H), 7.85 (m, 2H).

2-(7-Bromoheptyl)isoindole-1,3-dione (41b). Following the previous procedure and starting from 1,7-Dibromoheptane (6 mL), 41b was obtained as a solid (40% yield). mp 23-25 °C. \(^1\)H NMR (CDCl\(_3\)) \(\delta\) : 1.3 (m, 6H), 1.65 (m, 2H), 1.85 (m, 2H), 3.35 (m, 2H), 3.7 (t, 2H), 7.75 (m, 2H), 7.8 (m, 2H).

2-(8-Bromooctyl)isoindole-1,3-dione (41c). Following the previous procedure and starting from 1,8-Dibromooctane (8.12 mL), 41c was obtained as a solid (60% yield). mp 39-41 °C. \(^1\)H NMR (CDCl\(_3\)) \(\delta\) : 1.4 (m, 8H), 1.7 (m, 2H), 1.85 (m, 2H), 3.5 (m, 2H), 3.7 (t, 2H), 7.7 (m, 2H), 7.8 (m, 2H).

2-(9-Bromononyl)isoindole-1,3-dione (41d). Following the previous procedure and starting from 1,9-Dibromononane (10.5 mL), 41d was obtained as a solid (60% yield). mp 31-32 °C. \(^1\)H NMR (CDCl\(_3\)) \(\delta\) : 1.4 (m, 10 H), 1.7 (m, 2H), 1.85 (m, 2H), 3.4 (m, 2H), 3.7 (t, 2H), 7.75 (m, 2H), 7.85 (m, 2H).

2-(10-Bromodecyl)isoindole-1,3-dione (41e). Following the previous procedure and starting from 1,10-Dibromodecane (9 g), 41e was obtained as a solid (65% yield). mp 34-36 °C. \(^1\)H NMR (CDCl\(_3\)) \(\delta\) : 1.4 (m, 12 H), 1.7 (m, 2H), 1.85 (m, 2H), 3.4 (m, 2H), 3.7 (t, 2H), 7.75 (m, 2H), 7.85 (m, 2H).

2-(6-Morpholin-4-yl-hexyl)isoindole-1,3-dione (42a). A solution of 41a (5.37 g, 17.3 mmol) and morpholine (3.03 mL, 34.6 mmol) in toluene was refluxed for 10 hours. The mixture was washed with water then extracted with diluted HCl. The extracts were made basic by K\(_2\)CO\(_3\) and the alkaline solution was extracted with DCM. The organic layer was dried and evaporated affording 42a as a solid (3.48 g, 70%). mp 47-51 °C. \(^1\)H NMR (CDCl\(_3\)) \(\delta\) : 1.3 (m, 4 H), 1.6 (m, 2H), 1.8 (m, 2H), 2.35 (m, 2H), 2.5 (m, 4H), 3.7 (m, 6H), 7.75 (m, 2H), 7.85 (m, 2H).

2-(7-Morpholin-4-yl-heptyl)isoindole-1,3-dione (42b). Starting from 41b and following the previous procedure, 42b was obtained as an oil (95% yield). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) : 1.3 (m, 6 H), 1.5 (m, 2H), 1.75 (m, 2H), 2.35 (m, 2H), 2.45 (m, 4H), 3.7 (m, 6H), 7.75 (m, 2H), 7.85 (m, 2H).

2-(8-Morpholin-4-yl-octyl)isoindole-1,3-dione (42c). Starting from 41c and following the previous procedure, 42c was obtained as an oil (75% yield). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) : 1.3 (m, 8 H), 1.5 (m, 2H), 1.75 (m, 2H), 2.35 (m, 2H), 2.45 (m, 4H), 3.8 (m, 6H), 7.75 (m, 2H), 7.85 (m, 2H).
2-(9-Morpholin-4-yl-nonyl)isoindole-1,3-dione (42d). Starting from 41d and following the previous procedure, 42d was obtained as a solid (60% yield). mp 47-51 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.3 (m, 10H), 1.5 (m, 2H), 1.7 (m, 2H), 2.35 (m, 2H), 2.45 (m, 4H), 3.7 (m, 6H), 7.75 (m, 2H), 7.85 (m, 2H).

2-(10-Morpholin-4-yl-decyl)isoindole-1,3-dione (42e). Starting from 41e and following the previous procedure, 42e was obtained as a solid (68% yield). mp 49-54 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.3 (m, 12H), 1.5 (m, 2H), 1.7 (m, 2H), 2.35 (m, 2H), 2.45 (m, 4H), 3.7 (m, 6H), 7.75 (m, 2H), 7.85 (m, 2H).

6-Morpholin-4yl-hexylamine (43a). A stirred solution of 42a (3.48 g) and hydrazine monohydrate (1.6 mL) in EtOH (38 mL) was refluxed for 3 hours. HCl (4 mL) was then added portion wise and the mixture was allowed to reflux for 30 minutes. The solvent was removed and the residue treated with water and made alkaline by K$_2$CO$_3$. The aqueous phase is extracted with DCM which is then dried over Na$_2$SO$_4$ and evaporated affording 43a as a solid (1.74 g, 85%) mp 57-60 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.3 (m, 4H), 1.5 (m, 4H), 2.35 (t, 2H), 2.45 (m, 4H), 2.7 (t, H), 3.75 (m, 4H).

