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Abstract: Although several research strategies have been developed in the last decades, the current therapeutic options for the treatment of Alzheimer’s disease are limited to three acetylcholinesterase inhibitors: galantamine, donepezil and rivastigmine. However, they have only offered a modest improvement in memory and cognitive function. Moreover, these drugs show side effects, and relatively low bioavailability among other problems. These features limit their use in medicine and they lead to a great demand for discovering new acetylcholinesterase inhibitors. In addition to its important role in cholinergic neurotransmission, acetylcholinesterase also participates in other functions related to neuronal development, differentiation, adhesion and amyloid-\(\beta\) processing. Acetylcholinesterase accelerates amyloid-\(\beta\) aggregation and this effect is sensitive to peripheral anionic site blockers. Both features have lead to the development of dual inhibitors of both catalytic active and peripheral anionic sites. These compounds are promising disease-modifying Alzheimer’s disease drug candidates. On the other hand, due to the pathological complexity of Alzheimer’s disease, multifunctional molecules with two or more complementary biological activities may represent an important advance for the treatment of this disease. All these features are described in detail in the present chapter.

Keywords: Alzheimer’s disease, donepezil, galantamine, huperzine, huprines, infractopicrin, inhibitors AChE, ladostigil, physostigmine derivatives, rivastigmine, tacrine, tacrine hybrid, tacripyrines and donepezil hybrids, TAK-147.

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1. INTRODUCTION

Alzheimer’s disease (AD), the most common type of dementia worldwide, today represents a major public health issue and it is characterized by amyloid-β (Aβ) deposits, τ-protein aggregation, low levels of ACh and oxidative stress, among other events [1]. The first therapeutic strategy for the treatment of AD was mainly centered on the restoration of cholinergic functionality [2, 3]. Most of the AD treatments have been focused on the inhibition of AChE in order to enhance cholinergic neurotransmission by increasing ACh availability in the synaptic cleft. A decrease of ACh in the brain of AD patients appears to be a critical element in producing dementia [4]. Today, an acetylcholinesterase inhibitor (AChEI) is commonly used soon after the AD diagnosis [5].

Actually, the AChEIs approved by the United States Food and Drug Administration (FDA) for the symptomatic treatment of patients with mild or moderate AD include: Donepezil (a benzyl piperidine), Rivastigmine (a carbamate) and Galantamine (a tertiary alkaloid). However, they do not halt the progression of the disease or alter its final outcomes. Clinical experience has shown that AChE inhibition is a viable therapeutic approach to the palliative treatment of AD. One of the most common untoward effects of such therapy is gastrointestinal complaints resulting from stimulation of peripheral autonomic cholinergic system.

An ideal cholinesterase inhibitor (ChEI) should be well tolerated, highly selective in the brain for both the molecular enzyme forms and the specific region (cerebral cortex and hippocampus), minimal effects on the peripheral cholinergic system, and finally no organ toxicity. In addition, further compounds should be tested both in vitro and in vivo in the search for AChE molecular form-specific inhibitors for the treatment of AD [4]. Therefore, there is a great demand today in the medical community for a better cholinesterases inhibitor (ChEI).

A renewed interest in the search of AChEIs appeared when evidences suggested that AChE has an additional role in mediating the aggregation and deposition of Aβ peptide [6].
In the recent years, multifunctional compounds have been the subject of increasing attention by many investigators which have developed a number of compounds acting simultaneously of different target implicated in AD. These drugs could have more therapeutic efficacy than single-target compounds.

2. ACETYLCHOLINESTERASE

Cholinesterases (ChE) are a family of enzymes that catalyzes the hydrolysis of ACh, an essential process allowing for the restoration of the cholinergic neuron. The two types of ChE are: acetylcholinesterase (EC 3.1.1.7) and butyrylcholinesterase (BuChE; EC 3.1.1.8).

Acetylcholinesterase is one of the well-know enzyme, which plays an important role in the central nervous system (CNS). The availability of AChE crystal structures for various species with and without ligands provides a solid basis for structure-based design of novel AChE inhibitors [7].

The AChE structure has been extensively investigated since 1990s. The first experiment with X-ray was carried out on AChE in the electric eel, Torpedo californica (tcAChE), due to its availability [8]. The results obtained have lead to an informal model until the commercialization of human recombinant AChEs [9].

Target enzyme consists of a narrow gorge with two separate ligand binding sites: the catalytic active site (CAS) and the peripheral anionic site (PAS) Fig. (1) [6, 11]. The gorge itself is a narrow hydrophobic channel with a length of about 20Å, connecting the PAS to the active site [8]. It is surrounded by aromatic amino acids enabling a high selectivity for ACh. Substrate penetration is allowed by cation-π interactions between ACh quaternary ammonium atom and π electrons of phenylalanine (F), tryptophan (W) and tyrosine (Y) aromatic cores [12, 13]. These sites are described below.

The AChE catalytic active site is located at the bottom of the gorge and it contains serine (S)-histidine (H)-glutamate (E), the catalytic triad (the same in AChE and BuChE) within an esteratic site. The anionic site (also called α-anionic site)
is another part of the active site, and it is close to the esteratic site, Fig. (1). While the esteratic site hydrolyzes the ester bond, the anionic site interacts with the acetylcholine quaternary ammonium atom and it is responsible for its correct orientation [9].

**Figure 1**: AChE active site with two sites of ligand binding: CAS, at the base of the gorge and PAS near the gorge entrance (This figure is modified of Botti and co-workers) [10].

In Fig. (2) the participation of the catalytic triad in the acetylcholine hydrolysis is shown: the primary alcohol moiety of the serine residue (catalytic triad) participates in a transesterification reaction with ACh, resulting in acetylation of the enzyme. A neighboring group, the imidazol ring (part of a histidine residue) participates and facilitates the acetyl group transfer. The resulting acetylated serine moiety is extremely labile and rapidly undergoes spontaneous hydrolytic cleavage to liberate acetate anion and to regenerate the active catalytic surface [15].

The peripheral anionic site (also called β-anionic site) is located at the active center gorge entry, to a distance of approximately 14 Å from the main active site [16]. Tryptophan, tyrosine, and aspartate (D) amino acids residues are the most significant in the PAS [17]. This PAS encompasses binding sites for allosteric
ligands (activators and inhibitors) [6]. Ligand binding to the PAS affects enzymatic activity through a combination of both by steric blockade of ligands moving through the gorge, and by allosteric alteration of the catalytic triad conformation [18].

Figure 2: Catalytic active site. Electronic displacements reported in the acetylcholine hydrolysis (This figure is modified of Delgado and co-workers) [14].

Guo and co-workers [11] described a receptor-specific scoring function for predicting binding affinities for human AChE inhibitors. This method reported a list of those residues making the most important electrostatic and Van der Waals (VdW) contributions within the main active site, gorge area, acyl binding pocket and PAS.

It is well known that AChE exists in two different forms (with identical active sites): the globular forms, consisting of monomer (G₁), dimer (G₂) and tetramer
(G₄), and the asymmetric forms. In the human brain, the most abundant AChE forms are G₄ and G₁ [19]. A selective loss of the membrane-associated AChE molecular form G₄ has been observed in the AD brain, while the G₁ form is relatively preserved [4].

