Recent Development of Multifunctional Agents as Potential Drug Candidates for the Treatment of Alzheimer’s Disease

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Abstract: Alzheimer’s disease (AD) is a complex and progressive neurodegenerative disorder. The available therapy is limited to the symptomatic treatment and its efficacy remains unsatisfactory. In view of the prevalence and expected increase in the incidence of AD, the development of an effective therapy is crucial for public health. Due to the multifactorial aetiology of this disease, the multi-target-directed ligand (MTDL) approach is a promising method in search for new drugs for AD. This review updates information on the development of multifunctional potential anti-AD agents published within the last three years. The majority of the recently reported structures are acetylcholinesterase inhibitors, often endowed with some additional properties. These properties enrich the pharmacological profile of the compounds giving hope for not only symptomatic but also causal treatment of the disease. Among these advantageous properties, the most often reported are an amyloid-β anti-aggregation activity, inhibition of β-secretase and monoamine oxidase, an antioxidant and metal chelating activity, NO-releasing ability and interaction with cannabinoid, NMDA or histamine H3 receptors. The majority of novel molecules possess heterodimeric structures, able to interact with multiple targets by combining different pharmacophores, original or derived from natural products or existing therapeutics (tacrine, donepezil, galantamine, memantine). Among the described compounds, several seem to be promising drug candidates, while others may serve as a valuable inspiration in the search for new effective therapies for AD.

Keywords: Alzheimer’s disease, antioxidants, β-amyloid anti-aggregation properties, cholinesterase inhibitors, inhibitors of β-secretase, inhibitors of monoamine oxidase A/B, multi-target-directed ligands, neuroprotective properties.

INTRODUCTION

Alzheimer’s disease (AD), the most common form of dementia, is a progressive neurodegenerative brain disorder resulting in loss of memory and cognitive functions, often accompanied by behavioural disturbances like aggression and depression [1]. Although the disease is multifactorial and heterogeneous, it has certain common hallmarks, namely a massive loss of cholinergic neurons, deposition of neurofibrillary tangles and beta-amyloid (Aβ) aggregates [2]. The disease is age related and it affects about 6% of the population over the age of 65. Worldwide, it is estimated that there are about 35 million people suffering from AD. The incidence of AD is predicted to rise significantly in the next three decades, as the average age of the population increases [3, 4]. At present, there is no efficacious treatment available that allows the recovery or even slow the progression of the disease, therefore, effective therapeutics are needed. Over 20 years ago (in 1993), the first drug, tacrine (Cognex®) was approved by the U.S. FDA for the treatment of AD (Fig. 1). As an acetylcholinesterase (AChE) inhibitor, it was introduced for clinical use, based on the cholinergic hypothesis of AD. This hypothesis assumes that in AD the level of acetylcholine (ACh) is reduced due to the loss of the cholinergic neurons and decreased synthesis of ACh [5, 6]. Due to its hepatotoxicity, tacrine was soon withdrawn from the pharmaceutical market, however three other AChE inhibitors were approved as anti-AD drugs: rivastigmine, donepezil and galantamine (Fig. 1). These drugs also possess additional properties, although their importance is unknown at present. Rivastigmine is able to block butyrylcholinesterase (BuChE), while galantamine modulates nicotinic acetylcholine receptors [7]. Donepezil is a moderate inhibitor of Aβ self-aggregation and β-secretase (BACE1) responsible for the synthesis of Aβ. Donepezil also interacts with sigma-1 receptors, known for their anti-amnesic activity [8]. The current standard of AD treatment recommends combination of AChE inhibitors with memantine [9, 10]. Memantine is an N-methyl-D-aspartic acid (NMDA) receptor antagonist, which protects neuronal cells and reduces excitotoxicity by blocking pathologic stimulation of NMDA receptors (Fig. 1). The available therapy is considered as a short-term intervention only for the symptomatic treatment leading to a
temporary slowdown of the loss of cognitive functions [11].