7-Morpholin-4yl-heptylamine (43b). Starting from 42b and following the previous procedure, 43b was obtained as an oil in quantitative yield. $^1$H NMR (CDCl$_3$) $\delta$: 1.3 (m, 6H), 1.5 (m, 4H), 2.0 (br, 2H), 2.35 (t, 2H), 2.45 (m, 4H), 2.7 (t, H), 3.75 (m, 4H).

8-Morpholin-4yl-octylamine (43c). Starting from 42c and following the previous procedure, 43c was obtained as a solid in 70% yield. mp 63-65 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.3 (m, 8H), 1.5 (m, 4H), 2.3 (t, 2H), 2.45 (m, 4H), 2.7 (t, H), 3.75 (m, 4H).

9-Morpholin-4yl-nonylamine (43d). Starting from 42d and following the previous procedure, 43d was obtained as a solid in 70% yield. mp 36-39 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.3 (m, 10H), 1.5 (m, 4H), 2.3 (t, 2H), 2.45 (m, 4H), 2.65 (t, H), 3.7 (m, 4H).

10-Morpholin-4yl-decylamine (43e). Starting from 42e and following the previous procedure, 43e was obtained as a solid in 75% yield. mp 42-45 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.3 (m, 12H), 1.5 (m, 4H), 2.3 (t, 2H), 2.45 (m, 4H), 2.65 (t, H), 3.7 (m, 4H).

9-Chloro-1,2,3,4-tetrahydroacridine (44). To a cooled mixture (ice bath) of 2-Aminobenzoic acid (2.00 g, 14.58 mmol) and cyclohexanone (1.5 mL, 14.58 mmol), POCl$_3$ (12 mL) was carefully added. The mixture was heated under reflux for 2 h, then cooled at room temperature, and concentrated to give a slurry. The residue was diluted with EtOAc, neutralized with aqueous
K₂CO₃, and washed with brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated to furnish 45 as a pale brown solid (1.74 g, 55%); mp 85-87 °C. ¹H NMR (CDCl₃) δ: 1.63-1.71 (m, 2H), 2.13-2.42 (t, 2H), 2.67-2.82 (s, 4H), 7.24-7.39 (m, 4H).

(6-Morpholin-4-yl-hexyl)-(1,2,3,4-tetrahydroacridin-9-yl)amine (45a). A stirred suspension of 44 (0.35 g, 2.76 mmol) and Phenol (1.50 mL) was heated at 85-90 °C until a solution was obtained. 43a (0.6 g, 5.53 mmol) was then added and the reaction mixture heated to 130 °C for 4 hours. The crude was washed with NaOH 2N solution and extracted with EtOAc, dried over Na₂SO₄ and evaporated. Flash chromatography (DCM/MeOH/NH₄OH 70:30:0.7) afforded 45a as a brownish oil (0.26 g, 26%). ¹H NMR (CDCl₃) δ: 1.20-1.58 (m, 6H), 1.60-1.75 (m, 2H), 1.80-1.99 (s, 4H), 2.20-2.38 (t, 2H), 2.39-2.45 (s, 4H), 2.60-2.68 (s, 2H), 2.98-3.17 (s, 2H), 3.40-3.57 (t, 2H), 3.60-3.79 (m, 4H), 3.81-4.01 (br, 1H), 7.22-7.38 (m, 1H), 7.42-7.60 (t, 1H), 7.80-8.00 (t, 2H).

(7-Morpholin-4-yl-heptyl)-(1,2,3,4-tetrahydroacridin-9-yl)amine (45b). Starting from 43b and following the previous procedure, 45b was obtained as a brownish oil. ¹H NMR (CDCl₃) δ: 1.20-1.58 (m, 8H), 1.60-1.75 (m, 2H), 1.80-1.99 (s, 4H), 2.20-2.38 (t, 2H), 2.39-2.45 (s, 4H), 2.60-2.68 (s, 2H), 2.98-3.17 (s, 2H), 3.40-3.57 (t, 2H), 3.60-3.79 (m, 4H), 3.81-4.01 (br, 1H), 7.22-7.38 (m, 1H), 7.42-7.60 (t, 1H), 7.80-8.00 (t, 2H).

(8-Morpholin-4-yl-octyl)-(1,2,3,4-tetrahydroacridin-9-yl)amine (45c). Starting from 43c and following the previous procedure, 45c was obtained as a brownish oil. ¹H NMR (CDCl₃) δ: 1.20-1.58 (m, 10H), 1.60-1.75 (m, 2H), 1.80-1.99 (s, 4H), 2.20-2.38 (t, 2H), 2.39-2.45 (s, 4H), 2.60-2.68 (s, 2H), 2.98-3.17 (s, 2H), 3.40-3.57 (t, 2H), 3.60-3.79 (m, 4H), 3.81-4.01 (br, 1H), 7.22-7.38 (m, 1H), 7.42-7.60 (t, 1H), 7.80-8.00 (t, 2H).

(9-Morpholin-4-yl-nonyl)-(1,2,3,4-tetrahydroacridin-9-yl)amine (45d). Starting from 43d and following the previous procedure, 45d was obtained as a brownish oil. ¹H NMR (CDCl₃) δ: 1.20-1.58 (m, 12H), 1.60-1.75 (m, 2H), 1.80-1.99 (s, 4H), 2.20-2.38 (t, 2H), 2.39-2.45 (s, 4H), 2.60-2.68 (s, 2H), 2.98-3.17 (s, 2H), 3.40-3.57 (t, 2H), 3.60-3.79 (m, 4H), 3.81-4.01 (br, 1H), 7.22-7.38 (m, 1H), 7.42-7.60 (t, 1H), 7.80-8.00 (t, 2H).