The AChE’s primary function is the rapid splitting of ACh and thus terminating cholinergic neurotransmission. However, a new noncholinergic role has been discovered for AChE, related to neuronal development, differentiation, adhesion and Aβ peptide processing. The presence of the PAS seems to be fundamental for some of its so called “non-classical action”. This unique structural feature of the enzyme (PAS) may be responsible for the aggregation-promoting action of AChE. The AChE promotes amyloid fibrils assembly in the brain, by binding to Aβ through the PAS. This fact gives stable AChE-Aβ complexes that are more toxic than single Aβ peptides [20]. Amyloid β peptide interacts with the PAS inducing a conformational transition to the amyloidogenic conformation with the subsequent amyloid fibril formation [21], and consequent damage of cholinergic neurons. Several ligands that bind to the PAS have shown to retard aggregation [22]. Prefibrillar oligomers of the Aβ are recognized as potential mediators of AD pathophysiology. AChE is one of the several proteins associated with Aβ aggregation and amyloid plaque deposits. Modulation of Aβ-induced toxicity by increasing its degradation in order to reduce brain Aβ burden would be a rational strategy for the treatment and prevention of AD.

The fact that AChE accelerates Aβ aggregation, and this effect is sensitive to PAS blockers, has led to the development of dual inhibitors of both CAS and PAS. These compounds are promising modifying drug candidates because they can simultaneously improve cognition and slow the rate of Aβ-elicited neurodegeneration [23]. The interest in these dual-site inhibitors has recently emerged.

3. INHIBITORS

Molecular modeling has been often used to design novel enzyme inhibitors. In this way the research of prior AChE inhibitor has focused on using ligand-based design methods. Molecular docking can also be very useful in characterizing ligand-receptor binding by providing predictions of the bound conformation for
the ligand and a scheme for energetically ranking the ligand-receptor interaction [10, 24, 25].

Molecular modeling techniques include: the investigation of AChE-drug complexes, the ligand-binding sites calculation within the active site of the enzyme, the virtual screening toxicophorical analysis, and the estimation of pharmacokinetics properties. The toxicophoric and pharmacokinetic predictions constitute valuable tools helping the continuous search for pharmaceutical interesting molecules with low toxicity and suitable pharmacokinetic profile [26].

On the other hand, x-ray crystallographic analysis of AChE from *Torpedo californica* [8] followed by x-ray determination of the complexes of the enzyme with several structurally diverse inhibitors such as tacrine (THA) [27], donepezil (E2020) [16], galantamine [28, 29] and Huperzine A (Hup A) [30] provides crucial information with respect to orientation of these inhibitors in the active site of the enzyme.

Three classes of acetylcholinesterase inhibitors have been described: reversibles, pseudoirreversibles, and irreversibles [15]. In this chapter, we have only considered the pseudoirreversible and reversible inhibitors. Several inhibitors with synthetic and natural origins are available today in drug market. However, the side effects (due to lack of selectivity) and the relatively low bioavailability, among others factors, limit their use in medicine.

Early inhibition research was mainly focused on ligands binding in the active site. The research was later focused on finding novel ligands that bind to both sites in order to search more potent reversible inhibitors, that selectively favor the inhibition of AChE rather than the related BuChE [11]. Now, inhibition of the PAS can be considered the most promising AD treatment. The deposition of amyloid plaque in AD may be accelerated or even triggered by interaction of β-amyloid with PAS. Therefore, by interfering with PAS, the aggregation and neurotoxic effects of Aβ may be reduced. The PAS inhibitors are considered not only symptomatic drugs for AD, but also probably causative ones [23]. In Table 1 a resume of the most interesting AChEIs included here is shown.
In addition, there are studies indicating that AChEIs have the ability to affect the expression of nicotinic acetylcholine receptors (nAChRs) that play a major role in cognitive functions [31].

### Table 1. Summary of the Most Interesting AChEIs Described in the Present Chapter

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Significant Features</th>
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<tbody>
<tr>
<td><strong>PSEUDOIRREVERSIBLE INHIBITORS</strong></td>
<td></td>
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<tr>
<td>Physostigmine</td>
<td>Prototype of AChE. First drug investigated by the AD treatment. It has been shown to APL of nAChRs. It was discontinued after phase III clinical trials due to side effects.</td>
</tr>
<tr>
<td>Physostigmine Derivatives</td>
<td></td>
</tr>
<tr>
<td>Epastigmine</td>
<td>Less toxic than physostigmine. Adverse hematologic effects lead to suspension of clinical trials.</td>
</tr>
<tr>
<td>Phenserine</td>
<td>Selective AChEI. It is a dual AChE and β-APP inhibitor. The lack of significant efficacy leads to abandon clinical trials.</td>
</tr>
<tr>
<td>Tolserine</td>
<td>A phenserine derivative. Preclinical studies show a highly potent inhibitor of h-AChE compared to physostigmine and phenserine.</td>
</tr>
<tr>
<td>Rivastigmine</td>
<td>It is an inhibitor of both AChE and BuChE. It was approved in 2000 for the treatment of mild-to-moderate AD.</td>
</tr>
<tr>
<td>Ladostigil</td>
<td>It is a propargylamine and rivastigmine derivative, which combines neuroprotective effects with MAO-A and –B and cholinesterase inhibitory activities. Actually in phase IIb clinical trials.</td>
</tr>
<tr>
<td><strong>REVERSIBLE INHIBITORS</strong></td>
<td></td>
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<tr>
<td>Acridines</td>
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<tr>
<td>Tacrine</td>
<td>First AChEI approved in 1993. It has been abandoned because of remarkable side effects, including hematoxicity.</td>
</tr>
<tr>
<td>Bis(7) Tacrine</td>
<td>Dimer of tacrine. Interesting lead compounds.</td>
</tr>
<tr>
<td>Cystamine-tacrine dimer</td>
<td>It has lower toxicity than bis(7) Tacrine. It acts as a radical scavenger. Potentially useful in AD treatment.</td>
</tr>
<tr>
<td>N-benzylpiperidines</td>
<td></td>
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<tr>
<td>Donepezil</td>
<td>Approved in 1996 for the treatment of mild-to-moderate and latterly to severe AD. Selective AChEI. Side effects on the cardiovascular system are still unclear.</td>
</tr>
<tr>
<td>TAK-147</td>
<td>It presents high selectivity for AChE and BuChE, and it acts selectively on the CNS. Actually it is under clinical trials.</td>
</tr>
<tr>
<td>Isoquinoline Alkaloids</td>
<td></td>
</tr>
<tr>
<td>Galantamine</td>
<td>Approved for the treatment of mild-to-moderate AD in 2007. It is an AChEI and APL of the nAChER. It has excellent pharmacological and pharmacokinetics profiles and it exhibits low side effects.</td>
</tr>
<tr>
<td>Indole Alkaloids</td>
<td>They could be useful candidates for investigation as AChEI and as AD-drugs.</td>
</tr>
<tr>
<td>Infractopicrin and 10-hydroxy derivative</td>
<td></td>
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<tr>
<td>Other Alkaloids</td>
<td></td>
</tr>
<tr>
<td>Huperzine A</td>
<td>It has been approved for the symptomatic AD treatment in China. In European Union and USA it is yet in clinical trials.</td>
</tr>
<tr>
<td>Huperzine B</td>
<td>It is less potent than HupA. It exhibits other benefits. It appears to be a promising lead compound.</td>
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Table 1. contd....

