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Scuola di Dottorato di Ricerca in
"Scienza del Farmaco e delle Sostanze Bioattive"

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"New non peptidic inhibitors of β-Secretase as potential anti Alzheimer agents"

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SUMMARY

Chapter 1

Alzheimer's Disease

The pathology of Alzheimer's Disease (AD) is the most common form of dementia characterized by a progressive neurodegenerative disorder affecting the elderly that cause a slow insidious decline in cognitive ability, with an eventually fatal deadly outcome.

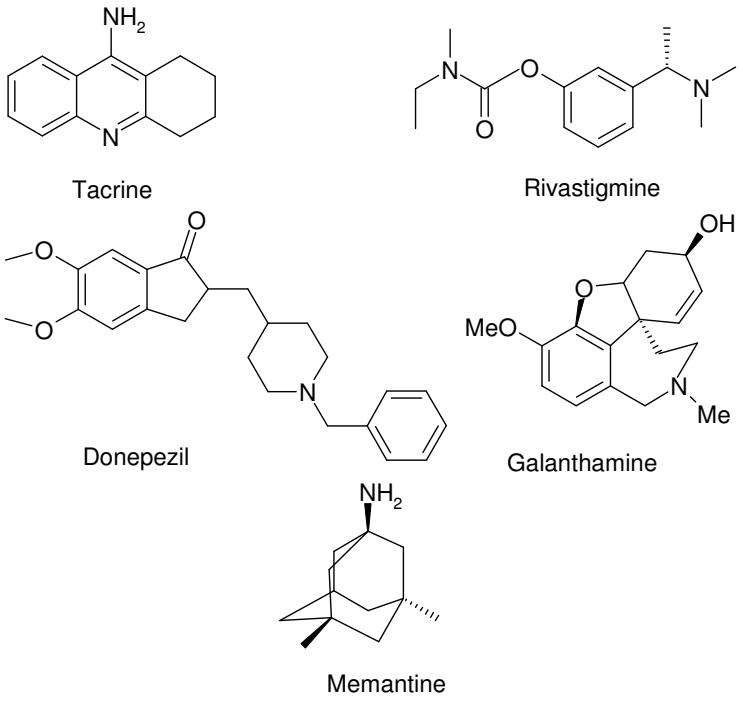
An estimated 10 percent of Americans over the age of 65 and half of those over age 85 have AD. More than four million Americans currently suffer from the disease, and the number is projected to balloon to 10-15 million over the next several decades. Alzheimer's Disease is now the third most expensive disease to treat in the U.S. The annual national direct and indirect costs of caring for AD patients have been estimated to be as much as $100 billion. People with AD live an average of 8 years after diagnosis, but may survive anywhere from 3 to 20 years.

This pathology has no known single cause, but in the last 15 years scientists have learned a great deal about factors that may play a role. Late-onset AD, which chiefly affects individuals over age 65, is the more common form of the illness that is most often associated with the term "Alzheimer's disease." The greatest known risk factors for late-onset AD are increasing age and a family history of the disease. The likelihood of developing late-onset AD approximately doubles every five years after age 65. By age 85, the risk reaches nearly 50 percent. Scientists have so far discovered one gene that increases risk for late-onset disease. Rare, familial types of AD found in a few hundred families worldwide have been linked to specific genes. Individuals who inherit these genes are virtually certain to develop the disease, usually before age 65, and sometimes as early as their 30s or 40s. This is a rare form of AD known as early-onset familial AD.

Researchers are working to discover other factors that affect AD risk. Some of the most exciting preliminary evidence suggests that strategies for general healthy aging may also help reduce the risk of developing AD. These measures include controlling blood pressure, weight and cholesterol levels; exercising both body and mind; and staying socially active.

Presently, researchers cannot definitively say what causes Alzheimer's disease, and there is currently no cure. However, considerable progress has been made in the field of AD research in recent years, including the development of several medications for early-stage AD. While the ideal medication for AD would either prevent or cure it, have no side effects, be inexpensive and be readily available, researchers have not yet discovered the ideal treatment. It is possible, however, to reduce some of the common symptoms of early-stage AD with medications. As of January 2002, the Food and Drug Administration (FDA) had approved four drugs designed to improve memory and slow the progression of AD. The first one, approved in 1993, Tacrine (Cognex ®) in Figure 1.1 has many side effects, including potential liver damage and has shown disappointing memory results. For these reasons it is seldom prescribed. Three newer drugs (Figure 1.1), Donepezil (Aricept ®), Rivastigmine (Exelon ®) and Galantamine (Reminyl ®), have proved beneficial in improving memory, and have fewer side effects. Unfortunately, these drugs are not effective for everyone, and their effectiveness is limited to the early and middle stages of AD. Another drug, Memantine HCl (Namenda® in Figure 1.1) is available.

Figure1.1



Originally considered for use in moderate to severe stage AD, it is sometimes prescribed for use earlier. None of the therapies currently available offer significant benefits in terms of slowing the progression of the disease. All can be considered be largely "symptomatic" treatments as opposed to "disease modifying".

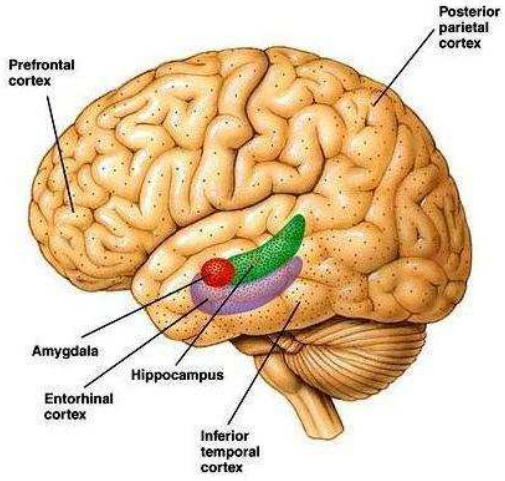
It currently is also possible to reduce some of the common emotional and behavioral symptoms associated with AD. For example, it is possible to prescribe drugs such as tranquilizers to reduce agitation, anxiety and unpredictable behavior. Drugs also can be prescribed to improve sleeping patterns and treat depression. Physicians may recommend taking Vitamin E, which may have some positive effects without unwanted side-effects if taken in reasonable quantities.

In the popular imagination AD is equated with an impaired memory, but the disease includes a number of other changes in brain function ; experts have documented common patterns of symptom progression that occur in many individuals with Alzheimer's Disease and developed several methods of "staging" based on these patterns. Progression of symptoms corresponds in a general way to the underlying nerve

cell degeneration that takes place in AD. Nerve cell damage typically begins with cells involved in learning and memory and gradually spreads to cells that control every aspect of thinking, judgment and behavior. The damage eventually affects cells that control and coordinate movement.

From the histological point of view the earliest damage occurs in the entorhinal cortex, hippocampus and basal forebrain, which are small, specialized structures in the brain that play a critical role in memory. The most striking early symptom is, in fact, loss of short term memory (amnesia), which usually manifests itself as minor forgetfulness that becomes steadily more pronounced with illness progression, with relative preservation of older memories. As the disorder progresses, cognitive (intellectual) impairment extends to the domains of language (aphasia), skilled movements (apraxia), recognition (agnosia), and those functions (such as decision-making and planning) closely related to the frontal and temporal lobes of the brain as they become disconnected from the limbic system, reflecting extension of the underlying pathological process.

Figure 2.1: Neurofibrillary Tangles and Amyloid Plaques



From the clinical point if view, AD overlaps with many other conditions leading to dementia. Advanced age in particular makes accurate diagnosis difficult if not possible.[1] [2] [3] [4] Even at necropsy, macroscopic examination of AD brains again shows an extensive overlap with changes encountered in normal aging. Thus, at the present, AD can be diagnosed positively only at necropsy on microscopic examination using silver impregnation stains, amyloidophilic dyes, or antibodies for visualisation.

The Pathology of Alzheimer's Disease

The pathology must take precedence, and in terms of pathology, the common theme of aberrant protein deposition is emerging with respect to a number of different neurodegenerative diseases such as Parkinson's disease (Table 1), Huntington's disease, prion diseases and amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease)(Table 1.1).

In Parkinson's disease, neuronal deposits called Lewy bodies containing R-synuclein are found in the substantia nigra, the subregion of the brain in which dopaminergic neurons are selectively lost. In Huntington's disease, the mutated huntingtin protein possesses a long string of glutamates that leads to the formation of intracellular inclusions in disease-affected neurons. In the prion diseases, such as Creutzfeldt-Jakob disease in humans, scrapie in sheep, the prion protein not only aggregates but also the aggregated protein is apparently infectious in the absence of genetic material. In amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), mutations in superoxide dismutase lead to astrocytic inclusions in transgenic mice.

Table1.1

disorder	protein
Alzheimer's disease	Aβ, tau
Parkinson's disease	α-synuclein
Huntington disease	huntingtin
prion diseases	PrP
amylotrophic lateral sclerosis	SOD1

In AD, one finds in the cerebral cortex the extraneuronal plaques and intraneuronal tangles first described by Alois Alzheimer nearly a century ago. The

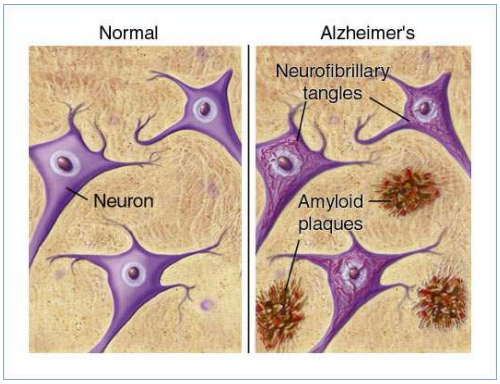
major protein component of the plaques is the amyloid-β protein (Aβ), and the tangles are composed of filaments of the microtubule-associated protein tau (τ). The Aβ is so named because of the β-pleated sheet secondary structure of the amyloid protein, while τ proteins are critical for polymerization and stabilization of tubulin in axonal microtubules and the cross-linking of microtubules to other cellular structures.[5 6]

These protein aggregates cause the degeneration of a specific subpopulation of neurons either because these aggregates are formed primarily in certain regions of the brain (i.e., where conditions are conducive to aggregate formations) or because certain neuronal subtypes are more sensitive to these toxic aggregates than other neurons leading to a slow but progressive increase of dementia.

Whether plaques and tangles independently or jointly are the causative agents of neuronal degeneration or, instead, a marker of the disease is still an issue of some debate. Plaques and tangles may be present in other diseases and even normal aging, [7] but AD does not develop in the absence of both. Plaques and tangles can occur independently of each other in diseases other than AD,[8 9 10 11 12 13] but there is increasing evidence that Aβ and τ are linked in AD [14 15 16] and there is some evidence that Aβ might precede τ formation.

The amyloid plaques and neurofibrillary tangles (Figure 3.1) of the AD brain have been subjected to rigorous pathological analysis at the cytological and biochemical levels, with the hope that further clues to disease etiology might be gleaned. In AD, dense deposits containing fibrillar forms of Aβ are intimately associated with dystrophic (degenerating) axons and dendrites (together referred to as neurites),[17] and these neuritic plaques are found in cerebral and midbrain regions associated with cognition and memory.[18 19 20]

Figure 3.1



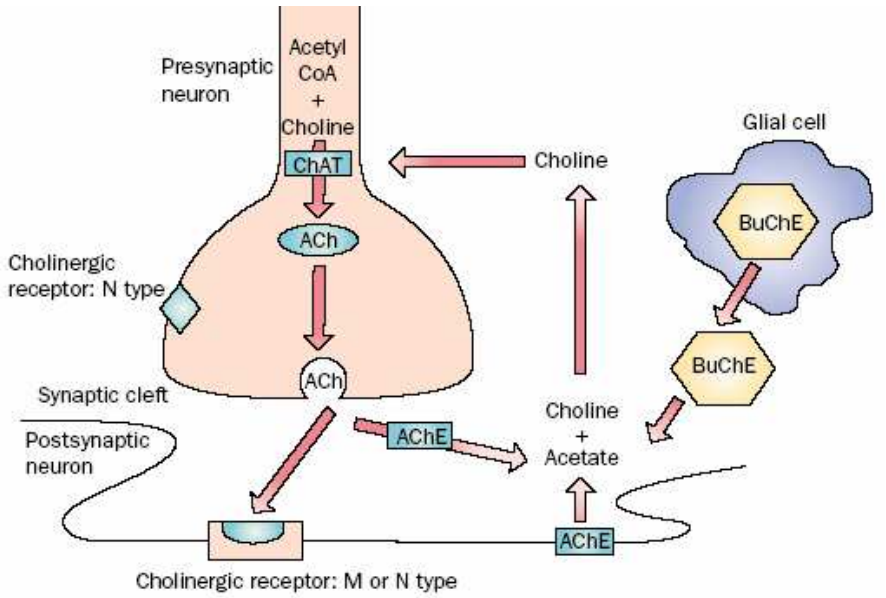
Neuritic plaques contain amorphous, nonfibrillary Aβ and are found in areas of the brain generally not implicated in clinical AD. Moreover, the diffuse plaques are often found in abundance in elderly, cognitively normal people, leading to the suggestion that these diffuse plaques may be the precursors to pathogenic dense plaques. Neurofibrillary tangles are also found in the brain regions critical to higher brain function. Biochemical analysis reveals that the filamentous form of the τ protein found in these tangles is hyperphosphorylated. Tau hyperphosphorylation renders insoluble this otherwise highly soluble cytosolic protein, and this modified form of τ is also found in many plaque-associated dystrophic neurites. Interestingly, tau-containing neurofibrillary tangles occur in a number of other, uncommon neurodegenerative diseases. In contrast, the amyloid containing neuritic plaques are unique to AD.

The Cholinergic Hypothesis

Another constant observation in post mortem AD brain is the loss of cholinergic markers such as acetylcholine (Ach) and choline acetyltransferase (ChAt).

First established in 1976 the "cholinergic hypothesis" postulates that many of the cognitive, functional and behavioral symptoms derive from a deficiency in cholinergic neurotransmission, linked to the loss of cholinergic neurons in the nucleus basalis and other nuclei and that cause an inability to transmit nerve cell impulses across cholinergic synapses.[21] [22] The cholinergic hypothesis of AD evolved from original observations by Davies and Maloney in 1976, who first reported decreased numbers of cholinergic neurons in autopsy brain tissue from patients with AD.[23] [24] It was postulated that a deficiency of the neurotransmitter, ACh, in the brain and subsequent reduced neurotransmission at central cholinergic experienced by AD patients.

Figure 4.1: ***Functional features of the cholinergic system. Ach= acethylcoline; Ache= acetylcholinesterase, BuChE= butyrylcholinesterase; ChAT= choline acethyltransferase; CoA= coenzyme A.***

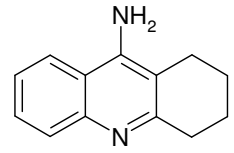


Because cholinergic function is required for short-term memory function, it was determined that cholinergic deficit in AD was also responsible for much of the short-term memory deficit.[25] Markers for cholinergic neurons such as choline acetyltransferase (ChAt) and acetylcholinestrase (AChE), which are enzymes responsible for synthesis and degradation of ACh, respectively, are decreased in the cortex and hippocampus, areas of the brain involved in cognition and memory. [26] In particular, the earliest loss of neurons occurs in the nucleus basalis and the entorhinal cortex, where cholinergic neurons are preferentially affected. As the illness progresses, up to 90 percent of cholinergic neurons in the nucleus basalis of Mynert may be lost. [26] [27] The resultant decrease in ACh-dependent neurotransmission is thought to lead to the functional deficits of AD, much as dopaminergic deficits underlie Parkinson's disease and its clinical manifestations.[26] [27]

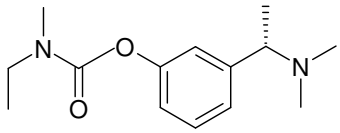
To improve cholinergic neurotransmission, different strategies have been investigated including the increasing of acetylcholine synthesis, the augmentation of presynaptic acetylcholine release, the stimulation of cholinergic postsynaptic muscarinic and nicotinic receptors, and the reduction of acetylcholine synaptic degradation with cholinesterase inhibitors. Clinical drug trials in patients with AD have focused on drugs that augment levels of ACh in the brain to compensate for losses of cholinergic function in the brain. These drugs have included ACh precursors, muscarinic agonists, nicotinic agonists, and cholinesterase inhibitors (AChEIs).[28] [29]The best developed and most successful approaches to date have used cholinesterase inhibition.[30] [31]

The first drug approved for general clinical use in AD was Tacrine (Cognex ®). However, three new cholinesterase inhibitors (AChEIs) are currently available: Donepezil (Aricept ®), Rivastigmine (Exelon ®), and Galantamine (Reminyl ®).[32] [33]

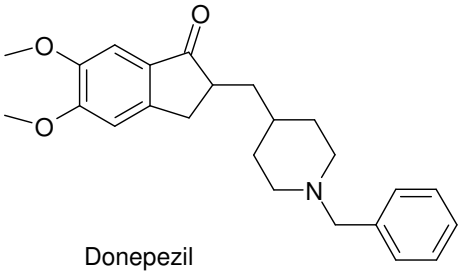
Figure 5.1



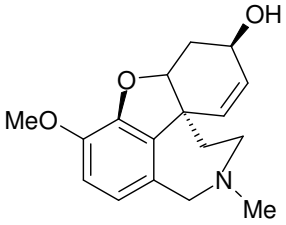
Tacrine



Rivastigmine



Donepezil



Galanthamine

All of these drugs have been tested primarily in patients with AD, with most trials studying treatment in patients with mild to moderately severe illness. Each of these compounds has its specific mode of action and inhibition constants (IC_{50}) (Table 2.1), which does not completely abolish all the functions of AChE and include some non-desirable side effects. Some of them present hepatotoxicity, nausea or vomiting, diarrhoea, abdominal pain, anorexia, weight loss, dizziness and bradycardia.

The clinical trials using the three main marketed compounds, Donepezil, Rivastigmine and Galanthamine have shown that the first one appears to be effective, of simple use and the best tolerated. The other two compounds are of similar effectiveness but less well tolerated.

Table 2.1

Compound	**IC_{50} (nM) AChE**	**IC_{50} (nM) BuChE**	**BuChE: AChE ratio**
Donezepil	23±5	7420±390	322
Rivastigmine	4150±160	37±5	0.009
Tacrine	190±40	47±10	0.247
Galanthamine	800±60	7300±830	9.125

Tacrine (THA), a competitive inhibitor of AChE interacting at the active site of the enzyme, has a substantial lipid solubility concentrating itself in the brain. It interacts with muscarinic receptors and inhibits monoaminooxidases. Some studies have suggested that the improvement of cognitive function by THA is associated with an increase in the number of nicotinic binding sites or glucose metabolism among other effects.[34] This reversible non-selective inhibitor causes cholinergic toxicity, due to its specificity towards butyrylcholinesterase (BuChE), another type of cholinesterase able to hydrolyse ACh among other ester compounds and that participates in the cholinergic regulation at human CNS. The use of Tacrine approved as the first AChE inhibitor by the U.S. Food and Drug Administration is limited because of its severe, though reversible, hepatotoxicity.

Galanthamine (GAL) is a plant alkaloid which is known to diminish cognitive deficits in animal models of learning and memory. GAL directly inhibits AChE activity, showing a weaker activity on BuChE. However peripheral side effects have been suggested.[35]. GAL stimulates preand post-synaptic nicotinic receptors acting as an allosteric modulator enhancing the production of ACh and other neurotransmitters,[36] although the clinical significance of this finding is not yet clearly understood.

Donepezil interacts with the active site of the enzyme.[37]. The piperidine derivative Donepezil, approved for clinical use in 1996, is a specific reversible inhibitor of AChE, which increases the concentration of Ach in the CNS for synaptic transmission. Donepezil seems to be selective towards the cerebral cortex[38] and well tolerated. Mild transient adverse effects are observed and appear to be dose related.

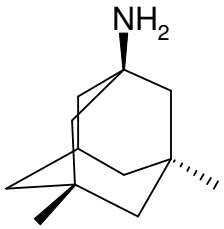
Rivastigmine, as well as Tacrine, inhibits both AChE and BuChE. It was approved for clinical treatment of AD on April 21, 2000.[39] It forms a carbamoylated complex with the active-site serine [40] inactivating it for about 10 hours producing

"pseudo-irreversible" inhibition. It is described as brain selective with particular activity in the cortex and hippocampus. The most common side effect seen in clinical trials is the one affecting the gastrointestinal tract. In these studies, the treatment with Rivastigmine proved to be economically beneficial only after two years of treatment because it finally delayed the progression of the disease, but did not stop it.

Although most AChEIs tests how a positive effect over the cognitive loss seen in AD patients, the major differences seen in a variety of clinical studies can be explained on the basis of the different criteria used to evaluate the compounds (selection criteria of patients, age of subjects, severity of disease, concomitant illnesses and medications, variable instruments of assessment and side effect evaluation).[41] These criteria depend on the severity and frequency of the side effects, as well as, on the percentage of responding patients and dropouts. Clinical studies have shown that ending the administration of the AChEIs in patients is related to a slow return towards the deterioration state, suggesting an additional unknown effect of the inhibitors. [41 42]

The newest drug for AD, Memantine (Namenda ®), breaks out of the ACh-enhancing mode and focuses on a different receptor complex: the NMDA receptor.

Figure 6.1



Memantine

Glutamate is the main excitatory neurotransmitter in the CNS and has a role in neurotransmission and plasticity. Glutamate receptors are divided into NMDA, AMPA, and kainate subtypes. The NMDA receptor has a complex structure with several binding sites for NMDA and glutamate and a central ion channel capable of binding phencyclidine. NMDA-receptor activation generates a longlasting influx of Ca^{2+} into neurons, which is thought to be involved in long-term potentiation a cellular process that underlies learning and memory. [43] [44] In pathogenesis, such as the neurodegeneration of AD, an increase of extracellular glutamate is thought to lead to excessive activation of NMDA receptors with consequent intracellular accumulation of Ca^{2+}. This intracellular accumulation of calcium then initiates a cascade of events that results in further neuronal death.[45] [46]

Memantine (Namenda ®) is a non-competitive, moderate-affinity, phencyclidine-site, NMDA antagonist that might protect neurons from glutamate-mediated excitotoxicity without preventing physiological activation of the NMDA receptor. It blocks the effects of abnormal glutamate activity hat may lead to neuronal cell death and cognitive dysfunction.

Memantine was approved for the US market in October 2003 and has been used in Europe for many years. It is a drug specially developed for use in moderate-to-severe dementia. It is not a AChEIs and therefore, is different from other drugs currently used for AD treatment. Several studies have been done to establish the consequence of the association between Memantine and other cholinesterase inhibitors.

Farlow and colleagues [47] recently reported on preliminary results from a 6 month, multi-centre, randomised controlled trial of Memantine combined with Donepezil compared with Donepezil and placebo in 400 patients with moderate to severe AD. The Memantine–Donepezil combination therapy led to improvement from baseline and

significant benefit over the Donepezil–placebo combination. Additional data are needed on Memantine as monotherapy, in combination therapy in mild-to-moderate AD, and in early stage dementia when it might, on the basis of its neuropharmacological properties, be most effective.

The Amyloid Hypothesis

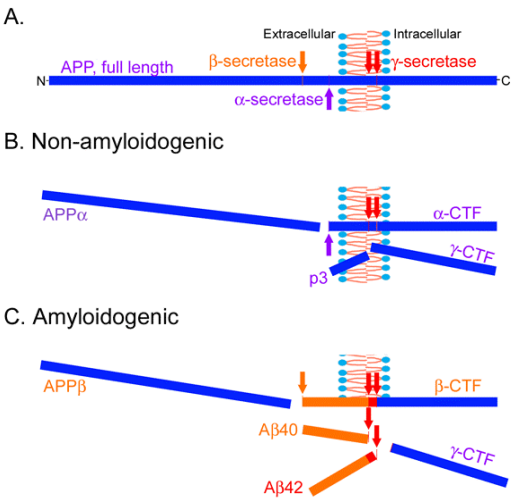
The common theme of Aβ production and deposition has bolstered the "amyloid hypothesis" of AD pathogenesis , which states that production and deposition of Aβ in the form of fibrils leads to neuronal cell death and eventually to the clinical presentation and progression of AD.[48]

Amyloid plaques are deposits composed primarily of β-amyloid insoluble peptides of approximately 4 kDa generated from the precursor Amyloid Precursor Protein (APP), a type Ia transmembrane protein, characterized by a large NH2-terminal extracellular/cytosolic domain and a small intracellular/luminal COOH-terminal domain and expressed in neurons and glia where it is synthesized and contranslationally inserted into the endoplasmatic reticulum (ER).[49]

The APP is enzymatically cleaved by three different forms of proteases (enzymes that break down protein molecules) named α-, β-, and γ-secretase. [50] These proteases are a heterogeneous group located in various cellular regions. The order in which these proteases cleave APP determines whether Aβ is formed. [51] [52] For example, cleavage of APP by α-secretase produces fragments that are substrates for γ-secretase, but processing of these fragments by γ-secretase does not lead to Aβ. [53] [54] In contrast, cleavage of APP by β-secretase produces a peptide fragment that is a substrate for γ-secretase that is converted by γ-secretase to Aβ 40 or Aβ 42.[55] There is likely to be an additional step that determines which form of Aβ is produced. [56]

Two distinct paths carry out the proteolysis of APP. Both appear to be regulated by numerous factors including stimulation of acetylcholine, serotonine, glutamate and neuropeptide receptor.[57] [58]

Figure 7.1: The APP processing



The dominant nonamyloidogenic α-pathway involving the α-secretase (metalloproteases such as the tumor necrosis alpha converting enzyme TACE and Adam10); and represents the most important APP proteolysis (90%). APP is cleaved between Lys16 and Leu 17 of the Aβ domain in the extracellular space and produce a soluble APPα fragment (sAPPα) and the αC- terminal fragment (α CTF) composed by 88 aa and subsequently cleaved by γ-secretase to produce fragment p3 (3-kDa molecular weight). The sAPPα fragment was reported to have neurotrophic and neuroprotective properties against glucose deprivation and glutamate toxicity. Besides it stimulates neurite outgrowth and regulates synaptogenesis. [59] [60] When APP undergoes non-amyloidogenic processing, there is no release of intact β-amyloid peptide, and the generated stubs do not aggregate and do not have amyloidogenic activity.

The second amyloidogenic β-pathway employs β-secretase (BACE1 or β-site APP Cleaving Enzyme 1) which is largely present in the distal Golgi membrane.[61] β-secretase is responsible for cleaving APP at the *N*-terminus of Aβ between Asp 1 and

Met-1. Thus the generated soluble APPβ fragment (sAPPβ) leaves the scene, whereas the membrane-bound β fragment (β CTF) is in turn cleaved by γ-secretase to produce Aβ of 40 or 42 aa length. The γ-secretase cleavage of β CTF leaves an unstable CTFγ composed by 57-59 aa residues. [62] Both Aβ-40 and Aβ-42 are produced in normal individuals but in proportions of 9:1 .The longer and more hydrophobic Aβ-42 is much more prone to fibril formation than is Aβ-40 and even though Aβ-42 is a minor form of Aβ it is the major species found in cerebral plaques.

AD causing mutations in APP near the γ and β-secretase cleavage sites, alters the way in which APP is processed in favour of increasing the ratio of amyliodigenic Aβ-42 fragment that has a greater propensity to self aggregate and may provide a nidus for extracellular amyloid deposition.

(The identification of the biological mechanism that cause the formation of amyloid plaques and neurofibrillary tangles typical of AD and the identification o the amyloid-β precursor protein (APP) as the main precursor of these plaques, lead scientific research to focus its attention on the two strong therapeutic targets γ -and β -secretases that are directly responsible of the Aβ peptide generation.)

The pharmacological inhibition of these enzymes could provide a targeted therapy for amyloidopathy in AD and in particular β-secretase that catalyses the first step of Aβ production is thought to be an ideal therapeutic target.

γ-Secretase

γ-Secretase is a high molecular weight multimeric protein complex that carries out its proteolytic function within the membrane. How exactly the substrate is hydrolyzed within the membranous environment is uncertain since it requires the presence of a water molecule bound to the enzyme in this hydrophobic environment, it represents an unusual transformation.

After either β or α-secretase release the APP ectodomain, the resulting C83 and C99 nAPP C-terminal fragments are clipped in the middle of their transmembrane regions by this enzyme.[63] Normally, about 90% of the proteolysis occurs between Val 40 and Ile 41 (Aβ numbering) to give Aβ40; roughly 10% takes place between Ala 42 and Thr 43 to produce Aβ42. Minor proportions of other C-terminal variants, such as Aβ39 and Aβ43, are formed as well. For this reason γ-secretase is central to AD pathogenesis, because of its importance in determining the ratio of Aβ40 to Aβ42 , but the exact mechanism of its action remains elusive.

It has been hypothesised that γ-secretase is a multiprotein complex and some studies suggest that presenilin proteins are the catalytic site of this complex and in particulary presenilin-1 (PS-1). [64]

However, it is unclear whether PS-1 is γ-secretase, or whether PS-1 is not γ-secretase but instead interacts with the enzyme to release Aβ. In either case PS-1 is essential to γ-secretase activity, and inhibition of PS-1 function is a potential strategy for developing Alzheimer's disease therapies.

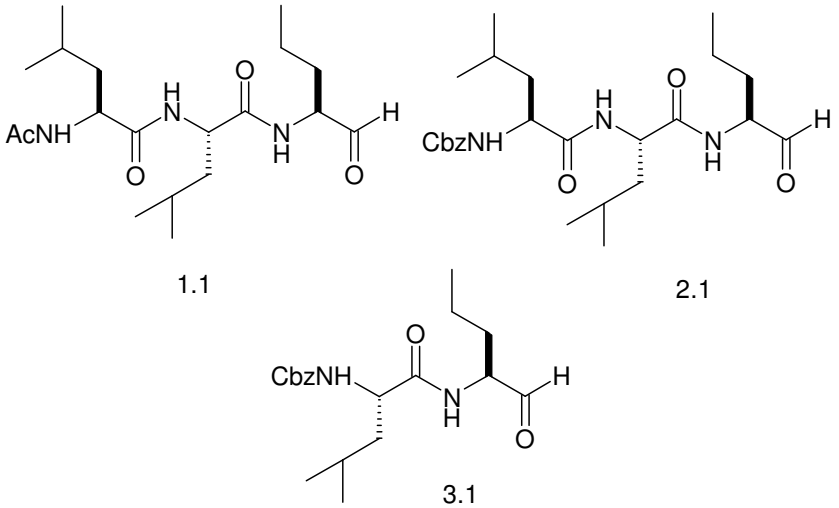
γ-Secretase inhibitors

The development of γ-secretase inhibitors began providing information about the characteristics of this mysterious protease and considering that γ-secretase does not have a specific aminoacid sequence requirement for substrate recognition but rather has a loosely defined preference for hydrophobic amino acid sequences.

Taking into account of this many γ-secretase inhibitors have been reported. Most of this development occurred in the absence of any knowledge of the nature of the enzyme complex since the key discoveries were made only more recently. Following the initially reported peptidic compounds similar to many "traditional" aspartyl protease inhibitors, more recent efforts have allowed the identification of a range of structurally diverse, nonpeptidic inhibitors which often attain high levels of potency in cell-based assays of inhibition of γ-secretase cleavage.

The first reported inhibitors were short, hydrophobic peptide aldehydes originally designed as calpain inhibitors (**1.1-3.1**, Figure 8.1), and these compounds blocked Aβ production at the γ-secretase level in APP-transfected cells with IC_{50}s of 5-200 μM.[65 66]

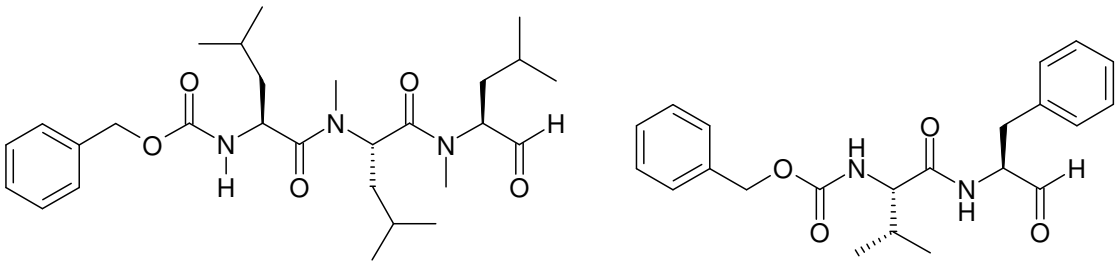
Figure 8.1



1.1 2.1 3.1

Peptide aldehydes are in fact able to inhibit serine-cysteine and aspartyl acid proteases. At low concentrations, peptide aldehydes are reported to increase Aβ production and at higher concentrations to inhibit proteases.[67] Authors explain this property by the inhibition, at low concentrations, of proteosomal degradation of α and β secretase-cleaved APP making more substrate available for γ secretase cleavage, al higher concentrations Aβ secretion would be explained by blocking cysteine protease having roles in protein processing and trafficking in the secretory pathway.[68]

Figure 9.1



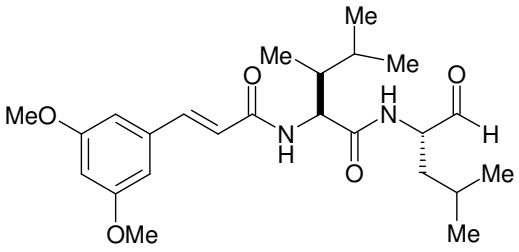
MG 132
4.1

MDL 28170
5.1

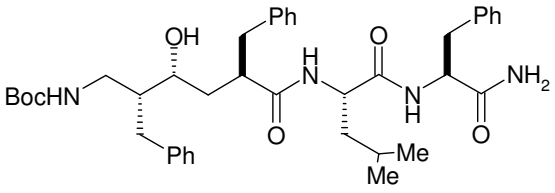
Initially known to act on calapin, MG132 compound 1.4 in Figure 8.1 was the most potent peptide aldheyde prototype with IC $_{50}$ of about 5μM against Aβ-40 and 25-50 μM against Aβ-42 in a cell based assay. Another calpain inhibitor MDL 28170 in Figure 8 inspired the syntesis of a series of different aldehyde derivatives.

Further optimization of these inhibitors has been carried out by the Scios group utilizing combinatorial chemistry to provide analogs such as **6.1** in Figure 10.1 (IC50 9.6μM – N9 cells).[69] Questions remain however as to the applicability of reactive functional groups such as these aldehydes to therapeutically relevant molecules, owing to their instability and potential for generating toxicity.