(10-Morpholin-4-yl-decyl)-(1,2,3,4-tetrahydroacridin-9-yl)amine (45e). Starting from 43e and following the previous procedure, 45e was obtained as a brownish oil. ¹H NMR (CDCl₃) δ: 1.20-1.58 (m, 12H), 1.60-1.75 (m, 2H), 1.80-1.99 (s, 4H), 2.20-2.38 (t, 2H), 2.39-2.45 (s, 4H), 2.60-2.68 (s, 2H), 2.98-3.17 (s, 2H), 3.40-3.57 (t, 2H), 3.60-3.79 (m, 4H), 3.81-4.01 (br, 1H), 7.22-7.38 (m, 1H), 7.42-7.60 (t, 1H), 7.80-8.00 (t, 2H).
1,2,3,4-Tetrahydroacridin-9-ylamine (46a). A stirred mixture of 2-Aminobenzonitrile (5 g, 42.4 mmol), Cyclohexanone (4.15 g, 42.4 mmol) and ZnCl$_2$ (11.44 g, 84.7 mmol) was heated neat at 130 °C for 3 hours. The reaction mixture was quenched with ice/water and made alkaline by NaHCO$_3$ saturated solution. The solid residue was filtered and crystallized from EtOH affording 46a as a pale yellow solid (3.78 g, 58%). mp 284-286 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.87-1.89 (m, 4H), 2.84-2.87 (m, 2H), 3.01 (s, 2H), 7.30-7.34 (m, 2H), 7.80-7.85 (m, 2H).

6-Chloro-1,2,3,4-tetrahydro-acridin-9-ylamine (46b). Following the previous procedure and starting from 2-Amino-4-chlorobenzonitrile (5 g, 32.9 mmol), 46b was obtained as a pale yellow solid (3.58 g, 47%). mp 172-173 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.85-1.87 (m, 4H), 2.83-2.85 (m, 2H), 3.06 (s, 2H), 7.28-7.33 (m, 1H), 7.76-7.82 (m, 2H).

11H-Indeno[1,2-b]quinolin-10-ylamine (47a). Following the previous procedure and starting from 2-Aminobenzonitrile (5 g, 42.4 mmol) and indanone, 47a was obtained as a pale yellow solid (2.5 g, 25%). mp 264-265 °C. $^1$H NMR (CDCl$_3$) $\delta$: 4.07 (s, 2H), 7.42-7.63 (m, 4H), 7.70-7.81 (t, 1H), 8.18-8.31 (m, 3H).

7-Chloro-11H-indeno[1,2-b]quinolin-10-ylamine (47b). Following the previous procedure and starting from 2-Amino-4-chlorobenzonitrile (5 g, 32.9 mmol) and indanone, 47b was obtained as a pale yellow solid (1.4 g, 16%). mp 124-125 °C. $^1$H NMR (CDCl$_3$) $\delta$: 4.09 (s, 2H), 7.51-7.64 (m, 4H), 8.17-8.26 (m, 3H).

(7-Bromoheptyl)-(11H-indeno[1,2-b]quinolin-10-yl)amine (48a). A suspension of 47a (0.65 g, 2.8 mmol), 1,7-dibromoheptan (0.57 mL, 3.4 mmol) and KOH (0.25 g, 4.5 mmol) in dry DMSO (10 mL) was stirred for 16 hours at room temperature under nitrogen in the presence of freshly activated 4 Å molecular sieves (100 mg). The mixture was filtered through Celite® and the filtrate diluted with water (250 mL), extracted with EtOAc (3 x 30 mL), anhydrified over Na$_2$SO$_4$ and concentrated to dryness. Flash chromatography (DCM/MeOH 98:2) afforded 0.11 g of 48a as a yellowish oil (10%). $^1$H NMR (CDCl$_3$): $\delta$ 1.38-1.60 (m, 6H), 1.62-1.83 (m, 4H), 3.60-3.78 (m, 4H), 4.12 (s, 2H), 7.40-7.61 (m, 4H), 7.72-7.83 (t, 1H), 8.16-8.29 (m, 3H).

(7-Bromoheptyl)-(7-chloro-11H-indeno[1,2-b]quinolin-10-yl)amine (48b). Following the previous procedure and starting from 47b (0.5 g, 1.88 mmol), 48b was obtained as a brownish oil (0.18 g, 22%). $^1$H NMR (CDCl$_3$): $\delta$ 1.37-1.59 (m, 6H), 1.63-1.85 (m, 4H), 3.58-3.76 (m, 4H), 4.11 (s, 2H), 7.42-7.63 (m, 4H), 7.71-7.82 (t, 1H), 8.15-8.32 (m, 2H).
N-(11H-Indeno[1,2-b]quinolin-10-yl)-N’-(1,2,3,4-tetrahydroanthracen-9-yl)heptane-1,7-diamine (49a). Following the previous procedure and starting from 48a (0.1 g, 0.24 mmol) and 46a (0.048 g, 0.24 mmol) 49a was obtained as a pale brown oil (0.03 g, 24%). $^1$H NMR (CDCl$_3$): $\delta$ 1.20-1.60 (m, 8H), 1.62-1.97 (m, 6H), 2.59-2.67 (m, 2H), 3.02-3.18 (m, 2H), 3.60 (t, 2H), 3.80 (t, 2H), 4.08 (s, 2H), 5.60 (br, 1H), 7.20-7.70 (m, 7H), 7.85-8.17 (m, 4H), 8.32-8.41 (m, 1H). ES-MS m/z: 527 (M + 1).