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<tr>
<th>Compounds</th>
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<tr>
<td><strong>REVERSIBLE INHIBITORS</strong></td>
<td></td>
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<tr>
<td>Tacrine Hybrid Derivatives</td>
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</tr>
<tr>
<td>Huprines</td>
<td>Highly potent AChEI. Huprine-tacrine heterodimers can be considered very promising compounds.</td>
</tr>
<tr>
<td>Antioxidant hybrids</td>
<td>They have antioxidant and neuroprotective and dual inhibition of AChE and BuChE. They are very interesting multifunctional prototypes.</td>
</tr>
<tr>
<td>- Tacrine-8-hydroxyquinoline</td>
<td>Display ChE inhibition, neuroprotection and less hematotoxicity. Good candidates for the development of novel drugs for AD.</td>
</tr>
<tr>
<td>- Mercapto-tacrine</td>
<td>Interesting in vitro biological activities: inhibition of hAChE and the β-secretase, radical scavenger activity. Actually further studies are being carried out.</td>
</tr>
<tr>
<td>- Tacrine-4-oxo-4H-chromene</td>
<td>One of these significantly inhibited Aβ aggregation induced by AChE and blocked the ROS. It may be a promising multifunctional drug candidate.</td>
</tr>
<tr>
<td>- Tacrine-feluric acid</td>
<td>Potent and selective hAChEI. Protect efficiently against free radicals. They are neuroprotective agents. Interesting new candidates for the treatment of AD</td>
</tr>
<tr>
<td>- Tacripyrines</td>
<td></td>
</tr>
<tr>
<td>Donepezil Hybrids</td>
<td>Highly potent inhibitors human AChE and BuChE compounds. They have a potential disease-modifying role in the AD treatment. They could represent new templates for further optimization studies.</td>
</tr>
<tr>
<td>Hybrids MAO and Cholinesterase Inhibitors</td>
<td>Selective for both AChE inhibitors activity and MAO-A. They can be new, attractive and promising drugs for the AD treatment.</td>
</tr>
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</table>

4. PSEUDOIRREVERSIBLE INHIBITORS

In this group are included agents with a carbamate ester moiety which is hydrolyzed by AChE, but much more slowly than ACh. It should be pointed that in stress conditions [32] they can enhance carbamate diffusion into the CNS.

These compounds interact with OH-serine in the catalytical triad of active site in essentially the same manner as ACh does, providing stable esters. The resulting carbamylated enzyme is much more stable than the acetylated enzyme. The carbamoyl moiety can be split from cholinesterase by spontaneous hydrolysis [33]. From the chemical point of view, these carbamates are in general N-alkyl and N,N-dialkyl derivatives. The natural product is physostigmine.

4.1. Physostigmine

Fig. (3), also known as eserine, is an alkaloid isolated from the seeds of the calabar bean, Physostigmina venenosum. It is the prototype of pseudoirreversible inhibitors of AChE and it has been the first ChEI investigated by the treatment of
AD [15]. This alkaloid exhibits equal inhibitory activity against AChE and BuChE. The inhibition of both cholinesterases is highly enentioselective [34].

![Chemical structure of Physostigmine]

Figure 3: Pseudoirreversible inhibitors: Physostigmine, epastigmine, fenserine, analogs phenserine and carbamate phystostigmine derivative.

There is evidence suggesting that in the AD brain the AChE activity remains unchanged or declined, while the BuChE activity rises in an attempt to modulate ACh levels in the cholinergic neurons. Therefore, both enzymes are likely involved in regulating ACh levels and consequently, they may represent therapeutic targets for the development of agents that, with the ability to inhibit BuChE in addition to AChE, should lead to improved clinical outcomes [35].

Physostigmine has memory enhancing effects in patients with AD [36], because it passes through the blood-brain barrier (BBB). Besides, the physostigmine has
been shown to act as an agonist on nAChRs from muscle and brain, by binding to sites that are distinct from those for the natural transmitter ACh [37]. Allosteric modulators interact with the receptor through binding sites, that they are distinct from those for ACh and for nicotinic agonist and antagonist [38, 39]. Thus, physostigmine is an allosterically potentiating ligand (APL), which may act as a low potency agonist and modulator of the nicotinic receptor [40]. The interest of nicotinic APLs is described in detail in section 5.3.1.

In spite of the first encouraging results, physostigmine was discontinued after the completion of phase III clinical trials for AD, because of an effect too diminutive in duration, variable bioavailability and narrow therapeutic index [41]. These considerations have stimulated the interest in designing new physostigmine-related inhibitors with improved pharmacokinetic properties.

4.2. Physostigmine Derivatives

The removal of the carbamate function in physostigmine has no effect on potency as an APL, but this removal reduces significantly the potency of physostigmine’s AChE inhibition [42]. It is well established that several structural elements concur to determinate AChE inhibitory activity of carbamate derivatives. For instance, the alkyl substituent carbamoyl nitrogen strongly affects the affinity profile [6]. One useful modification to the structure of physostigmine has been the replacement of the methyl group of carbamate moiety by aliphatic alkyl group or phenyl group. It leads to the derivatives: epastigmine and fenserine.

**Epastigmine** Fig. (3) (heptyl-physostigmine tartrate) is a carbamate derivative of physostigmine in which the carbamoylmethyl group has been substituted by a carbamoylheptyl group. This compound is less toxic than physostigmine [43] and it shows greater potency for BuChE [44]. In vitro and ex vivo results suggest that epastigmine has a long-lasting reversible brain ChE (i.e. AChE and BuChE) inhibitory effect. Specially, it preferentially inhibits the G₁ form of AChE [45, 46].

Clinical investigations demonstrated that epastigmine significantly improved cognitive performance. Also, pharmacokinetic studies have revealed that after oral administration it is rapidly distributed to the tissues and readily enters the CNS,
where it can be expected to inhibit AChE for a prolonged period. It is generally well tolerated and the majority of adverse events (cholinergic) are mild to moderate in intensity. Although its cholinergic tolerability was found to be favorable, however the adverse hematologic (granulocytopenia) effects reported in two studies have led to the suspension of further clinical trials [47, 48].

**Phenserine** Fig. (3), phenylcarbamate derivative of physostigmine, is a selective inhibitor of AChE with minimal effect on BuChE [49]. This carbamate is a dual AChE and β-amyloid precursor protein (β-APP) inhibitor being developed to treat mild to moderate AD [50]. It has a quick absorption rate and it is less toxic than physostigmine. Although phase III clinical trials have been conducted during 2003-2004, however, further clinical trials for AD have been abandoned [41].

Results in the short-term study by Winblad and co-workers [51] have shown the phenserine potentially benefiting mild to moderate AD symptomatically, but this derivative has not addressed possible amyloid metabolic mediated effects on disease processes in AD. Therefore, phenserine may represent an important new catalog of compounds for the treatment of AD, because the inhibitors available in the market do not reduce the β-APP level [52].

**Analogs of phenserine** were also synthesized and their cholinesterase inhibition activities were evaluated [52]. These derivatives, Fig. (3), contain an electron-withdrawn substituent in each position of the phenyl group in the phenylcarbamoyl moieties, and they showed less inhibition against AChE and BuChE than phenserine. By contrast, an electron-donor substituent as the methyl/methoxyl groups, maintained or improved their AChE potencies. The results obtained clearly showed that small electron-donor substituents in *meta-* or *ortho-* position of the phenyl ring are better choices for the retention of AChE inhibition potency.

One of these analogs, Tolserine with a 2’-methyl group on the phenylcarbamoyl moiety of phenserine, proved to be a highly potent inhibitor of human AChE (hAChE) compared to physostigmine and phenserine. Preclinical studies were initiated in 2000 and they were shown to be more selective against hAChE than against BuChE [53]. Another compound, the cyclohexylcarbamate derivative
exhibited extremely higher selectivity inhibiting AChE over BuChE, compared with phenserine [54].

Other complex molecules have been synthesized by replaced the pyrroline ring of physostigmine with a dihydropyran ring. Thus, the hexahydropyrrrolo[2,3-b]indole moiety of physostigmine can be replaced by hexahydrochromeno[4,3-b]pyrrole without affecting the affinity for AChE. The carbamate physostigmine derivative, Fig. (3), has been found as potent as physostigmine in vitro against human AChE and BuChE. However, the benzene ring position isomers of the carbamate moiety are much less potent. The position of the carbamate moiety in the phenyl ring has a pivotal role in determining anticholinesterase activity [6, 15].