Figure 10.1



6.1

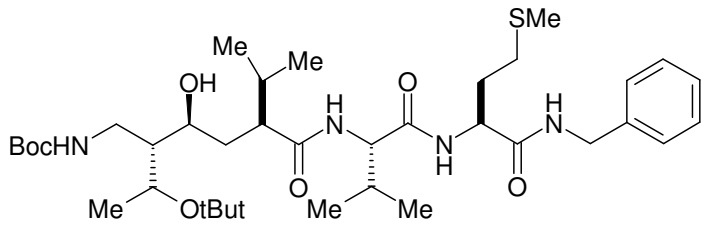


7.1

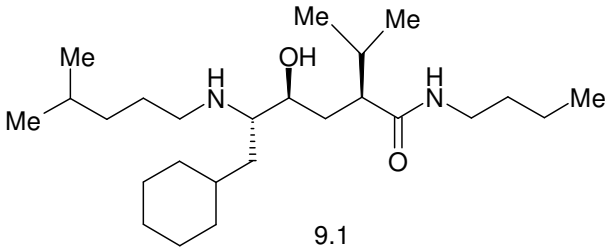
The utility of potential transition state isosteres has been extended by the Merck group with the identification of the dipeptide, hydroxyethylene isostere **7.1** in Figure 10.1. [70] [71] In contrast to previous compounds, this inhibitor showed potent inhibition of Aβ generation in a range of cell lines with Aβ40 IC_{50} values in the range 48-402 nM with similar levels of potency for inhibition of Aβ42 production. A series of structurally-related statine derivatives (e.g. **9.1** in Figure 11.1) have been disclosed by Bristol Myers Squibb.[72] [73]

More recently this area has been revisited with the disclosure of isosteres such as **8.1** [74] in Figure 11.1 and related hydroxyamides [75] and succinates. [76]

Figure 11.1



8.1

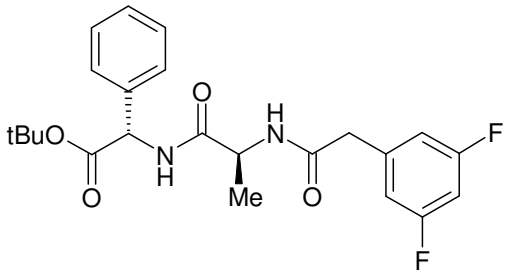


9.1

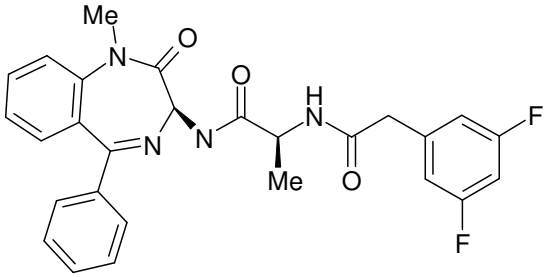
The use of semi-peptidic inhibitors has also been extensively exploited in a range of disclosures from the Elan/Lilly group.[77] [78] Initial reports detailed 3,5-difluorophenylacetate- capped dipeptide esters including **10.1** in Figure12.1 (AN-37124, DAPT) which is a potent γ-secretase inhibitor *in vitro* and has also demonstrated robust efficacy *in vivo*.[79] Modification of these early compounds resulted in benzodiazepine-containing analogs such as **11.1** (Figure 12.1). [80]

Further elaboration, taking advantage of the increased activity observed with several metabolites bearing an additional benzylic hydroxyl group, led to the discovery of mandelate analogs such as **12.1** and **13.1** in Figure 12.1(LY- 411575) which have shown high levels of potency in whole cell assays (e.g **13.1** in Figure 12.1; IC_{50} 119pM).[81]

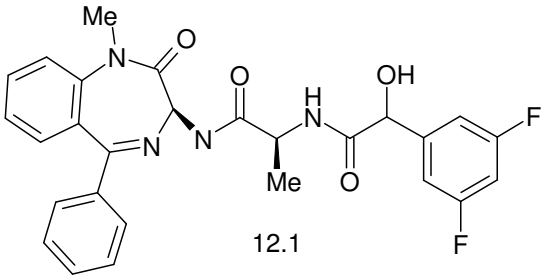
Figure 12.1



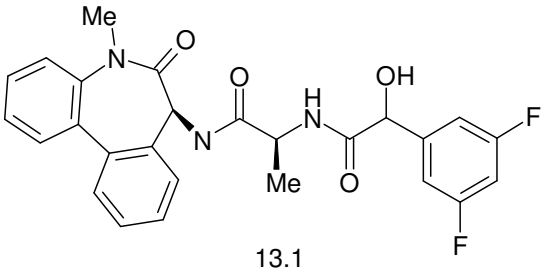
10.1 (DAPT)



11.1



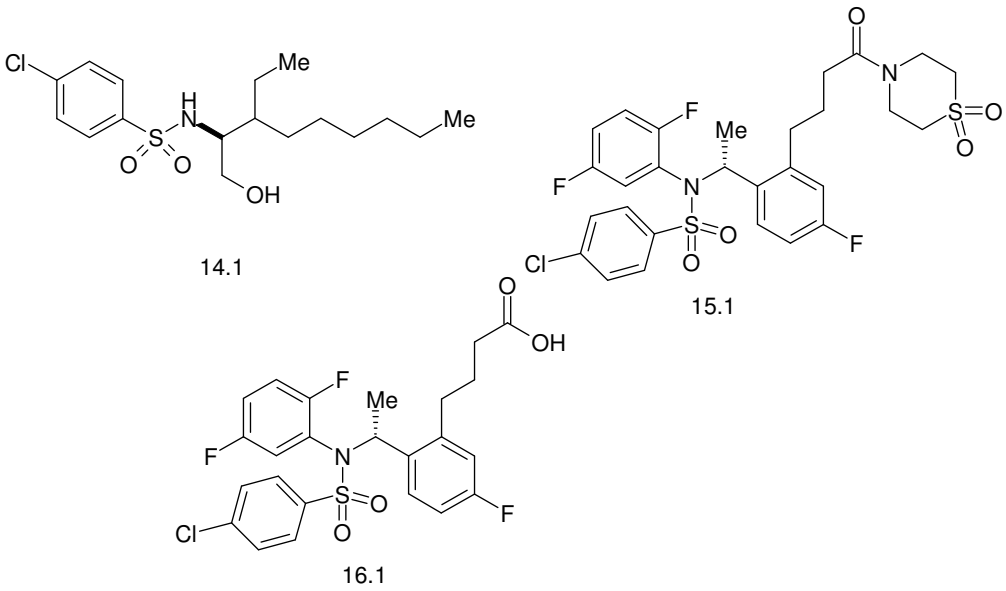
12.1



13.1

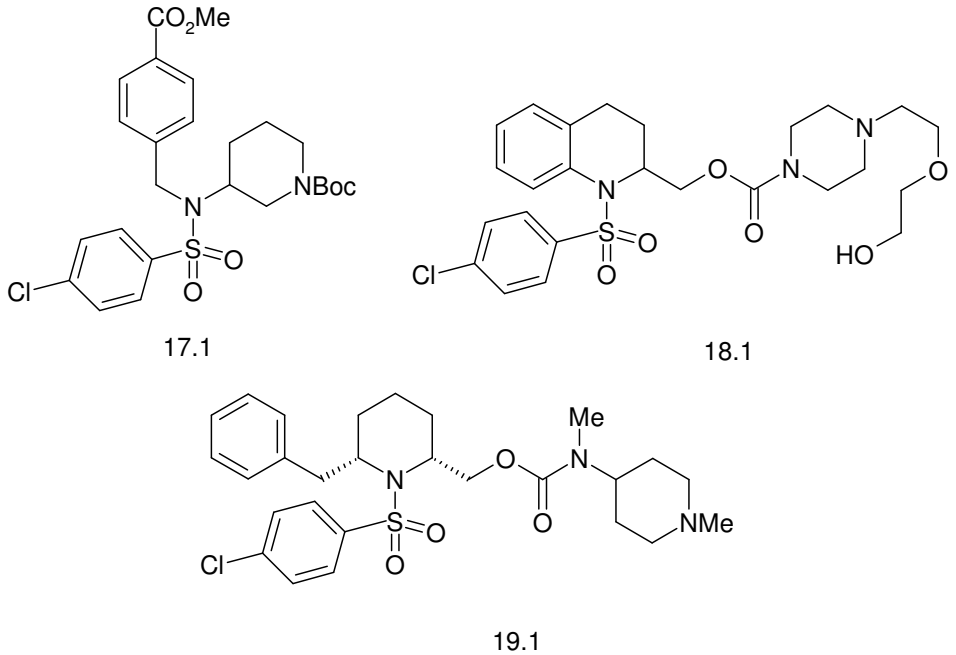
In contrast to the previously described amide-containing inhibitors, more recent disclosures have shown the utility of lipophilic motifs based around a sulfonamide moiety. The Amgen group has utilized fenchylamine sulfonamides as moderately potent inhibitors. Sulfonamides (e.g. **14.1** in Figure 13.1) prepared from amino alcohols have also been claimed by the Wyeth/Arqule groups.[82] [83] An alternative series of diaryl sulfonamides exemplified by **15.1** and **16.1** in Figure 13.1 (IC_{50} 7-13nM solubilized enzyme assay [84]) has also been disclosed by the BMS group. [85]

Figure 13.1



In a series of closely related disclosures, the Schering group have utilized a variety of sulfonamides, for example piperidine **17.1**, [86] tetrahydroquinoline **18.1** (IC_{50} 30-535nM in DKF167 cells expressing C99),[87] and 2, 6-disubstituted piperidine **19.1** (IC_{50} 0.2nM).[88]

Figure 14.1



β-Secretase

β-Secretase-1 (BACE1) is a 501 residue transmembrane aspartyl protease also known as Asp2 (for novel aspartyl protease 2) [89] [90] and memapsin2 (for membrane aspartyl protease / pepsin 2),[91] closely related to the pepsin protein family. It is characterized by a large extracellular domain, where two aspartyl residues involved in *β*-secretase activity reside and by a short intracellular domain containing a sorting sequence that was shown to be involved in the trafficking of the protein.

Since BACE1 co-localizes to endosomes and requires an acidic pH for its activity it is supposed that the endosomes are the cellular compartment where BACE1 is mostly active. Interestingly, endosomal dysfunction has been linked to Alzheimer's disease and neurodegeneration, and it was previously reported that *β*-secretase cleavage of APP occurs upon internalization. It cannot be excluded that BACE1 is active also in intracellular compartment distinct from the endosomes; in fact, BACE1 recycles from the endosomes to the trans-Golgi network and localizes and is active within the lipid rafts, invaginations of the membrane rich in cholesterol and other lipids [92] in proximity of membrane structures.

Soon after the discovery of BACE1, searches identified a homologous novel aspartyl protease, BACE2 (also termed Asp1, mempapsin1, and DRAP).[89] [91] [93] [94]

BACE2, which is expressed in highly vascularized tissues such as heart, kidney, and placenta has been identified on chromosome 21 [95] and share 64% amino acid similarity with BACE1. Cell transfection studies demonstrate that BACE2 cuts APP at the *β*-secretase site. [96] [97] [98]

However, BACE2 cleaves with higher efficiency at two other positions within the Aβ domain near the α-secretase cleavage site. Thus, BACE2 may play a role in the pathogenesis of familial forms of AD (FAD). However, BACE2 usually functions like an alternative α-secretases so that the processing of wild-type APP by BACE2 diminishes Aβ production in cells.

Bace inhibitors

Since it has been established that γ-secretase cleaves various substrates, some of which might have important physiological roles (Notch cleavage), γ-secretase inhibitors might have insurmountable mechanism-based toxicity.[99]

Taking into account this observation developing β-secretase inhibitors appears to be more encouraging. In fact BACE1 appears to have a single substrate APP, and inhibiting BACE1, and therefore preventing the abnormal generation of the Aβ peptides represents a rational biochemical approach to preventing disease progression in AD. Accordingly, BACE1 might be considered the primary target for lowering Aβ.

Transition-state analog inhibitors

Few inhibitors have been described and the majority of compounds designed are transition state analogues derived from the amino acid sequence around the cleavage site of APP. They represent a peptidomimetic BACE inhibitors substrate-based and were designed using the knowledge of the specificity and kinetics of BACE1 and they

can be divided in statine isosteres (Figure 15.1) and hydroxyethylene isosteres (Figure 16.1).

Figure 15.1: Statine isostere. Hydrogen bonding pattern based on Piana and Carloni [100] and Northrup [101]

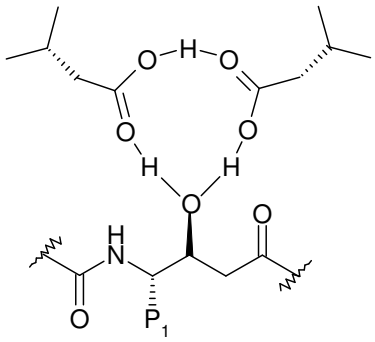
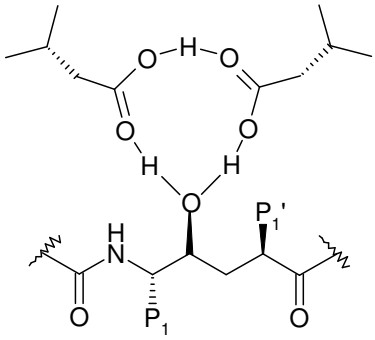


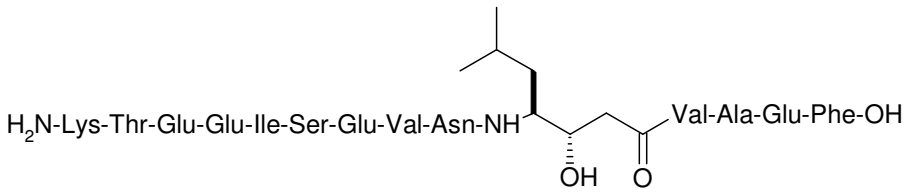
Figure 16.1: Hydroxyethylene isostere



The first compound synthesized was based on two residues of the Swedish mutant APP, replacing the aminoacid Leucine with Statine. This peptide analogue inhibited β-secretase activity, albeit poorly, with an IC_{50} of 40 μM in solubilized extracts of human brain membranes. Modifications of the statine hydroxyl or replacement of *S*- with the *R*-statine diastereomer led to loss of inhibitory activity, indicating that, as in other statine-containing inhibitors of aspartyl proteases, inhibitory potency is dependent on an

unmodified hydroxyl residue in the appropriate configuration. However, replacement of the Asp with the aminoacid valine resulted in a substantial increase in potency (IC_{50}~30 nM), providing a suitable reagent (**20.1**, Figure 17.1) for affinity purification of β-secretase.

Figure 17.1

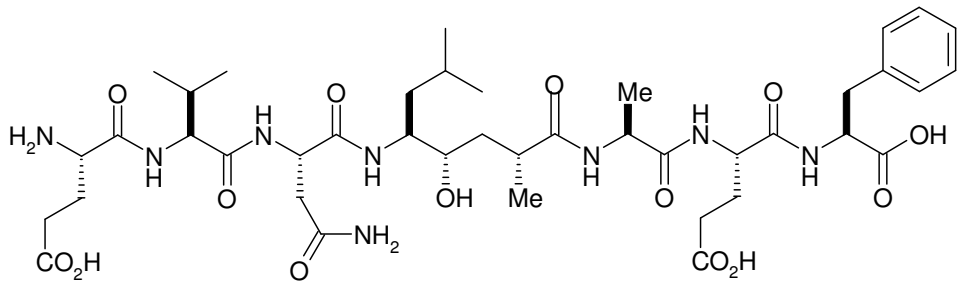


20.1

Not surprisingly though, given its size and the presence of hydrophilic residues, this compound displays no inhibition of Aβ production in whole cells.

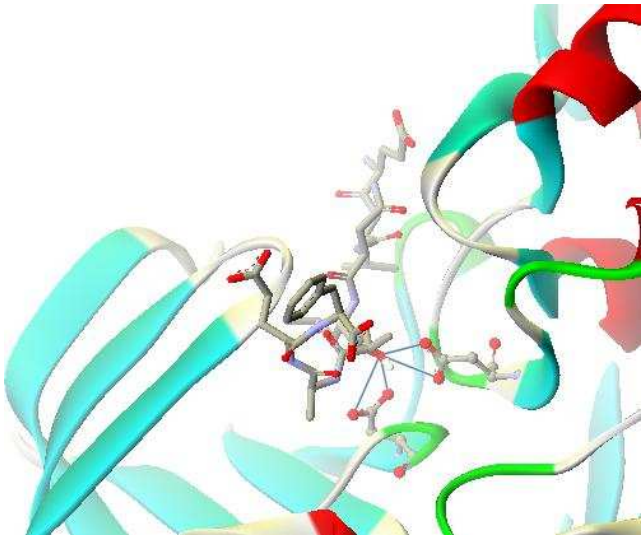
In order to reduce the molecular weight of inhibitors, Tang and colleagues at the University of Oklahoma in collaboration with Ghosh and co-workers have reported the development of peptidic BACE inhibitor **OM99-2** in Figure 18.1 which has a BACE IC_{50} of 0.002 μM.[102] This compound, an octapeptide based on the cleavage site of Swedish mutant APP, represents the main hydroxyethylene dipeptide isostere in which registration of the amino acid side-chains exactly mimics a peptide substrate.

Figure 18.1



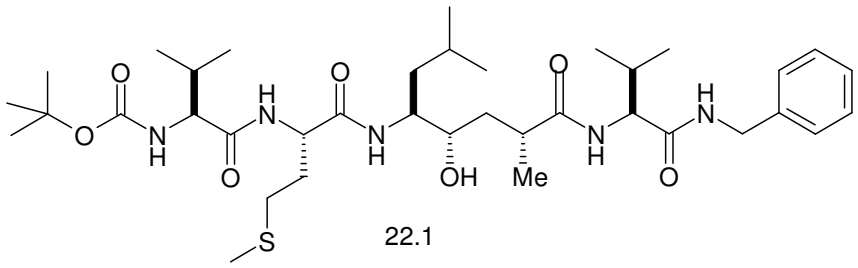
21.1 (OM99-2)

Figure 19.1: ***Peptidic hydroxyethylene*** **BACE** ***inhibitor*** **OM 99-2** ***and its binding mode in*** **BACE (Brookhaven entry 1FKN)**



They have further reported on using X-ray structure-based modification of the lead **OM99-2** that led to the discovery of a series of potent and considerably low molecular weight peptidomimetic BACE inhibitors such as **22.1** (Figure 20.1).

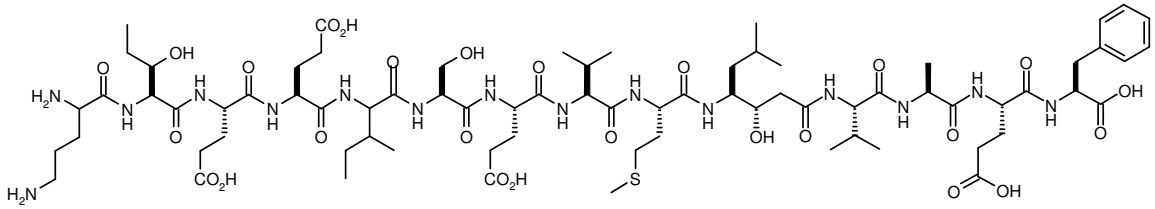
Figure 20.1



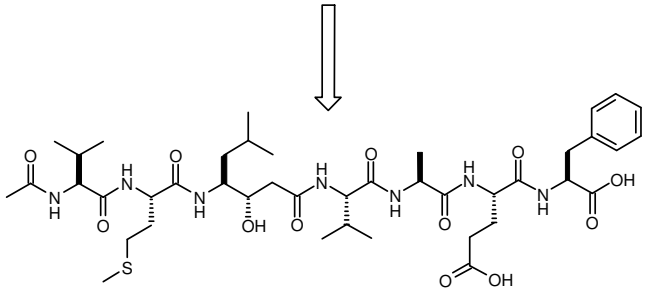
22.1

The Elan/Pharmacia team has reported the development of other cell-permeable BACE inhibitors. Replacement of the Leucine residue with a noncleavable Statine (sta) residue and replacement of the Asp with the Valine residue resulted in analogue **23.1** (IC_{50} = 0.03 μM; Figure 21.1). Further truncation of the N-terminus and C-terminus resulted in the identification of the smaller peptidic inhibitor **24.1** (IC_{50} = 0.3 μM; Figure 21.1).[103]

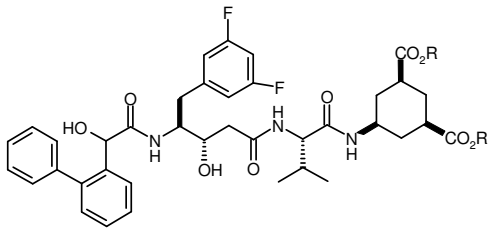
Figure 21.1



23.1



24.1



R= H 25.1
R =CH_3 26.1

Further modification of the central core region and combination of the optimal N- and C-terminal mimetics resulted in a potent but cell-permeable BACE inhibitor **26.1** (IC_{50}=0.12 μM; EC_{50}=4 μM; Figure 21.1).[104] The corresponding diacid **25.1** (Figure 21.1) was a potent inhibitor of the enzyme (IC_{50}=0.02 μM) but had no inhibition of Aβ in human embroyonic kidney cells presumably because of poor cellular permeability.

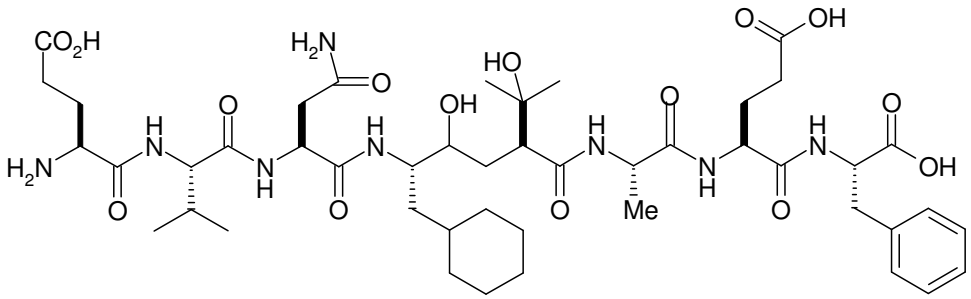
Further work in both the statine and hydroxyethylene series has been directed toward optimizing the early lead inhibitors to improve pharmaceutical properties, focusing first on increasing cell penetration by reducing molecular weight.

Researchers at Pfizer have prepared an inhibitor using a unique hydroxyethylene isostere with a cyclohexylmethyl group instead of leucine and a dimethyl carbinol substituent instead of Asp.[105]Compound **27.1** in Figure 22.1 has a BACE1 IC_{50} = 49 nM as determined by ELISA.

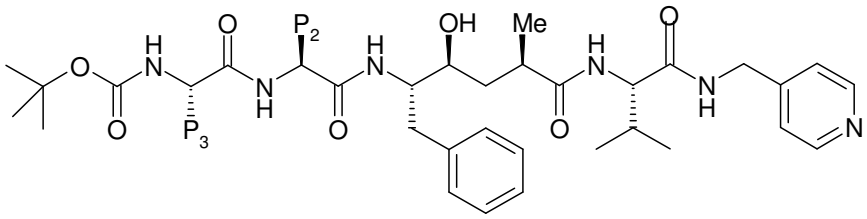
In efforts to increase solubility, a series of inhibitors of structure **28.1** (Figure 22.1) with a C-terminal pyridylmethyl group was prepared and more recently Takeda has published an application describing compounds utilizing an oxidized hydroxyethylamine isostere-type template.[106] A variety of benzylic amines are utilized on the prime side as indicated in structure **29.1** in Figure 22.1.

A number of these compounds are reported to have IC50s < 1 μM against the enzyme and activity in a cellular assay of Aβ lowering.

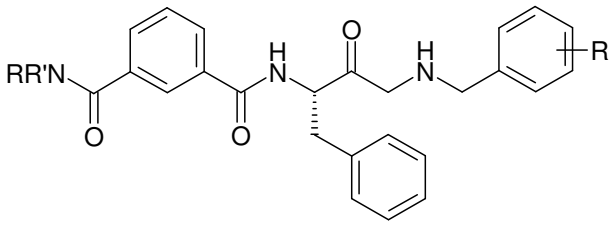
Figure 22.1



27.1



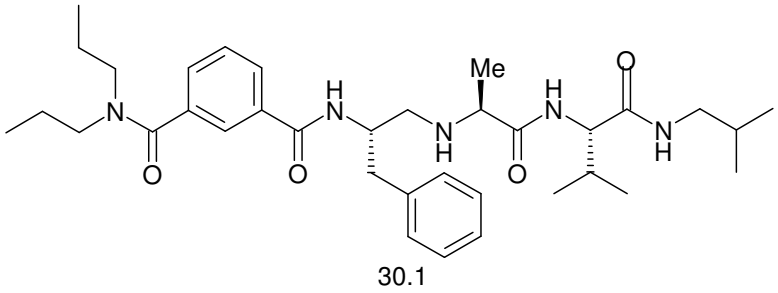
28.1



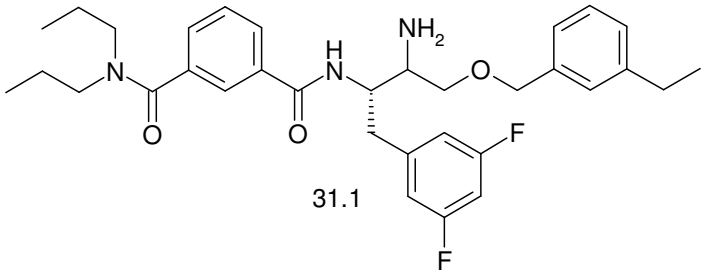
29.1

Additional isosteres claimed as BACE inhibitors include reduced amides such as **30.1** (Figure 23.1) in which the carbonyl of the scissile amide has been removed.[107] Another recent application claims a variation with an amino binding element similar to the compounds described by Sunesis, but with an ether-linked prime side binding group as in amine **31.1** in Figure 23.1.[108]

Figure 23.1



30.1



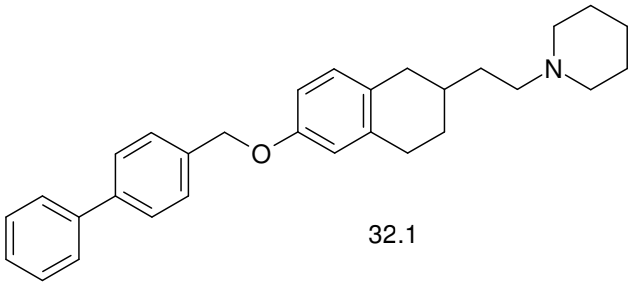
31.1

Non transition-state analog inhibitors

In addition to compounds containing transition state mimics as well as peptide and amino acid derived structures, several carbocyclic and heterocyclic BACE inhibitors have been disclosed. In general these compounds do not contain an obvious transition state mimic.

Takeda for example just recently described a non-peptidic class of BACE inhibitors, most probably identified from a screening approach. The tetraline derivatives discovered have a trisubstituted long chain-amines with more than 1 aromatic ring in the chain. In particular the substituted tetralin compound **32.1** in Figure 24.1was reported to have a BACE1 IC_{50} = 250 nM.

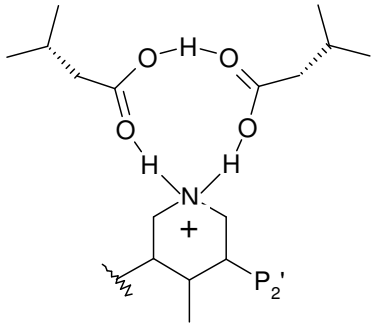
Figure 24.1



32.1

The ability of piperidine derivatives to function as inhibitors of the aspartyl protease renin was first discovered by the Roche group through a high throughput screening effort.[109] [110] [111] [112] X-ray crystal structure data for piperidine inhibitors complexed to renin showed that the protonated piperidine nitrogen was positioned between the two catalytic aspartyl acid residues (Figure **25.1**).

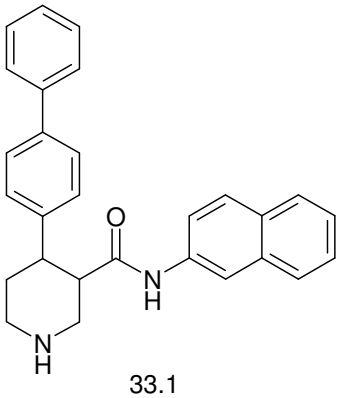
Figure 25.1: ***Piperidine inhibitors***



Interestingly, binding of the piperidines required conformational changes within the renin active site relative to binding of peptide-derived peptidomimetic inhibitors. It has therefore been proposed that the piperidines stabilize an alternate conformation of renin.[113] Incorporation of the piperidine scaffold into BACE inhibitors has been disclosed in patent applications by several groups.

Piperidines were included in a Vertex patent that focused on inhibitors having required distances between key hydrogen bonding functionalities.[114] Compound **33.1** in Figure 26.1 was indicated to be amongst the more potent BACE inhibitors, having a Ki ≤ 3 μM. Patent applications have appeared from Elan that encompass piperidine and other cyclic amine BACE inhibitors.[115 116]

Figure 26.1

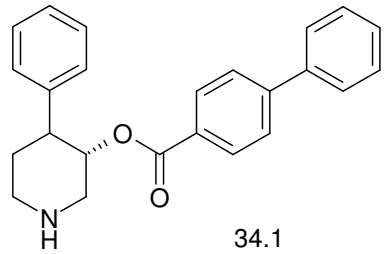


33.1

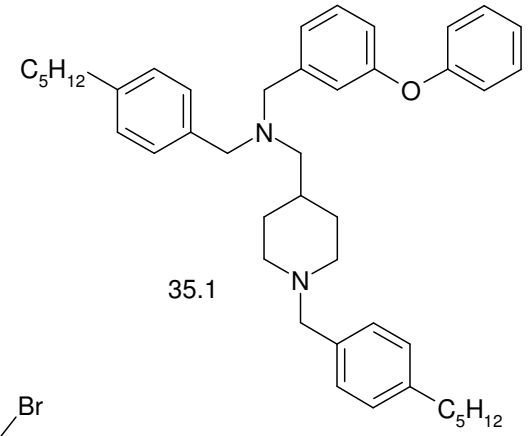
Ester **34.1** in Figure 27.1 is one illustrated example with modest BACE inhibitory potency (IC_{50}=11 μM). More recently, two patent applications have appeared from Actelion covering 3- or 4-aminomethyl piperidines [117] and 3- or 4-aminopiperidines.[118] Aminomethyl piperidine **35.1** (Figure 27.1), which is described as having an $IC_{50} \leq 3$ μM, is a representative of the Actelion chemotype.

Researchers at De Novo have described a similar series of piperazine inhibitors exemplified by compound **36.1**, which also has an $IC_{50} = 3$ μM.[119]

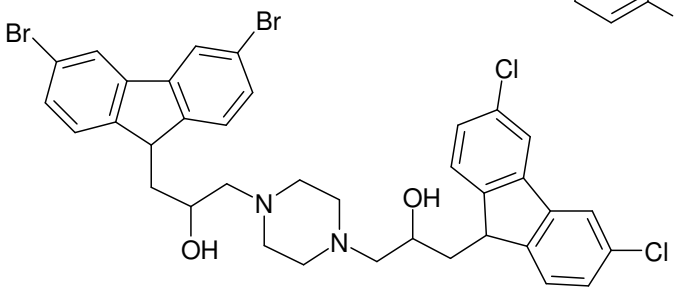
Figure 27.1



34.1



35.1



36.1

Chapter 2

Aim of the thesis

The studies of this Ph.D thesis, carried out in collaboration with Siena Biotech S.p.A, involve the identification and the design of novel small molecules with the potential of modulating Alzheimer's Disease (AD). The pathology is a progressive, neurodegenerative disease characterized by behavioural disturbance and a general decline in cognitive function. Current therapies rely on the use of cholinesterase inhibitors which temporarily improve cognitive function but do little to address the progression of AD.[120]

Many therapeutic strategies to this end have been proposed, including the suppression of amyloid-β peptide (Aβ) formation by inhibition of the proteases that cleave Amyloid-β Precursor Protein (APP).[121]

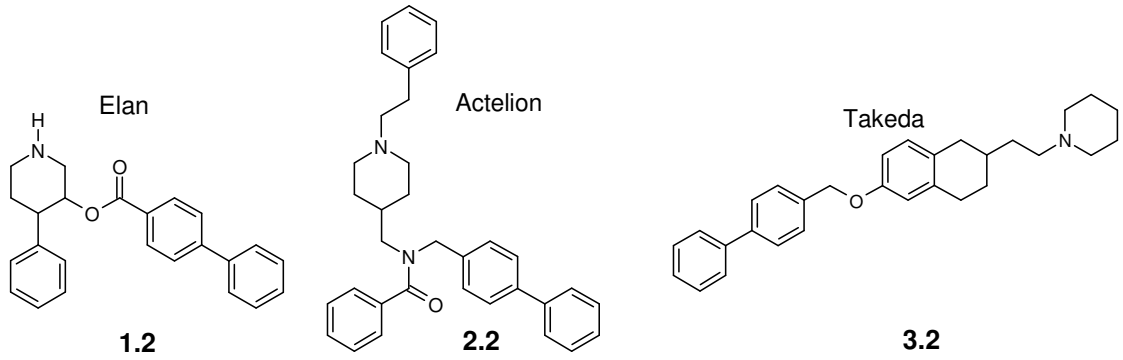
The more aggregable, less-soluble Aβ (1-42) is the main component of the amyloid plaques found in diseased brain tissue, despite comprising only about 10% of total secreted Aβ, and hence compounds selective for the inhibition of Aβ (1-42) production may be of particular interest. Consequently, since the discovery of β-secretase (BACE), the aspartyl protease responsible for the initial cleavage of APP, intensive efforts have focused on the synthesis of the new enzyme inhibitors.

The identification of non peptidomimetic BACE inhibitors has proven to be a challenging undertaking. To augment the disclosure of peptide-derived BACE inhibitors, a few companies have published non peptidomimetic BACE inhibitors and the knowledges to date are few and limited to published patent applications.

Companies like Elan and Actelion disclosed several series of compounds that posses good BACE inhibitor proprieties with associated values of IC_{50} in the

micromolar range (compounds **1.2**, **2.2** in Figure 1.2). These compounds spanned multiple classes of heterocyclic templates such as piperidines. [122] [123] [124] Moreover, Takeda disclosed a new series of aminoethyl-substituted tetralins (compound **3.2** in Figure 2.1) as BACE inhibitors. [125]

Figure 1.2



1.2 **2.2** **3.2**

Based on these new compounds, the aim of this PhD Thesis was to identify new BACE inhibitors that possess appropriate properties for drug development, such as good potency, small size, and high selectivity.

The present work can be divided in four parts :

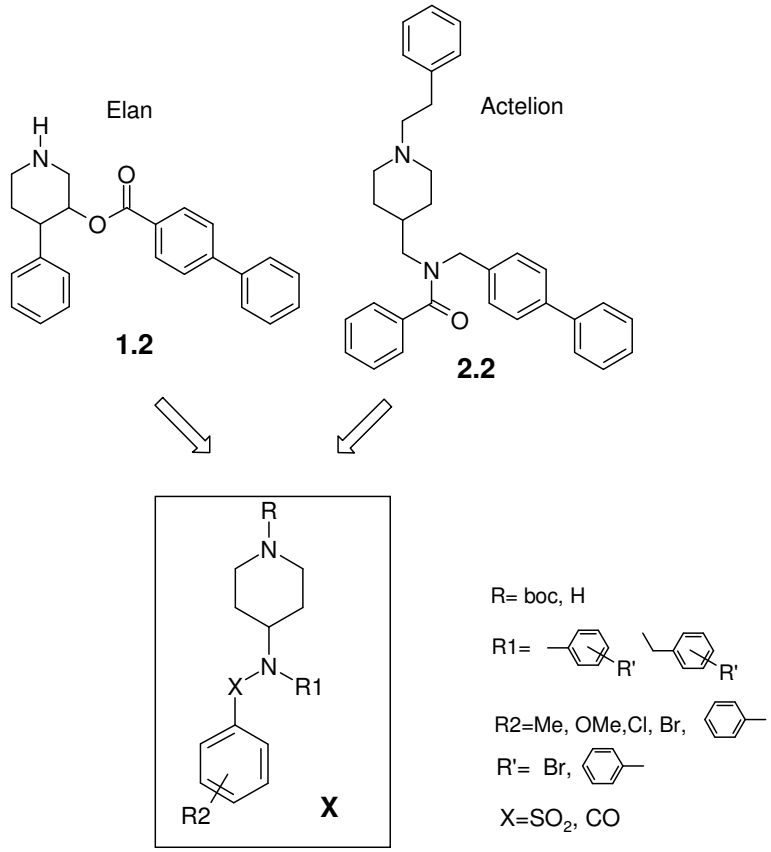
- ***Synthesis of patented compounds***

The two patented derivatives **1.2** and **3.2** in Figure 1.2 were synthesized and then taken as model compounds for the next studies.

- ***4-aminopiperidine derivatives***

A series of new piperidine derivatives of a generic structure **X** in Figure 2.2 were designed and synthesized. These new compounds are characterized by the presence of a 4-aminopiperidine scaffold and they are a form of hybrid of the two known reference compounds **1.2** and **2.2** in Figure 1.2.

Figure 2.2: ***New 4-piperidine derivatives of generic structure*** **X**

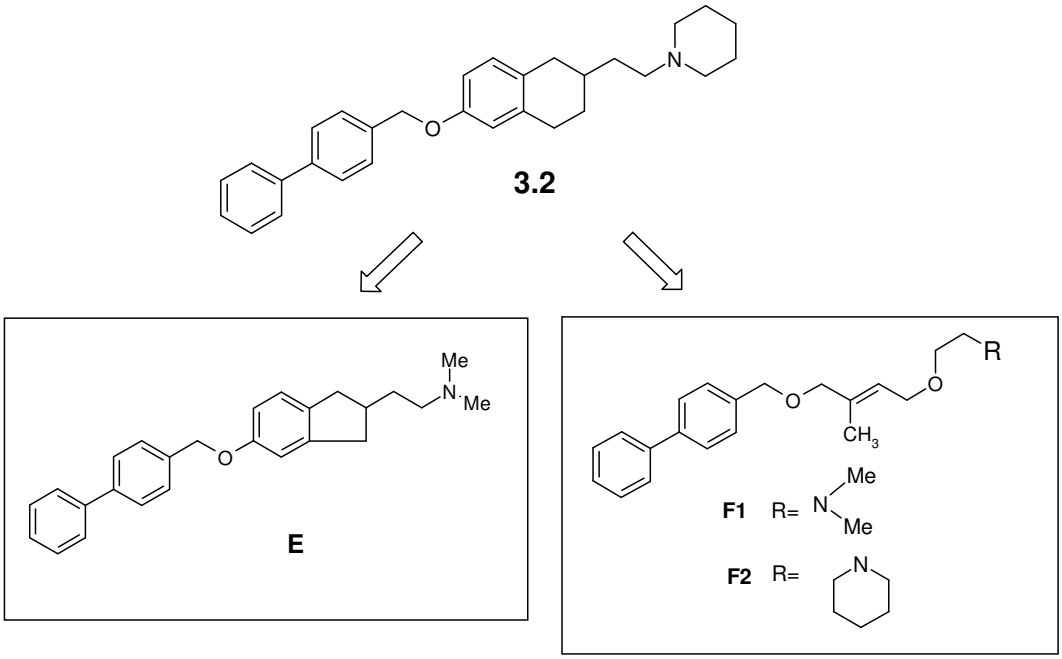


These molecules are characterized by the presence of an aromatic amido or sulphonamido moiety in which the two groups R1 and R2 can be simultaneously or alternately aryl halides or biphenylic groups. The N atom of the piperidine ring has also been substituted with Boc.