N-(6-Chloro-1,2,3,4-tetrahydroanthracen-9-yl)-N’-(11H-indeno[1,2-b]quinolin-10-yl)heptane-1,7-diamine (49b). Following the previous procedure and starting from 48a (0.1 g, 0.24 mmol) and 46b (0.055 g, 0.24 mmol) 49b was obtained as a pale brown oil (0.07 g, 51%). $^1$H NMR (CDCl$_3$): $\delta$ 1.27-1.58 (m, 6H), 1.59-1.80 (m, 4H), 1.81-1.96 (m, 4H), 2.57-2.65 (m, 2H), 3.00-3.11 (m, 2H), 3.55 (t, 2H), 3.70 (t, 2H), 3.98 (s, 2H), 7.20-7.70 (m, 6H), 7.85-8.17 (m, 4H), 8.32-8.41 (m, 1H). ES-MS m/z: 561 (M + 1).

N-(3-Chloro-11H-indeno[1,2-b]quinolin-10-yl)-N’-(6-chloro-1,2,3,4-tetrahydroanthracen-9-yl)heptane-1,7-diamine (49c). Following the previous procedure and starting from 48b (0.15 g, 0.34 mmol) and 46b (0.078 g, 0.34 mmol) 49c was obtained as a pale brown oil (0.05 g, 15%). $^1$H NMR (CDCl$_3$): $\delta$ 1.27-1.58 (m, 6H), 1.59-1.80 (m, 4H), 1.81-1.96 (m, 4H), 2.57-2.65 (m, 2H), 3.00-3.11 (m, 2H), 3.55 (t, 2H), 3.70 (t, 2H), 3.98 (s, 2H), 7.20-7.70 (m, 6H), 7.85-8.17 (m, 4H). ES-MS m/z: 595 (M + 1).

N,N’-Bis-(11H-indeno[1,2-b]quinolin-10-yl)heptane-1,7-diamine (49d). Following the previous procedure and starting from 48a (0.1 g, 0.24 mmol) and 47a (0.056 g, 0.24 mmol), 49d was obtained as a pale brown oil (0.05 g, 37%). $^1$H NMR (CDCl$_3$): $\delta$ 1.35-1.59 (m, 6H), 1.60-1.77 (m, 4H), 3.59-3.72 (m, 4H), 4.11 (s, 2H), 4.80 (br, 2H), 7.05-7.71 (m, 12H), 8.00-8.17 (m, 2H), 8.20-8.32 (m, 2H). ES-MS m/z: 561 (M + 1).

7-(7-Bromoheptyloxy)-9-oxa-1-azaanthracen-10-one (51). A stirred suspension of 50 (0.8 g, 3.7 mmol), 1,7-Dibromohexane (1.2 mL, 7.5 mmol) and K$_2$CO$_3$ (0.97 mg) in acetone (30 mL) was refluxed for 20 h. The reaction mixture was hot filtered and the filtrate evaporated. The residue was purified by flash chromatography (toluene/acetone 90:10), affording 51 as an oil (0.65 g, 45%). $^1$H NMR (CDCl$_3$): $\delta$ 1.35-1.57 (m, 6H), 1.79-1.97 (m, 4H), 3.38-3.50 (t, 2H), 4.05-4.17 (t, 2H), 6.91-7.03 (m, 2H), 7.39-7.45 (m, 1H), 8.16-8.23 (m, 1H), 8.60-8.75 (m, 2H).

7-[7-(1,2,3,4-Tetrahydroanthracen-9-ylamino)heptyloxy]-9-oxa-1-azaanthracen-10-one (52). A mixture of 46a (0.078 g, 0.39 mmol), 51 (0.31 g, 0.79 mmol) and a catalytic amount of tetra-n-
butylammoniumhydrogensulphate (0.0195 g) were stirred at room temperature for 4 hours in a biphasic mixture of DCM/NaOH 50% (7.5 / 5 mL). The organic layer was separated and washed with water, then dried and evaporated. The crude was purified by flash chromatography (DCM/MeOH 97:3) affording as a brownish oil (0.015 g, 7.6%). \( ^1H\) NMR (CDCl\(_3\)): \( \delta \) 1.42-1.65 (m, 6H), 1.77-2.05 (m, 8H), 2.60 (t, 2H), 3.30 (t, 2H), 3.90 (t, 2H), 4.15 (t, 2H), 5.80 (br, 1H), 7.05-7.71 (m, 8H), 8.00-8.17 (m, 2H). ES-MS m/z: 508 (M + 1).