4.3. Rivastigmine

(ENA-713), Fig. (4), is other carbamate which shows an inhibitory action toward AChE, but less marked than physostigmine. However, it has superior global pharmacological profile, including a good combination of longer duration of action, good tolerability and lower toxicity. Rivastigmine was approved by FDA for the treatment of mild-to-moderate AD in 2000 [6]. Recent investigation aims at developing films capable of delivering the drug in vivo in a sustained manner with reduction in gastrointestinal side adverse effects [55].

In addition rivastigmine is referred as a “brain-region” selective ChEI [56] since it preferentially inhibits AChE and BuChE of hippocampus and cortex [57]. It is stronger inhibitor of AChE G1 than G4 [4], and it is an inhibitor of both AChE and BuChE. This inhibition improves the cognition in elderly patients with late onset AD [58]. Clinical trials have demonstrated that patients treated with this drug have not shown the widespread cortical atrophic changes in parietotemporal regions invariable reported in untreated AD patients, and which were detectable in the subgroups treated with selective acetycholinesterase inhibitors [59, 60]. These findings are consistent with the hypothesis that inhibition of both enzymes may have neuroprotective and disease-modifying effects [61]. However, further longitudinal and long-term studies on this issue are needed.
**Figure 4:** Pseudoirreversible inhibitors: rivastigmine; [1]benzopyrano[4,3-b]pyrrole derivatives and Lagostigil.

### 4.4. Rivastigmine Related Inhibitors

Conformationally restricted analogs of rivastigmine, the [1]benzopyrano[4,3-b]pyrrole derivatives have been synthesized [6] by inserting the dimethylaminoethyl-phenyl moiety of rivastigmine, Fig. (4), in different tricyclic systems related to carbamate physostigmine derivative, Fig. (3).

A superimposition between the conformation of rivastigmine and the carbamate physostigmine derivative \((X=\text{CH}_2; \ R=\text{CH}_3)\), as obtained from Monte Carlo simulations, has been reported. In this study, several low energy conformations of
each molecule were selected and fitted. The overlap was satisfactory with these forms confirming that the tricyclic derivatives might act as rigid analogs of rivastigmine.

This study demonstrated that the potency towards AChE is generally increased in the rigidified \[1\]benzopyran[4,3-b]pyrrole derivatives Fig. (4) as compared to the flexible prototype rivastigmine. In this series of rigid compounds, the most potent inhibitors resulted methyl derivatives.

### 4.5. Ladostigil

(TV 3326), [(N-propargyl-(3R)aminoindan-5yl)-ethyl methyl carbamate] Fig. (4) is another synthetic carbamate, a propargylamine and rivastigmine derivative. It has been synthesized by combining the carbamate moiety of rivastigmine in the aminooindan structure of rasagiline. Rasagiline is one anti-Parkinsonian irreversible, selective monoamine oxidase (MAO-B) inhibitor [62]. Monoamine oxidase MAO is an important target to be considered for the treatment of AD. MAO is an enzyme bounded to mitochondrial outer membrane of neuronal glial and of other cells that exist as two isozymes, MAO-A and MAO-B.

Ladostigil combines neuroprotective effects with MAO-A and –B and cholinesterase inhibitory activities in a single molecule, presently in phase IIb clinical trials for the treatment of AD [63]. It has neuroprotective and antioxidant activities in cellular models at much lower concentrations than those inhibiting AChE. It also prevents both the age-related reduction in cortical AChE activity and the increase of BuChE activity in the hippocampus. A molecule that has such a selective effect on processes associated with aging could provide an ideal treatment against the progression of neurodegeneration in AD [64]. Moreover, recent finding demonstrated that the major metabolite of ladostigil, hydroxyl-1-(R)-aminoindan has also a neuroprotective activity and thus, it may contribute to the over activity of its parent compound [65, 66].

### 5. REVERSIBLE INHIBITORS

The currently most accepted AD therapy is the application of mild and reversible AChEI to restore ACh levels and therefore cholinergic brain activity. Reversible
inhibitors combine with the substrate cation-binding site of the catalytic surface of AChE and thus they deny acetylcholine’s access to this site. These compounds have a short duration of action due to the facile reversibility of their binding [15].

AChE structural complexity accounts for the large diversity of reversible inhibitors which can interact with either the active site, the peripheral site or both, and with the aromatic gorge, making use of distinct sets of interactions [9]. The chemical structures of these inhibitors are very different which fall into several classes.

5.1. Acridines

Cholinesterase inhibitors binding to the $\alpha$-anionic site are a group of chemical compounds containing certain common motives: firstly, these compounds typically contain condensed aromatic cores; and secondly, they should have quaternary ammonium or nitrogen included as a heteroatom. Interesting examples are acridines and tetrahydroacridines.

5.1.1. Tacrine

Fig. (5) (1,2,3,4-tetrahydro-9-acridinamine) has been described in 1961 [67] as a reversible inhibitor of AChE. It binds into the $\alpha$-anionic site and selectively it inhibits the $G_1$ form in the rat brain [4]. Also, THA inhibits BuChE, and it shows greater potency for BuChE as compared with AChE [44].

Tacrine has been the first cholinesterase inhibitor approved by the FDA for the treatment of AD in 1993 [68]. Clinical efficacy in relief of the symptoms of AD has been claimed for THA, but this positive finding is tempered by its tendency to produce hepatotoxicity. Thus, its use has been largely abandoned because of a high incidence of side effects including nausea, vomiting, dizziness, diarrheas, seizures and syncope. The serious hepatotoxicity of THA has been the main limitation for its clinical use [69]. Tacrine can cause reactive oxygen species (ROS) production stimulation and glutathione depletion in the human liver cell. Cytotoxic studies point out that oxidative stress might be involved in THA hepatotoxicity [70].
5.1.2. Tacrine Derivatives

To find less toxic derivatives of tacrine, several compounds have been synthesized. The most interesting are:

1-Hidroxytacrine, Fig. (5) (Velnacrine) was designed in the hope that the OH group performs the glucuronidation and subsequently facilitated elimination. This compound is somewhat less potent against AChEI \textit{in vitro} than the THA [15].

7-Methoxytacrine, Fig. (5) is less toxic than tacrine, and \textit{in vitro} as \textit{in vivo} test it proves to be superior to tacrine [71, 72]. \textbf{N-alkyl-7-methoxytacrine hydrochloride} derivatives are also of interest [73].
Bis(7)tacrine, Fig. (5). Several strategies have been employed to design high affinity dual inhibitors with dimeric structure. Recent studies have demonstrated that its homo- and hetero- dimers can improve and enlarge its biological profile with less side-effects [68]. Bis(7)tacrine is a heptamethylene-linked dimer of tacrine, and it has been designed taking into account that there are two AChE binding sites. It is more potent than THA in inhibition of rat AChE and it is more selective for AChE than for BuChE. The importance of ligand hydrophobicity has been indicated for effective cation-π interaction of the homodimer with PAS [15].

Bis(7)tacrine represents an interesting lead compound to design novel dual binding AChEIs. In this context, Minarini and co-workers [74] focused on the important biological properties of cystamine Fig. (3) as antioxidant, cyto- and neuroprotective agent [75]. These authors replaced the heptamethylene linker of bis(7)tacrine by the structure of cystamine, leading to cystamine-tacrine dimer Fig. (3), which appears to be characterized by a disulfide bridge. This dimer is able to inhibit human AChE, BuChE, self- and AChE-induced Aβ aggregation in the same range of the reference compound. It has lower toxicity than bis(7)tacrine, and it acts as a radical scavenger. All these results allowed us to consider the cystamine-tacrine dimer as potentially useful in AD treatment [74].

5.2. N-Benzylpiperidines

In this group of inhibitors the prototype is the Donepezil compound.

5.2.1. Donepezil

Fig. (6) was approved in 1996 for the treatment of mild-to-moderate AD and latterly it was also approved for severe AD [76]. In addition, Howard and co-workers [77] detected in double-blind, placebo-controlled trial involving patients with moderate or severe AD, significant benefits of continued donepezil therapy with respect to cognitive and functional outcomes over the course of 12 months.