- ***Tetraline derivatives***

Based on the patent reported from Takeda company in which an aminoethyl-substituted tetraline as a BACE inhibitor is shown, in the third part of this study we synthesized other non peptidomimetic derivatives using the compound **3.2** in Figure 3.2 as a starting point.

Figure 3.2: ***New derivatives*** **E, F1** ***and*** **F2**

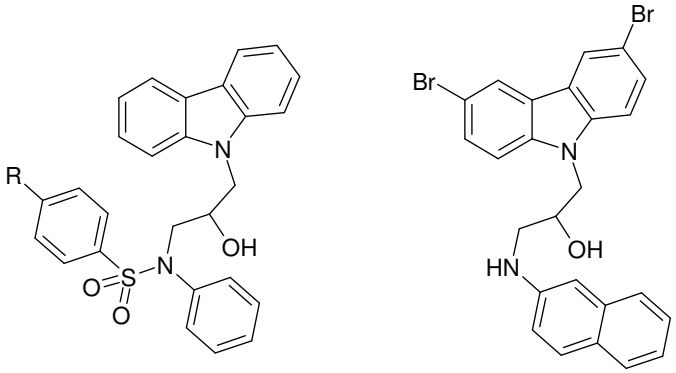


In particular poly-aromatic substituted compounds (**E, F1** and **F2** in Figure 3.2) have been designed and synthesized by replacing the central tetralinic moiety of patented compound with an indane cycle and with a isoprenoid moiety.

- ***Carbazole derivatives***

From a screening performed at Sienabiotech a series of commercially available carbazole derivatives (compounds **4.2, 5.2** and **6.2** in Figure 4.2) were discovered to have a significant inhibitory activity on BACE.

Figure 4.2

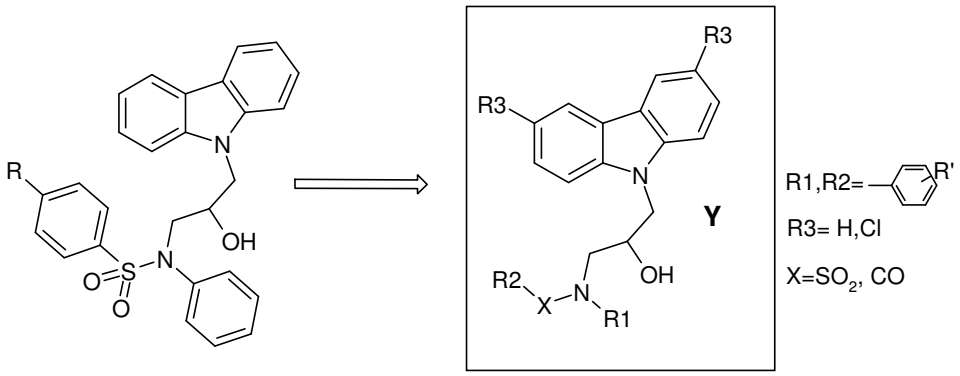


4.2 R= Me
5.2 R= H

6.2

Inspired by screened compound **4.2** and **5.2** we have accomplished the synthesis of a new series of compounds by designing a generic structure **Y** in Figure 5.2 where modifications have been brought both on the carbazole and on the sulphonamido moieties and various substituents were introduced on the aryl R1 and R2 groups.

Figure 5.2: ***New carbazole derivatives of generic structure*** **Y**

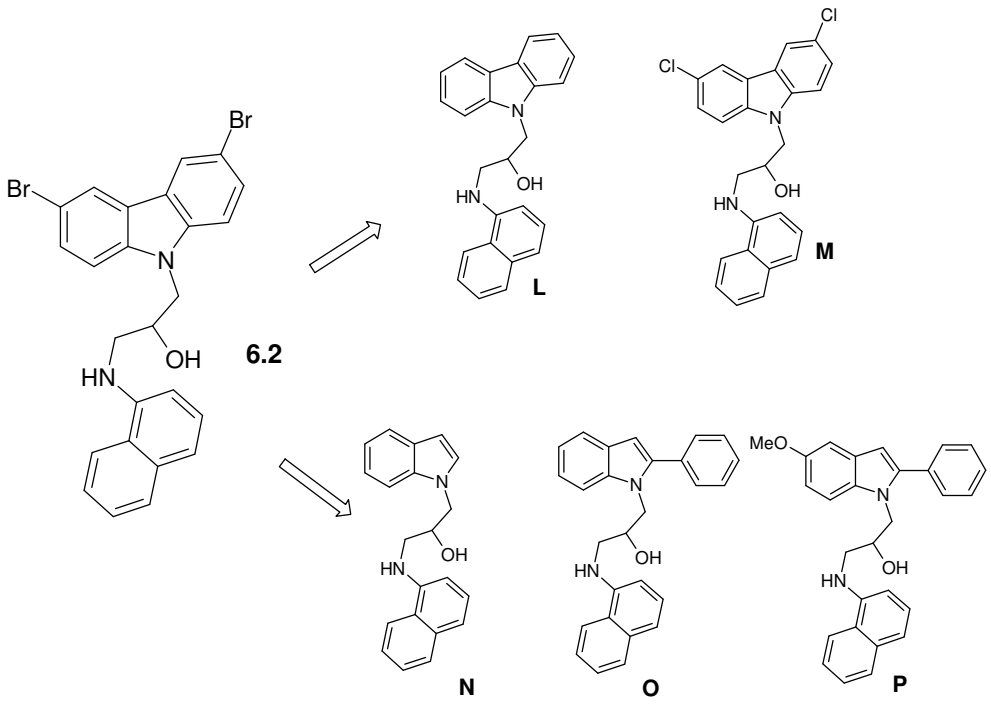


4.2 R= Me
5.2 R= H

Taking compound **6.2** as a reference, we therefore accomplished the synthesis of derivatives **L** and **M** showed in Figure 6.2 in which the carbazole moiety is unsubstituted (compound **L**) or di-chloro substituted (compound **M**) and derivatives **N**-

P in which the carbazole moiety has been replaced with indole moieties, maintaining the naphtylamino moiety as the common portion.

Figure 6.2: ***New carbazole derivatives*** **L-P**

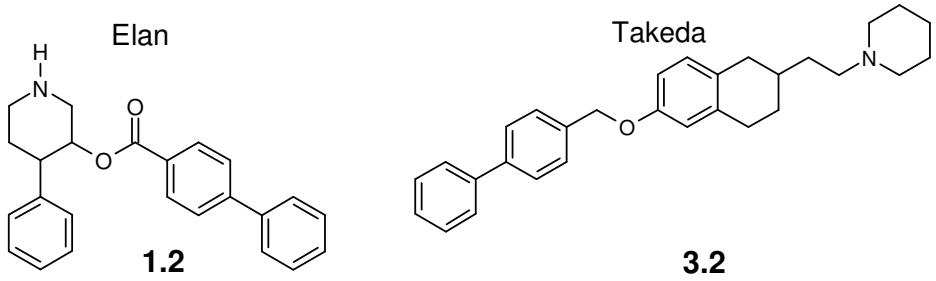


Furthermore all the synthesized compounds have been tested with a time resolved fluorescence quenching assay at Siena Biotech laboratories in order to evaluate their inhibitor activity on BACE.

Synthesis of Patented Compounds

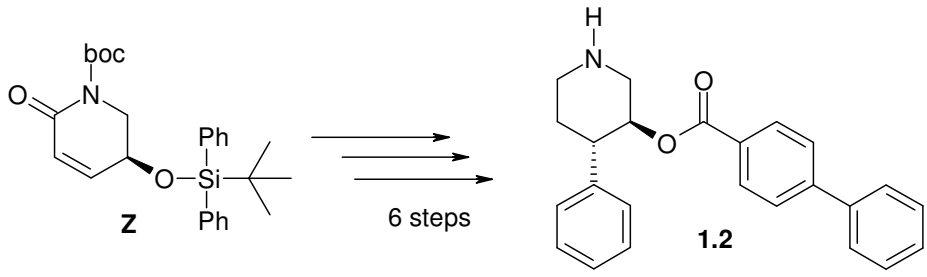
In a first stage of this project we accomplished the synthesis of compounds **1.2** and **3.2** (Figure 7.2) that represent two model compounds for the next studies.

Figure 7.2: ***Patented compouds***



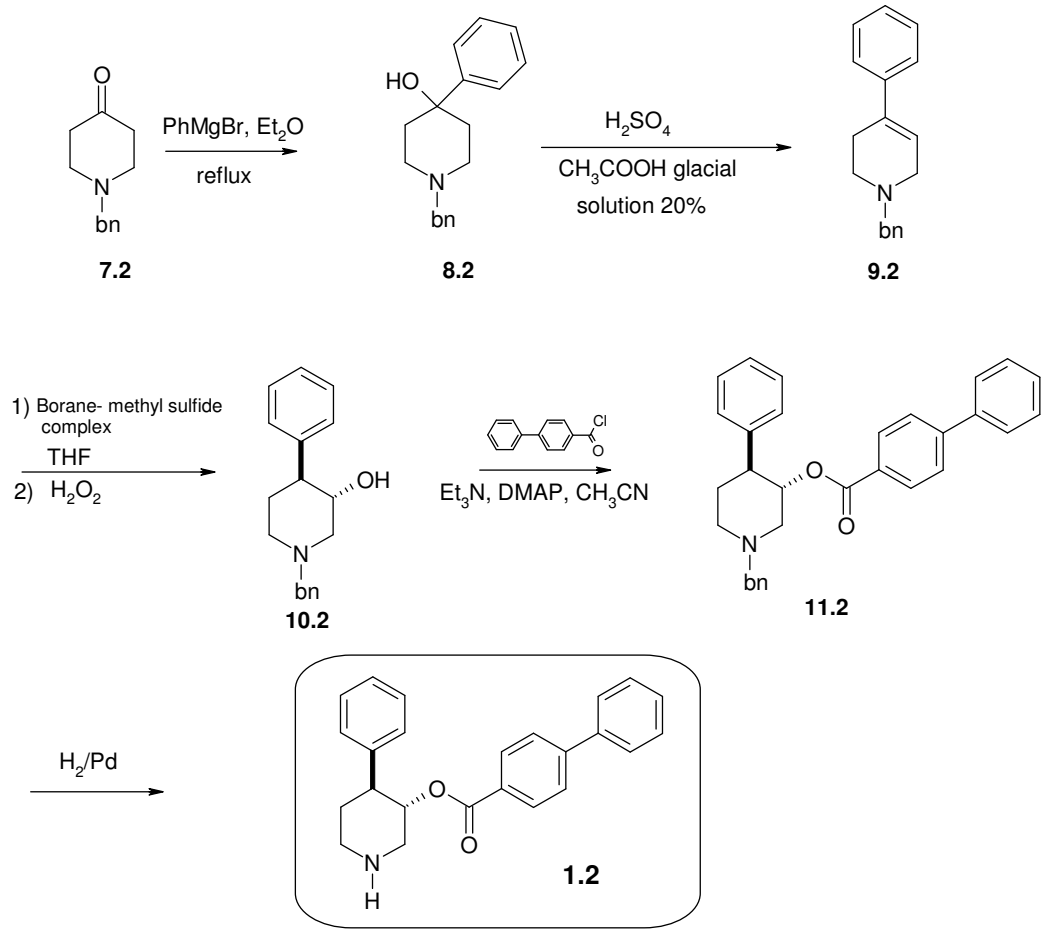
For compound **1.2** we did not apply the conventional strategy described in the patent but a different synthetic procedure planned in our research laboratory using appropriate methods also known in literature. [126] [127] [128] [129] The original patent in fact described a well-constructed synthesis which would have involved many steps showing disadvantages in term of yields. Moreover, it would have started from a non commercially available compound **Z** (Scheme 1.2) which would have to be synthesised from appropriate precursors without clear literature references reported in the patent.

Scheme 1.2 ***Original synthesis of compound*** **1.2**



The synthesis of compound **1.2** was then performed as shown in Scheme 2.2.

Scheme 2.2: ***Synthesis of compound*** **1.2**



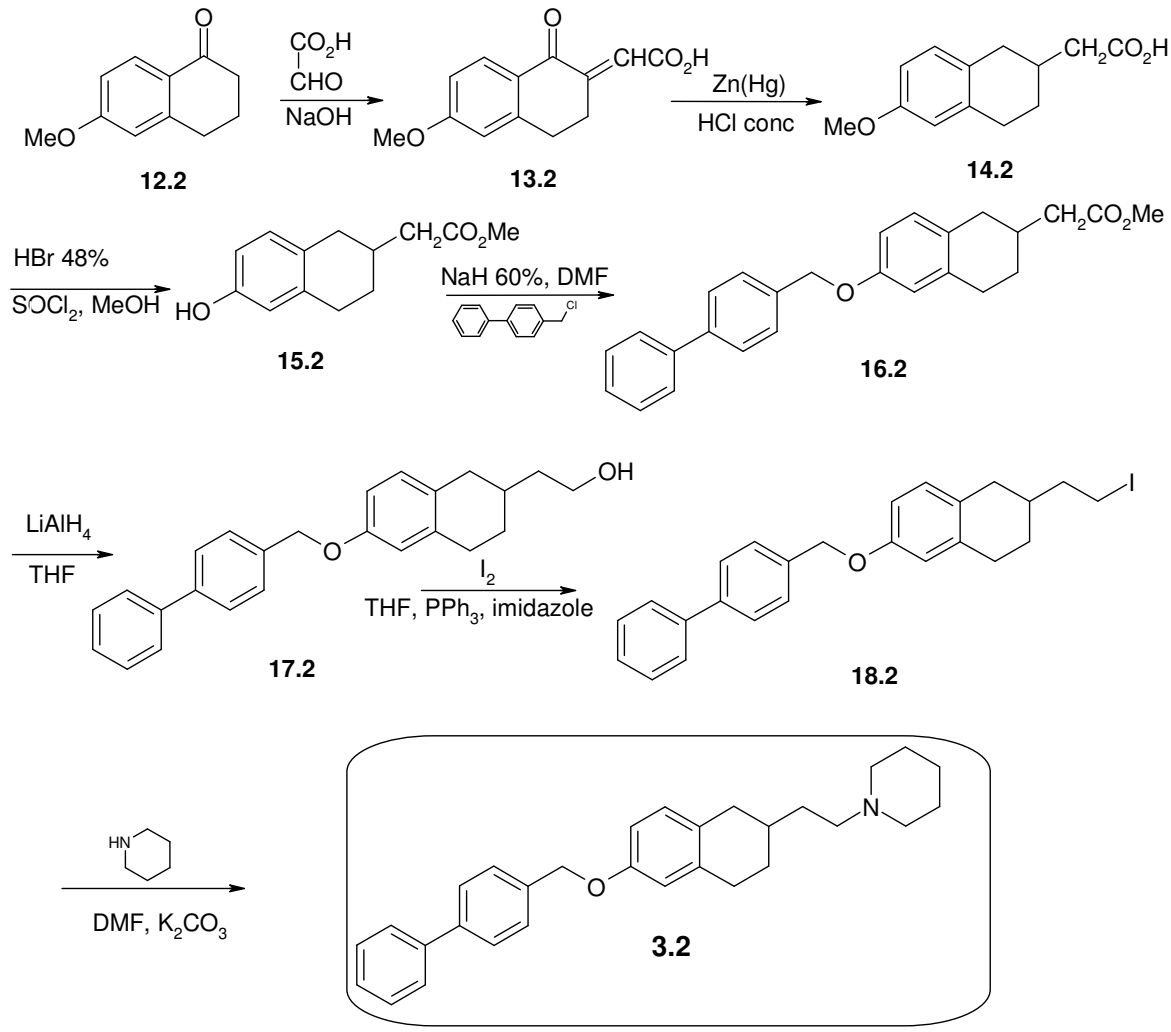
Compound **1.2** was prepared in a racemic *trans* form in five steps starting from the commercial available N-benzyl-piperidone **7.2** that was treated with PhMgBr in a Grignard reaction [126] to obtain the alcohol **8.2**. This compound was subsequently treated with sulphuric acid and glacial acetic acid to obtain the resulting product of dehydration **9.2**. Hydroboration-oxidation reaction of **9.2** in the presence of BH_3-sulfide-complex and H_2O_2 [127] yield the desired alcohol **10.2** that was obtained in good yield. Compound **10.2** was then submitted to reaction with biphenyl carbonyl chloride[128] in the presence of triethylamine as the base and dimethylamino piperidine as the catalyst to yield the

ester **11.2**. Finally, catalytic hydrogenation [129] reaction of compound **11.2** removed the benzylic group to yield the desired unprotected compound **1.2**.

The use of the synthetic procedure described above allowed us to obtain the desired compound **1.2** with a better yield with respect to that indicated in the patent.

Synthesis of compound **3.2** followed the procedure described in the original patent with the exception of the early on two steps in which we applied different synthetic procedures appropriately modified in our laboratory.[130] The synthesis of compound **3.2** is shown in Scheme 3.2.

Scheme 3.2: ***Synthesis of compound*** **3.2**



Compound **3.2** was prepared in a racemic form starting from the commercial available tetralone **12.2** according to the Marshall's work.[130] Compound was initially treated with glyoxylic acid in the presence of NaOH and dry EtOH. The reaction was

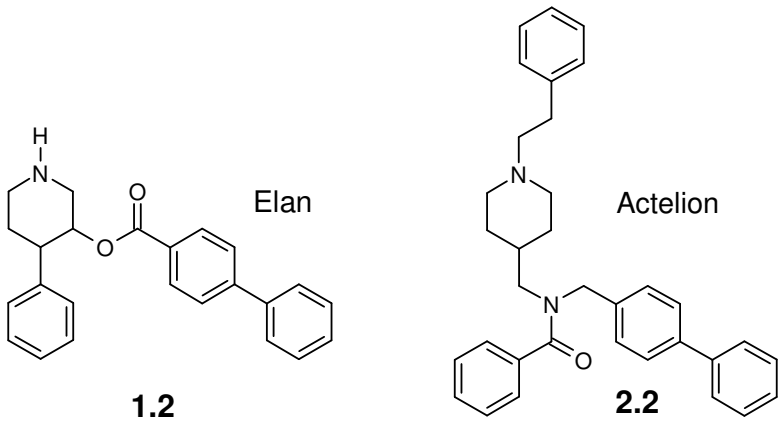
left stirring under reflux to obtain the desired product **13.2**. After Clemmensen reaction [132] of **13.2** with zinc amalgam we obtained the desired reduction product **14.2**. The synthesis proceeded according to the patent scheme and compound **14.2** was therefore treated with HBr 48% followed by reaction with $SOCl_2$ and MeOH to get the desired unprotected alcohol **15.2**.

Compound **15.2** was subsequently treated with 4-phenylbenzyl chloride in the presence of NaH 60% to yield the product of condensation **16.2** that was submitted to a reduction reaction with $LiAlH_4$ to obtain the alcohol **17.2**. After reaction of **17.2** with I_2 in the presence of triphenylphosphine and imidazole the resulting iodine product **18.2** was treated with piperidine and K_2CO_3 as base to get the desired tetraline derivative **3.2** with 72% of yield.

4-aminopiperidine derivatives

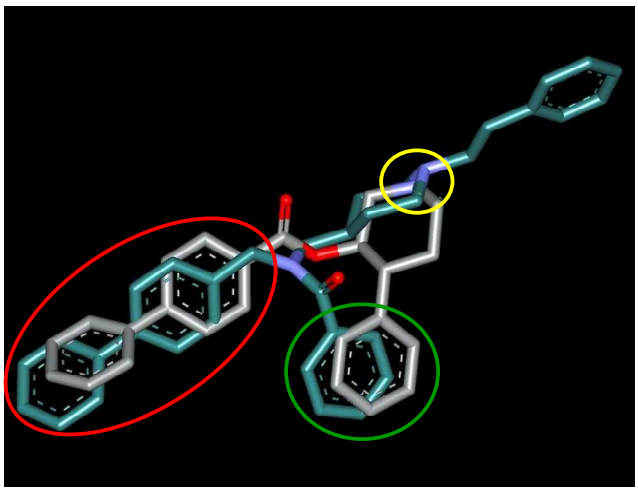
The PhD work was focused on the analysis of the patented compounds **1.2** and **2.2** showed in Figure 8.2 that represent two considerable examples of heterocyclic BACE inhibitors.

Figure 8.2



In order to explore the possible structural analogies between compounds **1.2** and **2.2** in Figure 8.2 a theoretical study was performed in Siena Biotech laboratories. To this scope Accelrys DS Pro software was used and compound alignment was carried out. Putative common features were first tethered and a flexible overlay carried out. Subsequently the molecules were minimised prior to a second rigid overlay to give the alignment hypothesis shown in Figure 9.2.

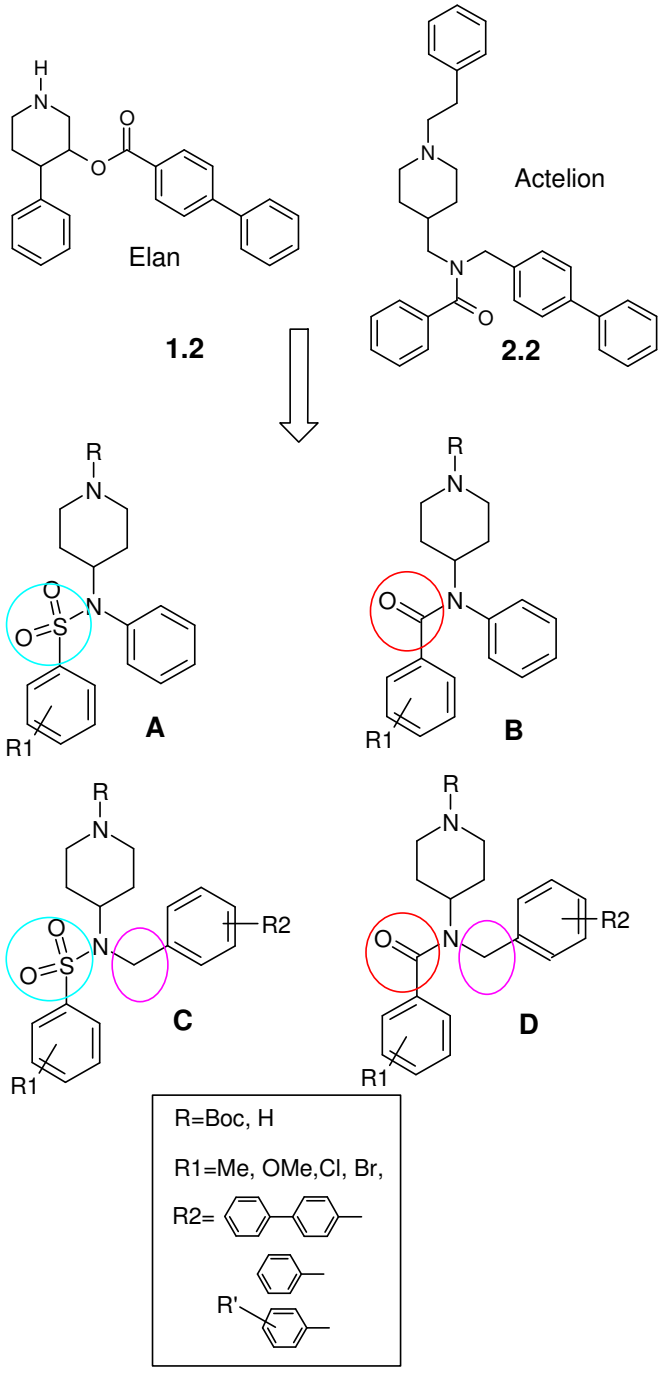
Figure 9.2: ***Comparison of the Actelion (green) and Elan (grey) molecules. Heteroatoms are coloured by atom type (N blue, O red).***



The resulting 3D molecular model obtained from the program allowed us to analyse in detail the spatial conformation of the two references compounds and their structural analogies. From the spatial superimposition of the two compounds we can observe that i) the two piperidine rings are aligned; ii) the two biphenylic groups of compounds **1.2** and **2.2** are perfectly superimposable in spite of the fact that they are in different positions inside the original molecules; iii) the phenylic ring of Elan patent (compound **1.2**) resulted aligned to the benzoyl one of the Actelion (compound **2.2**) albeit in the first one this scaffold is directly linked to the piperidine ring and in the second one is sensitively far away.

Looking at the Elan and Actelion structures we planned the synthesis of new piperidine derivates **A, B, C, D,** showed in Figure 10.2: new compounds are characterized by the presence of a 4-aminopiperidine scaffold and they are a form of hybrid of the two known reference compounds **1.2** and **2.2** in Figure 10.2.

Figure 10.2: ***4-aminopiperidine derivatives***



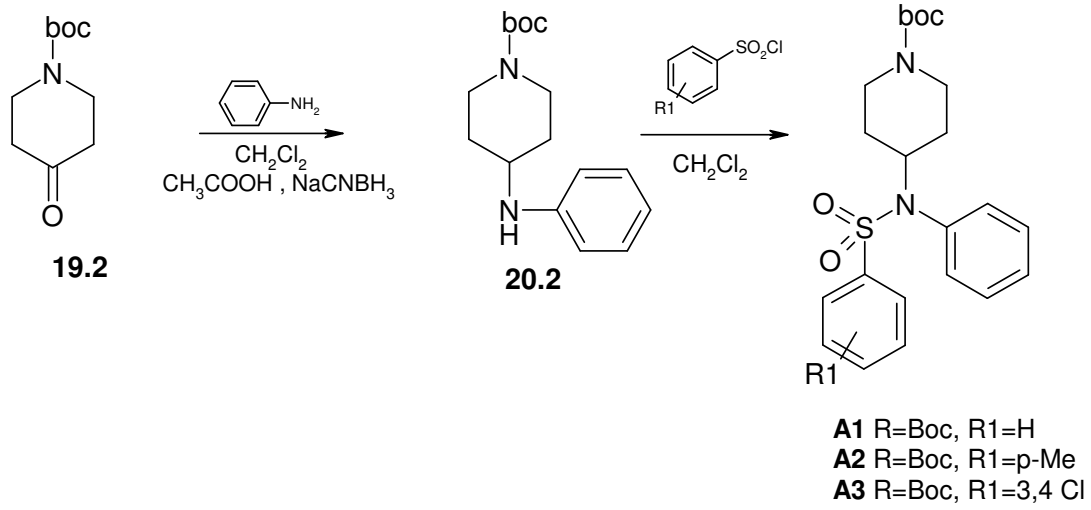
Compounds **A** are characterized by the presence of a phenyl group directly linked to the N atom in position 4 of the piperidine ring. They have a benzensulphonamido scaffold that can be variously substituted with R1 groups as shown in Figure 10.2.

Compounds **B** are derivatives related to the previous 4-aminopiperidine compounds, since they contain a phenyl group directly linked to the N atom in position 4 of the ring and the sulphonamido group is replaced by an arylamidic moiety.

Compounds **C** and **D** are 4-aminopiperidine derivatives that differ from the previous **A** and **B** compounds by the presence of a benzylamine scaffold variously substituted with R2 groups linked to the N atom in position 4 of the piperidine scaffold. In particular compounds **C** are benzensulphonamido derivatives and compounds **D** are arylamidic derivatives.

Compounds **A1-A3** were synthesized with new synthetic methodologies learned during a work experience in Siena Biotech laboratories; reagents supported on solid phase were used and new parallel procedures both for their preparation and purification were introduced. The synthetic procedure applied for compounds **A** is showed in scheme 4.2.

Scheme 4.2: ***Synthesis of compounds*** **A1, A2, A3**

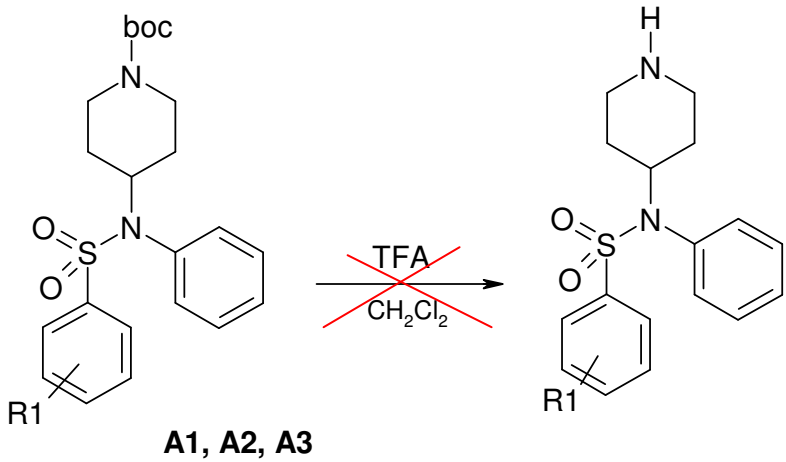


The commercial available *N*-boc-substituted piperidone **19.2** was dissolved in CH_2Cl_2 and treated with aniline in the presence of sodium cyanoborohydride and acetic acid. The reaction mixture was left under stirring for 3h at room temperature and then a solution of K_2CO_3 1M was added and the reaction extracted to obtain the desired secondary amine **20.2** in good yield. Product **20.2** was then dissolved in CH_2Cl_2 and treated with the various sulphonyl chlorides. A catalytic amount of DMAP was added in each reaction mixture and a solid phase-supported amine (PS-DIEA) was used to capture HCl released in the reaction mixtures. Condensation reaction of product **20.2** required too much time and afforded only small amounts of desired sulphonamide products. Purifications of these derivatives moreover was cumbersome because of their

trend to decompose. Only the three compounds **A1**, **A2** and **A3** showed in Table 1.2 were obtained after chromatography purification in poor yields.

Sulphonamides **A1**, **A2** and **A3** were therefore submitted to reaction with trifluoroacetic acid in CH_2Cl_2 in order to remove the Boc protecting group as showed in Scheme 5.2 but also in this case the reactions did not afford the hoped results probably on account of the instability of these compounds.

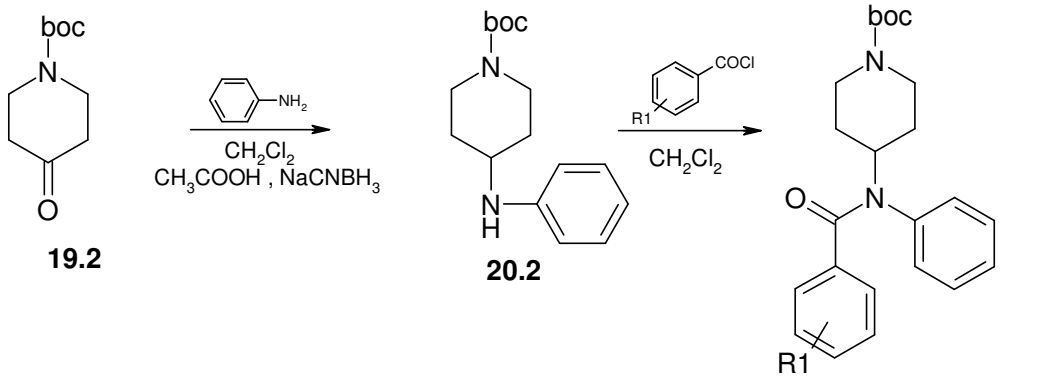
Scheme 5.2: ***Synthesis of N-unprotected derivatives***



A1, A2, A3

Compounds **B1-B5** were prepared by the same synthetic methodologies used for the previous compounds **A** as showed in Scheme 6.2.

Scheme 6.2. ***Synthesis of compounds*** **B1-B5**

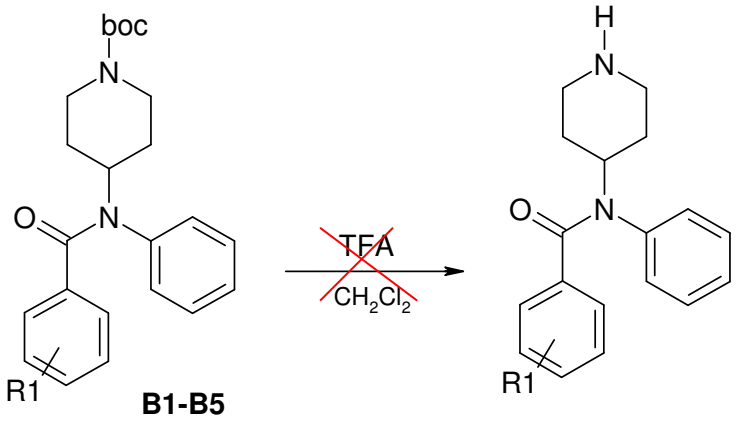


B1 R=Boc, R1= H
B2 R=Boc, R1=*p*-Me
B3 R=Boc, R1=*p*-Cl
B4 R=Boc, R1=3,4 Cl
B5 R=Boc, R1=*p*-OMe

The commercial available *N*-boc-substituted piperidone **19.2** was treated with aniline in the presence of sodium cyanoborohydride and acetic acid and the resulted secondary amine **20.2** was treated with various acyl chlorides. Also in this case a catalytic amount of DMAP was added in each reaction mixture and a solid phase-supported amine (PS-DIEA) was used to capture HCl released in the reaction mixtures. The resulting amides **B1-B5** were therefore obtained in good yields after chromatography purification with silica and ionic exchange pre-packed cartridges.

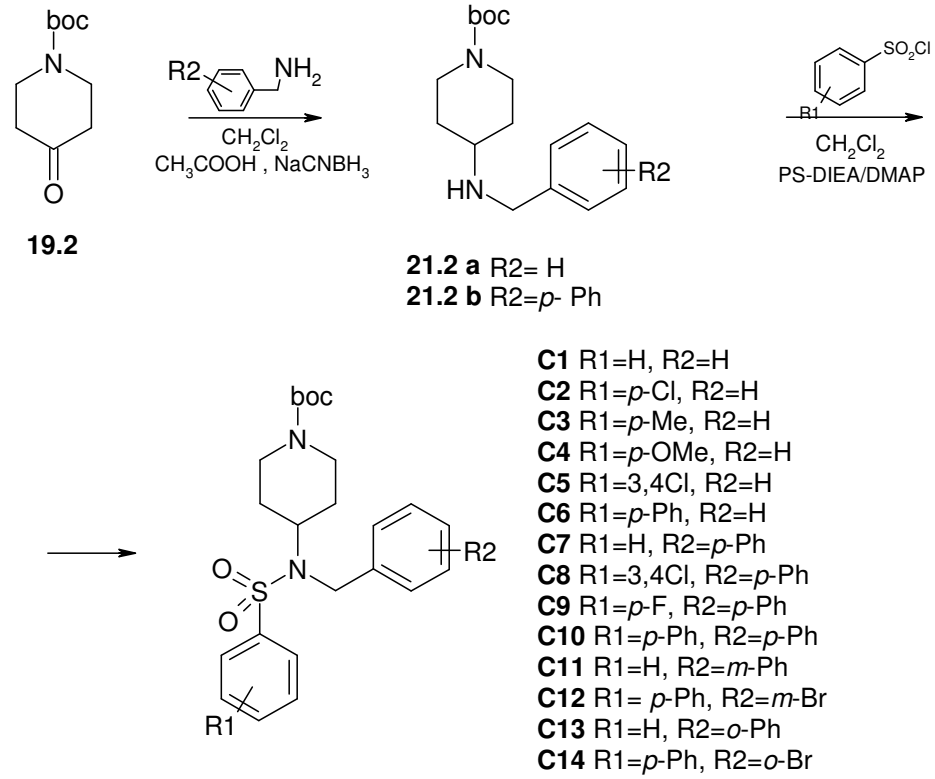
Compounds **B1-B5** were then submitted to reaction with trifluoroacetic acid in CH_2Cl_2 in order to remove the Boc protecting group as showed in Scheme 7.2 but also in this case reactions did not afford the unprotected amides because of their instability.

Scheme 7.2: ***Synthesis of N-unprotected derivatives***



Sulphonamido derivatives **C1-C14** have been prepared with parallel synthesis following the general procedure showed in Scheme 8.2.