[7-(4-Benzofuran-2-yl-phenoxy)heptyl]-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amine (53a). A solution of 46b (0.15 g, 0.65 mmol), 3c (0.3 g, 0.78 mmol) and KOH (0.06 g, 1.04 mmol) in dry DMSO (8 mL) was stirred for 16 hours at room temperature under nitrogen in the presence of freshly activated 4 Å molecular sieves (100 mg). The mixture was filtered through Celite\({\textsuperscript{®}}\) and the filtrate diluted with water (300 mL), extracted with EtOAc (3 × 25 mL) anhydrified over Na\(_2\)SO\(_4\) and concentrated to dryness giving 0.26 g of crude. Flash chromatography (DCM/MeOH 98:2) afforded as a yellowish oil (0.18 g, 51%). \( ^1H\) NMR (CDCl\(_3\)) \( \delta \): 1.28-1.52 (m, 6H), 1.54-1.92 (m, 8H), 2.52-2.68 (m, 2H), 2.92-3.12 (m, 2H), 3.38-3.45 (t, \( J = 7.0 \) Hz, 2H), 3.89-3.95 (t, \( J = 6.2 \) Hz, 2H), 5.24 (s, 1H), 6.82 (s, 1H), 6.88-6.92 (d, \( J = 8.8 \) Hz, 2H), 7.18-7.25 (m, 3H), 7.45-7.49 (m, 2H), 7.71-7.75 (d, \( J = 8.8 \) Hz, 2H), 7.83-7.88 (d, \( J = 10.6 \) Hz, 2H). \( ^{13}C\) NMR (CDCl\(_3\)) \( \delta \): 22.48, 22.74, 24.37, 25.79, 26.66, 28.93, 31.52, 33.90, 40.77, 49.33, 67.72, 99.41, 110.76, 114.56, 115.50, 118.23, 120.38, 122.66, 122.93, 123.54, 123.93, 124.49, 126.18, 127.41, 129.32, 133.71, 148.00, 150.57, 150.79, 154.48, 155.89, 159.31. ES-MS m/z: 539 (M + 1).

[7-(3-Benzofuran-2-yl-phenoxy)heptyl]-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amine (53b). Following the previous procedure and starting from 46b (0.15 g, 0.65 mmol) and 3f (0.3 g, 0.78 mmol), 53b was obtained as a yellowish oil (0.05 g, 14%). \( ^1H\) NMR (CDCl\(_3\)) \( \delta \): 1.38-1.58 (m, 6H), 1.60-1.93 (m, 8H), 2.60-2.65 (m, 2H), 2.98-3.10 (m, 2H), 3.45-3.52 (t, 7.0 Hz, 2H), 3.99-4.06 (t, 6.6 Hz, 2H), 5.28 (s, 1H), 6.81-6.92 (m, 1H), 7.00 (s, 1H), 7.21-7.59 (m, 8H), 7.87-7.91 (m, 2H).

2-Hydroxymethyl-4-methoxyphenol (54). A stirred solution of 2-Hydroxy-5-methoxy benzaldehyde (5 g, 32.86 mmol) in EtOH (66 mL, 0.5 M) was cooled to 0 °C and NaBH\(_4\) (1.24 g, 32.86 mmol) was added portionwise. The resulting suspension was then allowed to reach room temperature and stirred for about 1 hour. The solvent was removed and the residue was cooled to 0 °C, made acid with HCl 1N and extracted with Et\(_2\)O. The organic layers were collected, dried over Na\(_2\)SO\(_4\) and concentrated to afford as a white crystalline solid (5 g, 99%). (R.f. 0.18; petroleum ether/ethyl acetate 60:40 as eluent). mp 76 °C. \( ^1H\) NMR (CDCl\(_3\)) \( \delta \): 3.99 (s, 3H), 5.28 (s, 2H), 6.81-6.92 (m, 1H), 7.00 (s, 1H), 7.21-7.59 (m, 1H).
(2-Hydroxy-5-methoxybenzyl)-triphenylphosphonium bromide (55). To stirred solution of 54 (0.47 g, 3.05 mmol) in acetonitrile (325 mL, 0.1 M), triphenyl-phosphonium bromide (1.05 g, 3.05 mmol) was added. The resulting mixture was refluxed for 1 hour until the formation of an heavy white precipitate. The solid was filtered off and washed with cold acetonitrile affording 55 as a white solid (1.14 g, 78%). mp 298 dec. 

\[ \text{H NMR (d}_6\text{DMSO)} \delta : 3.99 (s, 3H), 5.68 (s, 2H), 6.97-7.15 (m, 8H), 7.32-7.54 (m, 3H), 8.35-8.59 (m, 2H), 8.85-8.91 (m, 5H). \]

4-Benzylxybenzoic acid ethyl ester (56). A stirred suspension of Ethyl-p-hydroxybenzoate (5 g, 30 mmol), K$_2$CO$_3$ (8.30 g, 60 mmol) and benzylbromide (5.35 mL, 45 mmol) in acetone (60 mL, 0.5 M) was refluxed for 10 hours, then hot filtered. The filtrate was concentrated to dryness. To the residue, petroleum ether was added and the flask kept in the freezer overnight. The formed white solid was filtered off affording 56 (6.06 g, 79%). mp 43-44 °C. 

\[ \text{H NMR (CDCl)}_3 \delta : 1.56-1.87 (t, 3H), 4.05 (q, 2H), 5.23(s, 2H), 6.78-6.90 (m, 3H), 7.01-7.12 (d, 2H), 7.23-7.42 (d, 2H), 8.02-8.14 (m, 2H). \]

4-Benzylxybenzoic acid (57). To a stirred solution of 56 (6.06 g, 24 mmol) in EtOH (50 mL), NaOH (1.42 g, 0.035 mmol) previously solubilized in 2.5 mL of water was added. The mixture was refluxed for 3 hours, then cooled to room temperature. EtOH was removed under vacuum and the residue was diluted with water then cooled to 0 °C and made acid with HCl 6N. The white solid that formed was filtered off affording 57 (5.24 g, 96%). mp 189-190 °C. 

\[ \text{H NMR (CDCl)}_3 \delta : 4.98 (s, 2H), 6.77-6.92 (m, 3H), 7.03-7.15 (d, 2H), 7.26-7.43 (d, 2H), 7.98-8.10 (m, 2H). \]

4-Benzylxybenzoyl chloride (58). 57 (5.24 g, 23 mmol) was refluxed in SOCl$_2$ (16.67 mL, 230 mmol) with stirring for 3 hours. The resulting solution is cooled to room temperature and the excess of SOCl$_2$ removed under vacuum affording 58 as a greyish solid (5.65 g, 100%). mp 99-100 °C. The intermediate is used for the next step without any further characterization.