Donepezil, a benzylpiperidine is a selective reversible inhibitor of AChE. Its affinity for this enzyme is greater than for BuChE [44]. The three-dimensional structure of AChE in complex with donepezil shows that donepezil interacts with
both the anionic site in the bottom of the gorge and the PAS near its entrance [78].
Molecular modeling based on the crystal structure has illustrated the complementarity between this class of inhibitors and the narrow gorge of the enzyme [79].

![Donepezil](image1.png)

![TAK-147](image2.png)

**Figure 6:** Reversible inhibitors. N-benzylpiperidines.

Donepezil is well absorbed with a relative oral bioavailability of 100% [80], with good penetration through the BBB and slow excretion [16]. There is some evidence that it may be better tolerated, with less gastrointestinal side effects, than rivastigmine or galantamine [81]. Its cholinergic adverse side effects on the cardiovascular system are still unclear. although a recent study demonstrated that donepezil is not associated with increased negative chromotropic, arrhythmogenic or hypotensive effects for elderly patients with AD [82].

### 5.2.2. TAK-147

Based on a working hypothesis of the enzyme’s active site, Ishiara and co-workers [83] designed a series of benzylamino compounds and they established several structure-activity relationships. As consequence, these studies lead to the discovery of TAK-147 Fig. (6).
Preclinical pharmacological and pharmacokinetic studies have shown that TAK-147 presents high selectivity for AChE and BuChE, and it acts selectively on the central nervous system. Thus, it exerts ameliorating effects on cholinergic deficits without showing excessive peripheral effects. On the basis of these and other results obtained, it is expected that TAK-147 would not only ameliorate the clinical symptoms in AD, but also that it can prevent or slow the progression of the disease [83, 84]. Actually, TAK-147 is under clinical trials as a therapeutic drug for AD.

5.3. Alkaloids

Natural sources provide a variety of structurally distinct and biologically active metabolites. Numerous plants have been used to treat neurodegenerative diseases and different neuropharmacological disorders. Plant extract is one of the major sources for discovery of new compounds with AChE inhibitory activity. We have described below several of the most interesting alkaloids.

5.3.1. Isoquinolines Alkaloids

**Galantamine**, Fig. (7), is the latest anticholinesterase drug used against AD, which has been approved for the treatment of mild-to-moderate AD in 2001 [80]. It is a tertiary alkaloid, having isoquinoline skeleton, found in the bulbs and flowers of the common snowdrop (*Galanthus nivalis*) and of other members of the *Amaryllidaceae* family. *Amaryllidaceae* alkaloids exhibited several types of pharmacological activities including on central-nervous system [85]. The difficulty of isolating galantamine from its natural source hindered its commercial use or even as a starting material for the synthesis of derivatives. Actually, there are several synthesis procedures described and it has been biosynthetically obtained [86].

Galantamine is among the very few drugs that exhibit a dual activity being both an inhibitor of AChE and an allosteric potentiator of the nicotinic response induced by ACh and by competitive agonist [42]. There is much evidence indicating that neural nAChR plays an important role in learning and memory. Moreover, the density/activity of brain nAChR is substantially reduced in AD
patients compared with a control group of the same age [87]. Studies of human brain tissue collected during postmortem [88] and brain imaging studies in living AD patients [89] demonstrated specific loss of nicotinic cholinergic receptors in AD [90]. These abnormalities are closely associated with increased levels of neuritic plaques and neurofibrillary tangles in the AD patients [91]. A means to up-modulate or to potentiate the activity of nicotinic receptors in response to ACh is to use allosterically potentiating ligands. Several problems originated by administration of nicotinic cholinergic agonists can be avoided with allosteric modulators [90].

Figure 7: Reversible inhibitors. Isoquinolines alkaloids.
Compared with conventional AChE inhibitors, galantamine produces relatively less AChE inhibition; it is less potent than physostigmine, tacrine and donepezil, but it has excellent pharmacological and pharmacokinetics profiles and it exhibits low side effects, \textit{i.e.} less toxic \cite{92, 93}.

**Galantamine Derivatives**

Bartolucci and co-workers \cite{29} determined the correct orientation and interactions of galantamine within the active site gorge AChE. The observed binding mode explains the affinities of a series of structural analogs of galantamine and it provides a rational basis for structure-based drug design aimed at developing synthetic analogs of galantamine with improved pharmacological properties.

A docking procedure has also been described which can be applied to produce models of ligand-receptor complexes for AChE and other macromolecular targets of drug design. In this study a galantamine derivative was included, which has a N-propylpiperidine substituent at the nitrogen atom instead of a methyl group. A molecular model of the complex between tcAChE and this galantamine derivative has been reported. The side chain of this ligand is predicted to extend along the enzyme active site gorge from the anionic site, at the bottom, to the peripheral anionic site, at the top \cite{94}.

Therefore, structure-activity relationship (SAR) studies reveal that substitution on the nitrogen atom of galantamine is favorable for AChE inhibitory activity. May be these substituents display interactions with the PAS. Thus, derivatives that contain a long substituent on the nitrogen are of particular importance. Such structures potentially span the whole binding cavity of tcAChE and they interact with several amino acids at the PAS, which should lead to increased potency of AChE inhibition \cite{94}.

Jia and co-workers \cite{95} developed a new series of galantamine derivatives capable of interacting with both, the active and the peripheral sites of AChE. They have designed, synthesized and evaluated as AChE inhibitors several \textbf{N-substituted galantamine derivatives} Fig. (7) by selecting benzyl-amino groups and modified benzyl-amino moieties as pharmacophoric units and by
incorporating them into the galantamine molecule. Besides, different lengths of the alkyl chain between galantamine and benzylamino moieties have been also explored.

Structure-activity studies showed that the potency of AChE inhibition has been mainly influenced by the function at the end of the linker, as well as, the length of the connecting units. Especially, the incorporation of a phenyl ring between the alkyl chain and the terminal nitrogen-containing moieties provided additional sites of interactions between the inhibitor and the enzyme. These results suggested that the benzyl-piperidine moiety might be a potent segment for fishing the PAS. This group binds better to the PAS of AChE than to the other moieties, which could be used in the development of novel bivalent ligands. The results of this study provided a basis for future design and development of bivalent AChE inhibitors [95].

Galantamine derivatives are predicted to improve interactions with AChE, in particular with the PAS. Several N-alkyl-phenyl substituents appear as favorable since the PAS is predominantly composed of hydrophobic aromatic residues. In addition, virtual-screening simulations have been used to select novel inhibitor candidates containing different structural scaffolds in order to find novel structural patterns with potential AChE inhibitory activity [26].

**Montamine**

After the discovery of galantamine, the isolation and characterization of alkaloids from *Amaryllidaceae* have increased in order to find better AChEIs. Pagliosa and co-workers [96] studied the activity of isoquinolines alkaloids isolated from *Hippeastrum* species (*Amaryllidaceae*). One of them, montamine Fig. (5), significantly inhibits AChE activity, although this alkaloid requires further investigations.

**5.3.2. Indole Alkaloids**

Indole-containing compounds play a role in diverse pharmacological actions. Various indole-structures have been discovered from plants extract and they have showed good AChE inhibitory activity. A natural source for new drugs is the
fungal fruiting bodies (macromycetes). Several groups of macromycetes yield diversity of bioactive secondary metabolites and they constitute a valuable complementary source for novel lead compounds.