Scheme 8.2: ***Synthesis of compounds*** **C1-C14**

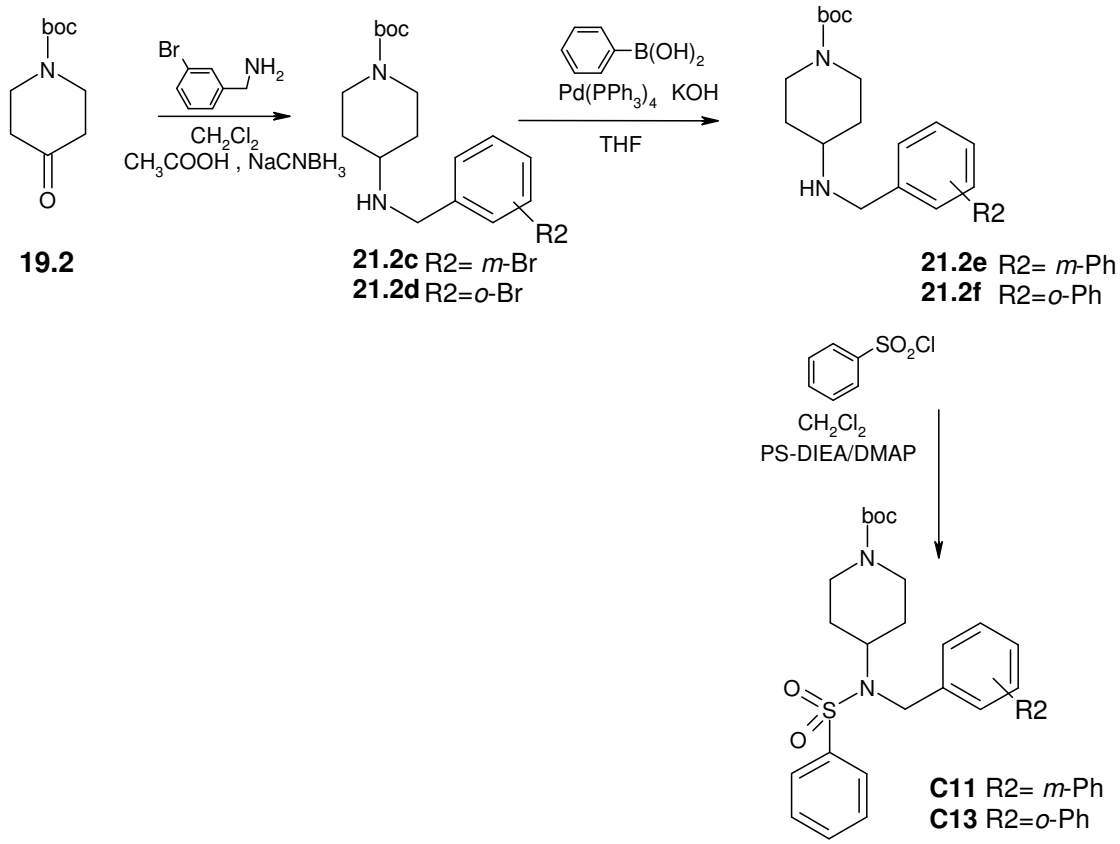


The commercial available *N*-substituted piperidone **19.2** was treated with assorted benzylamines in the presence of sodium cyanoborohydride and acetic acid to obtain the desired secondary amines **21.2a** and **21.2b** in good yield.

Compounds **21.2a** and **21.2b** were then dissolved in CH_2Cl_2 and subsequently the various sulfonyl-chlorides and catalytic amount of DMAP were added using a solid phase-supported amine (PS-DIEA) to capture HCl released in the reaction mixture.

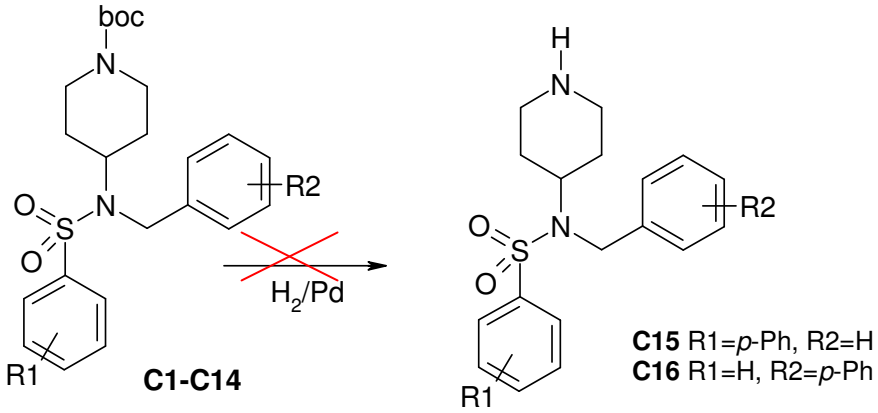
Preparation of compound **C11** and **C13** on the contrary required a different synthetic procedure showed in Scheme 9.2. Compound **21.2c** and **21.2d** in fact were treated with phenylboronic acid in a cross coupling reaction in the presence of $Pd(PPh_3)_4$ to yield the secondary amine **21.2e** and **21.2f** that were therefore treated with benzensulphonyl chloride and catalytic amounts of DMAP and PS-DIEA to produce the desired compounds **C13** and **C11** in good yield.

Scheme 9.2: ***Synthesis of compounds*** **C11** ***and*** **C13**



The piperidine derivatives **C1-C14** were also submitted to a catalytic hydrogenation with Pd/C to yield the related unprotected compounds as showed on scheme 10.2.

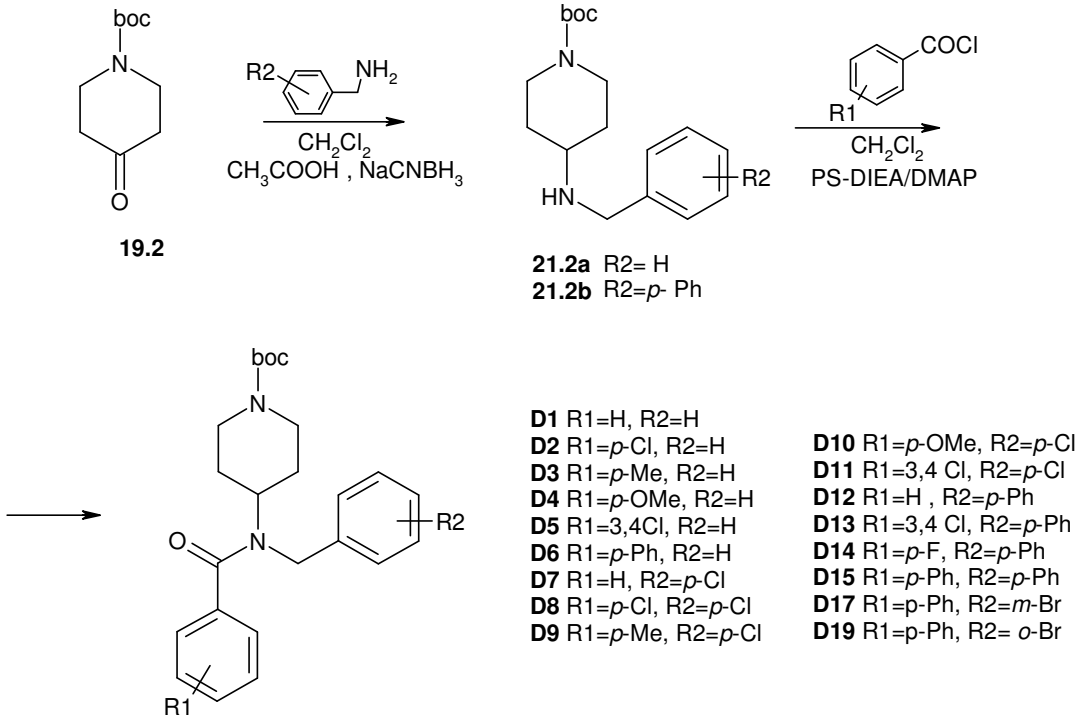
Scheme 10.2: ***Synthesis of N-unprotected derivatives***



Only the two unprotected derivatives **C15** and **C16** in Table 4.2 were obtained and then purified.

Aryl amidic compounds **D** are the related aryl amidic derivatives of previous compounds **C**. They have been prepared with parallel synthesis in the same manner following the general procedure as shown in Scheme 11.2.

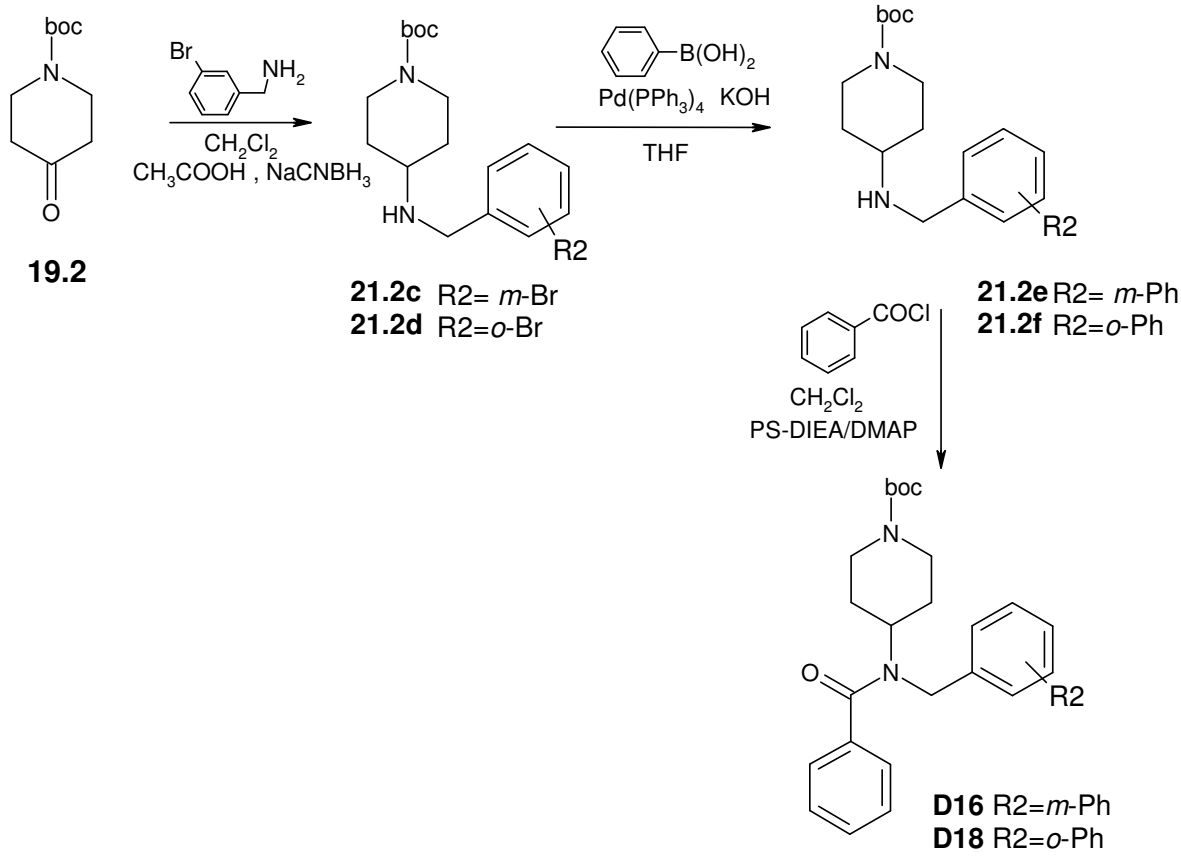
Scheme 11.2: ***Synthesis of compounds*** **D1-D19**



Compounds **21.2a** and **21.2b** obtained from reaction of the commercial available *N*-substituted piperidone **19.2** with assorted benzylamines in the presence of sodium cyanoborohydride and acetic acid were then dissolved in CH_2Cl_2 and subsequently the various acyl-chlorides were added. A catalytic amount of DMAP was used and a solid phase-supported amine (PS-DIEA) added to capture HCl released in the reaction mixture. Resulted products **D1-D19** are showed in Table 7.2.

Preparation of compound **D16** and **D18** as seen before for the related sulphonamidic compounds, required a different synthetic procedure showed in Scheme 12.2.

Scheme 12.2: ***Synthesis of compound*** **D16** ***and*** **D18**

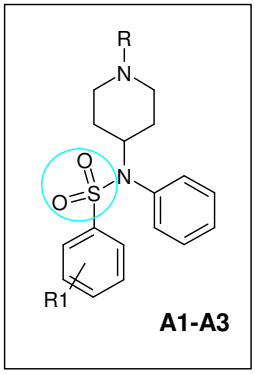


Compound **21.2c** and **21.2d** in fact were treated with phenylboronic acid in a cross coupling reaction in the presence of $Pd(PPh_3)_4$ to yield the secondary amine **21.2e** and **21.2f** that was therefore treated with benzoyl chloride and catalytic amounts of DMAP and PS-DIEA to produce the desired compound **D16** and **D18** in good yields.

BACE inhibition assay of 4-aminopiperidine derivatives

Tables 1.2-5.2 show all the synthesized compounds **A-D** and their percentage of inhibitory activity on BACE, when tested with the TR-FRET technology at the highest concentration of 20 μM.

Table 1.2:Compounds A1-A3

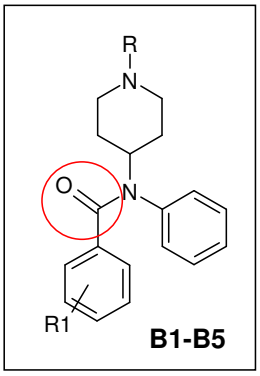


A1-A3

Compd	R	R1	% inhibition
A1	Boc	H	No activity*
A2		*p*-Me	
A3		3,4 Cl	

* No activity detected up to 20μM

Table 2.2:Compounds B1-B6

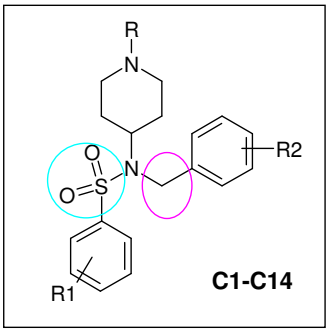


B1-B5

Compd	R	R1	% inhibition
B1	Boc	H	No activity *
B2		*p*-Me	
B3		*p*-Cl	
B4		3,4 Cl	
B5		*p*-OMe	

* No activity detected up to 20μM

Table 3.2:Compounds C1-C14

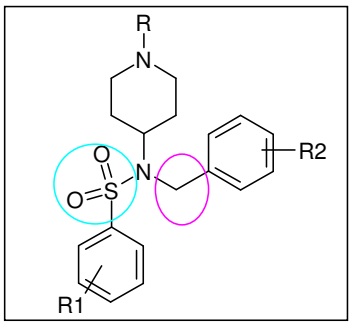


C1-C14

Compd	R	R1	R2	% inhibition
C1	**Boc**	H	H	No Activity*
C2		*p*-Cl		
C3		*p*-Me		
C4		*p*-OMe		
C5		3,4 Cl		
C6		*p*-Ph		
C7		H	*p*-Ph	33%
C8		3,4 Cl		No Activity*
C9		*p*-F		
C10		*p*-Ph		
C11		H	*m*-Ph	37%
C12		*p*-Ph	*m*-Br	26%
C13		H	*o*-Ph	No Activity*
C14		*p*-Ph	*o*-Br	

* No activity detected up to 20μM

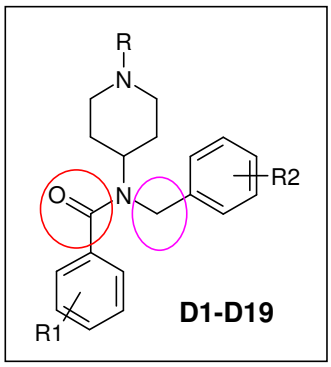
Table 4.2:Compounds C16 and C15



Compd	R	R1	R2	% inhibition
C15	H	*p*-Ph	H	No Activity*
C16	H	H	*p*-Ph	

* No activity detected up to 20μM

Table 5.2: Compounds D1-D19



Compd	R	R1	R2	% inhibition
D1	Boc	H	H	No Activity*
D2		*p*-Cl		
D3		*p*-Me		
D4		*p*-OMe		
D5		3,4 Cl		
D6		*p*-Ph		
D7		H	*p*-Cl	
D8		*p*-Cl		
D9		*p*-Me		
D10		*p*-OMe		
D11		3,4 Cl		

Compd	R	R1	R2	% inhibition
D12	Boc	H	*p*-Ph	No Activity*
D13		3,4 Cl		
D14		*p*-F		
D15		*p*-Ph		
D16		H	*m*-Ph	
D17		*p*-Ph	*m*-Br	
D18		H	*o*-Ph	
D19		*p*-Ph	*o*-Br	

* No activity detected up to 20μM

On the basis of the data resulting from biological tests we can point out that the presence of inhibitory activity on BACE of the herein reported 4-aminopiperidines seems to be essentially correlated with the presence of a benzylamine moiety. Aniline derivative **A** and **B** in Table 1.2 and 2.2 did not show any activity whatsoever.

Moreover, it is clear that the sulphonilic group represents an essential portion within this series of compounds in order to have activity. As a matter of fact all the three active compounds **C7**, **C11**, **C12** are sulphonamido derivatives, although they =show only a modest percentage of activity at the highest concentration of 20 μM (Table 3.2), whereas the benzamido derivatives are devoid of any inhibitory properties (**D1-D19**, Table 5.2).

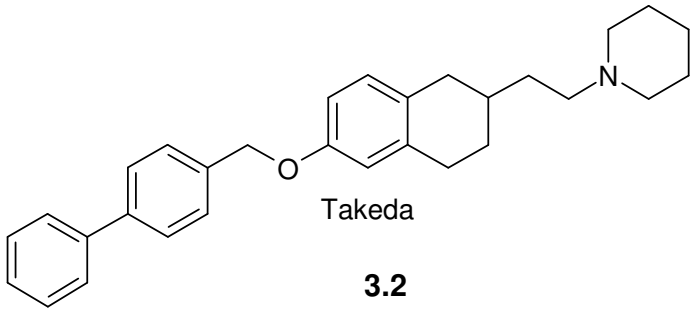
Another common feature to all the active compounds **C7**, **C11**, **C12** is the presence of a Boc group. In fact, the analog of **C7** which is devoid of the N-Boc portion, that is compound **C16** has no inhibitory activity up to a concentration of 20 μM (Table 4.2).

Finally, the activity may be associated with the steric hindrance of the two aromatic scaffolds and with their respective position inside the molecule. In fact, some activity is only found when at least three phenyl rings are present, as in compounds **C7** and in **C11** where a *para*-phenyl or a *meta*-phenyl benzylic portions are respectively present, accompanying the remaining benzene-sulfonyl moiety. In **C12**, a *para*-phenyl benzene-sulfonyl substituent coexists with the single ring benzylamino portion, summing up a total of three aromatic rings, here again. On the other hand, when only two aromatic rings are present, as in the sulfonamido derivatives **C1**-**C5** the BACE inhibitory activity is lost (Table 3.2).

Tetraline derivatives

In the present section of the PhD thesis we report the synthesis of non peptidomimetic derivates potentially active on BACE planned using compound **3.2** in Figure **11.2** as a starting point.

Figure 11.2

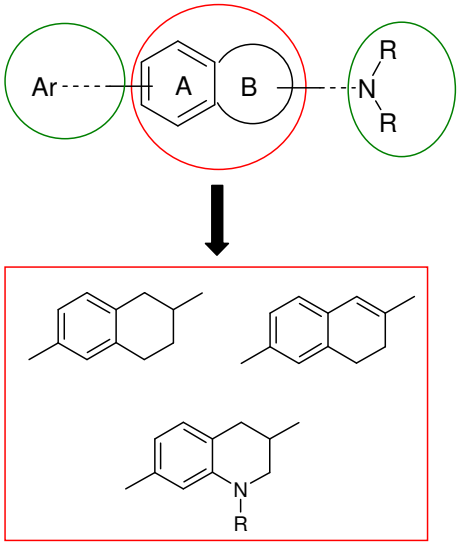


Takeda

3.2

In particular, during the exam of the patent we noticed that the side chains in the general structure of the patent (Figure 12.2) were extensively represented and varied, while the central core AB of the generic structure in Figure 12.2 was only represented by the three substructures showed below.

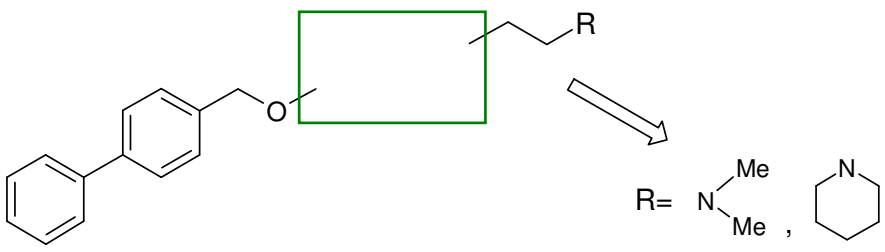
Figure 12.2: ***Generic structure of Takeda compound and the three claimed substructures.***



On these basis we therefore planned new molecules inserting different heterocycles or other moieties instead of the tetralinic core of Takeda compound (Figure 13.2) with the aim of increasing the polarity of the patented compound that possesess a good value of IC_{50} but most likely cannot be developed as a drug because of its accentuated lipofilicity that can compromise oral bioavailability.

The insertion of heteroatoms like oxygen, nitrogen and sulphur inside the core should increase the polarity and accordingly improve the ability to cross the blood-brain-barrier that represent the most important obstacle for CNS drugs.

Figure 13.2



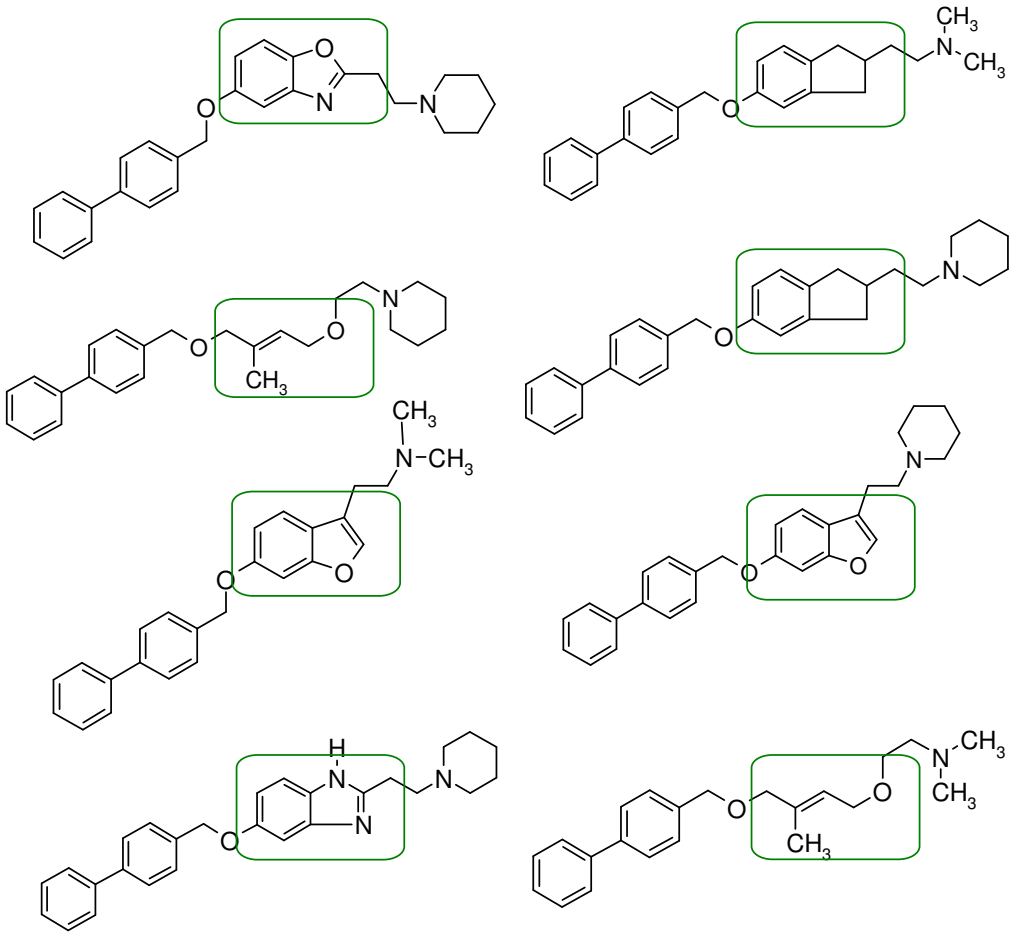
We designed molecules in which the biphenylic scaffold present in the original molecule was maintained and we made changes in the amino moiety. We inserted alternatively the piperidine ring, already present in the patent but also a dimethylamino group already present in a further compound reported in the patent that possessed a higher value of IC_{50} in comparison to compound **3.2** in Figure 11.2 but that was definitely more lipofilic. We therefore designed new structures using both the combinations.

With the aim of explore in details the new projected molecules, compounds planned were also profiled for their predicted physicochemical properties[131] to give good pharmacokinetic and CNS permeability parameters.

All compounds designed were therefore submitted to a theoretic analysis basing on the Lipinski, Veber and Norinder's rules using Spotfire2 program in Siena Biotech Laborartories. In the ambit of this study, restrictions of three of the most important parameters ***RBN*** (number of rotatable bonds), ***MlogP*** (parameterization of the hydrophobicity) and ***BBB*** (blood-brain barrier permeability) were made to their optimum value in order to obtain compounds with better physicochemical properties.

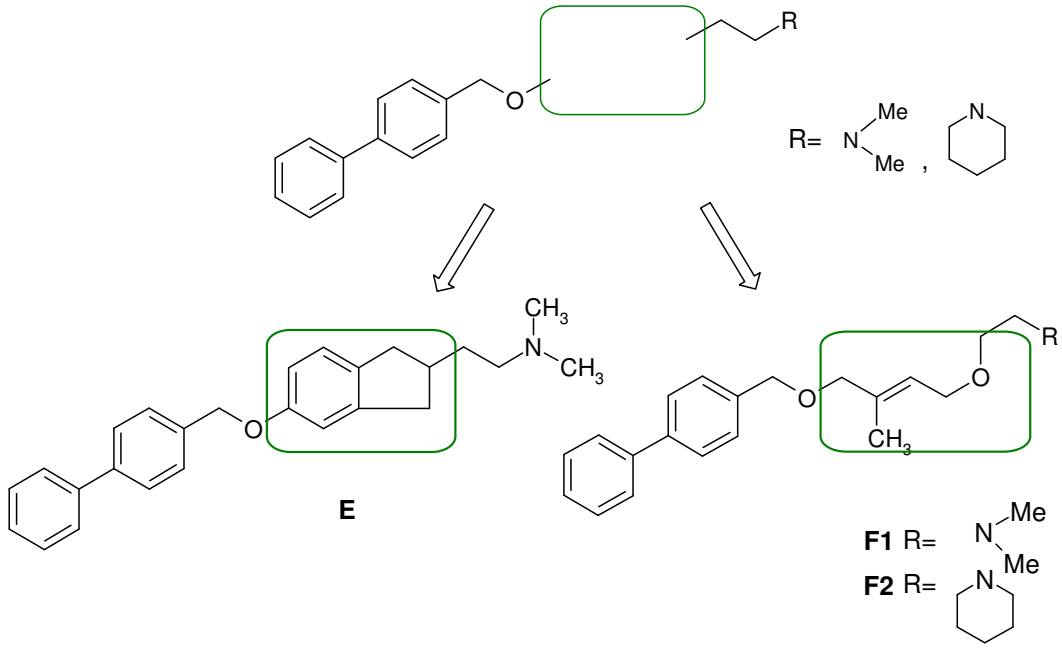
Approximately 80 compounds were planned and designed in our research laboratory and for each one the synthetic feasibility was definite as a result of a detailed bibliographic research. Among these compounds we finally selected for our study the 8 compounds showed in Figure 14.2.

Figure 14.2: ***New structures related to Takeda disclosed compounds***



Among compounds showed in Figure 14.2 compounds **E** and **F1** and **F2** in Figure 15.2 have been finally chosen as the best candidates for development on the basis of their synthetic feasibility and their calculated CNS permeability parameters.

Figure 15.2

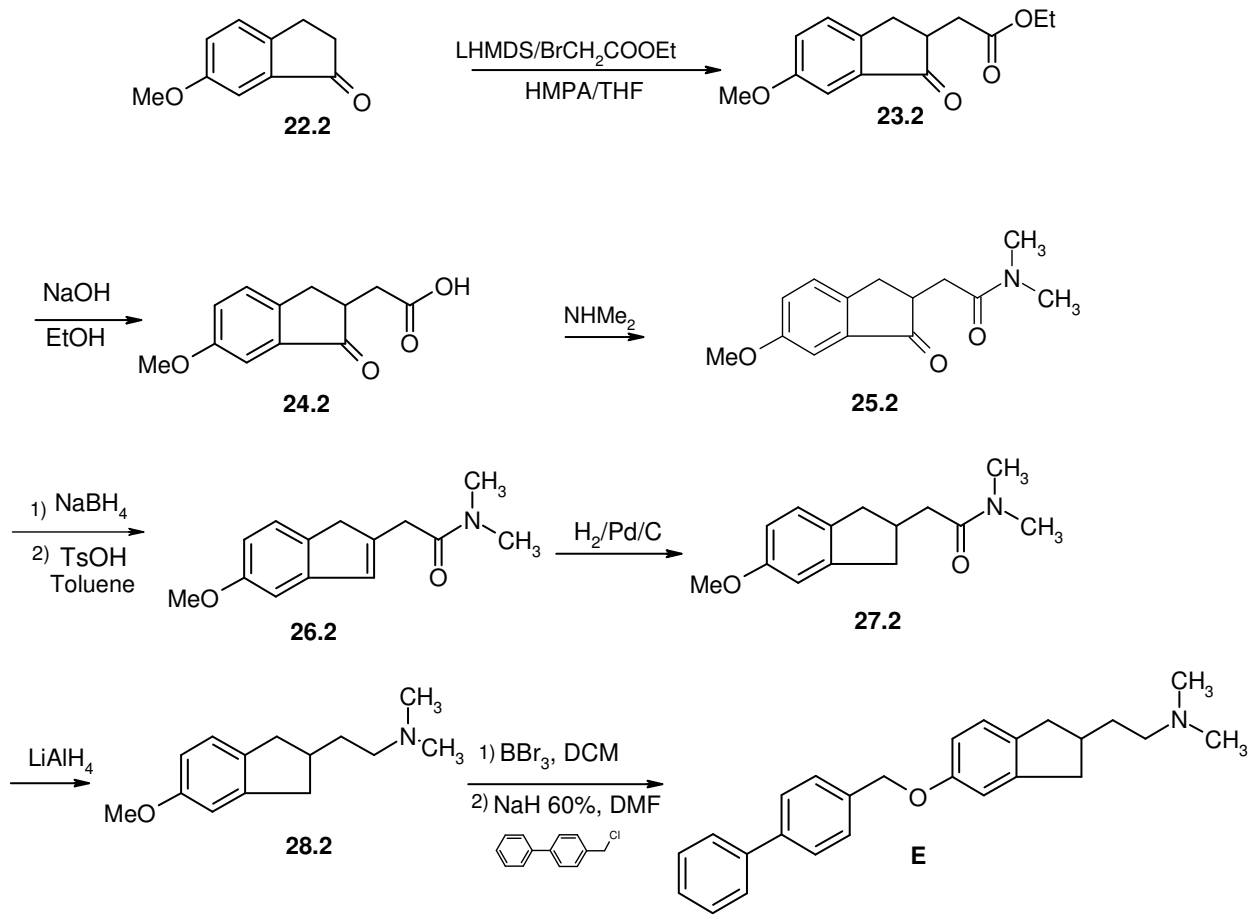


The indane **E** and the isoprenoid derivatives **F1** and **F2** have been finally synthesized.

Considering the structural similarity between compound **E** and compound **3.2** we planned his preparation on the basis of the synthetic procedure described for the patented compound making some synthetic modifications where necessary.

Derivate **E** was therefore prepared in seven steps as shown in Scheme 13.2.

Scheme 13.2: ***Synthesis of compound*** **E**



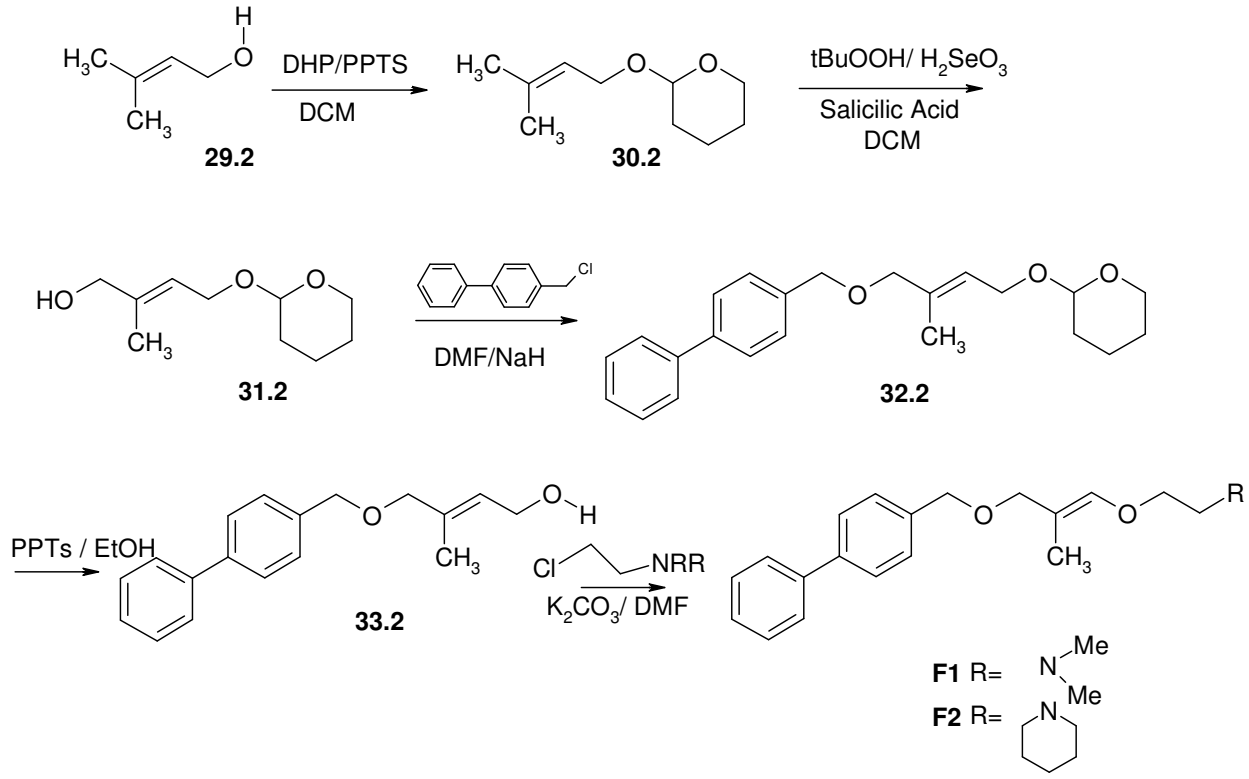
Commercially available 5-methoxy-1-indanone **22.2** was esterified with $BrCH_2COOMe$[132] in the presence of LHMDS to obtain the desired ester **23.2** in good yield. The following hydrolysis of **23.2** with NaOH in EtOH yielded acid **4.2** and amidation reaction of **24.2** with *N,N*-dimethylamine produced amide **25.2**. The

following reduction-elimination reaction of compound **25.2** with $NaBH_4$ and *p*-TsOH resulted in the formation of indene derivative **26.2**. The catalytic hydrogenation of **26.2** followed by reduction of the remaining amide **27.2** with $LiAlH_4$, afforded the desired amino derivative **28.2**. Finally, deprotection reaction of compound **28.2** with BBr_3 followed by etherification with 4-phenylbenzyl chloride in the presence of NaH 60% produced the desired aminoalkyl-substituted indane **E** in good yield.

In order to make further structural modifications in the central core of the patented compound reported from Takeda company, a non cyclic scaffold instead of the tetralinic one was designed. We therefore accomplished the synthesis of derivatives **F1** and **F2** using synthetic procedures already developed in our research laboratory.

Compounds **F1** and **F2** were synthesized in six steps starting from commercially available 3-methyl-2-buten-1-ol **29.2** as shown in Scheme 14.2.

Scheme 14.2: ***Synthesis of compounds*** **F1** ***and*** **F2**



The hydroxyl group of alcohol **29.2** was protected by reaction with 3,4-dihydropyran and pyridinium *p*-toluenesulfonate (PPTs)[133] to generate a tetrahydropyranyl ether in high yield. The resulting ether **30.2** was oxidized with *tert*-butylhydroperoxide and catalytic H_2SeO_3 in the presence of and salicylic acid to obtain

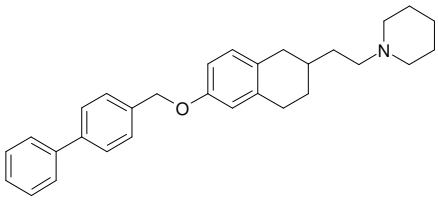
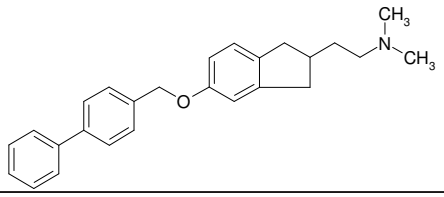
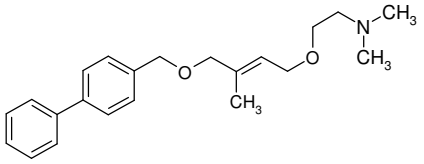
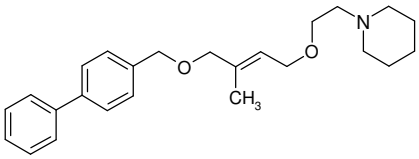
compound **31.2** that was then treated with NaH 60% and 4-phenylbenzyl chloride in a DMF solution to generate compound **32.2**.

After deprotection reaction of **32.2** with pyridinium *p*-toluenesulfonate in EtOH, the resulting alcohol **33.2** was then treated with chloroethyl *N,N*-dimethylamine or chloroethyl piperidine in the presence of K_2CO_3 to respectively obtain the two desired final compounds **F1** or **F2** in good yield.