![Fig. (1). Drugs approved for the treatment of AD.](image)

Although AD pathogenesis is complex and remains unclear, a large number of biological targets for potential therapeutics have been identified [12, 13]. The main targets in current AD research include: Aβ protein, tau protein, receptors (cholinergic, glutamatergic, serotonergic, dopaminergic, noradrenergic, histaminergic), enzymes (AChE, BuChE, α-, β- and γ-secretase, monoamine oxidase A (MAO-A), monoamine oxidase B (MAO-B)). There is also a number of processes involved in the pathomechanism of AD which are considered as promising directions in the search for AD treatment. The most important are excitotoxicity, oxidative stress, calcium and metal dyshomeostasis, neuroinflammation and mitochondrial damage. According to Gril and Cummings [14], current therapeutic targets for the treatment of AD have been classified into two groups: symptomatic and disease-modifying. It is worth noting, that such classification is too simplistic, and some of the targets are involved in different mechanisms. The symptomatic therapeutic targets include mostly receptors whereas the disease-modifying targets are closely connected to Aβ, tau production and neuroprotection [15, 16].

Over the years, extensive scientific research has focused on potential disease-modifying therapies for AD [17-19].

The role of medicinal chemistry is to design, synthesize and develop novel bioactive molecules, and for this purpose different drug discovery strategies may be applied. The drug discovery paradigm, “one-target, one-drug, one-disease” approach dominates in the pharmaceutical industry. However, during last decade, new paradigm, so called multitarget directed ligand (MTDL), called also designed multiple ligands (DMLs) or multiple ligand strategy (MLS) has been developed as innovative approach dedicated for complex diseases [20-24]. It arises from an observation that some well-known drugs with good clinical efficacy are promiscuous molecules able to interact with more than one target. On the other hand, it has been observed that selective ligands with high specificity for a single target, often lacked clinical efficacy. MTDL approach may be achieved either by connecting different molecules endowed with high potency for different targets or a single agent able to modulate multiple targets simultaneously [25-29]. Complexity of AD and a lack of effective treatment of the disease prompted many research teams to search for multiple ligands.

Over the years, many potential multifunctional agents for the treatment of age-related neurological disorders have been developed [30-36]. The MTDLs for AD are a combination of pharmacophores interacting with symptomatic and/or disease-modifying targets. The selected examples of the most interesting or representative ligands are presented below. The largest group of these agents comprises dual binding site cholinesterase inhibitors often with additional properties such as Aβ anti-aggregating activity [34-39], neuroprotective and antioxidant activity [40, 41], calcium channel blocking [42, 43], cannabinoid CB1 receptor antagonism [44], BACE-1 inhibition [45, 46], histamine H1 receptor antagonism [47, 48], NMDA receptor channel blocking [49, 50], serotonin 5-HT3 receptor antagonism [51], serotonin transporter inhibition [52]. Other examples of dual-acting ligands are MAO-B inhibitors with iron-chelating agents [53], metal chelators with BACE-1 inhibitors [54], metal chelators with antioxidants [55, 56] and modulators of γ-secretase with PPARγ activities [57]. Most of these multifunctional ligands have been shown to display biological activity in vitro and require verification in animal models. However, several compounds like bis(7)-tacrine [58], ladostigil [59] and memoquin [60] (Fig. 2) showed promising activity in vivo and in preclinical or even clinical studies.

The purpose of this review is to update the most recent reports on the development of multifunctional agents as potential drug candidates for the treatment of AD. The topic is very attractive for both the academia and the industry, therefore the number of original papers published each year is increasing. Moreover, several review papers have been presented during the last few years [61, 62] including our one published in November 2011 [63]. This review will focus on recent disclosures of multifunctional compounds from the medicinal chemistry point of view published within the last three years. Multifunctional ligands are classified based on the biological targets, then chemical leads and their modifications. Biological properties of these ligands are presented and their structure-activity relationship (SAR) is discussed.

**CHOLINESTERASE INHIBITORS WITH β-AMYLOID ANTI-AGGREGATION PROPERTIES**

AChE and BuChE are enzymes involved in cholinergic neurotransmission through the hydrolysis of acetylcholine (ACh) [64]. In healthy brain tissue, AChE is the main enzyme responsible for acetylcholine hydrolysis, while BuChE plays a supportive role [65]. As AD progresses, the activity of AChE decreases while that of BuChE shows a progressive and significant increase. It was reported that BuChE is able...
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Fig. (2). Structures of selected multifunctional ligands, potential anti-Alzheimer’s drugs.