Geissler and co-workers [97] studied two indole alkaloids, infractopicrin and 10-hydroxy-infractopicrin, Fig. (8), isolated from fruiting bodies of *Cortinarius infractus* Berk (*Cortinariaceae*). These alkaloids have shown inhibitory activity against AChE whilst having low cytotoxicity, and also they possess a higher selectivity than galantamine. Docking studies suggest possible reasons for the selectivity to the AChE active site versus BuChE, such as the lacking of \( \pi-\pi \) interactions in BuChE. Also *in vitro* fibril formation is positively influenced viz. reduced formation. These results, as well as the positive pharmacokinetic properties, suggest that these alkaloids could be useful candidates for further investigation as AChEI and as AD-drugs.

Recently, the AChE inhibitory activity of quinoline (biostere of the indole ring) derivatives Fig. (8) and \( \beta \)-carboline derivatives Fig. (8) has been studied to understand the SAR of indole structure. The simple indoles with substitutions of electron donating (methoxy and hydroxy) and electron withdrawing (carboxyl) groups on the benzene ring show a low percent of inhibitory activity in tcAChE. It implies that the structures might be too small and they are not appropriate to fit within AChE binding pocket. Adding a side chain at the pyrrole ring, \( \beta \)-carbolines and quinolines improves the inhibitory activity significantly. Both, \( \beta \)-carboline and quinoline analogs bound in the same binding site with some slightly overlapped structure. These two compound types could be used for developing AChE inhibitors in the future [98].

### 5.3.3. Other Alkaloids

**Huperzine A**, Fig. (9) is an alkaloid isolated from the *Huperzia serrata*. This plant has been used in Chinese traditional medicine against memory deficits, contusions, strains, swellings, *etc.* since ages [99]. The natural compound is a strong AChE inhibitor, preferentially the G4 form [4], binding to the PAS [9]. It is three times as potent as physostigmine against AChE but it is less potent against BuChE [100]. In addition, other effects of neurodegenerative disorders have been
studied such as link between huperzine and oxidative stress [101, 102] and mechanisms of neuroprotection by huperzine A [103].

Figure 8: Reversible inhibitors. Indole alkaloids.

Huperzine A is the most-promising drug candidate with potent anticholinesterase effect. Clinical trials at phase IV in China showed that this alkaloid significantly improved memory shortages in aged people with benign senescent forgetfulness and in patients with AD or vascular dementia, with minimal peripheral cholinergic side effects and no unexpected toxicity [104]. It has been approved as a new drug for the symptomatic treatment of AD in China [105]. However, in the European Union and United States it is yet in clinical trials [106].
Total synthesis of the compound has been achieved [107], although the synthetic racemic mixture of huperzine A has less AChE inhibitory effects than the natural kind [108]. Several analogs and derivatives of huperzine A have been prepared and tested for their inhibitory activities towards AChE. Many of these derivatives demonstrated lower potency than the natural Hup A [109].

Other interesting derivative is bis(12)-hupyridone (B12H), a novel dimeric AChE inhibitor derived from a naturally occurring monomeric analog Hup A, which was investigated in vitro and in vivo [110]. Compared with Hup A, B12H has twice the potency in inhibiting rat brain AChE in vitro. Moreover, B12H shows other interesting biological activities. Although more experiments are needed, the authors indicate that B12H might provide greater therapeutic efficacy for the treatment of AD, and it might exert neuroprotection via acting on multiple targets [110].
Huperzine B

Other alkaloid isolated from the *Huperzia serrata* is huperzine B (Hup B), Fig. (9) an effective and reversible inhibitor of AChE. The inhibitory activity of Hup B is less potent than Hup A. However, Hup B exhibits a higher therapeutic index and other benefits. Thus, it appears to be a promising lead compound for developing novel multifunctional AChE inhibitors.

Shi and co-workers [111] described the synthesis, inhibitory activities and preliminary pharmacological results of 16-substituted bifunctional Hup B derivatives, which are derived from the 16-methyl group. Their main structural characteristic is that Hup B moiety from the 16-position is connected through a tether chain with a terminal aromatic ring. These derivatives are more potent inhibitors for both, AChE and BuChE, than the parent Hup B. Preliminary pharmacological evaluation indicated that 16-substituted derivatives of Hup B are potential new drug candidates for AD treatment, and further exploration is needed to evaluate their pharmacological and clinical efficacies [111].

5.4. Hybrids

Anticholinesterase drugs such as rivastigmine, donepezil and galantamine, have shown only a modest improvement in memory and cognitive function. Due to the pathological complexity of AD, it is unlikely that a unitary mechanism of action will provide a comprehensive therapeutic approach to such multifaceted neurodegenerative disease [112]. Efficient therapy is more likely to be achieved by drugs that incorporate several pharmacological effects into a single chemical entity. Thus, molecules with two or more complementary biological activities may represent an important advance in the treatment of the disease [113].

This novel therapeutic strategy in which drug candidates are designed to possess diverse pharmacological properties and to act on multitude targets has led recently to the discovery of several anti-AD drug candidates such as ladostigil, mentioned above in section 4.5, and the hybrid derivatives, that they are described below.
5.4.1. Tacrine Hybrid Derivatives

Tacrine has been extensively studied, being the first marketed product. Today, it remains as a reference structure even if the adverse troubles induced, such as hepatotoxicity and gastrointestinal disorders led to its withdrawal from the marked. The structure of tacrine has been widely used as scaffold to provide new molecules endowed with additional properties beyond simple AChE inhibition. Several tacrine hybrids of interest are described below:

**Huprines**

Huprines are hybrid molecules of tacrine and Hup A and they have been described as highly potent AChE inhibitors. Synthesis, SAR, and docking calculations of several huprines derivatives have been reported based on recombinant human AChE inhibitory activity [114].

One of these hybrids, huprine X (HX), Fig. (10) binds to AChE with high affinity. It has been shown to tightly bind to the active site [115] and it has also been suggested to interact with the PAS [116]. HX inhibits the amyloidogenic process induced by AChE *in vitro*, and moreover, it presents agonist activity on muscarinic acetylcholine receptors (mAChRs) [117] and potential allosteric activity on nAChRs [118].

While *in vitro* pharmacological characterization of HX is relatively complete, its *in vivo* effects are needed to be investigated. Thus, Hedberg and co-workers [119] studied whether HX could affect the AD-related neuropathology *in vivo* in two mouse models. Their results provide further evidence that drugs targeting AChE affect some of the fundamental processes that contribute to neurodegeneration, but whether HX might act in a disease-modifying manner in AD patients remains to be proven.

A type of huprine derivatives heterodimeric, Fig. (10), consists of: (i) a unit of racemic or enantiopure huprine Y Fig. (10), with high-affinity reversible AChEI [116]; (ii) a unit of tacrine Fig. (3), which is a known AChEI and with reported affinity for both the active and PAS of AChE [120], or for 6-chlorotacrine, and
(iii) a linker between (i) and (ii) of suitable length, an oligomethylene chain of 6-10 methylene groups or a 4-methyl-4-aza-heptamethylene chain.

**Figure 10:** Reversible inhibitors. Tacrinde hybrid derivatives.

These heterodimers have been designed to simultaneously interact with both the active and peripheral sites of AChE, and in some cases also with the aromatic residues at the midgorge of the enzyme. Their dual site binding for AChE, supported by kinetic and molecular modeling studies, gives rise to a very potent inhibition of the catalytic activity of human AChE, and moreover, to an *in vitro*
neutralization of the effect of this enzyme towards Aβ aggregation. These compounds are also able to cross the BBB, as predicted in an artificial membrane model assay. Overall, huprine-tacrine heterodimers can be considered very promising lead compounds for AD [121, 122].

**Tacrine-Antioxidant Hybrids**

Currently, the synthesis of multifunctional compounds that combine neuroprotective effects has a special interest. In this way, a family of tacrine-antioxidant hybrids has shown hepatoprotective properties [123]. The tacrine-induced oxidative stress can be prevented by treating hepatocytes with a free radical scavenger such as vitamin E [124]. Thus, tacrine derivatives endowed with additional antioxidant properties might be beneficial by reducing its toxicity.