***BACE inhibition assay of* E, F1 *and* F2**

Compounds **E, F1** and **F2** were submitted to biological tests with the TR-FRET technology at Siena Biotech laboratories and compounds **E** showed an inhibitory activity on BACE in the μM range (Table 6.2).

Table 6.2

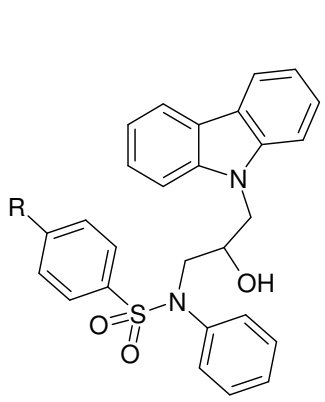
Compound	IC_{50}(μM)
3.2 	11
E 	12
F1 	>20
F2 	>20

The shrinkage of the saturated ring of the Takeda compound from a six- to a five-member ring, as in the indane derivative **E**, preserved the good inhibitory properties of the molecule. On the contrary, the replacement of the bicyclic core portion with an acyclic isoprenoid moiety caused the loss of activity, as in compounds **F1** and **F2**.

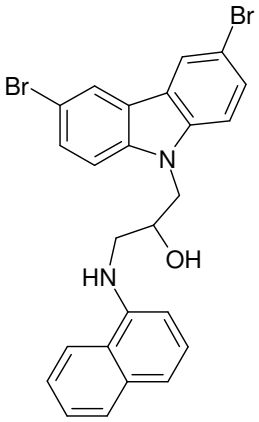
Carbazole derivatives

In order to find other new potential hits as BACE inhibitors, a considerable number of available compounds were screened at Sienabiotech Laboratories. During the screening, three carbazole derivatives **4.2-6.2** in Figure 16.2 were discovered to have interesting biological results, showing an inhibitory activity on BACE in the micromolar range.

Figure 16.2



4.2 R=Me
5.2 R=H



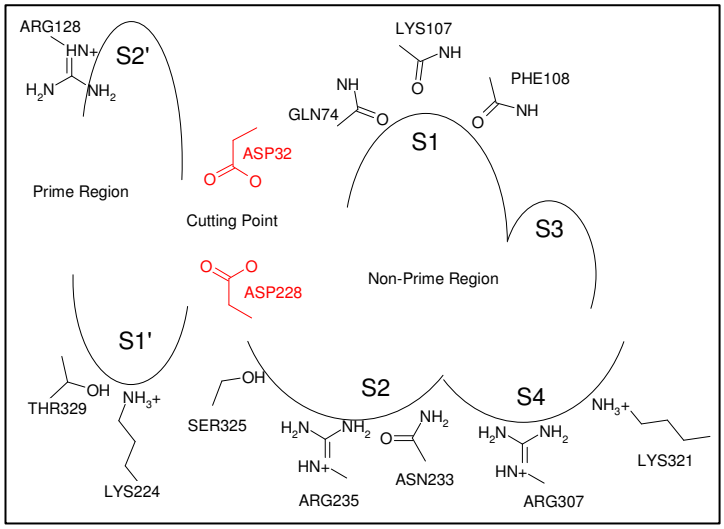
6.2

In order to explain the activity of compounds **4.2**, **5.2** and **6.2** docking studies have been carried out at Siena Biotech laboratories and were performed using the X-ray structure from the Protein Data Bank: 1FKN.

In the protease family the binding site is usually mapped using a sub-pocket organization. The binding site is considered to be divided in two main regions defined by the cutting point of the substrate peptide. The binding site part that recognizes the *C*-terminus region of the peptide is called prime region (S') in Figure 17.2. The side at the *N*-terminus of the peptide is termed non-prime region (S). The two regions are mapped in sub-pockets starting from the cutting point towards the periphery of the substrate

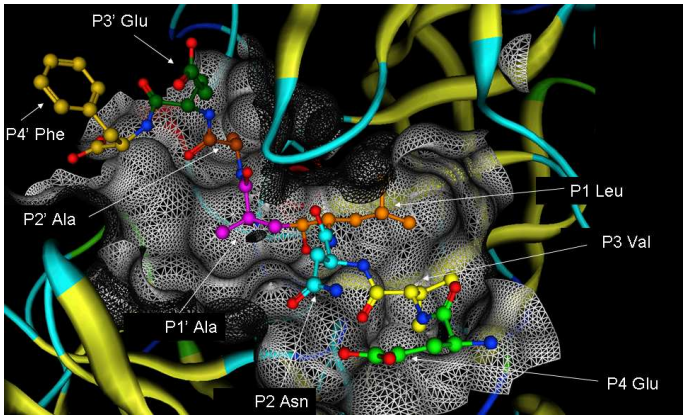
peptide. The S2', S1, and S2 pockets were mainly involved in lipophilic interactions. On the contrary, S1' and S4 pockets were mainly involved in hydrophilic interactions.

Figure 17.2: ***BACE Binding Site Map.***



The main pockets of the BACE binding site are shown in this schema. For each pocket hydrophilic interactions are also shown.

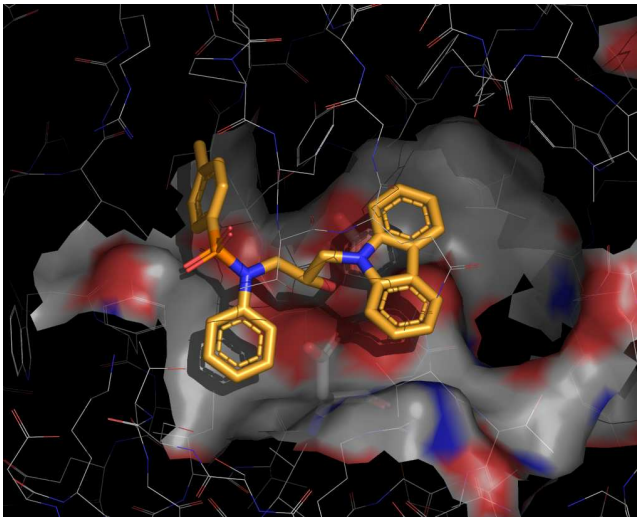
In red: the catalytic aminoacids Asp32 and Asp228 activate a water molecule that functions as the nucleophile responsible for amide cleavage:



3D Binding Site Map of BACE on 1FKN co-crystal structure. [134]

On these basis, two putative binding modes based on docking calculation were hypothesized. Due the high pseudo-symmetry of this class of molecules two opposite binding modes were found (**A** and **B** in Figures 18.2 and 19.2).

Figure 18.2: ***Carbazole binding mode*** **A**



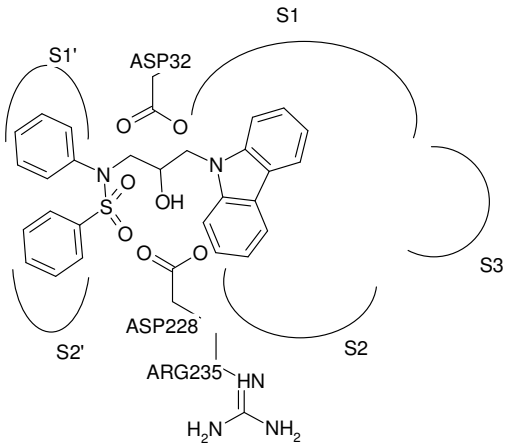
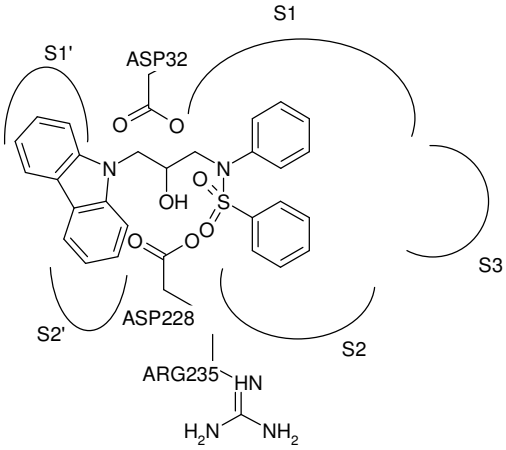
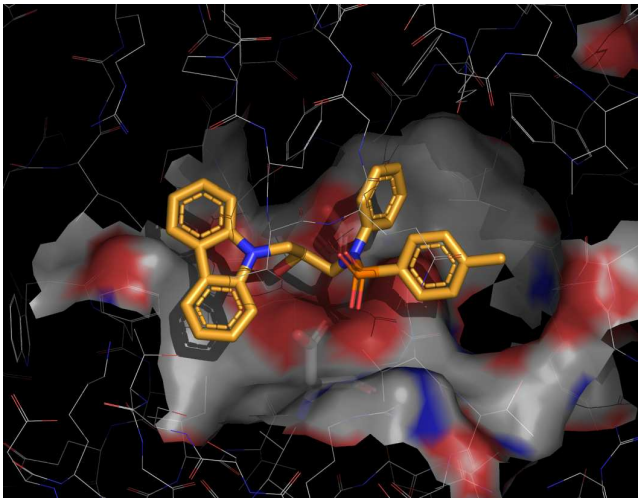


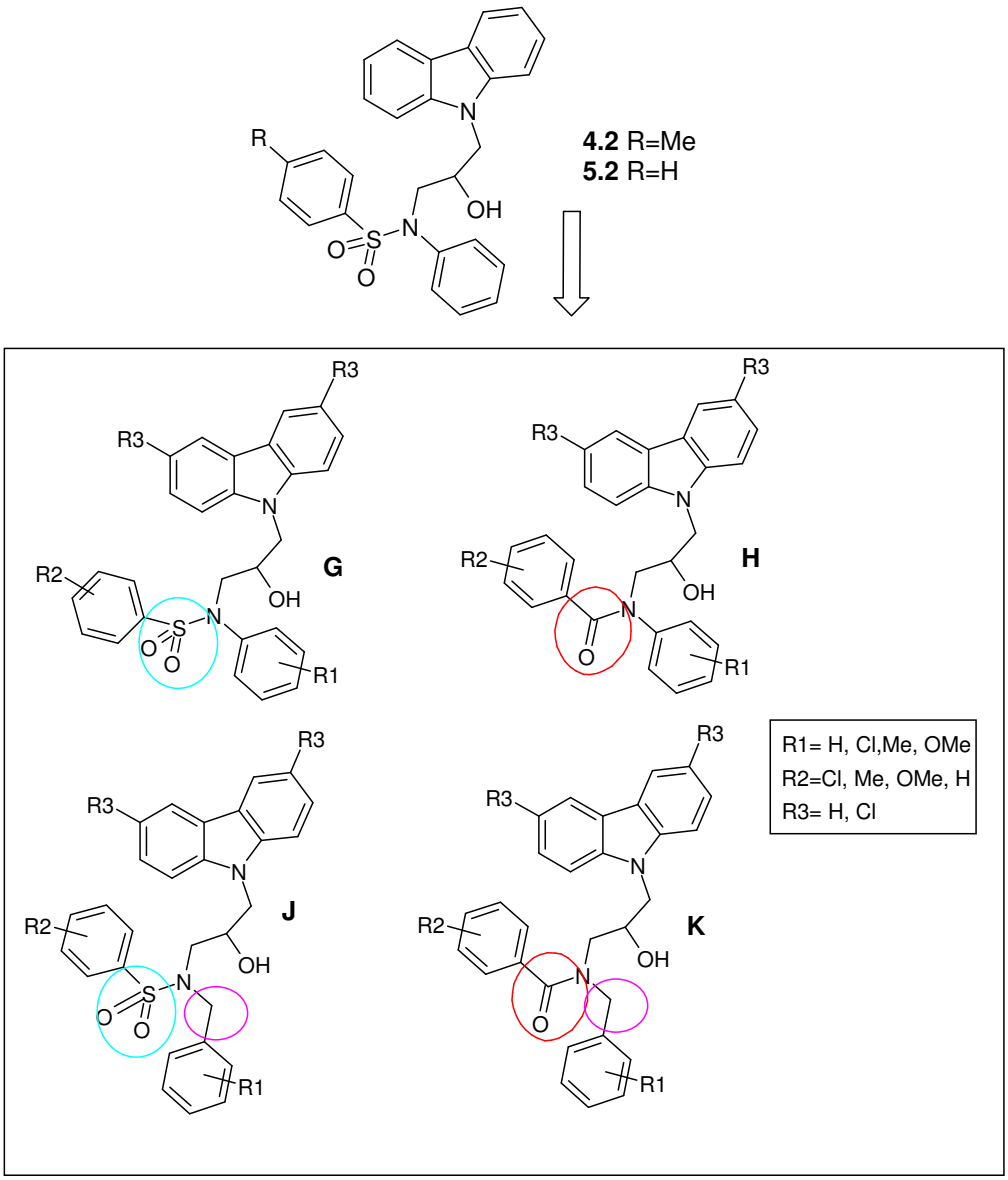
Figure 19.2: ***Carbazole binding mode*** **B**





With the aim of guiding the exploration of this class, new compounds related to the carbazole scaffold have been planned in our laboratory. In particular, taking compounds **4.2** and **5.2** as a reference, we accomplished the synthesis of new compounds **G, H, J, K** showed in Figure 20.2.

Figure 20.2: ***New carbazole derivatives***

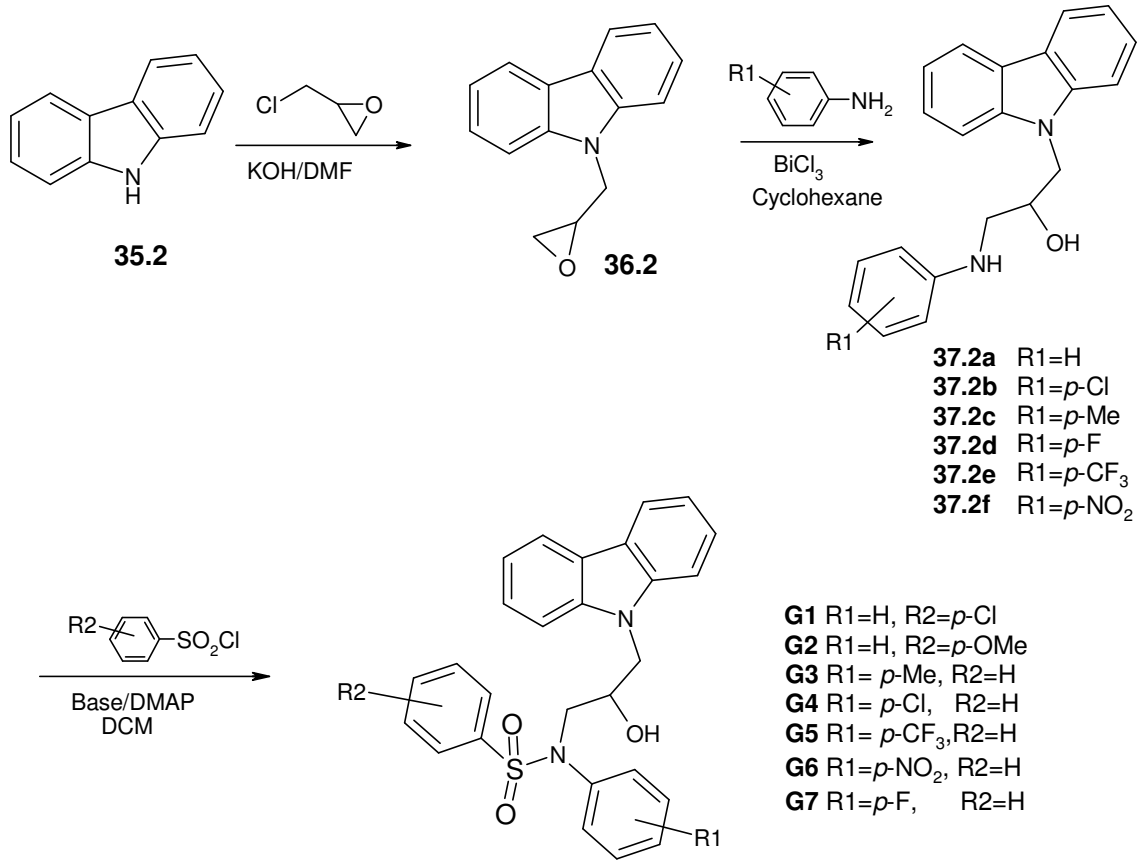


Compounds **G, H, J, K** are characterized by the presence of a carbazole moiety and differ on the basis of the presence of an aryl amidic (**H**, **K**) or a aryl sulphonamidic (**G**, **J**) functionalities. Furthermore, we made some structural modifications on the amino moiety by inserting several groups R1 and R2 represented by variously substituted aryl scaffolds.

Compounds **J** and **K** differ from previous compounds **G** and **H** (Figure 23.2) for the presence of a benzylamine portion linked to the N atom of the side chain of the molecule instead of the arylic one present in **G** and **H** derivatives. In particular, compounds **J** are aryl sulphonamidic derivatives while compounds **K** are aryl amidic derivatives. In both compounds **J** and **K** the aromatic scaffold can be substituted with R2 groups. Moreover, the carbazole moiety of compounds **J** and **K** can contain an halogen substituent (R3).

Carbazole derivatives **G1-G7** were synthesized following the procedure shown in Scheme 15.2.

Scheme 15.2: ***Synthesis of compounds*** **G1-G7**

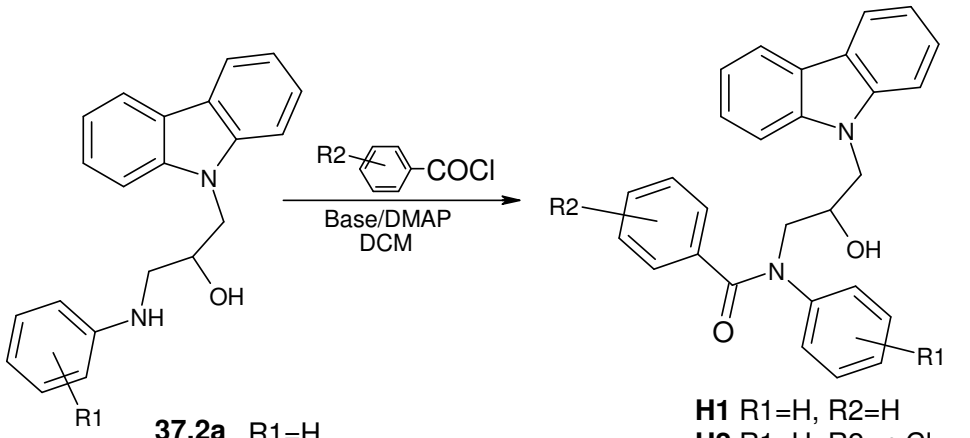


The various carbazole derivatives were easily prepared in a parallel way in three steps starting from commercially available carbazole **35.2** that was initially alkylated with epichloridrine in the presence of KOH to give the desired epoxide **36.2** in good yields. Subsequently the epoxide **36.2** was opened by reaction with various anilines in cyclohexane at room temperature in the presence of $BiCl_3$ as the catalyst and the secondary amines **37.2a-f** were easily obtained in excellent yields by crystallisation from Et_2O. The reaction of products **37.2a-f** with the appropriate differently *p*-substituted aromatic sulfonyl chloride led to the final compounds **G1-G7**. For all the

coupling reactions we used PS-DIEA (a solid phase-supported amine) to capture HCl released in the reaction mixtures, and DMAP as the catalyst.

The synthetic procedure followed for derivatives **H1-H9** is showed in Scheme 16.2.

Scheme16.2: ***Synthesis of compounds*** **H1-H9**



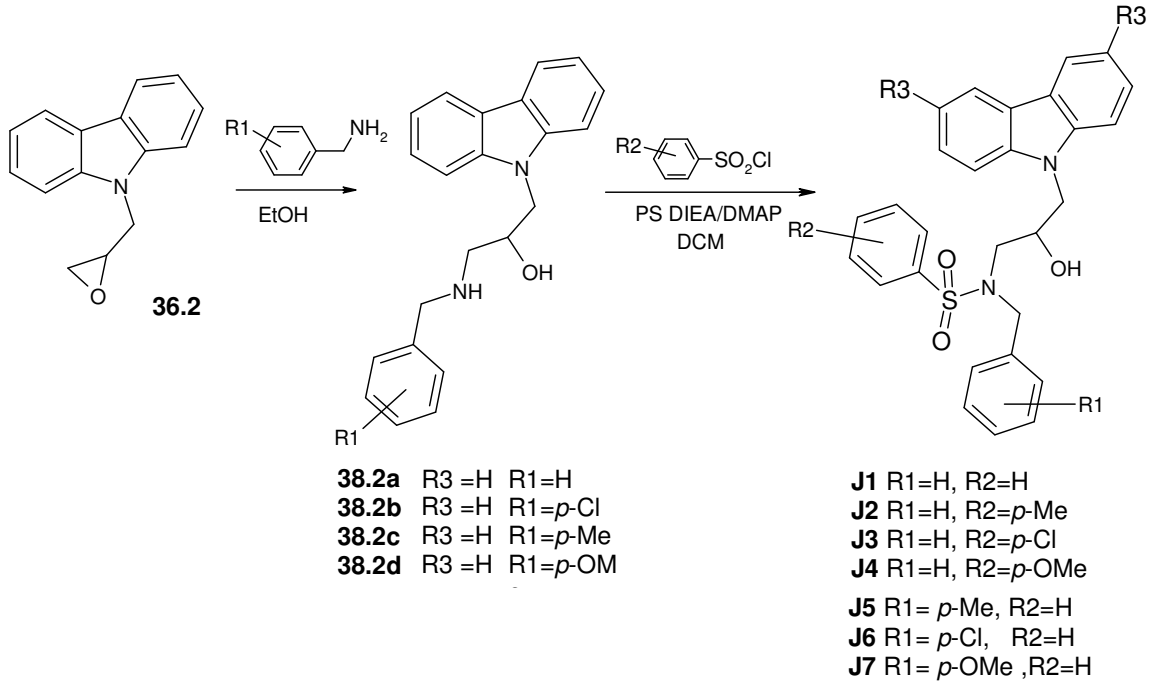
37.2a R1=H
37.2b R1=*p*-Cl
37.2c R1=*p*-Me
37.2d R1=*p*-F
37.2e R1=*p*-CF_3
37.2f R1=*p*-NO_2

H1 R1=H, R2=H
H2 R1=H, R2=*p*-Cl
H3 R1=H, R2=*p*-OMe
H4 R1=H, R2=3,4 Cl
H5 R1= *p*-Me, R2=H
H6 R1= *p*-Cl, R2=H
H7 R1= *p*-CF_3,R2=H
H8 R1=*p*-NO_2, R2=H
H9 R1=*p*-F, R2=H

Secondary amines **37.2a-f**, obtained as shown in Scheme 15.2, were submitted to condensation reactions with appropriate differently *p*-substituted aromatic aroyl chloride to yield compounds **H1-H9**. Also in this case we used a solid phase-supported amine (PS-DIEA) to capture HCl released in the reaction mixtures and DMAP as the catalyst.

The synthesis of sulphonamidic derivatives **J1-J7** followed the synthetic procedure already described for the previous series and is shown in Scheme 17.2.

Scheme 17.2: ***Synthesis of compounds*** **J1-J7**



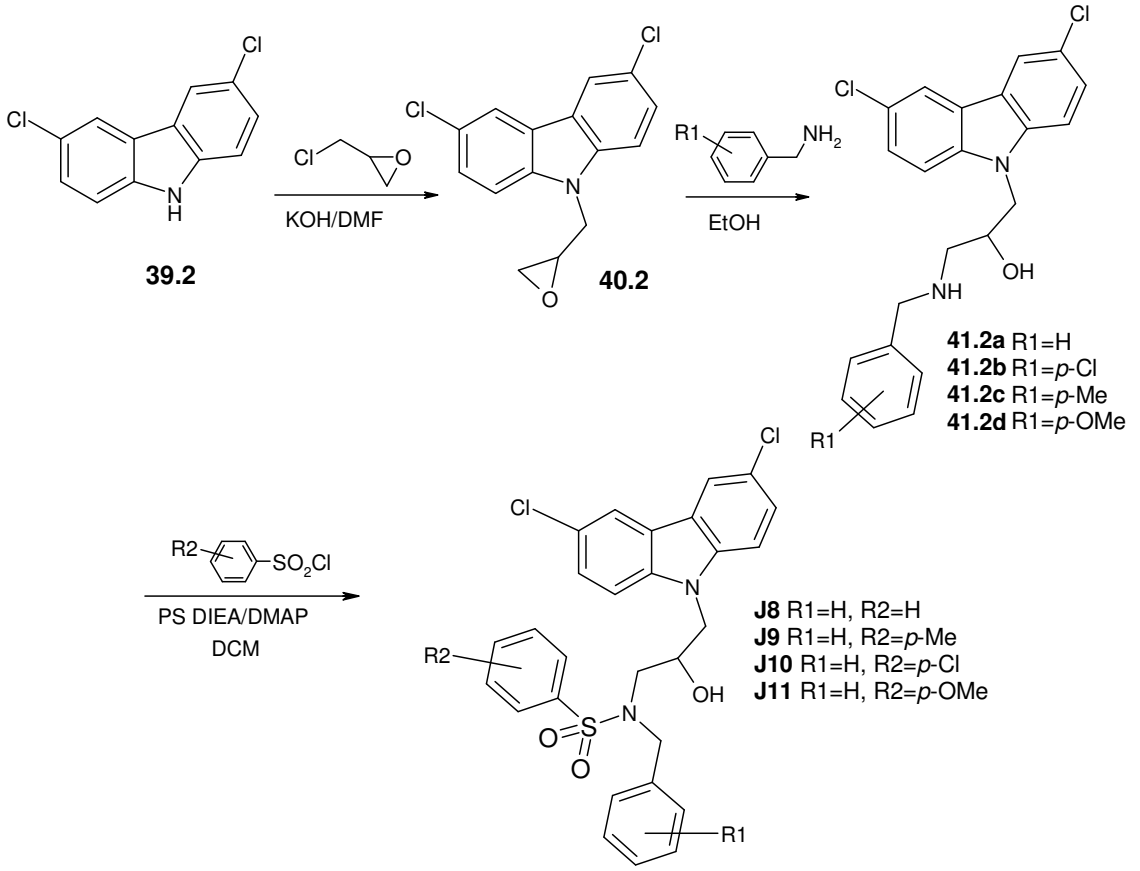
38.2a R3 =H R1=H
38.2b R3 =H R1=*p*-Cl
38.2c R3 =H R1=*p*-Me
38.2d R3 =H R1=*p*-OM

J1 R1=H, R2=H
J2 R1=H, R2=*p*-Me
J3 R1=H, R2=*p*-Cl
J4 R1=H, R2=*p*-OMe
J5 R1= *p*-Me, R2=H
J6 R1= *p*-Cl, R2=H
J7 R1= *p*-OMe ,R2=H

Carbazole derivatives **J1-J7** were easily prepared from epoxide **36.2** which was synthesized as seen above (Scheme 15.2). Epoxide **36.2** was opened by reaction with various *p*-substituted benzylamines in EtOH at room temperature and secondary amines **38.2a-d** were easily obtained in excellent yield by crystallisation from Hexane/Et_2O. The reaction of products **38.2a-d** with the appropriate differently *p*-substituted aromatic sulfonyl chloride led to the final compounds **J1-J7**. As usual for all the coupling reactions we used PS-DIEA to capture HCl released in the reaction mixtures and catalytic amounts of DMAP.

Compounds **J8-J11** were, on the contrary, synthesized starting from commercially available di-chlorocarbazole **39.2**, following the same procedure seen above as shown in scheme 18.2.

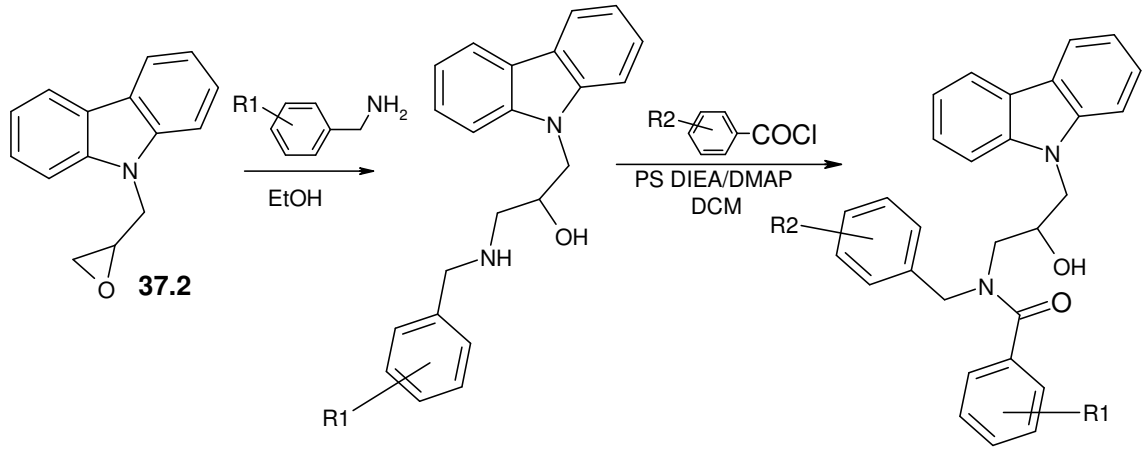
Scheme 18.2:*Synthesis of compounds* J8-J11



Commercially available carbazole **39.2** that was initially alkylated with epichloridrine in the presence of KOH to give the desired epoxide **40.2** in good yields. Subsequently the epoxide **40.2** was opened by reaction with various *p*-substituted benzylamines in EtOH at room temperature and the secondary amines **41.2a-d** were easily obtained in excellent yields by crystallisation from Et_2O and cyclohexane. The reaction of products **41.2a-d** with the appropriate differently *p*-substituted aromatic sulfonyl chloride led to the final compounds **J8-J11**

Compounds **K1-K7** were synthesized as showed in Scheme19.2.

Scheme 19.2:*Synthesis of compounds* K1-K7



41.2a R1=H
41.2b R1=*p*-Cl
41.2c R1=*p*-Me
41.2d R1=*p*-OMe

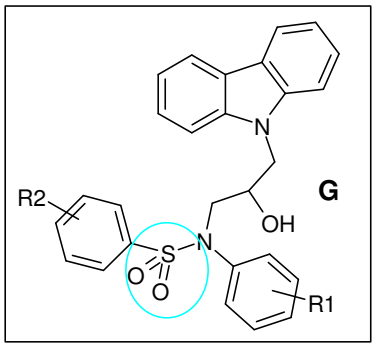
K1 R1=H, R2=H
K2 R1=H, R2=*p*-Cl
K3 R1=H, R2=*p*-OMe
K4 R1=H, R2=3,4 Cl
K5 R1= *p*-Me, R2=H
K6 R1= *p*-Cl, R2=H
K7 R1= *p*-OMe ,R2=H

The various carbazole derivatives **K1-K7** were prepared in a parallel way starting from epoxide **36.2**, prepared as seen before (Scheme 15.2). Epoxide **37.2** was opened by reaction with variously *p*-substituted benzylamines in EtOH at room temperature and secondary amines **41.2a-d** were easily obtained in excellent yields by crystallisation from Et_2O. The reaction of products **41.2a-d** with the appropriate differently *p*-substituted aromatic acyl chlorides yielded compounds **K1-K7**, under the usual conditions.

***BACE inhibition assay of carbazoles* G, H, J *and* K**

The various carbazole derivatives **G-K** obtained during this PhD research work and their IC_{50} values measured by the TR-FRET assay are shown in Tables 7.2-11.2.

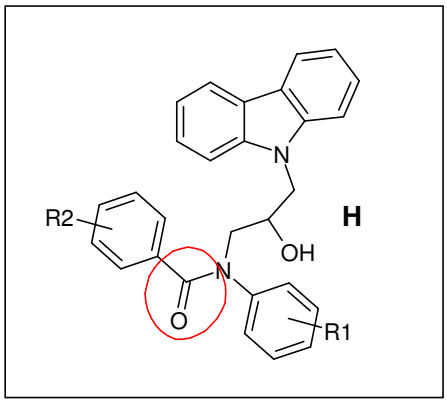
Table 7.2: Compounds G1-G7



G

Compd	R1	R2	IC_{50} µM
5.2	H	H	3.0
4.2		*p*-Me	2.6
G1		*p*-Cl	2.9
G2		*p*-MeO	2.4
G3	*p*-Me	H	7.1
G4	*p*-Cl		>10
G5	*p*-CF_3		3.6
G6	*p*-NO_2		3.8
G7	*p*-F		> 10

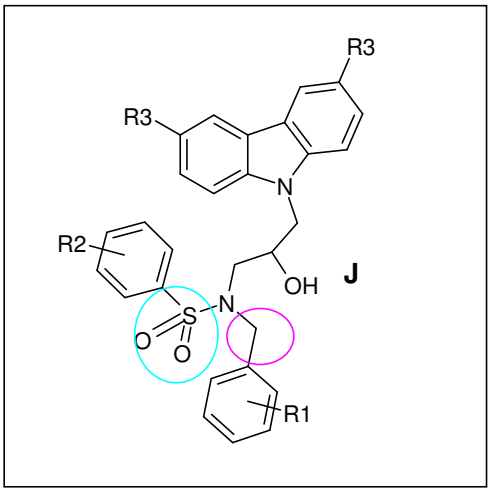
Table 8.2: Compounds H1-H9



Compd	R1	R2	IC_{50} µM
H1	H	H	>10
H2		*p*-Cl	4.7
H3		*p*-OMe	3.8
H4		3,4 Cl	2.4
H5	*p*-Me	H	>10
H6	*p*-Cl		>10
H7	*p*-CF_3		>10
H8	*p*-NO_2		>10
H9	*p*-F		>10

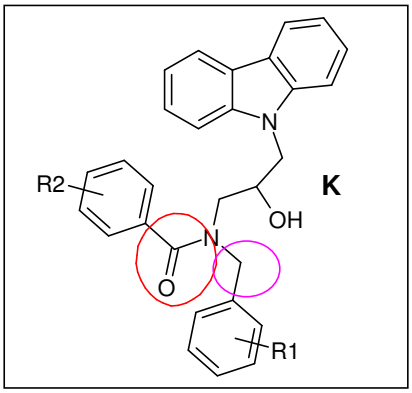
As showed in Tables 7.2 and 8.2 aniline-type sulphonamide derivatives **G** display a general trend of appreciable activities on BACE comparable to the reference compounds **4.2** and **5.2**. For these compounds the activity is maintained for almost every substitution of R1 and R2. On the contrary, the related aniline carboxamido compounds **H** showed in Table 8.2 show inhibitory activities on BACE only when the benzamidic portion is substituted (R2 ≠ H).

Table 9.2: Compounds J1-J11



Compd	R1	R2	R3	IC_{50} µM
J1	H	H	H	1.9
J2		*p*-Me		2.7
J3		*p*-Cl		1.7
J4		*p*-MeO		1.6
J5	*p*-Me	H		>10
J6	*p*-Cl			2.7
J7	*p*-OMe			5.7
J8	H	H	Cl	>10
J9		*p*-Me		2.3
J10		*p*-Cl		>10
J11		*p*-OMe		0.9

Figure 21.2: Compounds K1-K7



K1 R1=H, R2=H
K2 R1=H, R2=*p*-Cl
K3 R1=H, R2=*p*-OMe
K4 R1=H, R2=3,4 Cl
K5 R1= *p*-Me, R2=H
K6 R1= *p*-Cl, R2=H
K7 R1= *p*-OMe ,R2=H

IC_{50} > 10 µM

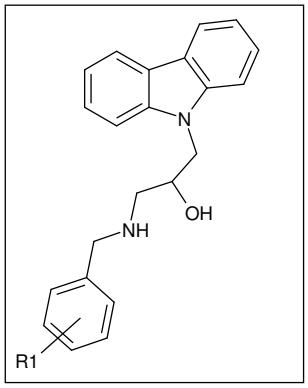
For benzylamine derivatives **J** and **K** showed in Tables 9.2 and Figure 21.2 the trend of activity on BACE is similar to the previous aniline-type compounds showed in Tables 7.2 and 8.2. In this case the situation is emphasized because only sulphonamido derivatives **J** in Table 9.2 show significant values of IC_{50} in the micromolar range, while the corresponding carbonyl derivatives **K**, on the contrary, did not show any activity at the highest concentration of 10μM. Moreover, it should be noticed that benzylamine-sulfonyl derivatives **J** generally showed slightly better inhibitory properties than their aniline-counterparts **G**.

In details, also in this case among various R2-substituents on the benzenesulphonamido ring, chloro and methoxy groups are associated with the most active inhibitors. Actually, the two most active compounds in Table 9.2 resulted to be the *p*-chlorobenzyl- (**J3**) and *p*-methoxybenzyl- (**J4**) substituted sulphonylic derivatives, which showed inhibitory activities on BACE of 1.7 and 1.6 μM, respectively.

The insertion of chlorine atoms into the carbazole moiety (R3 = Cl) of the sulfonamidic derivatives afforded efficient inhibitors only when it was associated with the concomitant presence of electron-donating substituents in the benzene-sulfonyl ring, such as a *p*-methyl in **J9** or a *p*-methoxy in **J11**, whereas unsubstituted (**J8**) or *p*-chloro-substituted (**J10**) derivatives resulted to be inactive (Table 9.2). In particular the lowest IC_{50} value of the whole series was found for di-chlorocarbazole derivative **J11,** (R2 = *p*-methoxy), whose value was as low as 0.9 μM.