to compensate for the lack of AChE, thus enabling continued regulation of cholinergic neurotransmission [66, 67]. Recent studies have shown that BuChE has an influence on the modulation of motor control, awareness, cognition and behaviour by regulation of acetylcholine level in the central nervous system (CNS) [68-70]. Additionally, cholinesterases display several non-classical properties associated with Aβ and neurofibrillary tangles, and therefore they are important in the pathogenesis of AD. AChE was reported to co-localize with Aβ in neuritic plaques and can enhance the rate of formation of Aβ fibrils, forming stable complexes with them. Moreover, AChE was suggested to be a pathological chaperone which induces a conformational transition in Aβ leading to aggregation and fibril formation [71, 72]. It is well-established that the peripheral anionic binding site (PAS) of AChE is involved in these processes. Compounds which are able to interact with the catalytic site (CAS) and PAS of the enzyme, so-called dual binding site inhibitors, are potential inhibitors of AChE and Aβ aggregation. Thus, cholinesterase inhibitors with Aβ anti-aggregation properties are potential multifunctional ligands [73-75].

Tacrine Derivatives

The structure of tacrine (9-amino-1,2,3,4-tetrahydroacridine) (Fig. 1) is widely used as a pharmacophoric moiety in the development of MTDLs endowed with an inhibitory activity against cholinesterases and Aβ fibril formation [76]. Tang et al. [77] continued their development of oxoisoaporphine-tacrine heterodimers based on the dual-site theory. Previous studies have revealed that oxoisoaporphine alkaloids isolated from the rhizome of Me-nispernum dauricum and their synthetic analogues displayed a high inhibitory activity and good selectivity against AChE. The 1-azabenzanthrone fragment of these inhibitors can interact with PAS [78, 79]. A new series of compounds was designed by linking a tacrine pharmacophore with an oxoisoaporphine moiety (Fig. 3). Both fragments were connected by an aminomethyl linker containing a secondary amine in the middle and an amide bond close to the oxoisoaporphine site. Differences between the molecules included the type and length of the linker and also modifications in the tacrine unit. The results of Ellman’s test [80] showed that the newly synthesized hybrids were EeAChE inhibitors with IC_{50} values in the nanomolar range, from 3.4 to 910 nM. SAR analysis revealed that the most potent AChE inhibitors were compounds with a non-modified tacrine unit. The activity of derivatives with a cyclopentyl ring instead of cyclohexyl ring was significantly decreased (more than 100-fold), while the activity of analogues with a cycloheptyl ring was comparable or weaker. The optimal linker contained six carbon atoms and also included a secondary amine and a carbonyl group. These hybrids also displayed activity against EqBuChE with IC_{50} values ranging from 21 to 1760 nM. The Aβ anti-aggregating activity of the novel compounds was estimated in the self-induced Aβ_{1-42} and the AChE-induced Aβ_{1-40}

Fig. (3). Tacrine heterodimers as cholinesterase and Aβ aggregation inhibitors.
thioflavin (ThT) aggregation assays [81]. All the compounds exhibited a high influence on self-induced Aβ-amyloid aggregation at 10 μM (35.5 - 85.8%). They also had the ability to inhibit AChE-induced Aβ1-40 aggregation at 100 μM (60.2 - 89.6%). In summary, among the novel series of oxoisoporphine-tacrine hybrids, the most interesting hybrid is compound 1, which contains the tetrahydroacridine pharmacophore and a six atom spacer (Fig. 3). The compounds are more potent inhibitors of Aβ aggregation than the reference, curcumin.

Other multifunctional agents based on the structure of tacrine are tacrine-benzothiazole hybrids [82]. Some benzothiazole derivatives are able to interact with Aβ peptides and have been used as Alzheimer’s brain imaging agents [83], while others possess anti-aggregating and neuroprotective properties [84, 85]. A new series of five hybrids, bearing two pharmacophoric groups: tacrine and a benzothiazole moiety, is connected by a different linker, containing an amide bond, an alkyl or arylalkyl chain was developed (Fig. 3). All the synthesized compounds were found to be EeAChE inhibitors, with IC50 values in the submicromolar to low micromolar range (0.34 - 1.84 μM). SAR analysis of the tacrine-benzothiazole hybrids indicated that the length of the linker, its composition and geometry are important for their AChE inhibitory activity. All compounds also showed an inhibitory activity against self-induced Aβ1-42 aggregation at 50 μM ranging from 22.3 to 61.3% with the most active compound 2 (Fig. 3).