It has been demonstrated that oxidative damage is an event that precedes the appearance of pathological hallmarks of the AD, namely, amyloid plaques and neurofibrillary tangles [125]. During aging, the endogenous antioxidant protection system progressively decays and may be further diminished in AD. Drugs that specifically scavenger oxygen radical could be useful for either the prevention or the treatment of AD [126]. The development of tacrine derivatives endowed with additional antioxidant properties is an active field in the current AD research. Several selected compounds of this type are:

**Tacrine-8-hydroxyquinoline Hybrids**

New multiactive neuroprotectants with antioxidant, metal-binding properties and dual inhibition of AChE and BuChE in a simple molecule, tacrine-8-hydroxyquinoline hybrids, Fig. (11), have been synthesized by using moieties with well-known properties for each biological activity, such as: (i) tacrine for the inhibition of ChE through its binding to the CAS; (ii) 8-hydroxyquinoline derivative (PBT2) for its metal-chelating, neuroprotective and antioxidant properties [127] as well as for its potential interaction with the PAS; and (iii) the binding of tacrine and quinoline fragments with chains of different lengths (from 6 to 12 carbons) or with a triamine skeleton. These flexible linkers could be lodged by the enzyme cavity, allowing simultaneous interaction between the heteroaromatic fragments and both, the CAS and PAS of AChE.
**Figure 11:** Reversible inhibitors. Tacrine-antioxidant hybrids and nimodipine.
It is noted that PBT2 inhibits the redox-dependent formation of toxic soluble oligomers of Aβ, prevents brain Aβ deposition, and promotes its clearance [128]. Recently, PBT2 has entered in phase IIb clinical test for AD showing promised results [129].

These tacrine-8-hydroxyquinoline hybrids display interesting \textit{in vitro} biological activities for the treatment of AD: (a) they are more potent inhibitors of human AChE and BuChE than the parent fragment tacrine; (b) they show better antioxidant properties than the aromatic portion of vitamin E, responsible for radical capture which displays neuroprotective properties against mitochondrial free radicals and; (c) they displace the PAS-specific ligand propidium from the PAS of the AChE and thus, these hybrids could be able to inhibit Aβ aggregation promoted by AChE.

Such biological properties, along with their ability to reach therapeutic targets in CNS, highlight these tacrine-PBT2 hybrids as very interesting multifunctional prototypes in the search for new disease-modifying drugs useful in the treatment of AD [130].

Recently, Antequera and co-workers [131] evaluated the efficacy of a novel tacrine-8-hydroxyquinoline hybrid, named IQM-622 Fig. (11), using \textit{in vitro} and \textit{in vivo} models. This study demonstrates the following: (a) IQM-622 has neuroprotective effects in neuronal and astrocytic cell cultures; (b) it has both antiamyloid and neuroprotective effects in a mouse model and; (c) It has proved to be a potent inhibitor of human AChE and BuChE. Moreover, IQM-622 has shown to have interesting \textit{in vitro} biological activities for the treatment of AD including cholinergic, antioxidant, copper-complexing and neuroprotective properties. The results of this study suggest that IQM-622 holds potential for the treatment of AD-associated brain damage.

\textbf{Mercapto-tacrine Hybrids}

Chemical entities containing mercapto group have attracted our interest because of their multiple pharmacological actions. Due to some exogenous sulfhydryl compounds could maintain the level of intracellular glutathione, the major
antioxidant in nerve cells when exposed to the ROS insults, they may also lead to neuroprotection in the CNS [132].

In this context, it has been proposed that tacrine derivatives cooperating with mercapto group in a single molecular entity may work in a synergistic manner. Thus, a series of mercapto-tacrine derivatives Fig. (11) has been designed and synthesized. These compounds displayed a synergistic pharmacological profile of long-term potentiation enhancement, ChE inhibition, neuroprotection and less hepatotoxicity. It is expected that these multifunctional compounds are more efficient to improve the memory and cognitive impairment, with less side effect, and to diminish the oxidative damage caused by free radicals. They might be good candidates for further studies directed to the development of novel drugs for AD [133].

Tacrine-4-oxo-4H-chromene Hybrids

By using fragments endowed with interesting and complementary properties for the treatments of AD, a new family of tacrine-4-oxo-4H-chromene hybrids Fig. (11) has been designed, synthesized and evaluated biologically. The flavonoid scaffold derived from 4-oxo-4H-chromene has been chosen for its antioxidant action among other activities, as well as for its potential interaction with AChE-PAS due to its aromatic character.

Flavonoids have attracted much attention in recent years because they can limit the neurodegeneration associated with a variety of neurological disorders [134]. Flavonoids mediate their effects by several routes, including their capacity to scavenge neurotoxic species, such as free radicals [135].

Among the possible structural modifications on the tacrine fragment, Fernández-Bachiller and co-workers inserted one or two chlorine atoms to study possible effects on ChE inhibition. To reach radical capture capacity, they envisaged introducing one or two phenol groups to the 4-oxo-4H-chromene fragments. In addition, they considered connecting tacrine and 4-oxo-4H-chromene fragments by alkylenediamine tethers of different lengths. The flexible linkers used could be lodged in the narrow enzymatic cavity, allowing simultaneous interaction of both the CAS and PAS of AChE.
These new hybrids showed interesting \textit{in vitro} biological activities for the potential treatment of AD, such as inhibition of the human AChE, BuChE (being more potent than the parent inhibitor, tacrine) and the β-secretase (BACE-1), as well as radical scavenger activity, and they could be able to penetrate into the CNS. Actually, further studies are being carried out [136].

\textbf{Tacrine-Feluric Acid Hybrids}

Feluric acid (4-hydroxy-3-methoxycinnamic acid), a bioactive component of traditional chinese medicine, has several properties such as antioxidant and anti-inflammatory effects, inhibition of Aβ fibril aggregation and hepatoprotective effects. Pi and co-workers [137] synthesized a series of hybrid compounds by linking feluric acid to tacrine as multifunctional agent. One of these hybrids, tacrine-6-feluric acid (T6FA), Fig. (11) has been recently evaluated. \textit{In vitro} results demonstrated that it significantly inhibited Aβ aggregation induced by AChE and it blocked the cell death and the intracellular ROS accumulation. T6FA may be a promising multifunctional drug candidate for AD.

\textbf{Tacripyrines}

Tacripyrines have been designed by combining an AChEI, tacrine, with a calcium antagonist such as nimodipine Fig. (11). These compounds are targeted to develop multitarget therapeutic strategy to confront AD. Because 1,4-dihydropyridines (DHPs) are compounds that selectively block L-type voltage-dependent Ca$^{+2}$ channel (VDCC), hybrid molecules that combine an AChEI and DHP, such as tacrine and nimodipine, might represent a promising approach to the AD treatment. This strategy is based on the fact that bis(7)tacrine Fig. (5) attenuates β-amyloid neuronal apoptosis by regulating L-type calcium channels [138].

Marco-Contelles and co-workers [139] have synthesized and evaluated a series of tacrine-DHP hybrids, named tacripyrines Fig. (11) that are potent and selective inhibitors of hAChE. These compounds have also been devoid of human BuChE inhibition activity, showing therefore, an extremely high selectivity. Besides inhibition of AChE and blockade of VDCC, tacripyrines protect much more efficiently against free radicals than the parent compounds, tacrine and
nimodipine, that have not shown any neuroprotective effect. Tacripyrines are neuroprotective agents, also they show moderate Ca\(^{2+}\) channel blocking effect and they cross the BBB. Therefore, they can be considered as interesting new chemical entity candidates for AD treating [139]. New tacripyrines are being currently studied [140].