Biological tests were made also for the synthetic precursors of compounds **G** and **H**, that are, the various secondary amines **42a-d** (Figure 22.2). When these compounds were tested with TR-FRET assay at the highest concentration of 20 μM, none of them showed a significant inhibitory activity on the enzyme.

Figure 22.2



41.2a R1=H
41.2b R1=*p*-Cl
41.2c R1=*p*-Me
41.2d R1=*p*-OMe

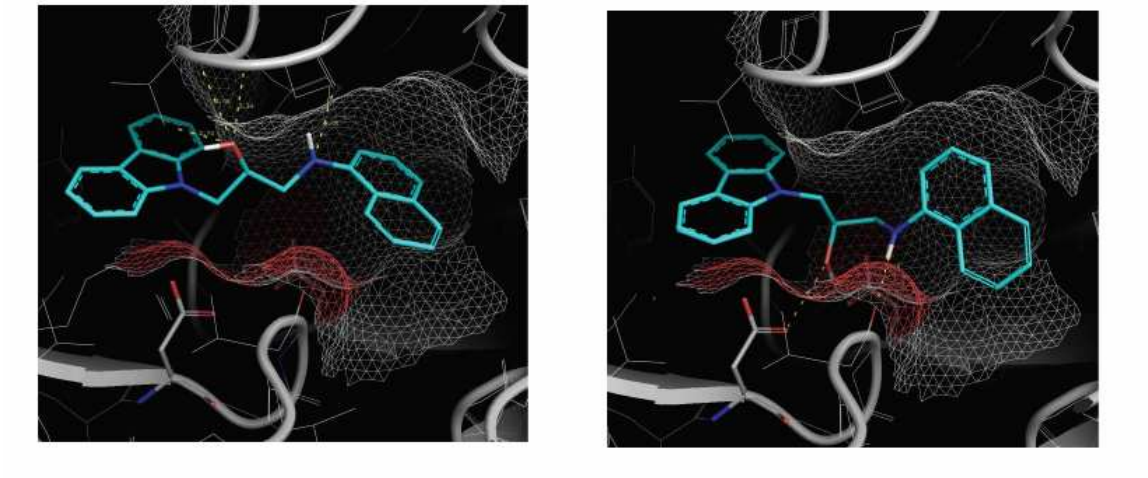
⟹ IC_{50} > 10 μM

As a continuation of these studies and based on the good inhibitory activity on BACE of the di-bromocarbazole derivative **6.2** (see Figure 16.2, IC_{50} =1.1 μM), we therefore accomplished the synthesis of other derivatives related to the carbazole series, still maintaining as a constant portion the α-naphthylamine moiety.

Also in this case in order to explain the activity of compound **6.2**, docking studies have been carried out at Siena Biotech laboratories and were performed using the X-ray structure from the Protein Data Bank: 1FKN.

Figure 23.2 shows the two opposite hypothesized binding modes **A** and **B** for compound **6.2** on the basis of docking calculation.

Figure 23.2 : ***Binding mode*** **A** ***and*** **B** ***hypotized for compound*** **6.2**

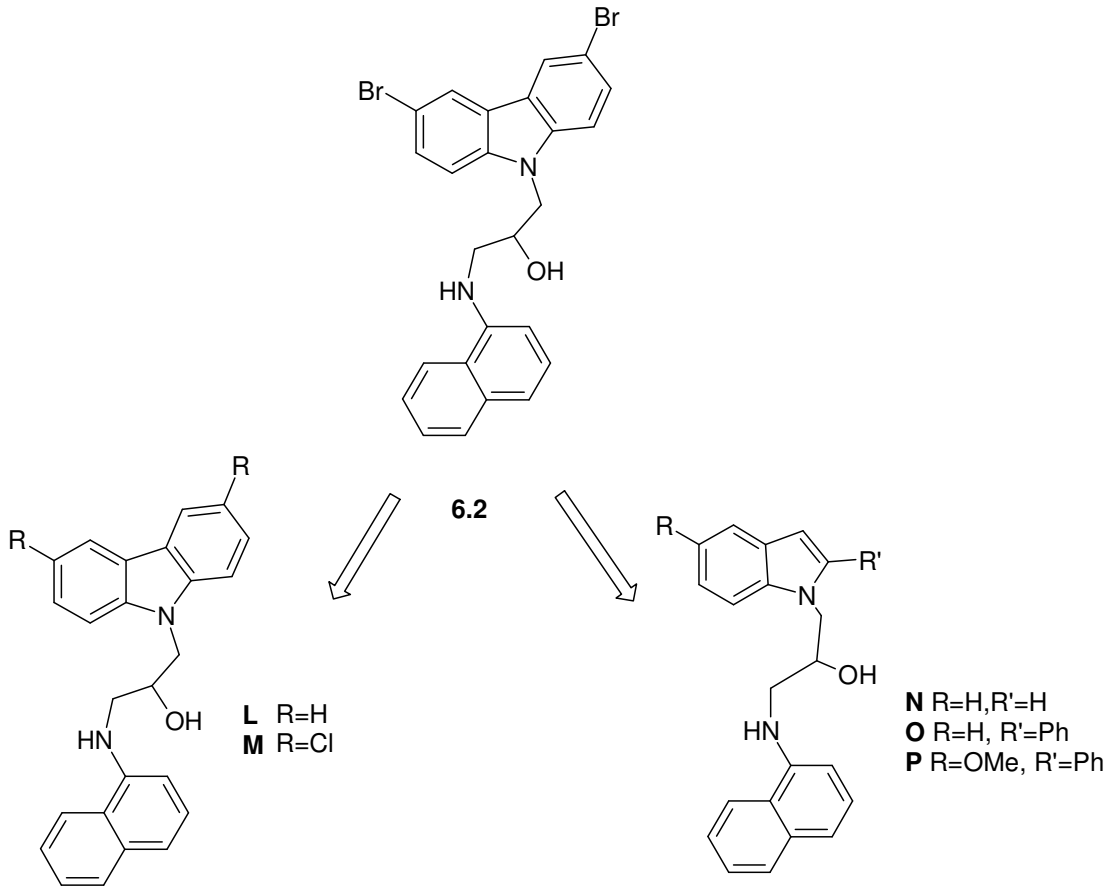


Binding mode **A**

Binding mode **B**

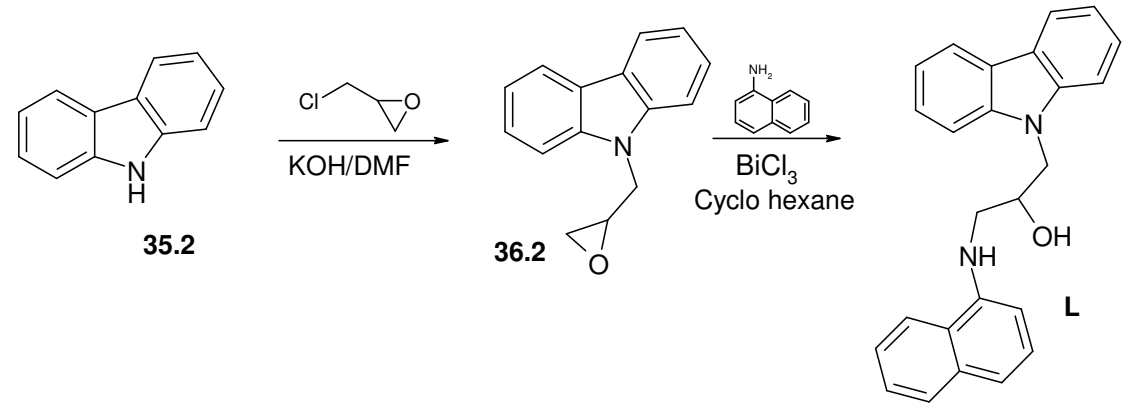
Taking compound **6.2** (Figure 24.2) as the reference and with the aim of initially exploring the role of the two halogen atoms in the carbazole scaffold of this compound, the unsubstituted carbazole derivative **L** and the di-chloro substituted **M** were synthesized. Furthermore other compounds, **N-P** (Figure 24.2,) were synthesized in which the carbazole moiety was replaced with indole (compound **N**), 2-phenylindole (compound **O**) and 5-methoxy, 2-phenyl indole (compound **P**) moieties.

Figure 24.2: ***New carbazole derivatives***



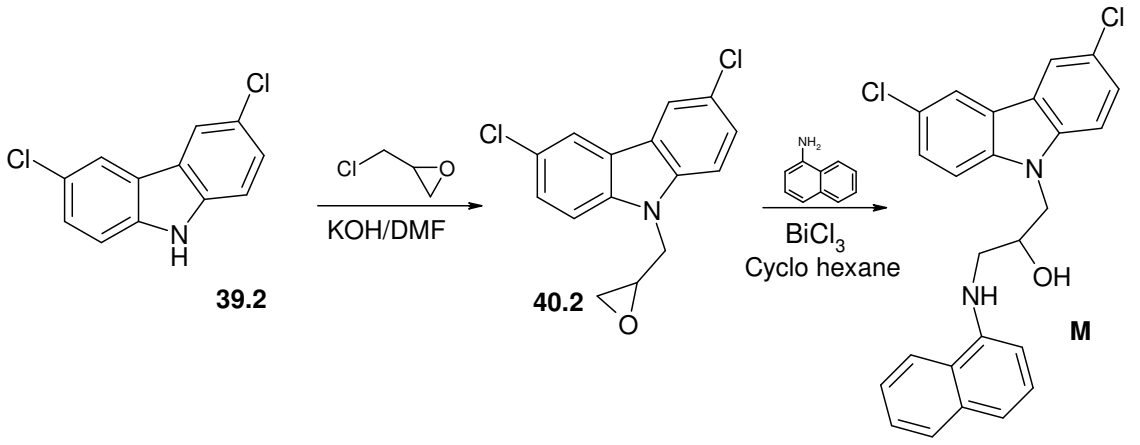
The syntheses of **L** and **M** were done on the basis of the synthetic procedures previously described for other carbazole derivatives and are shown in Schemes 20.2 and 21.2.

Scheme 20.2: ***Synthesis of compound*** **L**



Epoxide **36.2**, synthesized as reported in Scheme 15.2, was submitted to a ring-opening reaction with 1-naphtylamine in cyclohexane and in the presence of $BiCl_3$ as the catalyst and compound **L** was easily obtained in excellent yields by crystallisation from Hexane/Et_2O (Scheme 20.2).

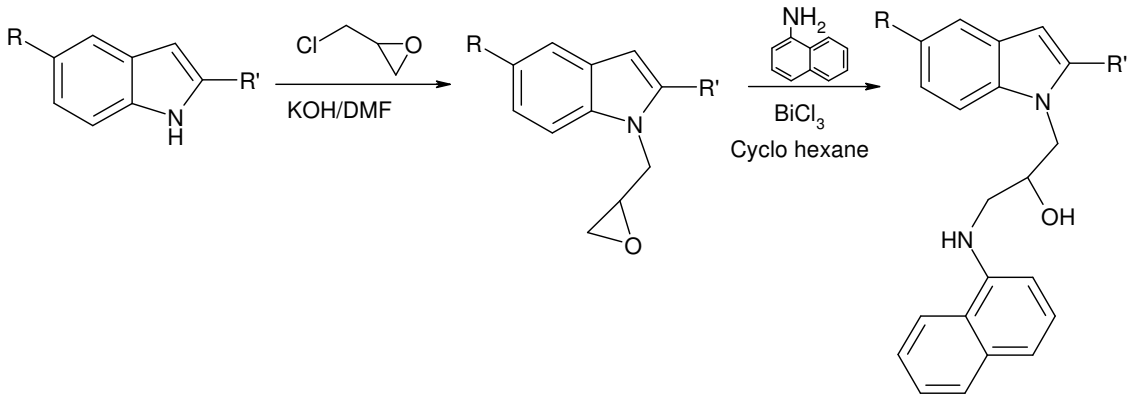
Scheme 21.2:***Synthesis of compound*** **M**



Synthesis of **M** started from commercially available carbazole **39.2** which was alkylated with epichloridrine in the presence of KOH to give the desired epoxide **40.2** in good yields. As seen before, epoxide **40.2** was then opened by reaction with 1-naphtylamine in cyclohexane and in the presence of $BiCl_3$ as catalyst and compound **M** was obtained in excellent yields by crystallisation from Hexane/Et_2O (Scheme 21.2).

Compounds **N-P** in Figure 24.2 were synthesized following the generic procedure showed in Scheme 22.2 using parallel methodologies as seen above for other derivatives previously described.

Scheme 22.2:*Generic synthetic procedure for compounds* N,O,P.



44 R=H,R'=H
45 R=H, R'=Ph
46 R=OMe, R'=Ph

48 R=H,R'=H
49 R=H,R'=Ph
50 R=OMe, R'=Ph

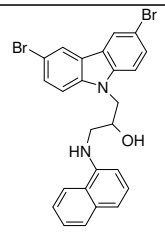
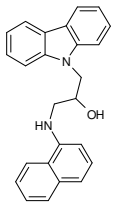
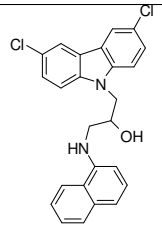
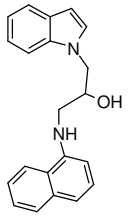
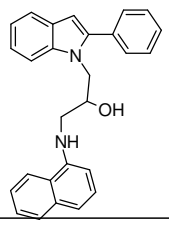
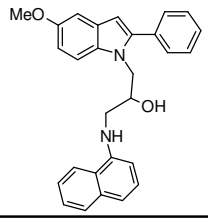
N R=H,R'=H
O R=H, R'=Ph
P R=OMe, R'=Ph

Commercially available indoles **44-46** were alkylated with ephychloridrine in the presence of KOH to give the desired epoxides **48-50** in good yields. Epoxides **48-50** were then opened by reaction with 1-naphtylamine in cyclohexane and in the presence of $BiCl_3$ as the catalyst. Subsequent crystallisation from Hexane/Et_2O afforded compounds **N-P** in excellent yields.

***BACE inhibition assay of derivatives* L-P**

All synthesized compounds **L-P** were submitted to biological tests with the TR-FRET technology at Siena Biotech laboratories. The IC_{50} values calculated for synthesized compounds are showed in Table 11.2.

Table 11.2

Compound	(IC_{50}μM)
6.2 	1.1
L 	3.0
M 	0.5
N 	>10
O 	1.6
P 	>10

Available data resulting from biological screening of synthesized compounds **L-P** showed inhibitory activities for these derivatives comparable to the reference compound **6.2**. Considering in details derivatives **L** and **M** in which the carbazole moiety was maintained, it should be noticed that the removal of Br atoms in the heterocyclic scaffold of the molecule caused a slight decrease of activity on BACE: the unsubstituted carbazole derivative **L** (Table 11.2) in fact showed an IC_{50} value of 3.0μM. On the contrary, the replacement of the original Br atoms with Cl substituents in the carbazole moiety afforded a noticeable improvement of activity: in fact the lowest IC_{50} value of the whole series of synthesized compounds was found for the di-chlorocarbazole derivative **M** whose value was as low as 0.5 μM.

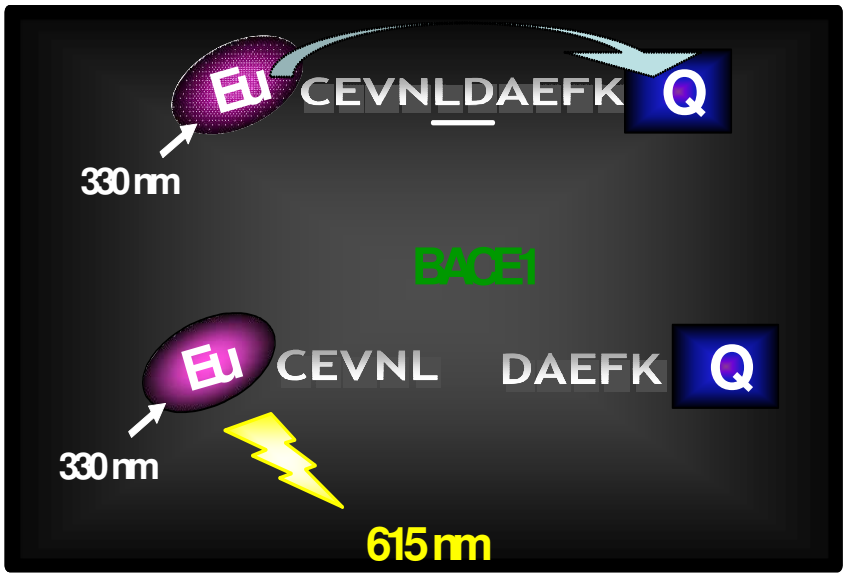
Among indoles derivatives **N-P**, only 2-phenyl indole derivative **O** (Table 11.2) showed an appreciable value of inhibitory activity on BACE exhibiting an IC_{50} value of 1.6 μM. On the contrary, the unsubstituted indole derivative **N** and the 5-methoxy, 2-phenyl indole derivative **P** did not show a significant inhibitory activity on the enzyme.

Chapter 3

Biological assay

All compounds synthesized during this PhD have been tested with a time resolved fluorescence quenching assay at Siena Biotech laboratories. The principle of the assay used for tests is shown in Figure 1.3.

Figure 1.3: ***Principle of TR-FRET assay.***
(Time Resolved-Fluorescence Resonance Energy Transfer)



An Eu^{+} chelate is coupled to the N terminus and a quenching organic fluorophore (Q) to the C-terminus of the peptide containing the Amyloid Precursor Protein Swedish mutation (underlined in white). The proteolitic cleavage of the substrate interrupts the energy transfer between the donor-acceptor pair and, upon excitation at 330 nm, produces a linear increase of emitted fluorescence at 615 nm. The fluorescence emitted is directly related to the enzymatic activity and can be followed by continuous TRF measurements.

Chapter 4

Experimental section

General details

Proton NMR spectra were recorded on a Bruker AC 200 (200 MHz) spectrometer as dilute solutions in deuterochloroform. The chemical shifts are recorded relative to tetramethylsilane as internal standard and the multiplicity of the signals is designated by one of the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; br, broad; m, multiplet; with all various possible combinations (e.g. dd is double doublet). All coupling constants *J*, are reported in Hertz.

Flash chromatography was performed on Merck silica gel 60 or on Isolute pre-packed silica columns and Isolute ionic exchanged pre-packed silica column as the stationary phase and the solvents employed were either of analytical grade or were distilled before the use.

All reactions were monitored by TLC using Merck silica gel 60 F_{24} pre-coated aluminium backed plates which were visualised with ultraviolet light and/or basic potassium permanganate solution and phosphomolybdic acid.

Dry organic solvents were stored under nitrogen and/or over sodium wire. The organic solvents were dried by distillation from the following: dichloromethane, pyridine, triethylamine, acetonitrile from calcium hydride; methanol from magnesium methoxide; benzene, toluene, THF and diethyl ether from sodium metal. *N,N*-dimethylformamide (DMF) Dimethylsulfoxide (DMSO) were purchased from Aldrich in SureSeal containers.

All other commercially available reagents were used as received from Fluka, Sigma-Aldrich, Merck, Lancaster.

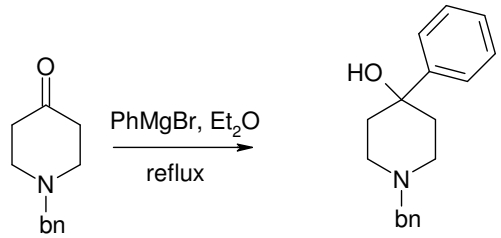
Solvents were removed by rotary evaporation *in vacuo*.

Where necessary, reaction requiring anhydrous conditions were performed in a flame or oven-dried apparatus under a nitrogen atmosphere.

All LCMS analyses were carried out using a Waters Alliance HT 2795 instrument with a Phenomenex C18 column and either a 5 or 10 minute gradient of acetonitrile/water containing 0.1% formic acid.

Actelion compound

Synthesis of compound 8.2

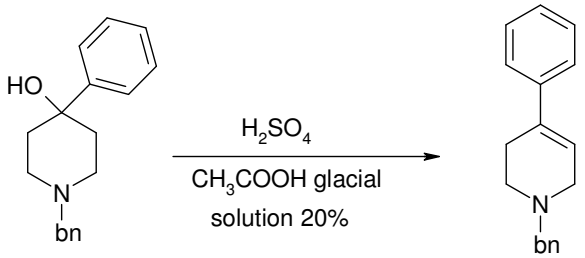


7.2 8.2

To a three neck flask under nitrogen with Mg (1.08g, 44.4 mmol) and I_2 (cat) and Et_2O (5 ml) a solution of Phenyl-bromide (5.80g, 36.9 mmol) in Et_2O (15 ml) was added dropwise. The mixture was refluxed for 0.5h. Then a solution of N-benzyl-piperidone **7.2** (2.00 g, 10.5 mmol) in Et_2O (30 ml) was added dropwise. The mixture was refluxed for 1h and then left overnight at room temperature and then NH_4Cl was added dropwise to neutralise the unreacted Mg. The product was extracted with $CHCl_3$ and the organic phase was evaporated under vacuum to give the desidered product (3.38g) as a yellow oil (99% yield).

^{1}H NMR (200 MHz) $CDCl_3$: δ, 1.74 (dd, 2H, *J*= 14.1 and 2.5 Hz), 2.50 (td, 2H, *J*=12.0 and 2.2Hz), 2.81 (td, 2H, *J*= 13.0 and 4.3 Hz), 2.81 (dd, 2H, *J*= 8.8 and 2.2 Hz,), 3.60 (s, 2H), 7.65-7.16 (m, 10H);

Synthesis of compound 9.2

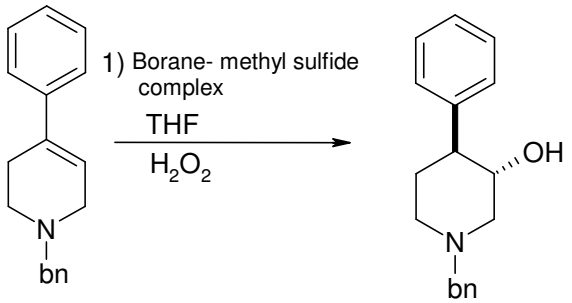


8.2 9.2

N-benzyl-4-phenyl-4-hydroxy-piperidine **8.2** (3.38g, 12.65 mmol) was treated with H_2SO_4 in CH_3COOH sol 20% (50 ml). The reaction mixture was stirred for 1h at room temperature and then NaOH 1N was added until the pH was adjust to basicity. The desired product was extracted with Et_2O and evaporated under vacuum. The crude was purified with flash chromatography (8:2 Hexane/AcOEt) to give a yellow oil (1.64g, 51.7% yield).

^{1}H NMR (200 MHz) CDCl3: δ 2. 61-2.47 (m, 2H), 2.72 (t, 2H, *J*= 5.20 Hz), 3.18 (dd, 2H *J*= 6.0 and 2.70 Hz), 3.65 (s, 2H) , 6.14 -6.00 (m, 1H), 7.58-7.18 (m, 10H);

Synthesis of compound 10.2

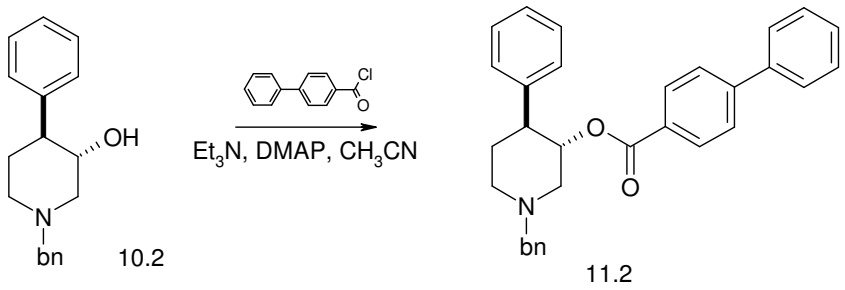


9.2 10.2

To a solution of olefine **9.2** (1.1g, 4.41 mmol) in THF (14 ml) BH_3-sulfide-complex (4.4ml, 8.8 mmol) was added dropwise and the reaction mixture was allowed to warm to reflux for 2h. After cooling to 0°C NaOH 1N (11 ml) was added dropwise and then H_2O_2 dropwise (16.5 ml). The reaction was allowed to warm to 100°C and stirred for 2h. After cooling to room temperature Et_2O was added and the reaction mixture was washed with NaOH 1N, Na-tiosulfate sol sat. and NH_4Cl sol sat. The organic phase was collected and evaporated in vacuo and a yellow solid was obtained. Purification by flash chromatography (1:1 Hexane/AcOEt) afforded a white solid (800 mg, 68% yield).

^{1}H NMR (200 MHz) CDCl3: δ 1.48-1.63 (m, 2H), 1.75-2.17 (m, 4H), 2.31-2.50 (m, 1H), 2.88-3.20 (m, 1H), 3.15-3.27(m, 1H), 3.60 (AB quartet, 2H, *J*= 15.90 and 13.10 Hz), 3.78-3.96 (m, 1H), 7.20-7.40 (m, 10H);

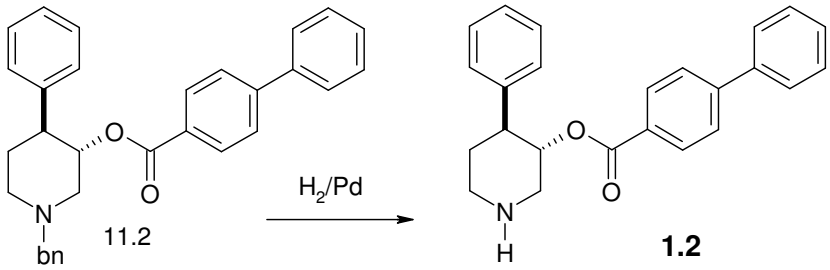
Synthesis of compound 11.2



N-benzyl-4-phenyl-3-hydroxy-piperidine **10.2** (520 mg, 1.94 mmol) was dissolved in CH_3CN and biphenyl carbonil chloride (2.10g, 9.70 mmol), Et_3N (2.11 ml, 15.52 mmol), DMAP (cat) were added. The mixture was stirred overnight at room temperature, then it was quenced with H_2O and NH_4OH sol 30% and extracted with AcOEt. The organic phase was washed with $NaHCO_3$ sol sat and evaporated under vacuum. Purification by flash chromatography (7:3 Hexane/AcOEt) afforded the 798 mg (92% yield) of desired product as a yellow solid.

^{1}H NMR (200 MHz) CDCl3: δ 2.26-1.84 (m, 4H) 2.93-2.75 (m, 1H), 3.04-2.93 (m, 1H), 3.55-3.48 (m, 1H), 3.64 (dd, *J*= 21.40 and 13.10 Hz, 2H), 5.39 (td, *J*= 10.30 and 4.60 Hz, 1H), 7.64-7.51 (m, 4H), 7.51-7.09 (m, 12H), 7.88 (AA'XX', 2H, *J*= 8.40 and 4.0 Hz);

Synthesis of compound 1.2

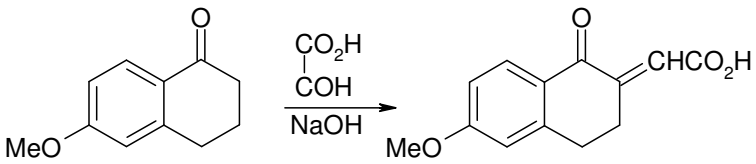


Compound **11.2** (200mg, 0.45 mmol) was dissolved in AcOEt (23 ml) and Pd /C 10% (281.1 mg, 0.60 mmol) was added. The reaction mixture was stirred under H_2 for 15 days and then filtered on Celite and washed with AcOEt. The organic phase was evaporated and purification by flash chromatography (9:1 Hexane/AcOEt) afforded the desired product (149 mg) as a white solid (80% yield).

^{1}H NMR (200 MHz) CDCl3: δ 2.10-1.70 (m, 2H) 2.78-2.60 (m, 2H), 2.97 (td, 1H, *J*= 11.30 and 3.80 Hz), 3.17 (d, 1H, *J*= 12.4 Hz), 3.50 (dd, 1H *J*= 11.70 and 4.50 Hz), 5.18 (td, 1H, *J*= 10.31 and 4.5 Hz), 7.50-7.12 (m, 8H), 7.64-7.50 (m, 4H), 7.8 (AA'XX', 2H, *J*= 8.42 and 4.01 Hz);

Takeda derivative

Synthesis of compound 13.2

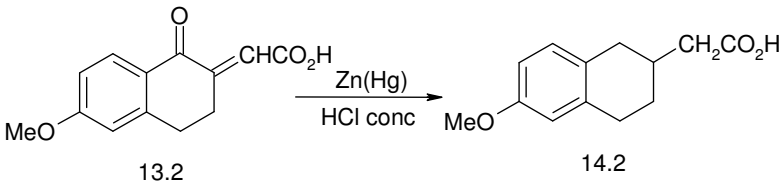


12.2 13.2

The commercial available 5- methoxy 1-tetralone **12.2** (5g, 28.37 mmol) was diluted in a solution of NaOH in water and dry EtOH and the glicossilic acid (5.22 g, 56.74 mmol) was added. The reaction was left under stirring under reflux for 18 h at 60 °C and than stopped after TLC analysis. The mixture was cooled and diluted with water Et_2O and after treated with a HCl 2N solution. The resulting product, a yellow precipitate (5.1 g), was filtered and collected (78 %yield).

^{1}H NMR (200 MHz) CDCl3: δ 1.99-1.37 (m, 1H), 3.00 (t, 2H, *J*= 6.50), 3.43 (td, 2H, *J*= 6.52 and 1.52 Hz), 3.88 (s, 3H), 6.72-6.75 (m, 1H), 6.91-6.85 (m,2H), 8.08 (d, 1H, *J*= 8.67);

Synthesis of compound 14.2

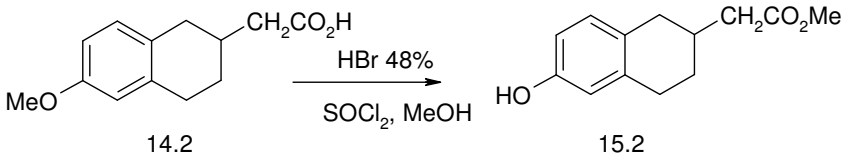


13.2 14.2

The Clemmensen reactive was prepared with Zinc dust (20g, 300mmol) and $HgCl_2$ (2.10g, 7.73mmol) in the presence of HCl 37% (26 ml) and water (30 ml). The mixture was left under stirring for 30 minutes and the resulting Clemmnsen reactive was cooled at 0 °C and then water (6 ml), HCl (2ml), toluene (30 ml) and glacial acetic acid (2ml) were added. The acid **13.2** resulting from the first step (2.5 g,10.77 mmol) was then added to the previous solution and the mixture was stirred under reflux at 100°C for 4h. The mixture was cooled and diluted with water, wash with brine and finally extracted with Et_2O. The resulting crude product **14.2**, a pink solid (1.45g, 62% yield), was then used for the next reaction.

^{1}H NMR (200 MHz) CDCl3: δ 1.60-1.38 (m,1H), 1.90-1.60 (m,1H), 2.36-2.18 (m, 1H), 2.51-2.38 (m, 2H), 2.85-2.79 (m, 3H), 2.93 (d, 2H, *J*= 14.62 Hz), 3.77 (s, 3H), 6.71-6.61 (m, 2H), 6.97 (d, 1H, *J*= 8.24);

Synthesis of compound 15.2

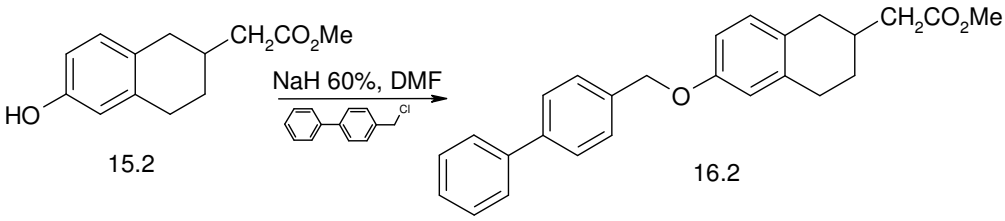


14.2 15.2

The acid **14.2** (1.25 g, 5.68 mmol) was added to a HBr 48% (8.19 ml) solution and the mixture was heated to reflux at 160 °C and stirred for 4h. After TLC analysis, the mixture was cooled and diluted with water and extracted with AcOEt. The organic phase was washed with water and brine and than dried over Na_2SO_4 and concentrated. The resulting brown oil was diluted in MeOH (17 ml) and the reaction was left under stirring at 0 °C and than $SOCl_2$ (856 mg, 7,20 mmol) was added dropwise to the mixture. The reaction was stirred at room temperature for 2h and stopped after TLC analysis: water and brine were added and the organic layer extracted, dried over Na_2SO_4 and concentrated. The resulting product **15.2**, a brown oil (1.10g, 88 % yield), was then used as a crude for the next reaction.

^{1}H NMR (200 MHz) CDCl3: δ 1.53-1.22 (m,2H), 2.02-1.85 (m, 1H), 2.48-2.33 (m, 3H), 2.88-2.73 (m, 3H), 3.70 (s, 3H), 4.80 (s, 1H), 6.62-6.56(m, 2H), 6.90 (d, 1H, *J*= 8.06).

Synthesis of compound 16.2

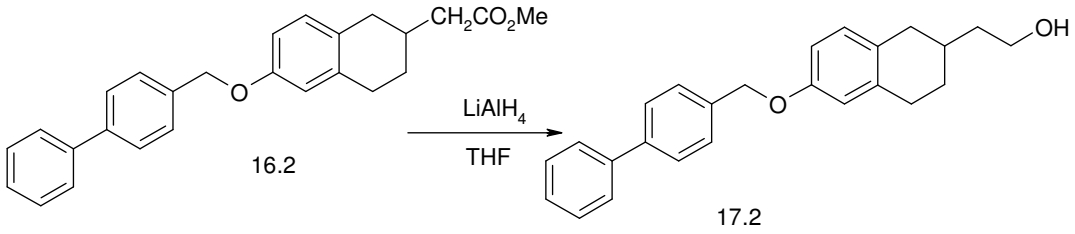


15.2 16.2

The alcohol **15.2** obtained from the previous reaction (1.10 g, 5.0 mmol) was dissolved in DMF(25 ml) and cooled at 0 °C. NaH 60% (255 mg, 10.26 mmol) was added and the mixture was left under stirring at 40 °C for 1h and than cooled at 0 °C. Then 4-phenylbenzyl chloride (1.11g, 5.47 mmol) was added and the reaction was stirred at room temperature for 15h and stopped after TLC analysis: water was added and the mixture extracted with AcOEt and than dried over Na_2SO_4 and concentrated. The resulting brown oil was purified by flash chromatography (1:1 Hexane/AcOEt). The purification afforded the desired product **16.2** (1.26g) as a yellow solid (65% yield).

^{1}H NMR (200 MHz) CDCl3: δ 1.58-1.22 (m,2H), 2.10-1.88 (m, 1H), 2.51-2.23 (m, 3H), 2.92-2.79 (m, 3H), 3.70 (s, 3H), 5.07 (s, 2H), 6.82-6.70 (m, 2H), 6.98 (d, 1H, *J*= 8.05), 7.63-7.35 (m, 9H).

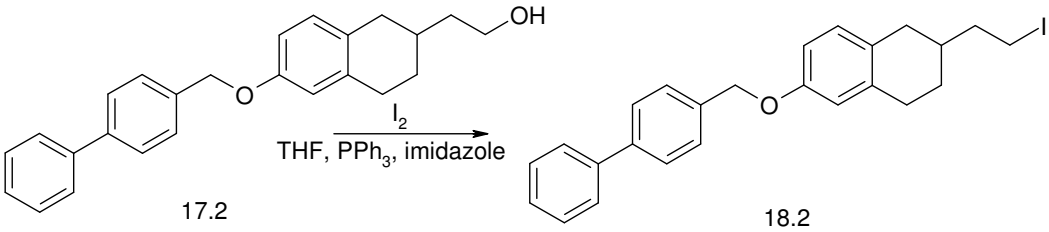
Synthesis of compound 17.2



Compound **16.2** (1.0g, 2.6 mmol) was added to a suspension of $LiAlH_4$ (196 mg, 5.21 mmol) and dry THF(15 ml) at 0 °C and the mixture was left under stirring at room temperature for 2h. After TLC analysis the mixture was diluted with water and treated with Sodium and Potassium tartrate and filtered on Celite and washed with AcOEt. The resulting product **17.2** , a white precipitate (940 mg), was collected (> 98%yield).

^{1}H NMR (200 MHz) CDCl3: δ 1.71-1.25 (m, 5H), 2.0-1.78 (m, 1H), 2.39 (t, 1H, *J*=13.12 Hz), 2.90-2.77 (m, 3H), 3.86-3.74 (m, 2H), 5.06 (s, 2H), 6.80-6.70 (m, 2H), 6.98 (d, 1H, *J*= 8.05), 7.62-7.34 (m, 9H).