### Donepezil-Related Compounds

Özer et al. [86] designed a new series of compounds based on a donepezil structure, which were expected to inhibit both cholinesterase and Aβ aggregation. This class of dual-acting compounds was a combination of 4-benzylpiperidine/piperazine and a differently substituted benzene ring connected by an N-acylhydroxyamine moiety (Fig. 4). The benzene fragment contained one or two methoxy/ethoxy groups. The results of Ellman’s test showed that these compounds were moderate and non-selective inhibitors of both AChE and BuChE, with IC50 values in the micromolar range (53.1-88.5 μM for hAChE and 48.8-98.8 μM for EbBuChE, respectively). Compound 3 (Fig. 4), bearing a 4-ethoxybenzyl fragment and 4-benzylpiperidine, was found to be the most potent hAChE inhibitor. All compounds were able to inhibit the aggregation of Aβ1-40 and Aβ1-42, in comparison with the reference substance - rifampicin (69 - 90% at 100 μM).

Dual-acting compounds with cholinesterase and Aβ aggregation inhibitory activities were identified in a series of 2-(aminoalkyl)-isodole-1,3-dione derivatives [87, 88]. Target compounds were designed from structural fragments using molecular modelling. Two pharmacophoric groups - an isodole-1,3-dione (phthalimide) fragment and an alkylamine moiety - were connected by an alkyl chain (Fig. 4). Synthesized compounds were found to be moderate and selective EeAChE inhibitors, with IC50 values ranging from 0.9 to 19.5 μM. They were also tested in the modified thioflavin T assay using a smaller peptide containing 11 amino acids instead of the whole Aβ [89]. The most promising compound was 4 (Fig. 4) (EeAChE IC50 = 1.1 μM), with a heptamethylene linker, which inhibits EeAChE (IC50 = 1.1 μM) and Aβ fibril formation in 39.4% at 80 μM.

![Donepezil-related derivatives with Aβ anti-aggregation activity.](image)

### Benzotriazinone and Triazafluoranthenone Derivatives

A new series of inhibitors of Aβ aggregation and AChE/BuChE was identified in two groups of derivatives: benzo[e][1,2,4]triazin-7(1H)-ones and [1,2,4]-triazino[5,6,1-j,k]carbazol-6-ones [90]. A quinoline moiety presented in both planar triazaheterocyclic systems is a structural element important for blocking the Aβ aggregation process by π-π hydrophobic and electrostatic/polar interactions [91]. Designed compounds possess a variety of alkylamine or arylalkylamine substituents attached at position C6 in benzotriazinone and C5 in triazafluoranthenone (Fig. 5). The majority of the compounds exhibited an Aβ1-40 anti-aggregating activity with IC50 values in the micro- or submicromolar range (Aβ1-40 IC50 = 0.37-65 μM). In the most cases, triazafluoranthenone derivatives were more potent than benzotriazinones with the same substituent. Results of Ellman’s assay showed that the tested compounds were weak or moderate inhibitors of EeAChE and EbBuChE, however, among both groups two interesting multifunctional ligands were selected. Compound 5 (Fig. 5), a benzotriazinone derivative with a dianmioalkyl chain and phenyl ring at the end of the linker, displayed balanced biological properties: an inhibition of Aβ1-40 aggregation with IC50 = 1.4 μM and inhibition of EeAChE and EbBuChE with IC50 values of 1.5 μM and 1.9 μM, respectively. Moreover, compound 5 showed Aβ1-42 anti-aggregating activity, which was suggested may result from the ability to disrupt β-sheet interactions. Compound 6 (Fig. 5), a triazafluoranthenone derivative with an octamethylene chain, was found to be a potent inhibitor of Aβ1-40 aggregation with IC50 value of 1.4 μM and a selective, very potent EbBuChE inhibitor with IC50 = 25 nM. These two leads (compound 5 and 6) are promising agents for further development as potential anti-AD drugs.