2-(4-Benzylxyphenyl)-5-methoxybenzofuran (59). A stirred suspension of 55 (10 g, 20.8 mmol), 58 (5.6 g, 22.9 mmol) and Et$_3$N (8.7 mL, 62.6 mmol) in Toluene (208 mL) was refluxed for 10 hours. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated to dryness, EtOH was added to the residue and the flask kept in the freezer overnight. The solid that formed was filtered off affording 2.35 g of crude which upon flash chromatography (DCM/petroleum ether 50:50) gave 59 as a white crystalline solid (0.74 g, 11 %). mp 76 °C. 

\[ \text{H NMR (CDCl)}_3: \delta 3.85 (s, 3H), 5.12 (s, 2H), 6.82 (s, 1H), 6.86-6.87 (d, 2.6 Hz, 1H), 7.02-7.06 (m, 3H), 7.35-7.43 (m, 6H), 7.74-7.79 (d, 8.8 Hz, 2H). \]
4-(5-Methoxybenzofuran-2-yl)phenol (60). 59 (0.2 g, 0.61 mmol) was dissolved in THF (100 mL) and Pd/C 10% was added catalytically. The flask was purged and filled with H₂. The reaction was stirred under H₂ (1 atm) at room temperature for 5 h. The catalyst was then removed by filtration through a pad of Celite® and the filtrate concentrated to dryness affording 0.19 g of crude. Flash chromatography (DCM/petroleum ether/acetone 50:45:5), afforded 60 (0.1 g, 69%) as a white solid. mp 189 °C. ¹H NMR (CD₃COCD₃): δ 3.81 (s, 3H), 6.82-7.09 (m, 5H), 7.37-7.41 (d, 8.4 Hz, 1H), 7.73-7.77 (d, 8.8 Hz, 2H), 8.73 (br, 1H OH).

2-[4-(7-Bromoheptyloxy)phenyl]-5-methoxybenzofuran (61). A stirred suspension of 60 (0.07 g, 0.29 mmol), K₂CO₃ anhydrous (0.08 g, 0.58 mmol) and 1,7-dibromoheptan (0.1 mL, 0.58 mmol) in acetone (20 mL) was refluxed for 10 hours, then filtered when hot. The filtrate was concentrated and petroleum ether was added to the resulting residue. After cooling overnight in a freezer a white solid formed which was filtered off affording 61 (0.07g, 58%). mp 123 °C. ¹H NMR (CDCl₃): δ 1.15-1.32 (m, 6H), 1.75-1.89 (q, 7.2 Hz, 4H), 3.37-3.44 (t, 6.6 Hz, 2H), 3.83 (s, 3H), 3.94-4.00 (t, 6.6 Hz, 2H), 6.80-7.00 (m, 5H), 7.34-7.39 (d, 8.8 Hz, 1H), 7.72-7.76 (d, 8.8 Hz, 2H); ¹³C NMR (CDCl₃): δ 25.83, 28.03, 28.47, 29.07, 32.65, 33.89, 55.83, 67.88, 99.73, 103.09, 111.29, 112.16, 114.69, 123.15, 126.25, 130.02, 149.65, 153.07, 155.94, 156.86, 159.43, 197.95.

Benzyl-[7-[4-(5-methoxybenzofuran-2-yl)phenoxy]heptyl]methyl-amine (62). A stirred solution of 61 (0.07 g, 0.17 mmol) and N-Benzylmethylamine (0.04 mL, 0.34 mmol) in toluene (20 mL) was refluxed for 20 hours. The mixture was washed with water and the organic layer dried over Na₂SO₄ and concentrated. The crude was then purified by flash chromatography (toluene/acetone 96:4), affording 62 as a white solid (0.05 g, 65%). mp 122 ºC. ¹H NMR (CDCl₃): δ 1.25-1.52 (m, 8H), 1.75-1.82 (m, 2H), 2.18 (s, 3H), 2.32-2.39 (t, 7.2 Hz, 1H), 3.47 (s, 2H), 3.84 (s, 3H), 3.94-4.01 (t, 6.4 Hz, 2H), 6.80- 6.87 (m, 2H), 6.92-7.00 (m, 3H), 7.24-7.39 (m, 6H), 7.72-7.77 (d, 8.4 Hz, 2H). ¹³C NMR (CDCl₃): δ 25.98, 27.31, 29.17, 29.28, 42.25, 55.85, 57.46, 62.32, 68.02, 99.71, 103.12, 111.30, 112.15, 114.72, 123.14, 126.27, 126.82, 128.13, 129.01, 130.05, 139.25, 149.67, 155.96, 156.92, 158.51, 159.50.

2-(4-Methoxy-benzyl)-benzofuran (63). Following the same procedure for the synthesis of 1a and starting from 4-Metoxybenzoylchloride (5 g, 27 mmol), 63 was obtained as a colorless oil (2.97 g, 46%). ¹H NMR (CDCl₃) δ: 2.47 (s, 2H), 3.93 (s, 3H), 6.89 (s, 1H), 6.91-6.99 (m, 2H), 7.21-7.32 (m, 2H), 7.33-7.61 (m, 2H), 7.72-7.83 (m, 2H).