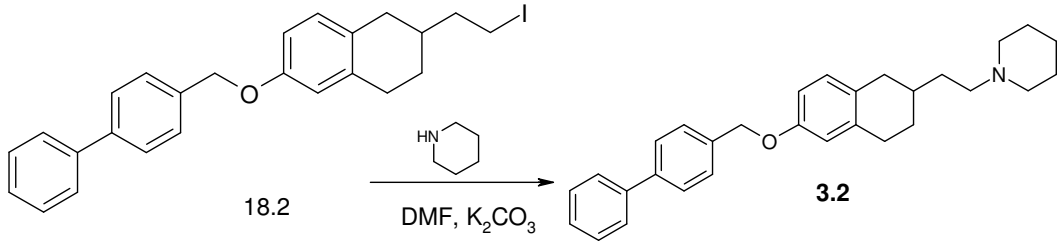
Synthesis of compound 18.2



A THF solution of compound **17.2** (940 mg, 2.62 mmol) was added to a solution of PPh_3, (860mg, 3.29 mmol) dry THF(30 ml), imidazole (210 mg, 3.0 mmol) and I_2 (830 mg, 3.26 mmol). After 1h the reaction was treated with water and extracted with AcOEt. The organic layers were washed with $Na_2S_2O_3$ and brine and than dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography (Hexane). The purification afforded the desired product **18.2** (570 mg) as a white solid (47% yield).

^{1}H NMR (200 MHz) CDCl3: δ 1.50-1.25 (m, 1H), 2.02-1.84 (m, 4H), 2.43-2.29 (m, 1H), 2.90-2.77 (m, 3H), 3.30 (t, 2H, *J*= 6.81 Hz), 5.06 (s, 2H), 6.80-6.70 (m, 2H), 6.98 (d, 1H, *J*= 8.05),7.64-7.35 (m, 9H).

Synthesis of compound 3.2

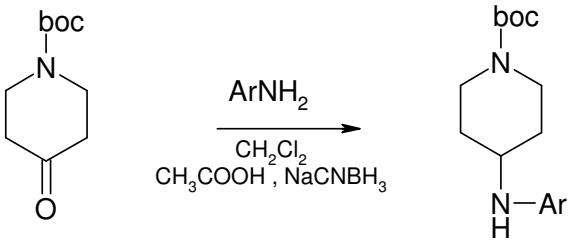


A DMF solution of piperidine (213 mg, 2.5 mmol) and K_2CO_3 (430 mg) were added to product **18.2** (570 mg, 1.25 mmol) and the mixture was left under stirring for 40h at room temperature. After TLC analysis, the mixture was stopped: water was added and the resulting precipitate collected and extracted with AcOEt, dried over Na_2SO_4 and concentrated. The resulting crude was purified with by chromatography (95:15 $CHCl_3$/MeOHt). The purification afforded the desired product **3.2** (370 mg) as a white solid (72% yield) that was finally treated with HCl and Et2O to obtain the hydrochloric derivative.

^{1}H NMR (200 MHz) CDCl3: δ 3.12-1.70 (m, 19H), 3.62-3.44 (m, 2H), 5.06 (s, 2H), 6.80-6.68 (m, 2H), 6.95 (d, 1H, *J*= 8.05), 7.62-7.34 (m, 9H).

4- aminopiperidine derivatives

General procedure for reductive amination



19.2

To a solution of commercial N-Boc-4-amino-piperidine **19.2** (1 eq) in DCM (5 ml), the various aromatic amines (1.2 eq), sodium cyanoborohydride (1.2 eq) and acetic acid (2.50 eq) were added. The reaction mixtures were stirred at room temperature for 3 hours. The desired secondary amines **21.2** and **21.2a-f** were recovered from extractive work up and purification by SCX column.

21.2 Yield 67 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.95-1.76 (m, 4H); 2.80-2.59 (m, 4H), 3.12-2.93 (m, 1H), 4.10-3.94 (m, 1H); 7.35-7.23 (m, 5H);

21.2a Yield 67 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.95 -1.76 (m, 4H); 2.84-2.60 (m, 4H), 3.08-2.93 (m, 1H); 3.82 (s , 2H), 4.10-3.94 (m, 1H); 7.38-7.22 (m, 5H);

21.2b Yield 50 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.56 -1.25 (m, 4H); 1.96-1.81 (m, 2H), 3.86 (s , 2H), 4.10-3.96 (m, 1H); 7.61-7.36 (m, 9H);

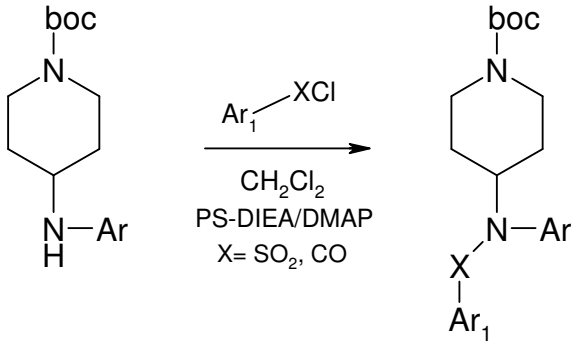
21.2c Yield 27 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.37-1.25 (m, 2H) ,1.44 (s, 9H), 1.92 - 1.78 (m, 2H), 2.88-2.57 (m, 3H), 3.79 (s, 2H), 4.08-3.91 (m, 2H) 7.29-7.14 (m, 2H), 7.40-7.35(m, 1H), 7.48 (s,1H);

21.2d Yield 55 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.51-1.25(m, 2H) ,1.44 (s, 9H), 1.95 - 1.80 (m, 2H), 2.86-2.57 (m, 3H), 3.88 (s, 2H),4.20-3.92 (m, 2H) 7.16-7.07 (dt, 1H, *J*= 7.69 and 1.83 Hz), 7.41-7.23 (m, 2H), 7.53 (d,1H, *J*= 7.87 Hz);

21.2e Yield 70 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38-1.19 (m, 2H) ,1.46 (s, 9H), 1.94 - 1.78 (m, 2H), 2.35 (s, 1H) 2.85-2.60 (m, 3H), 3.79 (s, 2H),4.10-3.94 (m, 2H), 7.56-7.15 (m, 8H), 8.18 (d,1H, *J*= 6.41 Hz);

21.2f Yield 70 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28-1.10 (m, 2H) ,1.43 (s, 9H), 1.68 - 1.57 (m, 1H), 2.50-2.36 (m, 1H) 2.80-2.62 (t, 2H, *J*= 11.31 Hz), 3.76 (s, 2H), 3.96-3.82 (m, 2H), 7.47-7.22 (m, 8H);

General procedure for cupping reactions



The desired secondary amine **21.2** and **21.2a-f** (1eq) were dissolved in CH_2Cl_2 and the various acyl/sulfonyl-chlorides (1.10 eq), PS-DIEA (1.50 eq) and DMAP cat. were added. After one night at room temperature the amidic products **A**, **B**, **C** and **D** were obtained from extractive work up and were purified by flash chromatography.

A1 Yield 67 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.37 (s, 9H), 1.95-1.76 (m, 1H), 2.75-2.67 (m, 1H), 4.20-3.89 (m, 2H), 4.45-4.26(m, 1H); 7.35-7.23 (m, 5H); 7.73-7.42 (m, 5H);

A2 Yield 58 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36 (s, 9H), 1.77 (d, 2H, *J*= 11.72 Hz), 2.43 (s, 3H), 2.73-2.60 (m, 1H), 4.24-4.00 (m, 2H), 4.40-4.25 (m, 1H), 7.00-6.98 (m, 2H), 7.29-7.24 (m, 2H), 7.38-7.30 (m, 3H), 7.60 (d, 2H, *J*= 8.42 Hz);

A3 Yield 64 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (s, 9H), 1.77 (m, 2H), 2.69-2.57(m, 1H), 4.22-3.98 (m, 2H), 4.41-4.23 (m, 1H), 7.23-7.00 (m, 2H), 7.76-7.30 (m, 6H);

B1 Yield 67 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.91 (d, 2H, *J*= 13.62 Hz), 2.85 (t, 2H, *J*=11.42 Hz), 4.25-4.10 (m, 2H), 5.30-4.89 (m, 1H), 6.98-6.96 (m, 2H), 7.24-7.10 (m, 8H);

B2 Yield 53 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 9H),1.46-1.52 (m, 2H), 1.89 (d, 2H, *J*= 13.60 Hz), 2.21 (s, 3H), 2.84 (t, 2H, *J*= 11.36 Hz), 4.17-4.15 (m, 2H), 5.30-4.85 (m, 1H), 7.02-6.88 (m, 4H), 7.17-7.11 (d, 2H, *J*= 7.69 Hz), 7.25-7.16 (m, 3H);

B3 Yield 68 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 9H),1.44-1.55 (m, 2H), 1.89 (d, 2H, *J*= 13.70 Hz), 2.85 (t, 2H, *J*= 11.42 Hz), 4.17-4.15 (m, 2H), 5.30-4.86 (m, 1H), 7.08-6.96 (m, 2H), 7.17-7.08 (m, 4H), 7.26-7.18 (m, 3H);

B4 Yield 45 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 9H),1.44-1.55 (m, 2H), 1.88 (d, 2H, *J*= 13.56 Hz), 2.89-2.87 (m, 2H), 4.19-4.16 (m, 2H), 4.92-4.82 (m, 1H), 7.06-6.96 (m, 2H), 7.17 (d, 1H, *J*= 8.43 Hz), 7.28-7.24 (m, 4 H), 7.36 (d, 1H, *J*= 1.83 Hz);

B5 Yield 58 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.87 (d, 2H, *J*= 13.60 Hz), 2.80-2.76 (m, 2H), 3.70 (s, 3H), 4.21-4.17 (m, 2H), 4.94-4.85 (m, 1H), 6.61 (d, 2H, *J*= 1.90 Hz), 6.98 (d, 2H, *J*= 1.83 Hz), 7.23-7.19(m, 5H);

C1 Yield 66 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.46-1.43 (m, 4H), 1.65 (s, 9H), 2.56-2.51 (m, 2H), 2.97 (d, 2H, *J*= 12.46 Hz), 3.90-3.76 (m, 1H), 4.45 (s, 2H) , 7.32-7.24 (m, 3H), 7.37 (d, 2H, *J*= 7.33 Hz), 7.56-7.47 (m, 3H), 7.82 (d, 2H, *J*= 6.96 Hz);

C2 Yield 73 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.48-1.41 (m, 4H), 1.39 (s, 9H), 2.56-2.51 (m, 2H), 3.90-3.76 (m, 1H), 4.10-3.88 (m, 2H), 4.40 (s, 2H), 7.44-7.26 (m, 5H), 7.46 (AA'/XX', 2H, J_{AX}=8.45 and $J_{AA'/XX'}$=2.2 Hz), 7.71 (AA'/XX', 2H, J_{AX}=8.45 and $J_{AA'/XX'}$=2.2 Hz);

C3 Yield 66 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35-1.25 (m, 4H), 1.38 (s, 9H), 2.43 (s, 3H), 2.59-2.56 (m, 2H), 3.89-3.99 (m, 1H), 4.10-3.97 (m, 2H), 4.40 (s, 2H) , 7.32-7.24 (m, 3H), 7.37 (d, 2H, *J*= 7.33 Hz), 7.37-7.23 (m, 7H), 7.70 (d, 2H, *J*= 8.42 Hz);

C4 Yield 73 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.48-1.41 (m, 3H), 1.41 (s, 9H), 2.62-2.57 (m, 2H), 3.82 (s, 3H), 4.10-3.90 (m, 2H), 4.37 (s, 2H), 7.38-7.26 (m, 5H), 6.96 (AA'/XX', 2H, J_{AX}=7.98 and $J_{AA'/XX'}$=1.91 Hz), 7.75 (AA'/XX', 2H, J_{AX}=7.98 and $J_{AA'/XX'}$=1.9 1Hz);

C5 Yield 45 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.46-1.43 (m, 2H), 1.65 (s, 9H), 2.58-2.55 (m, 3H), 3.01-2.98 (m, 3H), 4.03-3.79 (m, 1H), 4.43 (s, 2H) , 7.31-7.26 (m, 5H), 7.57-7.51 (m, 2H), 7.79 (s, 1H);

C6 Yield 42 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (s, 9H), 1.55-1.44 (m, 4H); 2.70-2.50 (m, 2H); 4.18-3.84 (m, 3H); 4.44 (s, 2H); 7.72-7.27 (m, 12H), 7.86 (d, 2H, *J*=8.4 Hz);

C7 Yield 35 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (s, 9H), 1.52-1.40 (m, 4H), 2.70-2.51 (m, 2H), 4.10- 3.84 (m, 3H), 4.46 (s, 2H), 7.60-7.34 (m, 12H), 7.82 (d, 2H, *J*=6.59 Hz) ;

C8 Yield 40 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.76-1.50 (m, 4H), 4.25-4.03 (m, 2H), 2.72-2.60 (m, 2H), 4.19-3.80 (m, 3H), 4.53 (s, 2H), 7.14 (dd, 2H, *J*=8.6 and 2 Hz), 7.56-7.23 (m, 10H) ;

C9 Yield 55 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.52-1.36 (m, 4H), 1.39 (s, 9H), 2.72-2.52 (m, 2H), 4.09-3.81 (m, 3H), 4.44 (s, 2H), 7.26-7.12 (m, 2H), 7.61-7.35 (m, 9H) 7.85-7.78 (m, 2H) ;

C10 Yield 80 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (s, 9H), 1.60-1.54 (m, 4H), 2.74-2.55 (m, 2H), 4.90-4.14 (m, 3H), 4.48 (sb, 2H), 7.62-7.34 (m, 14H), 7.69 (AA'/XX', 2H, J_{AX}=8.79 and $J_{AA'/XX'}$=2.3 Hz), 7.87 (AA'/XX', 2H, J_{AX}=8.79 and $J_{AA'/XX'}$=2.31 Hz);

C11 Yield 55 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.61-1.21 (m, 4H), 1.37 (s, 9H), 2.67-2.51 (m, 2H), 4.16-3.84 (m, 3H), 4.48 (s, 2H), 7.57-7.34 (m, 9H) 7.85-7.81 (m, 2H) ;

C12 Yield 80 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.48-1.14 (m, 4H), 2.70-2.57 (m, 2H), 4.09-3.97 (m, 3H), 4.40 (s, 2H), 7.64-7.14 (m, 9H), 7.70 (AA'/XX', 2H, J_{AX}=8.40 and $J_{AA'/XX'}$=2.51 Hz), 7.84 (AA'/XX', 2H, J_{AX}=8.40 and $J_{AA'/XX'}$=2.51 Hz);

C13 Yield 58 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29-0.98 (m, 4H), 1.40 (s, 9H), 2.58-2.36 (m, 2H), 3.98-3.67 (m, 3H), 4.35 (s, 2H), 7.55-7.15 (m, 12H), 7.78-7.70 (m, 2H),

C14 Yield 54 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.50-1.24 (m, 4H), 2.68-2.56 (m, 2H), 4.10-3.96 (m, 3H), 4.51 (s, 2H), 7.67-7.10 (m, 9H), 7.74 (AA'/XX', 2H, J_{AX}=8.60 and $J_{AA'/XX'}$=2.31 Hz), 7.93 (AA'/XX', 2H, J_{AX}=8.60 and $J_{AA'/XX'}$=2.31 Hz);

D1 Yield 45 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36 (s, 9H), 1.70-1.46 (m, 4H), 3.31 (m, 2H), 4.00-3.80 (m, 2H), 4.40-4.70 (m, 2H), 7.42-7.20 (m, 10H);

D2 Yield 56 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36 (s, 9H), 1.75-1.56 (m, 4H), 2.80-2.40 (m, 2H), 4.20-4.00 (m, 2H), 5.10-4.80 (m, 2H), 7.33-7.17 (m, 9H);

D3 Yield 56 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.58-1.56 (m, 4H), 2.35 (s, 3H), 2.70-2.52 (m, 2H), 4.10-3.95 (m, 3H), 4.70-4.50 (m, 2H), 7.30-7.17 (m, 9H);

D4 Yield 61 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.65-1.62 (m, 3H), 3.80 (s, 3H), 4.20-3.95 (m, 3H), 4.63 (s, 2H), 6.90-6.70 (m, 2H), 7.39-7.26 (m, 7H);

D5 Yield 23 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.80-1.62 (m, 4H), 2.60-2.41 (m, 2H), 4.20-4.00 (m, 3H), 4.42-4.85 (m, 2H), 7.65-7.26 (m, 8H);

D6 Yield 50 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.85-1.52 (m, 4H), 2.90-2.32 (m, 2H), 4.25-4.00 (m, 2H), 4.86-4.50 (m, 2H), 7.46-7.26 (m, 14H);

D7 Yield 56 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.58-1.56 (m, 4H), 2.80-2.43 (m, 2H), 4.00-3.89 (m, 1H), 4.30-4.12 (m, 2H), 4.80-4.60 (m, 2H), 7.68-7.17 (m, 9H);

D8 Yield 47 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (s, 9H), 1.75-1.56 (m, 4H), 2.80-2.40 (m, 2H), 3.89-3.80 (m, 1H), 4.20-4.00 (m, 2H), 4.80-4.60 (m, 2H), 7.50-7.10 (m, 8H);

D9 Yield 76 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36 (s, 9H), 1.80-1.62 (m, 6H), 2.40 (s, 3H), 3.89-3.80 (m, 1H), 4.20-4.00 (m, 2H), 4.80-4.60 (m, 2H), 7.48-7.24 (m, 8H);

D10 Yield 44 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.58-1.56 (m, 4H), 2.80-2.40 (m, 2H), 3.82 (s, 3H), 4.40-4.12 (m, 2H), 4.60 (s, 2H), 7.26-7.20 (m, 2H), 7.37-7.26 (m, 6H);

D11 Yield 76 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36 (s, 9H), 1.80-1.50 (m, 6H), 2.87-2.43 (m, 2H), 3.80-3.65 (m, 1H), 4.34-4.06 (m, 2H), 4.70-4.40 (m, 2H), 7.68-7.10 (m, 7H);

D12 Yield 60 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.56-1.54 (m, 4H), 2.80-2.43 (m, 2H), 4.20-4.00 (m, 2H); 4.80-4.50 (m, 2H), 7.67-7.35(m, 14H);

D13 Yield 35 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.80-1.65 (m, 3H), 2.54-2.31 (m, 1H), 2.90-2.68 (m, 1H), 3.59-3.38 (m, 1H), 4.26-4.00 (m, 2H), 4.38 (s, 1H), 4.75-4.64 (m, 1H), 4.77 (AB quartet, 2H, *J*=15.61 Hz), 7.14 (d, 2H, *J*=7.51 Hz), 7.62-7.30 (m, 10H);

D14 Yield 35 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.80-1.50 (m, 5H), 2.80-2.40 (m, 1H), 4.21-4.01 (m, 2H), 4.62 (sb, 2H), 7.20-7.08 (m, 2H), 7.60-7.26 (m, 12H);

D15 Yield 68 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.80-160 (m, 5H), 2.80-2.43 (m, 2H),4.22-4.14 (m, 2H), 4.80-4.60 (m, 2H), 7.61-7.34 (m, 18H);

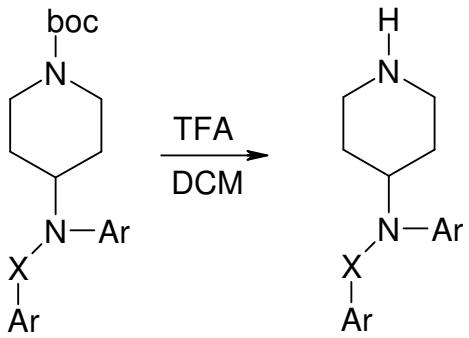
D16 Yield 77 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.80-160 (m, 5H), 2.80-2.43 (m, 2H),4.22-4.14 (m, 2H), 4.80-4.60 (m, 2H), 7.61-7.34 (m, 14H);

D17 Yield 56 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.80-1.46 (m, 4H), 2.80-2.42 (m, 2H), 4.20-4.09 (m, 3H), 4.80-4.45 (m, 2H), 7.67-7.10 (m, 13H);

D18 Yield 30 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.80-1.24 (m, 4H), 2.80-2.21 (m, 2H), 4.20-3.89 (m, 3H), 4.80-4.30 (m, 2H), 7.50-7.30 (m, 14H);

D19 Yield 39 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.80-1.46 (m, 4H), 2.80-2.42 (m, 2H), 4.20-4.09 (m, 3H), 4.80-4.45 (m, 2H), 7.67-7.10 (m, 13H);

General procedure for deprotection reaction



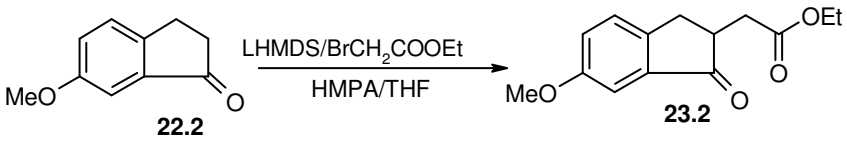
Boc-compounds **C1-C14** (1.0 eq) were dissolved in a solution of TFA/DCM (4 ml) and stirred for 2 hours at room temperature. The desired deprotected products **C15** and **C16** were collected after concentration under vacuum and purified with scx pre-packed cartridges.

C15 Yield 79 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29-0.98 (m, 1H), 1.70-1.44 (m, 3H), 2.65-2.53 (m, 2H), 3.07 (d, 2H, *J*= 12.45 Hz), 3.20-3.50 (m, 1H), 4.49 (s, 2H), 7.01-7.33 (m, 12H), 7.85-7.80 (m, 2H);

C16 Yield 43 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.92-0.76 (m, 2H), 1.68-1.17 (m, 2H), 2.68-2.55 (m, 2H), 3.21-3.07 (m, 2H), 4.10-3.96 (m, 1H), 4.48 (s, 2H), 7.88-7.15 (m, 14H);

Tetraline Derivative E

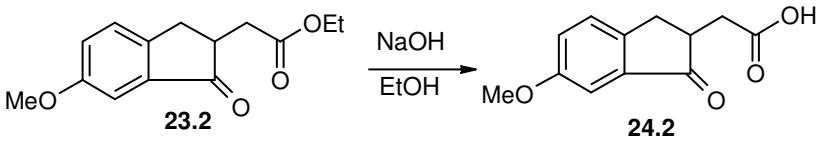
Synthesis of compound 23.2



A THF solution of the commercial available 6-methoxy indanone **22.2** (1.0 eq) was added dropwise to LHMDS (1.2 eq) at -60 °C and the mixture was stirred. After 30 minutes HMPA (1.2 eq) and $BrCH_2COOEt$ (2.0 eq) were added and the reaction was left under stirring for 1h at -60 °C. After TLC analysis, the mixture was stopped: NH_4Cl solution was added and the organic layer extracted with AcOEt, dried over Na_2SO_4 and concentrated. The desired product was purified with silica and ionic exchange pre-packed cartridges.

Yield 70% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.22 (t, 3H, *J*=7.14 Hz), 2.66 (dd, 1H, *J*=16.8 and 8.8 Hz), 2.87 (dd, 1H, *J*=15.22 and 3.91 Hz), 3.09-2.71 (m, 2H), 3.38 (dd, 1H, *J*=16.61 and 7.60 Hz), 3.83 (s, 3H), 4.13 (q, 2H, *J*=7.14 Hz), 7.37-716 (m, 3H);

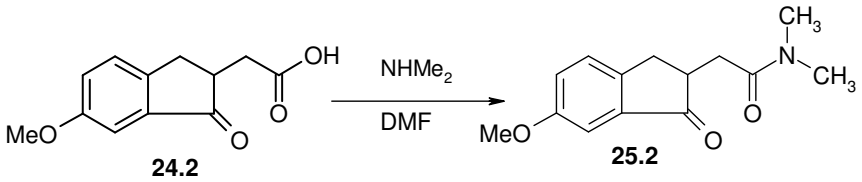
Synthesis of compound 24.2



Compound **23.2** (1.0 eq) was diluted in EtOH and a solution of NaOH 2N (10 eq) was added. The mixture was stopped after 5h at room temperature with HCl 10% and than the organic layer extracted with Et_2O, dried over Na_2SO_4 and concentrated. The desired acid was purified with silica and ionic exchange pre-packed cartridges to obtain the desire product **24.2**.

Yield 97% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.64 (dd, 1H, *J*=17.80 and 9.42 Hz), 2.81 (dd, 1H, *J*=16.70 and 3.92 Hz), 3.10-2.97 (m, 2H); 3.42 (dd, 1H, *J*=16.60 and 7.5 Hz), 3.84 (s, 3H), 7.38-7.15 (m, 3H);

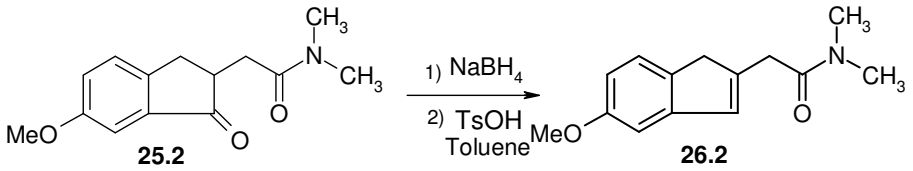
Synthesis of compound 25.2



To a stirred solution of carboxylic acid **24.2** (1.0 eq) in dry DMF were added at 0 °C 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride (1.1 eq), hydroxybenzotriazole (1.0 eq) and the N,N dimethyl amine (1.1 eq). The solution was stirred for 2h. After evaporation of the solvent, the residue was poured into water and extracted with AcOEt. The extracts were dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography with AcOEt as eluent.

Yield 40% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.63 (dd, 1H, *J*=17.10 and 9.21 Hz), 2.81 (dd, 1H, *J*=16.70 and 3.90 Hz), 2.94 (s, 3H), 3.02 (s, 3H), 3.09-2.97 (m, 2H); 3.41 (dd, 1H, *J*=16.70 and 7.40 Hz), 3.83 (s, 3H), 7. 21-7.14 (m, 2H), 7.33 (d, 1H, *J*=8.2 Hz);

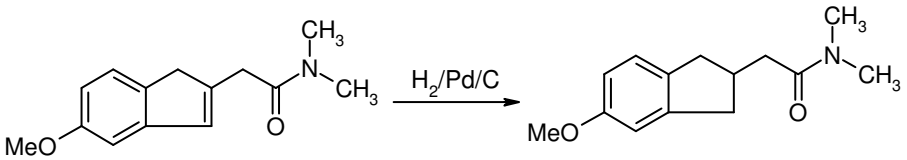
Synthesis of compound 26.2



To a stirred solution of amide **25.2** (1 eq) in MeOH at 0 °C $NaBH_4$ (2.5 eq) was added dropwise and the solution was stirred for 1h. After evaporation of the solvent, the residue was neutralized with HCl 1N and than extracted with DCM dried over Na_2SO_4 and concentrated. The residue was diluted in dry toluene and TsOH (cat) was added and the raction was heated to reflux at 120 °C and stirred for 1h . After TLC analysis, the mixture was stopped: $NaHCO_3$ solution and brine were added and the organic layer were dried over Na_2SO_4 and concentrated. The crude was used for the next step.

Yield 65% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.98 (s, 3H), 3.05 (s, 3H), 3.37 (s, 2H), 3.57 (s, 2H), 3.81 (s, 1H), 6.57 (bs, 1H), 6.69 (dd, 1H, *J*=8.01 and 2.38 Hz), 6.99-6.85 (m, 1H); 7. 28-7.14 (m, 2H);

Synthesis of compound 27.2

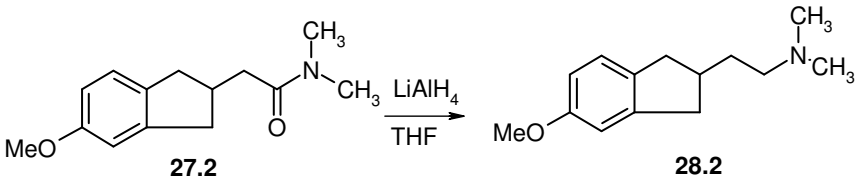


26.2 **27.2**

To a stirred solution of amide **26.2** (1.0 eq) in EtOH, Pd/C (0.1 eq) was added under vacuum and N_2 and the mixture was left stirring with H_2 for 13h. After TLC analysis, the mixture was filtered and the desired product was collected.

Yield 76% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.69-246. (m, 4H), 2.96 (s, 3H), 2.98 (s, 3H), 3.21-2.83 (m, 3H), 3.77 (s, 3H), 6.78-6.66 (m, 2H), 7.08 (d, 1H, *J*=8.0 Hz);

Synthesis of compound 28.2

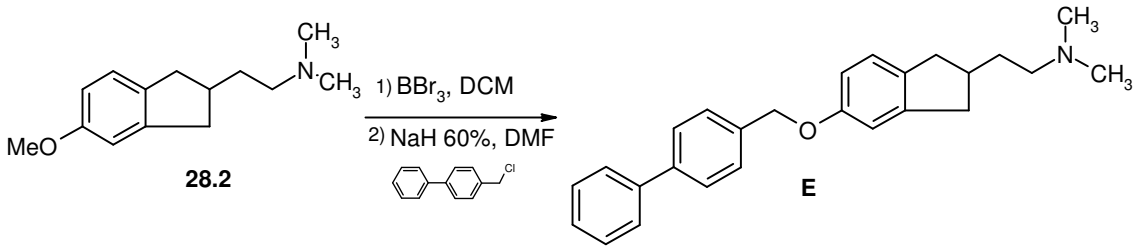


27.2 **28.2**

To a stirred solution of amide **27.2** (1.0 eq) in dry THF, a solution of $LiAlH_4$ (1.5 eq) was added and the mixture was heated to reflux at 70 °C for 1h. After TLC analysis water, NaOH were added and the organic layer were dried over Na_2SO_4 and concentrated. The residue was purified by silica and ionic exchange pre-packed cartridges.

Yield 63% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.76-1.65 (m, 2H), 2.29 (s, 6H), 2.63-2.37 (m, 4H), 2.84-2.68 (m, 1H), 3.07-2.92 (m, 2H), 3.77 (s, 3H), 6.73-6.64 (m, 2H), 7.06 (d, 1H, *J*=8.0 Hz);

Synthesis of compound E



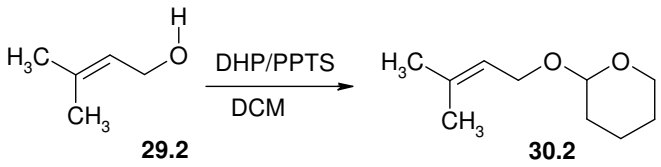
A solution of BBr_3 1M in DCM (3 eq) was added dropwise at -78 °C to a stirred solution of amine resulting from sixth step (1.0 eq) in DCM. After 30 minutes the mixture was heated to room temperature and left stirring for 1h. After TLC analysis $NaHCO_3$ was added and the organic layer were extracted with DCM and dried over Na_2SO_4 and concentrated. The residue was purified by SCX pre-packed cartridges. To a stirred solution of alcohol resulting from the previous step (1 eq) in dry DMF at 0 °C NaH 60% (2.5 eq) was added and the mixture was heated at 50 °C and stirred for 1h.The mixture was cooled to 0 °C and than 4-phenylbenzyl chloride (1.1 eq) was added and the reaction was stirred at room temperature for 2h. After evaporation of the solvent water was added and the organic layer were extracted with AcOEt and dried over Na_2SO_4 and concentrated. The residue was purified by silica and ionic exchange pre-packed cartridges.

Yield 41% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.46-1.84 (m, 2H), 2.36 (s, 6H), 242-2.66 (m, 5H), 2.90-3.10 (m, 2H), 5.07 (s, 2H), 6.74-6.88 (m, 2H), 7.08 (d, 1H, *J*=8.41 Hz), 7.34-7.63 (m, 9H);

Yield 63% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.65-1.76 (m, 2H), 2.29 (s, 6H), 2.37-2.63 (m, 4H), 2.68-2.84 (m, 1H), 2.92-3.07 (m, 2H), 3.77 (s, 3H), 6.64-6.73 (m, 2H), 7.06 (d, 1H, *J*=8.0Hz);

Isoprenoid derivatives F1 and F2

Synthesis of compound 30.2

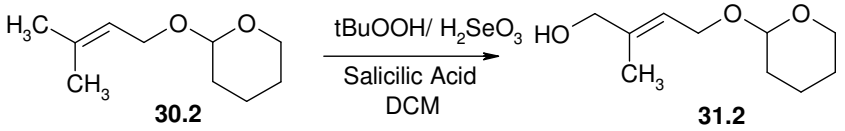


29.2 **30.2**

To a DCM solution of the commercial available 3-methyl-2-buten-1-olo (1.0 eq) were added 3,4-dihydropyrane (1.5 eq) and PPTs (0.1 eq) and the mixture was stirred for 4h at room temperature. After TLC analysis, the reaction mixture was partially evaporated, diluted with Et_2O and washed with saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated to yield the desired product that was purified with silica and ionic exchange pre-packed cartridges.

Yield 87% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.85-1.40 (m, 6H), 1.64 (s, 3H), 1.70 (s, 3H), 3.51-3.40 (m, 1H), 3.86-3.80 (m, 1H), 3.92 (dd, 1H, *J*=12.10 and 6.02 Hz), 4.17 (dd, 1H, *J*=12.10 and 6.02 Hz), 4.58 (t, 1H, *J*=4.02 Hz), 5.28 (t, 1H, *J*=6.02 Hz);

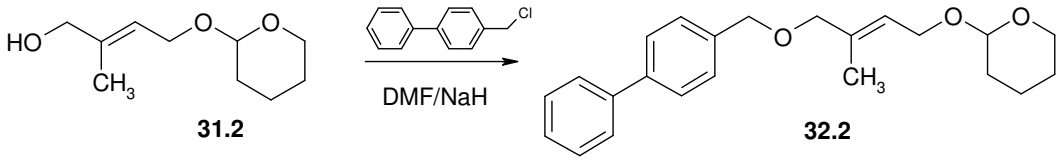
Synthesis of compound 31.2



Compound **30.2** (1.0 eq) and *tert*-butyl hydroperoxide (3.20 eq) were stirred in the presence of H_2SeO_3 (0.11 eq) and salicylic acid (0.11 eq) in DCM for 14 h at room temperature. The DCM was removed under reduced pressure and *tert*-butyl hydroperoxide was removed by repeated washing with toluene. The residue was dissolved in Et_2O, washed with $NaHCO_3$ to remove H_2SeO_3, dried over Na_2SO_4 and concentrated. The residue was purified by silica and ionic exchange pre-packed cartridges.

Yield 40% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.85-1.54 (m, 9H), 3.57-3.47 (m, 1H), 3.94-3.83 (m, 1H), 4.10-4.01 (m, 4H), 4.29 (dd, 1H, *J*=12.10 and 6.42 Hz),4.64 (t, 1H, *J*=3.29 Hz), 5.63 (t, 1H, *J*=6.10 Hz);

Synthesis of compound 32.2

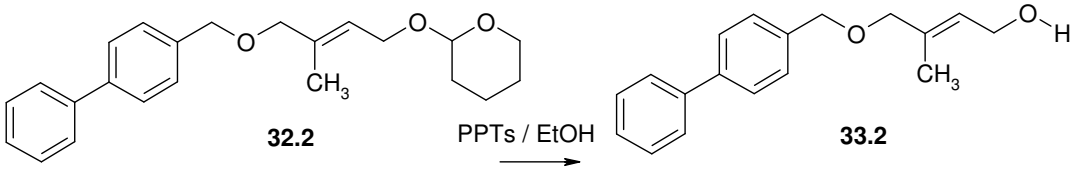


31.2 **32.2**

Alcohol **31.2** (1.0 eq) was dissolved in dry DMF and cooled to 0 °C prior to NaH 60% (2.5 eq) addition and the mixture was heated to 50 °C and stirred. After 1h 4-phenylbenzyl chloride (1.1 eq) was added at 0 °C and the mixture stirred for 24h at room temperature. The reaction mixture was poured over water and extracted with AcOEt. The combined extracts were dried over Na_2SO_4 and concentrated.

Yield 30% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.82-1.70 (m, 9H), 3.54-3.48 (m, 1H), 6.9-3.80 (m,1H), 3.98 (s, 2H), 4.20-4.10 (m, 2H), 4.40-4.24 (m, 1H), 4.52 (s,1 H), 4.60 (dd, 2H, *J*=14.80 and 2.92 Hz), 5.80-5.66 (m, 1H), 7.48-7.33 (m, 5H), 7.62-7.55 (m, 4H);

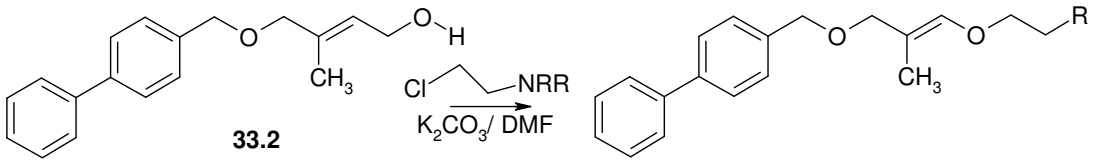
Synthesis of compound 33.2



To a solution of compound **32.2** (1.0 eq) in EtOH was added PPTs (0.10 eq) and the reaction stirred at 55 °C for 6h. The solvent was partially evaporated in vacuo and the resulting solution was diluted with Et_2O and washed with half-saturated brine to remove the catalyst. The organic layer was dried over Na_2SO_4 and concentrated to yield the desired product that was purified with silica and ionic exchange pre-packed cartridges.