### Diarylimidazole Derivatives

Promising dual-acting diarylimidazole hybrids have recently been reported [92]. The main objective of the project was to obtain a lead compound, as a selective and potent
EqBuChE and Aβ-aggregation inhibitor. The chemical library of nearly seven hundred (696) natural and synthetic compounds containing flavonoids, alkaloids, coumarins, chalcones, imidazoles, benzimidazoles or thiophenes was screened for inhibition of EqBuChE. The screening assay led to the selection of three hits, imidazole derivatives which displayed EqBuChE inhibitory activities with IC\textsubscript{50} values in the range of 0.2 to 4 μM. The most potent and selective BuChE inhibitor was found to be compound 7 (Fig. 6). A series of a following generation of imidazole derivatives was developed based on this lead and the results of molecular modelling. Compound 8 (Fig. 6) was an analogue of compound 7 with a thienyl group and a thioethyl substituent instead of a methoxy group. It was found to be the most active EqBuChE inhibitor (IC\textsubscript{50} = 0.10 μM). Extended biological studies revealed that compound 8 displays a high potency for inhibition of Aβ\textsubscript{1-40} fibril formation with an IC\textsubscript{50} value 5.8 μM. It was proposed that due to the presence of a thioephene moiety, this compound participates in the binding to prefibrils and therefore might prevent or delay the formation of Aβ assemblies.

Isaindigotone Derivatives

Isaindigotone (Fig. 7) is a naturally occurring alkaloid, the structure of which is based on a deoxyvasicinone moiety linked with a substituted benzylidene fragment [93]. The similarity of tacrine structure to deoxyvasicinone led to the discovery of a novel series of cholinesterase inhibitors among isaindigotone derivatives [94]. Continuing on from their previous studies, Yan et al. [95] designed and synthesized a series of novel deoxyvasicinone derivatives with additional Aβ anti-aggregation properties. Knowing that the introduction of a chlorine atom at position 6 in tacrine improves the activity of its analogues, they modified a deoxyvasicinone moiety in the same manner. The deoxyvasicinone fragment was connected with an amine fragment by two different linkers. The first group of derivatives had an N-phenylalkanamide linker and the second had an N-alkylbenzamide moiety, in order to enlarge their hydrogen bonding interaction (Fig. 7). Some of the compounds were modified by expanding a cyclopentane ring in a deoxyvasicinone, from five to six carbon atoms, which changed the planarity and linearity of the pharmacophore and therefore enabled the observation of their interactions with cholinesterases, Aβ and influence on their activity. Synthesized isaindigotone derivatives displayed an inhibitory activity against EeAChE in the nanomolar range and EqBuChE in the micromolar range. SAR studies revealed that more active and selective compounds were found to be those with a five-membered ring in the deoxyvasicinone structure. The findings suggest that the more planar structure was preferential for blocking AChE. The majority of the active compounds was derived from the group containing the N-phenylalkanamide side chain. The most potent inhibitor, selective towards EeAChE vs. EqBuChE, was compound 9 (Fig. 7) with IC\textsubscript{50} = 41.0 nM and with a selectivity ratio about 93. All the compounds were tested for their ability to inhibit self-induced Aβ\textsubscript{1-40} aggregation. Their activity was between 33.96 and 62.31% at 10 μM. Compound 9 was found to be the most potent inhibitor. Assays performed using a circular dichroism spectroscopy and electron microscopy confirmed that compound 9 reduces β-sheet structure formation and Aβ\textsubscript{1-40} fibril formation. The presented results indicate that this compound is a promising multifunctional agent for further development.

Chelerythrine

Numerous multifunctional cholinesterase inhibitors have been discovered in plants. Naturally occurring substances like coumarins, flavonoids and stilbene derivatives are an important source of AChE inhibitors [96]. Brunhofer et al. [97] presented the results of their screening tests for activity against cholinesterases in a library containing 502 natural
and natural-based compounds. Among the tested compounds, 23 were identified as cholinesterase inhibitors with the most promising agent, called chelerythrine (10) (Fig. 8) - an isoquinoline alkaloid. Chelerythrine (10) showed a moderate inhibitory activity against hAChE and hBuChE with IC50 values in the micromolar range (hAChE IC50 = 1.54 μM, hBuChE IC50 = 10.34 μM). This compound was also a potent inhibitor of self-induced Aβ1-40 aggregation (IC50 = 4.20 μM) and showed the high activity in disaggregating test performed on Aβ1-40 aggregates with IC50 of 13.03 μM. Moreover, chelerythrine (10) displayed inhibition of AChE-induced Aβ1-40 fibril formation at 5, 10 and 100 μM with 48.5%, 65.0% and 88.4%, respectively.