### 5.4.2. Donepezil Hybrids

A new series of donepezil-tacrine hybrids related derivatives Fig. (12a) has been synthesized as dual AChEIs, that could bind simultaneously to the peripheral and to the catalytic sites of the enzyme. The molecular structure of these hybrids contains: (i) the tacrine heterocyclic ring or 6-chlorotacrine, which is recognized as a catalytic site AChEI, (ii) the indanone ring (the heterocyclic present in donepezil) or the related heterocyclic, the phthalimide moiety, as responsible for the binding to the PAS of the enzyme, and (iii) the linker connecting (i) and (ii) through a different tether length.

Biological activity and molecular modeling studies have been performed in these compounds to explore their binding to the enzyme. Thus, it has been suggested that the phthalimide moiety acts as an efficient ligand for the PAS. As it has been found for the AChE inhibition, the best binding to the PAS of AChE corresponds to a tether length between the two anchoring groups (9-aminoacridine and phthalimide) of nine units [141].

The synthesis, pharmacological evaluation (AChE and BuChE inhibition and Aβ-antiaggregating effect) and molecular modeling of other class of highly potent donepezil-tacrine hybrids have also been described, Fig (12b). On the basis of the binding modes of donepezil [78] and tacrine [27] within tcAChE, these novel hybrids have been designed by combining the 5,6-dimethoxy-2-[(4-piperidinyl) methyl]-1-indanone moiety of donepezil with tacrine.

All these new compounds are highly potent inhibitors of bovine and human AChE and BuChE. The most potent AChEIs are those bearing an indanone system, a chloride atom at the tacrine unit and, a tether length of three methylenes. These
results suggest that the novel donepezil-tacrine hybrids herein reported may have a potential disease-modifying role in the treatment of AD [142].

**Figure 12**: Reversible inhibitors. Donepezil hybrids and AP2238.
Other Donepezil Hybrids

A series of hybrid compounds structurally derived from donepezil and AP2238 has been synthesized. AP2238, Fig. (12), has been designed to bind both anionic sites of human AChE for which the simultaneous inhibition of the catalytic and the Aβ pro-aggregating activities of AChE has been verified. The potency of AP2238 against AChE is comparable to that of donepezil, while its ability to contrast Aβ aggregation is higher. Docking studies of AP2238 at the hAChE gorge have shown several interactions [143]. Thus, the indanone core from donepezil is linked to the phenyl-N-methylbenzylamino moiety from AP2238 through a double bond. With this double bond the evaluation of the decreasing linker flexibility in the biological activities is possible.

SAR studies have been performed to evaluate the role of different substituents in the position 5 or 6 of the indanone ring in its interaction with the PAS, as well as of different alkyl chains of several lengths carrying diverse amines at one end. Furthermore, the indanone ring itself has been replaced by a tetralone scaffold. Two compounds proved to be the most active within the series and their potency against AChE in the same order of magnitude of the reference compounds. These compounds together with other derivatives, with a 5-carbon alkyl chain bearing an amino moiety at one end, contact in satisfactory way to the PAS. This binding remarkably improves the inhibition of AChE-induced Aβ aggregation with respect to the reference compounds. They have also shown activity against self-aggregation of Aβ42 peptide, while the reference compounds resulted ineffective. These three compounds mentioned could represent new templates for further optimization studies [80].

5.4.3. Hybrids MAO and Cholinesterase Inhibitors

Many research groups have developed a number of compounds acting simultaneously on different receptors implicated in AD. In this context, Samadi and co-workers [144] have designed new multipotent MAO and ChE inhibitors for the potential treatment of AD. They reported the synthesis and pharmacological evaluation of hybrids from donepezil and PF9601N, Fig. (13).
Last one is a well known MAO inhibitor [145], bearing N-benzyl piperidine and propargylamine moieties attached to a central pyridine or naphthyridine ring.

**Figure 13:** Reversible inhibitors. Hybrids MAO and ChEIs.
The compounds $h$ ($R_1=H; R_2=H; n=2$) and particularly $k$, Fig. (13), have shown a strong and selective AChE inhibitory activity and moderate, but selective MAO-A inhibitory profile. The authors conclude that the most sensitive moiety to modulate AChE inhibition is the length of the spacer, which would control the dual interaction of these molecules with both CAS and PAS sites, improving inhibition when both binding sites are spatially targeted at the same time [144].

In a latter communication, Samadi and co-workers [144] reported the synthesis, pharmacological evaluation and molecular modeling of heterocyclic substituted alkyl and cycloalkyl propargylamine type I and type II, Fig. (13), that were designed as multipotent inhibitors able to simultaneously inhibit MAO and ChE. Molecules type II are the result of a conjunctive approach that combines for the first time the structure of tacrine with the N-propargylamine moiety present in PF9601N.

In molecules type II the most attractive derivative within this series is that with $X=N$ and $Y=NC_5H_{10}$, which is a non-competitive inhibitor of tcAChE, that it preferentially binds the PAS of AChE. Molecular docking calculations reveal that the ability of this derivative to inhibit AChE is due to the cumulative effects of hydrogen bonds, $\pi$-$\pi$ interactions and hydrophobic interactions. Amino acid residues in different sub-sites are engaged to stabilize the docked complex. This compound is active on hAChE and it is able to weakly inhibit the pro-aggregating action exerted by hAChE on amyloid. Thus, it may also be considered for further development aimed at enlarging its biological activity.

The authors suggested that a hybrid molecule resulting from the juxtaposition of compounds of these series targeting an equipotent cholinesterase inhibition capacity, would possibly afford new attractive and promising drugs for the treatment of AD [146].

**CONCLUDING REMARKS**

The development of effective drugs against AD is an acute clinical need. The difficult task today of developing newer AChEIs is that they will need to be more effective than those actually FDA approved.
The search for AChEIs bind to its CAS and PAS has become an area of very active research. Particularly attractive is the multi-target-directed ligand, approach based on the “one-molecule, multiple-target” paradigm. This innovative strategy has provided a number of compounds acting simultaneously on different receptors and enzymatic systems implicated in AD.

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CONFLICT OF INTEREST

The authors confirm that this chapter contents have no conflict of interest.

ABBREVIATIONS

Aβ = amyloid-β
ACh = acetylcholine
AChE = acetylcholinesterase
AChEI = acetylcholinesterase inhibitor
AD = Alzheimer’s disease
APLs = allosterically potentiating ligands
BBB = blood-brain barrier
B12H = bis(12)-hupyridone
β-APP = β-amyloid precursor protein
BuChE = butyrylcholinesterase
CAS = catalytic active site
ChE = cholinesterases
ChEI = cholinesterases inhibitor
CNS = central nervous system
D = aspartate
DHPs = 1,4-dihydropyridines
E = glutamate
E2020 = donepezil
F = phenylalanine
FDA = Food and Drug Administration
G₁ = globular monomer form acetylcholinesterase
G₂ = globular dimer form acetylcholinesterase
G₄ = globular tetramer form acetylcholinesterase
H = histidine
hAChE = human acetylcholinesterase
Hup A = huperzine A
Hup B = huperzine B
HX = hupryne X
mAChRs = muscarinic acetylcholine receptors
MAO = monoamine oxidase
MAO-A = monoamine oxidase isozyme A
MAO-B  =  monoamine oxidase isozyme B
nAChRs  =  nicotinic acetylcholine receptors
PAS     =  peripheral anionic site
PBT2    =  8-hydroxyquinoline derivative
ROS     =  reactive oxygen species
S       =  serine
SAR     =  structure-activity relationship
tcAChE  =  *torpedo californica* acetylcholinesterase
T6FA    =  tacrine-6-feluric acid
THA     =  tacrine
VDCC    =  voltage-dependent Ca$^{+2}$ channel
W       =  tryptophan
Y       =  tyrosine

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Research Strategies Developed


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