4-Benzofuran-2-ylmethyl-phenol (64). Following the same procedure for the synthesis of 2a and starting from 63 (2.5 g, 11 mmol), 64 was obtained as a white solid (2.11 g, 90%). mp 137 ºC.
$^1$H NMR (CDCl$_3$) $\delta$: 2.45 (s, 2H), 6.86 (s, 1H), 6.89-6.97 (m, 2H), 7.18-7.29 (m, 2H), 7.34-7.62 (m, 2H), 7.71-7.85 (m, 2H).

2-[4-(7-Bromo-heptyloxy)-benzyl]-benzofuran (65). Following the same procedure for the synthesis of 3c and starting from 64 (2.0 g, 8.9 mmol), 65 was obtained as a white solid (2.57 g, 72%). mp 165 °C. $^1$H NMR (CDCl$_3$) $\delta$: 1.27-1.59 (m, 6H), 1.71–1.98 (m, 4H), 2.34-2.67 (s, 2H), 3.42-3.46 (t, 2H), 4.01-4.07 (t, 2H), 6.86 (s, 1H), 6.92-6.97 (d, 2H), 7.25-7.32 (m, 2H), 7.45-7.59 (m, 2H), 7.73-7.75 (d, 2H).

[7-(4-Benzofuran-2-ylmethyl-phenoxy)-heptyl]-benzyl-methyl-amine (66). Following the same procedure for the synthesis of 4c and starting from 65 (0.23 g, 0.57 mmol), 66 was obtained as a brownish oil. $^1$H NMR (CDCl$_3$) $\delta$: 1.26-1.61 (m, 8H), 1.72-1.87 (m, 2H), 2.20 (s, 3H), 2.32-2.43 (m, 4H), 3.45 (s, 2H), 3.96-4.05 (t, 2H), 6.82 (s, 1H), 6.92-6.98 (d, 2H), 7.21-7.34 (m, 7H), 7.42-7.53 (m, 2H), 7.74-7.79 (d, 2H). ES-MS $m/z$: 442 (M + 1).
Biology

Inhibition of AChE and BuChE activity.

The capacity of compounds to inhibit AChE activity was assessed using the Ellman’s method\textsuperscript{(33)} Initial rate assays were performed at 37 °C with a Jasco V-530 double beam Spectrophotometer: the rate of increase in the absorbance at 412 nm was followed for 5 min. AChE stock solution was prepared by dissolving human recombinant AChE (E.C.3.1.1.7) lyophilized powder (Sigma, Italy) in 0.1 M phosphate buffer (pH = 8.0) containing Triton X-100 0.1 %. Stock solution of BuChE (E.C. 3.1.1.8) from human serum (Sigma Italy) was prepared by dissolving the lyophilized powder in an aqueous solution of gelatine 0.1 %. Stock solutions of inhibitors (1 or 2 μM) were prepared in methanol. Five increasing concentrations of the inhibitor were used, able to give an inhibition of the enzymatic activity in the range of 20-80 %. The assay solution consisted of a 0.1 M phosphate buffer pH 8.0, with the addition of 340 μM 5,5'-dithio-bis(2-nitrobenzoic acid), 0.02 unit/mL of human recombinant AChE, or BuChE from human serum and 550 μM of substrate (acetylthiocholine iodide, ATCh or butyrylthiocholine iodide, BTCh, respectively). 50 μL aliquots of increasing concentration of the tested compound were added to the assay solution and preincubated for 20 min at 37 °C with the enzyme followed by the addition of substrate. Assays were carried out with a blank containing all components except AChE or BuChE in order to account for the non-enzymatic reaction. The reaction rates were compared and the percent inhibition due to the presence of tested inhibitor at increasing concentration was calculated. Each concentration was analyzed in duplicate, and IC50 values were determined graphically from log concentration–inhibition curves (GraphPad Prism 4.03 software, GraphPad Software Inc.).

Inhibition of β-amyloid aggregation.

Preparation of solutions: Stock solution of 1 μM was prepared by solubilizing the lyophilized Aβ\textsubscript{25-35} peptide by brief vortexing in sterile water at 4 °C, then by sonication for 1 min. The peptide stock solution was aliquoted and stored at -20 °C. All steps were carried out at 4 °C to prevent Aβ\textsubscript{25-35} polymerization. The new compounds were solubilized in MeOH solution to a concentration S5 of 1 M. The stock solution was diluted to obtain aliquots with concentrations between 0.1-50 μM, then stored at -20 °C.

Measurement of inhibitory activity by UV-Visible Spectroscopy: To study the kinetic of Aβ\textsubscript{25-35} polymerization alone, experiments were carried out by using a reaction mixture containing 80 μL phosphate buffer (10 mM final concentration) and 10 μL Aβ\textsubscript{25-35} (100 μM final concentration), pH
7.2. When Aβ25-35 was added to the buffer solution, we performed sonication for 1 min to avoid any peptide aggregation. 10 µL MeOH were added to the solution to have the same conditions for the experiments with the new compounds. To study the inhibitory activity of the new compounds, experiments were carried out by using a reaction mixture containing 80 µL phosphate buffer (10 mM final concentration), 10 µL MeOH containing 10 µM final concentration of one of the new compounds or 10 µL MeOH containing 0.1, 1, 10, 20 and 50 µM final concentration for IC50 determination and 10 µL Aβ25-35 (100 µM final concentration), pH 7.2. All steps were carried out at 4 °C to prevent Aβ25-35 polymerization. UV-Visible spectroscopy was performed on a Cary 300 bio UV-Visible spectrophotometer. Polymerization kinetics were monitored in the range of 190–380 nm between 0 and 6 hours. Data were collected 5 hours after incubation. For each inhibition experiment, one sample containing Aβ25-35 alone and another containing the new compound alone were used in parallel as controls in the same experimental conditions. Moreover, to rule out any influence due to the new compounds absorbance, their UV-visible spectra were subtracted from the Aβ25-35 absorption spectra. At least three independent measurements were made for all cases. All results are presented with means and standard deviation. IC50 was calculated by using a least-square fitting technique to match the experimental data with a sigmoidal curve. IC50 was the concentration of the new compound inhibiting the formation of Aβ fibrils to 50 % of the control value.