Yield 62% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.72 (d, 3H, *J*=7.30), 3.87 (d, 2H, *J*=18.01), 4.18 (dd, 2H, *J*=24.10 and 6.51 Hz), 4.55 (d, 2H, *J*=7), 5.80-5.66 (m, 1H), 7.48-7.31 (m, 5H), 7.61-7.56 (m, 4H);

Synthesis of compound F1 and F2



33.2

The desired alcohol obtained from step four (1.0 eq) was dissolved in dry DMF and cooled to 0 °C prior to NaH 60% (2.5 eq) addition and the mixture was heated to 50 °C and stirred for 1h. After chloroethyl amine (1.1 eq) was added at 0 °C and the mixture stirred for 18-24h at room temperature. The reaction mixture was poured over water and extracted with AcOEt. The combined extracts were dried over Na_2SO_4 and concentrated and then purified with silica and ionic exchange pre-packed cartridges (95:5 CH_2Cl_2/MeOH)

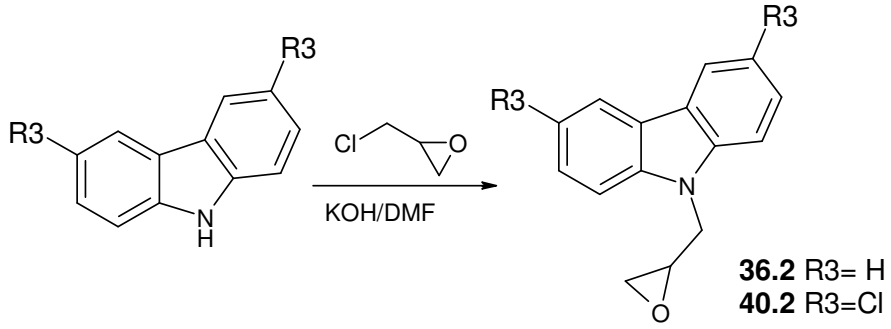
F1: Yield 30% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25 (s, 2H), 1.70 (d, 3H, *J*=10.21), 2.3 (s, 6H), 2.64-2.52 (m, 2H), 3.64-3.47 (m, 2H), 3.94 (d, 1H, *J*=9.10), 4.13-4.07 (m, 1H), 4.51 (s, 1H), 4.57 (d, 1H, *J*=6.70), 5.78-5.64 (m, 1H), 7.48-7.36 (m, 5H), 7.61-7.55 (m, 4H);

F2: Yield 25% ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.66-1.2 (m, 6H), 1.70 (d, 4H, *J*=11.30), 2.45 (sb, 3H), 2.57 (t, 2H, *J*=5.91), 3.68-3.50 (m, 2H), 3.93 (d, 1H, *J*=11.10), 4.10 (t, 2H *J*=6.45), 4.51 (s, 1H), 4.56 (d, 1H, *J*=4.72), 5.74-5.64 (m, 1H), 7.48-7.33 (m, 5H), 7.61-7.55 (m, 4H).

Carbazole Derivatives

Synthesis of carbazole derivatives

Generic synthetic procedure for reaction with epichloridrin

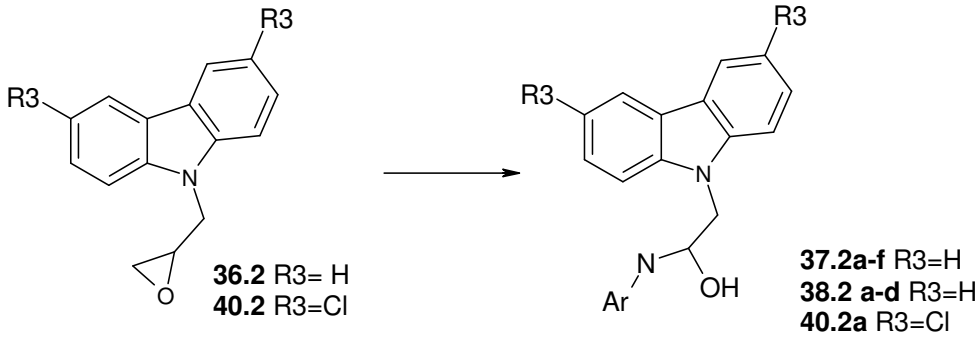


The carbazole (1.0 eq) was diluted in DMF and the KOH (1.2 eq) was added. The reaction was left under stirring for 1h at room temperature and after cooled -10 °C. The cooled resulting mixture was added very slowly to a mixture of the epichloridrin (2.5 eq) in DMF anhydrous and left under stirring for 4-6 hours. The reaction was stopped after TLC analysis: the mixture was threw to ice water and extracted with AcOEt and after dried over Na_2SO_4 and concentrated.The resulting products (**36.2** and **40.2**) have been purified with silica and ionic exchange pre-packed cartridges.

36.2 Yield 50% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.57 (dd, 1H, *J*=4.76 and 2.56 Hz), 2.81 (ps t,1H, *J*=4.29 Hz) , 3.39-3.32 (m, 1H), 4.40 (dd, 1H *J*=15.75 and 4.71 Hz), 4.63 (dd, 1H *J*=15.75 and 3.29 Hz), 7.30-7.22 (m, 2H),7.49-7.46 (m, 4H), 8.10(d, 1H, *J*=7.87);

40.2 Yield 50% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.52(dd, 1H, *J*=4.76 and 2.75 Hz), 2.81 (ps t,1H, *J*=4.02 Hz) , 3.37-3.28 (m, 1H), 4.29 (dd, 1H *J*=16.11 and 5.30 Hz), 4.66 (dd, 1H *J*=15.90 and 2.74 Hz), 7.47-7.36 (m, 6H), 7.99-7.97 (m, 2H);

General procedure for ring opening reaction



36.2 R3= H
40.2 R3=Cl

37.2a-f R3=H
38.2 a-d R3=H
40.2a R3=Cl

Procedure 1

To a mixture of epoxide (1.0 eq) in EtOH (heating at 50 °C is soluble) the amine (2.0 eq) was added and the reaction was heated to reflux overnight. The solvent was evaporated and the product was purified with silica and ionic exchange pre-packed cartridges.

Procedure 2

To a mixture of epoxide (1.0 eq) in ciclohexane at r.t. the amine (1.20 eq) and $BiCl_3$ (0.10 eq) were added and the reaction was left under stirring for 2-4 hours. The reaction was stopped: $NaHCO_3$ was added and the mixture extracted with DCM and than dried over Na_2SO_4 and concentrated. The products were purified with silica and ionic exchange pre-packed cartridges.

37.2a Yield 40% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.17-2.15 (m, 1H), 3.44-3.22 (m,2H), 4.47 (s, 2H), 6.78-6.61 (m, 3H),7.29-7.14 (m, 4H), 7.48-7.44 (m, 4H), 8.11 (d, 2H, *J*=7.69Hz);

37.2b Yield 68% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.17-2.15 (m, 1H), 3.44-3.22 (m,3H), 4.46 (s, 2H), 6.74 (AA'/XX', 2H, J_{AX}=8.7 and $J_{AA'/XX'}$=2.5 Hz), 7.11 (AA'/XX', 2H, J_{AX}=8.7 and $J_{AA'/XX'}$=2.5 Hz), 7.30-7.22 (m, 4H), 7.46 (d, 4H, *J*=3.66 Hz), 8.11 (d, 2H, *J*=7.69Hz);

37.2c Yield 63% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.23 (s, 3H), 3.20 (dd, 1H, *J*=6.4 and 12.8 Hz), 3.34 (dd, 1H, *J*=3.48 and 12.8 Hz), 4.4-4.5 (m,4H), 6.55 (AA'/XX', 2H, J_{AX}=8.4 and $J_{AA'/XX'}$=2.2 Hz), 6.98(AA'/XX', 2H, J_{AX}=8.4 and $J_{AA'/XX'}$=2.5 Hz), 7.21-7.29 (m,2H), 7.41-7.47 (m, 4H), 8.1(d, 2H, *J*=7.69 Hz);

37.2d Yield 43% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.17-2.15 (m, 1H), 3.36-3.16 (m, 2H), 4.46 (s, 2H), 6.58-6.52 (m, 2H), 6.92-6.83 (m, 2H), 7.29-7.22 (m, 2 H), 7.46 (d, 4H, *J*=3.6 Hz), 8.11 (d, 2H, *J*=7.87 Hz);

37.2e Yield 71% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.50-3.20 (m,2H), 4.42-4.36 (m,1H), 4.54-4.44 (m,2H), 6.61 (d, 2H, *J*=8.6 Hz), 7.47-7.23 (m, 8H), 8.11(d, 2H, *J*=7.69 Hz);

37.2f Yield 55% ^{1}H NMR (200 MHz, $CO(CD3)_2$ δ 2.07-2.02 (m,2H), 3.56-3.44 (m,2H), 4.67-4.48 (m, 5H), 6.77-6.74 (m, 2H), 7.19 (t, 2H *J*=7.15 Hz), 7.41 (t, 2H *J*=7.53 Hz), 7.62 (d, 2H *J*=8.00 Hz), 8.00 (d, 2H, *J*=9.30 Hz);8.13(d, 2H, *J*=7.51 Hz);

38.2a Yield 56% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.67 (dd, 1H, *J*=8.20 and 1.200 Hz), 2.83 (dd, 1H, *J*=3.50 and 12.00 Hz), 3.73 (AB quartet, 2H, *J*=13 Hz), 4.28-4.16 (m,1H), 4.36 (s,1H), 4.39 (d, 1H, *J*=2.38 Hz),7.31-7.20 (m, 7H), 7.46(d, 4H, *J*=4.39 Hz), 8.09 (d, 2H, *J*=7.69 Hz);

38.2b Yield 44% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.64 (dd, 1H, *J*=8.04 and 12.00 Hz), 2.8 (dd, 1H, *J*=3.60 and 12.00 Hz), 3.69 (AB quartet, 2H, *J*=13.01 Hz), 4.26-4.14 (m, 1H), 4.37(s,1H), 4.40 (d, 1H, *J*=2.56 Hz), 7.26-7.16 (m, 6H), 7.46 (d, 4H, *J*=3.67 Hz), 8.09 (d, 2H, *J*=7.87 Hz);

38.2c Yield 70% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.47 (s,3H), 2.78 (dd, 1H, *J* = 8.32 and 12.01 Hz), 2.92 (dd, 1H, *J*=3.61 and 12.01 Hz), 3.82 (AB quartet, 2H, *J*=13.01 Hz) 4.37-4.26 (m, 1H), 4.50 (s,1H), 4.53 (d, 1H, *J*=2.02 Hz), 7.41-7.35 (m, 6H), 7.60 (d, 4H, *J*=3.66 Hz), 8.23 (d, 2H, *J*=7.87 Hz);

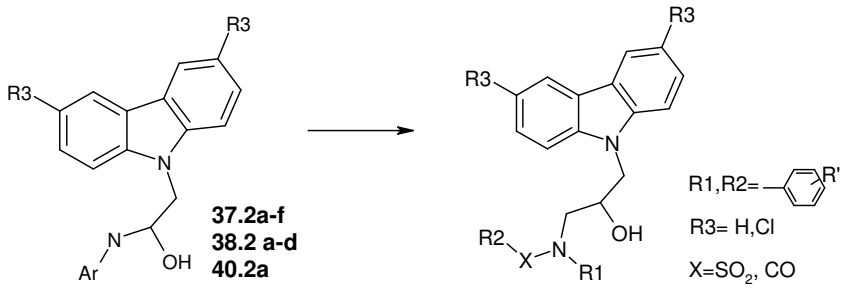
38.2d Yield 47% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.63 (dd, 1H, *J*=8.3 and 12 Hz), 2.8 (dd, 1H, *J*=3.60 and 12.00 Hz), 3.65 (AB quartet, 2H, *J*=13.01 Hz), 3.79 (s,3H), 4.23-4.11 (m, 1H), 4.37-4.35(m,1H), 4.39 (d, 1H, *J*=2.41 Hz), 6.83 (AA'/XX', 2H, J_{AX}=8.60 and $J_{AA'/XX'}$=2.50 Hz), 7.14 (AA'/XX', 2H, J_{AX}=8.60 and $J_{AA'/XX'}$=2.50 Hz), 7.28-7.21 (m, 2H), 7.46 (d, 4H, *J*=4.40 Hz), 8.09 (d, 2H, *J*=7.64 Hz);

41.2a Yield 73% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.58 (dd, 1H, *J*=12.00 and 8.30 Hz), 2.81 (dd, 1H, *J*=12.00 and 3.84 Hz), 3.73 (d, 2H, *J*=3.10 Hz), 4.16-4.06 (m,1H), 4.32 (d, 2H, *J*=5.49 Hz), 7.40-7.27 (m, 9H), 8.20-7.97 (m, 2H);

L Yield 70% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.30-2.21 (m, 1H), 3.40 (dd, 1H, *J*=12.60 and 6.49 Hz), 3.54 (dd, 1H, *J*=12.60 and 3.10 Hz), 4.74-4.55 (m, 3H), 6.57 (dd, 1H, *J*=5.78 and 2.60 Hz) 7.31-7.22 (m, 4H), 7.53-7.42 (m, 6 H), 7.85-7.76 (m, 2H), 8.12 (d, 2H, *J*=7.69 Hz);

M Yield 64 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.36 (dd, 1H, *J* = 3.84 and 12.0 Hz), 3.55-3.48 (m, 1H), 4.58-4.47 (m, 3H), 6.57-6.52 (m, 1H), 7.32-7.28 (m, 3H), 7.51-7.41 (m, 6H), 7.86-7.79 (m, 2H), 7.99-8.02 (m, 1H);

Generic synthetic procedure for cupping reaction with sulfonil and acyl chlorides



For each amine : To a solution of the amine (1.0 eq) in DCM the various sulfonil and acyl chlorides (1.0-1.50 eq) were added and after DMAP (cat) and PS–DIEA (1.20 eq) were added. The reaction mixtures were stirred at room temperature overnight and than filtered and evaporated and purified with silica and ionic exchange pre-packed cartridges.

G1 Yield 66 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.22 (s, 1H), 3.71-3.69 (m, 2H), 4.30-4.23 (m, 2H), 4.54-4.51 (m, 1H), 7.35-7.08 (m, 2H), 7.24-7.19 (m, 2H), 7.35-7.31 (m, 5H), 7.42-7.38 (m, 6H), 8.10 (d, 2H, *J*=7.69Hz);

G2 Yield 46 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.26-2.15 (m, 1H), 3.73-3.71 (m, 2H), 3.68 (s, 3H), 4.39-3.86 (m, 2H), 4.54-4.50 (m, 1H), 6.88 (d, 2H, *J*=7.69Hz), 7.09-7.05 (m, 2H), 7.25-7.21 (m, 3H), 7.35-7.32 (m, 5H), 7.46-7.42 (m, 4H), 8.06 (d, 2H, *J*=7.69Hz);

G3 Yield 57 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.24-2.19 (m, 1H), 3.73 (d, 4H, *J*=5.31 Hz), 4.46 (s, 3H), 4.54-4.20 (m, 4H), 7.69-7.21 (m, 15H), 8.08 (d, 2H, *J*=7.69 Hz);

G4 Yield 66 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.69 (d, 2H, *J*=6.20 Hz), 4.56-4.20 (m, 4H), 6.98 (d, 2H, *J* = 8.42 Hz), 7.59-7.21 (m, 13H), 8.07 (d, 2H, *J*=7.87 Hz);

G5 Yield 18 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.24-2.19 (m, 1H), 3.73 (d, 4H, *J*=5.31 Hz), 4.54-4.20 (m, 4H), 7.60-7.15 (m, 15H), 8.06 (d, 2H, *J*=7.69 Hz);

G7 Yield 77% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.72-3.68 (m, 2H), 4.56-4.23 (m, 3H), 7.02- 6.97 (m, 4H), 7.59-7.19 (m, 11H), 8.04 (d, 2H, *J* = 7.69 Hz);

H1 Yield 57% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.91 (dd, 1H, *J*=14.00 and 2.43 Hz), 4. 51-4.28 (m, 5H), 6.92-6.88 (m, 2H), 7.29 -7.05 (m, 10 H), 7.43 (d, 4H, *J*=3.81 Hz), 8.06 (d, 2H, *J*=7.69 Hz);

H2 Yield 57% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.64-3.61 (m, 1H), 3.92-3.88 (m, 1H), 4.39-4.23 (m, 2H), 4.48-4.47 (m, 1H), 6.90-6.86 (m, 2H), 7.11-7.08 (m, 4 H), 7.24 - 7.17 (m, 4 H), 7.43-7.41 (m, 4H), 8.05 (d, 2H, *J*=7.69 Hz);

H3 Yield 57% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.70 (s, 3H), 3.88-3.84 (m, 1H), 4.11-4.05 (m, 3H), 4.46-4.39 (m, 3H), 6.63-6.54 (m, 2H), 6.90-6.87 (m, 2H), 7.09-7.05 (m, 3H), 7.24 -7.22 (m, 4 H), 7.41-7.39 (m, 4H), 8.04 (d, 2H, *J*=7.69 Hz);

H4 Yield 40 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.43-3.41 (m, 1H), 3.92-3.88 (m, 1H), 4.18-4.09 (m, 1H), 4.38-4.35 (m, 2H), 4.48-4.43 (m, 1H), 6.97-6.90 (m, 3H), 7.14-7.12 (m, 4H), 7.25 -7.21 (m, 2 H), 7.47-7.39 (m, 5H), 8.05 (d, 2H, *J*=7.69 Hz);

H5 Yield 65% ^{1}H NMR (200 MHz, $CDCl_3$) 2.25(s, 3H), 3.62-3.42 (m, 2H), 3.93 (d, 1H, *J*=14.10 Hz), 4.22 (dd, 1H, *J*=714. and 10.75 Hz), 4.26-4.20 (m,4H), 6.88 (d, 2H, *J*=8.05 Hz), 7.27-7.13 (m,6H), 7.52-7.36 (m, 5H), 8.06 (d, 2H, *J*=8.05 Hz);

H6 Yield 43% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.30-2.21(m, 1H), 3.65 (d, 1H, *J*=3.6 Hz), 3.83 (dd, 1H, *J*=14.10 and 2.22 Hz), 4.24 (dd, 1H, *J*=14.10 and 7.70 Hz), 4.50-4.41 (m, 3H), 6.57 (dd, 1H, *J*=5.78 and 2.60 Hz), 6.75 (AA'/XX', 2H, J_{AX}=8.70 and $J_{AA'/XX'}$=2.51 Hz), 6.98 (AA'/XX', 2H, J_{AX}=8.70 and $J_{AA'/XX'}$=2.51 Hz) 7.31-7.22 (m, 4H), 7.26-7.16 (m, 6 H), 7.44-7.14 (m, 5H), 8.06 (d, 2H, *J*=7.69 Hz);

H7 Yield 65% ^{1}H NMR (200 MHz, $CDCl_3$) 3.62-3.42 (m, 2H), 3.93 (d, 1H, *J*=14.10 Hz), 4.22 (dd, 1H, *J*=714. and 10.75 Hz), 4.260-4.20 (m,4H), 6.88 (d, 2H, *J*=8.05 Hz), 7.27-7.13 (m,6H), 7.52-7.36 (m, 5H), 8.06 (d, 2H, *J*=8.05 Hz);

H8 Yield 80% ^{1}H NMR (200 MHz, $CDCl_3$) δ), 3.97 (dd, 1H, *J*=14.46 and 2.19 Hz), 4.23-4.12 (m, 1H), 4.58-4.41 (m, 3H), 6.84 (AA'/XX', 2H, J_{AX}=9.15 and $J_{AA'/XX'}$=2.30 Hz), 7.28-7.17 (m, 7H), 7.44-7.41 (m, 4H), 7.77 (AA'/XX', 2H, J_{AX}=9.15 and $J_{AA'/XX'}$=2.30 Hz), 8.02 (d, 2H, *J*=7.69 Hz);

H9 Yield 80% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.74-3.66 (m, 1H), 3.81 (dd, 1H, *J*=2.30 and 14.0 Hz), 4.28 (dd, 1H, *J*=14.0 and 8.0 Hz), 4.58-4.41 (m, 3H), 6.86-6.68 (m, 5H), 7.27-7.14 (m, 6H), 7.44 (d, 4H, *J*=3.48 Hz), 8.06 (d, 2H, *J*=7.87 Hz);

J1 Yield 59% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.63-2.65 (m, 1H), 3.09 (dd, 1H, *J*=14.90 and 3.70 Hz), 3.3 (dd, 1H, *J*=14.70 and 7.60 Hz), 4.15 (d, 4H, *J*=6.59 Hz), 4.20 (AB quartet, 2H, *J*=14.10 Hz),7.94-7.62 (m, 16 H), 7.80 (d, 2H, *J*=7.69 Hz), 8.06 (d, 2H, *J*=7.69 Hz);

J2 Yield 56% ^{1}H NMR (400 MHz) CDCl3: δ 8.06 (d, 2H, *J*= 7.2 Hz), 7.68 (d, 2H, *J*= 8.4 Hz), 7.39 (td, 2H, *J*= 7.7, 1.0 Hz), 7.30 (d, 2H, *J*= 7.6 Hz), 7.28-7.00 (m, 10 H), 4.35 (d, 1H, *J*= 14 Hz), 4.15 (d, 2H, *J*= 6.4 Hz), 4.00 (d, 2H, *J*= 14 Hz), 3.28 (dd, 1H, *J*= 14.8, 8.0 Hz), 3.08 (dd, 1H, *J*= 15.4, 3.8 Hz), 2.67 (m, 1H), 2.44 (m, 3H).

J3 Yield 38% ^{1}H NMR (400 MHz) CDCl3: δ 8.07 (d, 2H, *J*= 7.6 Hz), 7.69 (d, 2H, *J*= 8.8 Hz), 7.50-7.35 (m, 4H), 7.30-7.10 (m, 6H), 7.13-7.04 (m, 2H), 4.33 (d, 1H, *J*= 14.4 Hz), 4.17-4.03 (m, 4H), 3.30-3.27 (m, 1H), 3.25-3.08 (m, 1H), 2.53-2.52 (m, 1H).

J4 Yield 20% ^{1}H NMR (400 MHz) CDCl3: δ 8.05 (d, 2H, *J*= 7.6 Hz), 7.72 (d, 2H, *J*= 8.8 Hz), 7.38 (t, 2H, *J*= 7.6 Hz), 7.37-7.09 (m, 7H), 7.04-6.80 (m, 4H), 4.32 (d, 1H, *J*= 14 Hz), 4.17-4.10 (m, 2H), 4.08-4.00 (m, 2H), 3.87 (s, 3H), 3.29-3.23 (m, 1H), 3.08-3.03 (m, 1H), 2.71-2.70 (m, 1H).

J5 Yield 40% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.24 (s, 3H), 3.08 (dd, 1H, *J*=1.50 and 3.50 Hz), 3.29 (dd, 1H, *J*=15.0 and 7.70 Hz), 4.15 (d, 2H, *J*=6.20 Hz), 4.37-4.34(m,1H),

4.15 (AB quartet, 2H, *J*=13.90 Hz), 6.88-6.87 (m,4H), 7.26-7.15 (m,4H), 7.61-7.35 (m, 5H), 7.8 (d, 2H, *J*=8.20 Hz), 8.06 (d, 2H, *J*=7.69 Hz);

J6 Yield 73% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.84 (d, 1H, *J*=2.56 Hz), 3.03 (dd, 1H, *J*=15.0 and 3.0 Hz), 3.32 (dd, 1H, *J*=15.0 and 8.1 Hz), 4.09-3.96 (m,1H), 4.22-4.16 (m,2H), 4.13 (AB quartet, 2H, *J*=14.30 Hz), 6.84(AA'/XX', 2H, J_{AX}=8.40 and $J_{AA'/XX'}$=2.1 Hz), 6.98(AA'/XX', 2H, J_{AX}=8.40 and $J_{AA'/XX'}$=2.1 Hz), 7.27-7.14 (m, 4H), 7.62-7.36 (m, 5H), 7.80 (d, 2H, *J*=6.70 Hz), 8.07 (d, 2H, *J*=7.69 Hz);

J7 Yield 86% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.72 (d, 1H, *J* =2.91 Hz), 3.08 (dd, 1H, *J*=15.0 and 3.50 Hz), 3.29 (dd, 1H, *J*=15.0 7 and.69 Hz), 3.73 (s,3H), 4.13 (AB quartet, 2H, *J*=14.01 Hz), 4.17 (d, 2H, *J*=6.59), 6.60 (AA'/XX', 2H, J_{AX}=8.50and $J_{AA'/XX'}$=2.20 Hz), 6.91(AA'/XX', 2H, J_{AX}=8.50 and $J_{AA'/XX'}$=2.50 Hz), 7.24-7.15 (m,5H), 7.62-7.35 (m, 6H), 7.8 0(d, 2H, *J*=8.50 Hz), 8.06 (d, 2H, *J*=7.69 Hz);

J8 Yield 71% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.72 (d, 1H, *J*=3.10 Hz), 3.03 (dd, 1H, *J*=14.50 and 3.70 Hz), 3.24 (dd, 1H, *J*=14.50 and 7.40 Hz), 3.95-3.80 (m, 1H), 4.19 (AB quartet, 2H, *J*=14.0 Hz), 7.19-7.04 (m, 7H), 7.34 (dd, 2H, *J*=1.90 and 8.69 Hz), 7.65-7.52 (m, 3H), 7.81 (d, 2H, *J*=7.30 Hz), 7.95 (d, 2H, *J*=1.80 Hz);

J9 Yield 73% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.46 (s, 3H), 2.77 (d, 1H, *J*=3.10 Hz), 3.08 (dd, 1H, *J*=15.0 and 3.75 Hz), 3.21(dd, 1H, *J*=15.0 and 7.69 Hz), 4.13 0-3.85 (m, 3H), 4.11 (AB quartet, 2H, *J*=14.0 Hz), 7.04 (AA'/XX', 2H, J_{AX}=8.45 and $J_{AA'/XX'}$=2.20 Hz), 7.17-7.12 (m, 5H), 7.37-7.31 (m, 4H), 7.68 (AA'/XX', 2H, J_{AX}=8.45 and $J_{AA'/XX'}$=2.20 Hz), 7.94 (d, 2H, *J*=1.80 Hz);

J10 Yield 56% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.46 (s, 3H), 2.62 (m,1H), 3.03 (dd, 1H, *J*=14.70 and 3.10 Hz), 3.22 (dd, 1H, *J*=14.70 and 7.0 Hz), 3.98-3.84 (m, 1H), 4.13-4.02 (m, 2H), 4.19 (AB quartet, 2H, *J*=13.70 Hz), 7.02 (m, 9H), 7.51 (AA'/XX', 2H,

J_{AX}=8.50 and $J_{AA'/XX'}$=1.90 Hz), 7.72 (AA'/XX', 2H, J_{AX}=8.50 and $J_{AA'/XX'}$=1.90 Hz), 7.98-7.91 (m, 2H);

J11 Yield 43% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.79 (d, 1H, *J*=2.70 Hz), 2.97 (dd, 1H, *J*=14.80 and 3.70 Hz), 3.19 (dd, 1H, *J*=7.69 and 14.80 Hz), 3.89 (s, 3H), 4.14-3.93 (m, 3H), 4.19 (AB quartet, 2H, *J*=12.50 Hz), 6.99 (AA'/XX', 2H, J_{AX}=8.80 and $J_{AA'/XX'}$=2.00 Hz), 7.16-7.05 (m, 7H), 7.34 (dd, 2H, *J*=8.70 and 2.00 Hz), 7.71 (AA'/XX', 2H, J_{AX}=8.80 and $J_{AA'/XX'}$=2.0 Hz), 7.94 (d, 2H, *J*=2.01 Hz);

K1 Yield 90% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.28 (d, 1H, *J*=14.50 Hz), 4.29-3.86 (m, 1H), 4.40-4.26 (m, 5H) 6.76-6.64 (m, 2H), 7.10-6.94 (m, 2H), 7.46-7.20 (m, 12 H), 8.08 (d, 2H, *J*=7.69 Hz);

K2 Yield 48% ^{1}H NMR (200 MHz) CDCl3: δ 8.08 (d, 2H, *J*= 7.5 Hz), 7.46-7.00 (m, 13H), 6.80-6.65 (m, 2H), 4.45-4.13 (m, 5H), 3.94-3.86 (m, 1H), 3.30 (d, 1H, *J*= 13.9 Hz);

K3 Yield 30% ^{1}H NMR (200 MHz) CDCl3: δ 8.07 (d, 2H, *J*= 7.6 Hz), 7.45-7.19 (m, 7H), 7.10-6.90 (m, 3H), 6.87-6.73 (m, 5H), 4.40-4.13 (m, 5H), 4.05-3.80 (m, 1H), 3.80 (s, 3H), 3,25 (d, 1H, *J*= 13.9 Hz);

K4 Yield 74% ^{1}H NMR (200 MHz) CDCl3: δ 8.08 (d, 2H, *J*= 7.6 Hz), 7.52-7.10 (m, 12H), 6.80-6.60 (m, 2H), 4.30-3.83 (m, 6H), 3.36 (d, 1H, *J*= 13.7 Hz);

K5 Yield 57% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.21 (s, 3H), 3.26 (d, 1H, *J*=13.90 Hz), 4.40- 3.90 (m,5H), 6.55 (AA'/XX', 2H, J_{AX}=8.24 and $J_{AA'/XX'}$=2.20 Hz), 6.75 (AA'/XX', 2H, J_{AX}=8.24 and $J_{AA'/XX'}$=2.20 Hz), 7.46-7.23 (m, 11H), 8.08 (d, 2H, *J*=7.69 Hz);

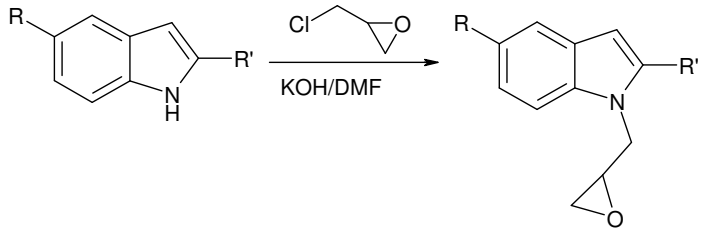
K6 Yield 73% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.18 (d, 1H, *J*=14.50 Hz), 4.40-3.84 (m,1H), 4.37-4.15 (m,4H), 6.54 (AA'/XX', 2H, J_{AX}=8.30 and $J_{AA'/XX'}$=2.40 Hz), 6.89

(AA'/XX', 2H, J_{AX}=8.30 and $J_{AA'/XX'}$=2.40 Hz), 7.47-7.21 (m, 11H), 8.09 (d, 2H, *J*=7.69 Hz);

K7 Yield 73% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.23 (d, 1H, *J* =14.46 Hz), 3.72 (s,3H), 4.04-3.9 (m, 1H), 4.34-4.16 (m, 4H), 6.60-6.45 (m, 4H), 7.47-7.20 (m, 11H), 8.08 (d, 2H, *J* = 7.69 Hz);

Synthesis of indole derivatives

Generic synthetic procedure for reaction with epichloridrin



44 R=H,R'=H
45 R=H, R'=Ph
46 R=OMe, R'=Ph

48 R=H,R'=H
49 R=H,R'=Ph
50 R=OMe, R'=Ph

For each indole:

The indole (1.0 eq) was diluted in DMF and the KOH (1.2 eq) was added. The reaction was left under stirring for 1h at room temperature and after cooled -10 °C. The cooled resulting mixture was added very slowly to a mixture of the epichloridrin (2.5 eq) in DMF anhydrous and left under stirring for 4-6 hours. The reaction was stopped after TLC analysis: the mixture was threw to ice water and extracted with AcOEt and after dried over Na_2SO_4 and concentrated. The resulting products have been purified with silica and ionic exchange pre-packed cartridges.

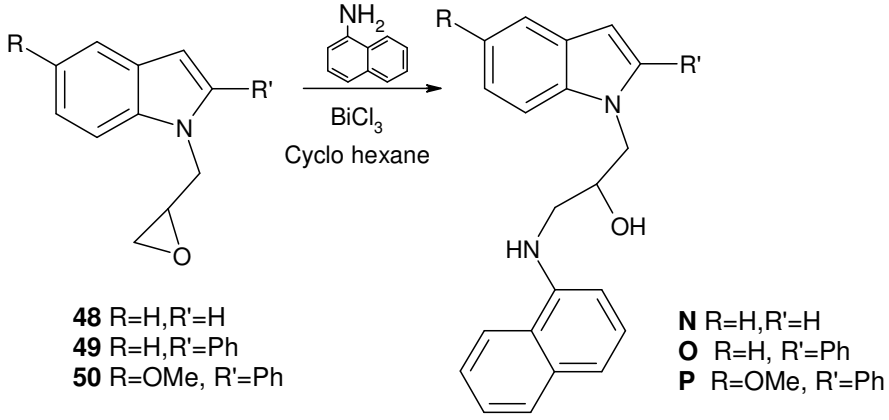
48 Yield 54 % ^{1}H NMR (200 MHz) CDCl3: δ 7.65 (d, 1H, *J*= 7.5 Hz), 7.39 (d, 1H, *J*= 8.19 Hz), 7.28-7.09 (m, 3H), 6.52 (dd, 1H, *J*=3.11, 0.73 Hz), 4.44 (dd, 1H, *J*= 15.3 and 3.2 Hz), 4.19 (dd, 1H, *J*= 15.2 and 5.12 Hz), 3.33-3.25 (m, 1H), 2.81 (ψ t, 1H, *J*= 4.2 Hz), 2.46 (dd, 1H, *J*= 4.6 and 2.6 Hz);

49 Yield >90 % ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.40 (dd, 1H, *J*=4.76 and 2.56 Hz), 2.74 (ps triplet, 1H, *J*=4.31 Hz), 3.17-3.25 (m, 1H), 4.26 (dd, 1H, *J*=15.56 and 4.57 Hz), 4.39 (dd, 1H, *J*=15.56 and 3.84 Hz), 6.58(s, 1H), 7.31-7.12 (m, 2H); 7.65-7.41 (m, 7H);

50 Yield 60% ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.39 (dd, 1H, *J*=4.76 and 2.50 Hz), 2.73 (ps triplet, 1H, *J*=4.76 Hz), 3.22-3.15 (m, 1H), 3.87 (s, 3H), 4.22 (dd, 1H, *J*=15.5 and

4.70 Hz), 4.36 (dd, 1H, *J*=15.5 and 3.60 Hz), 6.5 (s, 1H), 6.91 (dd, 1H, *J*=8.80 and 2.40 Hz), 7.12 (d, 1H, *J*=2.30), 7.54-7.36 (m, 6H);

General procedure for ring opening reaction



48 R=H,R'=H
49 R=H,R'=Ph
50 R=OMe, R'=Ph

N R=H,R'=H
O R=H, R'=Ph
P R=OMe, R'=Ph

Procedure 1

To a mixture of epoxide (1.0 eq) in EtOH (heating at 50 °C is soluble) the α-naphtylamine (1.0-4.0 eq) was added and the reaction was heated to reflux overnight. The solvent was evaporated and the products were purified with silica and ionic exchange pre-packed cartridges.

Procedure 2

To a mixsture of epoxide (1.0 eq) in ciclohexane at room temperature the α-naphtylamine (1.20 eq) and $BiCl_3$ (0.10 eq) were added and the reaction was left under stirring for 2-4 hours. The reaction was stopped: $NaHCO_3$ was added and the mixture extracted with DCM and than dried over Na_2SO_4 and concentrated. The products were purified with silica and ionic exchange pre-packed cartridges.

N Yield 55% ^{1}H NMR (200 MHz) CDCl3: δ 7.80 (m, 2H), 7.66 (d, 1H, *J*= 7.8 Hz), 7.50-7.10 (m, 8H), 6.62-6.50 (m, 2H), 4.66 (s, 1H), 4.45-4.24 (m, 3H), 3.51-3.43 (m, 2H), 3.28 (dd, 1H, *J*=12.7 and 6.8 Hz), 2.21 (s, 1H);

O Yield 57% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.04 (dd, 1H, *J*=12.80 and 6.70 Hz), 3.24 (dd, 1H, *J*=12.80 and 3.66 Hz), 4.58-4.26 (m, 3H), 6.60 (s, 1H), 7.30-7.14 (m, 4H), 7.59-7.38 (m, 11H), 7.76-7.64 (m,1H), 7.79-7.74 (m, 1H);

P Yield 57% ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.1 (dd, 1H, *J*=12.60 and 6.71 Hz), 2.26-2.45 (m, 3H), 3.66 (dd, 1H, *J*=12.60 and 3.10 Hz), 3.88 (s, 3H), 6.37 (dd, 1H, *J*=5.67 and 2.90 Hz), 6.52 (s, 1H), 6.92 (dd, 1H, *J*=8.80 and 2.40 Hz), 7.12 (d, 1H, *J*=2.40 Hz); 7.58-7.31 (m,1H), 7.79-7.73 (m, 1H);

Chapter 5

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