Electron Microscopy: The samples are taken again 5 min after measuring the kinetics. 10 µL of reaction mixtures were applied to carbon-coated collodium film on 200 or 400 mesh copper grid, negatively stained with 2 % (w/v) uranyl acetate and viewed on a Philips CM 10 transmission electron microscope operating at 100 KV (SERCOMI, Bordeaux, France).

The neuroprotective effects against Aβ25-35 peptide.

Chemicals: Aβ25-35 peptide and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Bachem (Bubendorf, Switzerland) and Sigma Chemical Co. (St. Louis, MO), respectively. All other reagents were of the highest grade of purity commercially available.

Aβ25-35 peptide preparation for neurotoxicity assay: Aβ25-35 peptide was dissolved in hexafluoro isopropanol (HFIP) to 1 mg/mL, sonicated and incubated at room temperature for 24 h to produce unaggregated Aβ peptide. The HFIP was dried under vacuum in a Speed Vac and the resulting peptide film was dissolved in DMSO to 1 mM. The unaggregated Aβ25-35 stock solution was then
aliquoted and stored at -20 °C until use. For neurotoxicity assay, the Aβ25-35 stock solution was diluted directly into cell culture media.

**Cell culture and neurotoxicity assay:** Human neuronal-like SH-SY5Y cells were routinely grown at 37 °C in a humidified incubator with 5 % CO2 in Dulbecco’s modified Eagle’s medium supplemented with 10 % fetal bovine serum, 2 mM glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin. To evaluate the protective effects of compounds against Aβ25-35 peptide induced neurotoxicity, the SH-SY5Y cells were seeded in 96-well plates at 3×10^4 cells/well, incubated for 24 h and subsequently treated with 10 μM of unaggregated Aβ25-35 peptide for 3 h at 37 °C in 5 % CO2, in presence or absence of various concentrations of compounds (1-30 μM). The neuronal viability in terms of mitochondrial metabolic function was evaluated by the reduction of MTT to formazan as previously described. The cellular reduction of MTT represents an indicator of the initial events underlying the mechanism of Aβ25-35 peptide neurotoxicity. Briefly, after removal of the treatment, SH-SY5Y cells were washed with phosphate buffered saline (PBS) and incubated with MTT (5 mg/mL) in PBS for 2 h at 37 °C in 5 % CO2. After further washing, the formazan crystals were dissolved with isopropanol. The amount of formazan was measured (570 nm, ref. 690 nm) with a spectrophotometer (TECAN®, Spectra model Classic, Salzburg, Austria). The neuronal viability was expressed as a percentage of control cells and calculated by the formula: (absorbance of treated neurons/absorbance of untreated neurons) × 100.

**Inhibition of BACE-1 activity**

Purified Baculovirus-expressed BACE-1 (β-secretase) and rhodamine derivative substrate were purchased from Panvera (Madison, WI, U.S). Sodium acetate and DMSO were from Sigma Aldrich (Milan, Italy). Purified water from Milli-RX system (Millipore, Milford, MA, USA) was used to prepare buffers and standard solutions. Spectrofluorometric analyses were carried out on a Fluoroskan Ascent multiwell spectrofluorometer (excitation: 544 nm; emission: 590 nm) by using black microwell (96 wells) Cliniplate plates (Thermo LabSystems, Helsinki, Finland). Stock solutions of the tested compounds were prepared in DMSO and diluted with 50 mM sodium acetate buffer pH=4.5. Specifically, 20 μL of BACE-1 enzyme (25 nM) were incubated with 20 μL of test compound for 60 minutes. To start the reaction, 20 μL of substrate (0.25 μM) were added to the well. The mixture was incubated at 37 °C for 60 minutes. To stop the reaction, 20 μL of BACE-1 stop solution (sodium acetate 2.5 M) were added to each well. Then the spectrofluorometric assay was performed by reading the fluorescence signal at 590 nm. Assays were done with a blank
containing all components except BACE-1 in order to account for non enzymatic reaction.. The reaction rates were compared and the percent inhibition due to the presence of test compounds was calculated. Each concentration was analyzed in triplicate. The percent inhibition of the enzyme activity due to the presence of increasing test compound concentration was calculated by the following expression: $100 - \left( \frac{v_i}{v_o} \times 100 \right)$, where $v_i$ is the initial rate calculated in the presence of inhibitor and $v_o$ is the enzyme activity. To demonstrate inhibition of BACE-1 activity, a peptido mimetic inhibitor (inhibitor IV, Calbiochem, Darmstadt, Germany) was used as reference inhibitor ($IC_{50}=13.61$ nM).

**Substrate:** Rhodamine-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-quencher

![Rhodamine](image)
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