Chalcone and Coumarin Derivatives

In a previously reported research, chalcone and coumarin fragments were essential for an anticholinesterase activity, and in some cases for Aβ anti-aggregating properties [98, 99]. A novel series of multifunctional compounds with the chalcone or coumarin moiety were developed [100]. The chalcone and coumarin fragments were connected with different amine fragments to obtain dual-acting compounds (Fig. 9). All the tested compounds showed activity against cholinesterases comparable or weaker than the reference compound, galantamine. The most potent EeAChE inhibitor was compound 11 (Fig. 9) with IC50 value of 1.76 μM. Derivatives of chalcone exhibited higher than coumarin inhibitory activity against EqBuChE with the most potent compound 12 (Fig. 9) with IC50 value of 8.27 μM. All the compounds were tested for their ability to inhibit Aβ fibril formation in the thioflavin T fluorescence assay. Both chalcone and coumarin derivatives were found to be moderate inhibitors of the self-induced Aβ aggregation (30 - 70% at 100 μM). For oligomer formation and disassembly the biotinyl-Aβ1-42 single-site streptavidin assay was used [101]. In the oligomer formation assay, coumarin derivatives exhibited good inhibitory properties with IC50 values in the micromolar range (1 - 36 μM), while chalcone derivatives were inactive. Similarly, in the disassembly test only coumarin derivatives were active (IC50 values in the range of 4 to 29 μM). Compound 13 (Fig. 9) gave the best results in the oligomer assembly and disassembly assay. The results obtained were in agreement with the assay performed using atomic force microscopy (AFM), which confirmed that the compounds...
acted as fibrillogenesis inhibitors. AFM also showed that in the case of compound 11 the formed fibrils were significantly shorter. Among tested compounds, coumarin derivatives were found to be most promising for further studies.

**HYBRIDS WITH AChE/BuChE AND BACE1 INHIBITORY PROPERTIES**

β-Amyloid is produced by the sequential proteolytic cleavage of the amyloid precursor protein (APP) by the aspartyl proteases: β- and γ-secretase. The β-secretase (memapsin-2, BACE1) catalyzes the first and key step in the production of β-amyloid peptide [102]. Thus, inhibition of BACE1 is seen as an attractive therapeutic target for the treatment and prevention of AD [103]. The identification and cloning of this enzyme led to better understanding of its physiological function and to the development of β-secretase inhibitors [104]. Recently, numerous BACE1 inhibitors have been discovered, moreover a few of them have been tested in the early stage of clinical trials [105-107].

**Tacrine Derivatives**

4-Oxo-4H-chromene, a flavone based compound, was connected with a tacrine pharmacophore in the novel heterodimers reported by Fernández-Bachiller et al. [108]. The synthesized compounds were tested for inhibition of hAChE, hBuChE and hBACE1, for antioxidant properties and also for the blood-brain barrier (BBB) penetration. The developed compounds contained tacrine and 4-oxo-4H-chromene connected by the linkers of the different lengths (from 7 to 12 carbon atoms). The structures of tacrine and 4-oxo-4H-chromene were modified by an introduction of various substituents. In the tacrine unit a chlorine atom was introduced at positions 6, 5 or 7. The presence of a chlorine atom at position 6 in the tacrine moiety improved AChE inhibition, while the introduction of a second chlorine atom decreased the inhibitory potency against both the enzymes. The presence or absence of hydroxyl and methoxy groups had only a little influence on the inhibition of both the enzymes.

The developed compounds showed a stronger inhibitory activity against EqBuChE than AChE from bovine erythrocytes with the IC₅₀ values ranging from 0.175 to 100 nM and 5 to 1000 nM, respectively. The most potent compounds were tested against hAChE and hBuChE. In these assays compound 14 (Fig. 10) displayed an excellent inhibitory activity against hAChE with IC₅₀ value of 35 pM and compound 15 (Fig. 10) against hBuChE with IC₅₀ value of 38 pM. Eight compounds were evaluated as inhibitors of hBACE1 using a fluorescence resonance energy transfer (FRET) assay [109, 110]. Among them, five compounds exhibited a good inhibitory activity against hBACE1 with an IC₅₀ values below 5 μM. Noteworthy is compound 16 (Fig. 10), which is a potent inhibitor of both cholinesterases and hBACE1 inhibitor with IC₅₀ = 2.80 μM. Compound 16 was also the best antioxidant in this series, being 1.3-fold more potent than Trolox (a vitamin E analogue used as the reference compound) in the oxygen radical absorbance capacity test (ORAC assay) [111]. All the compounds were also tested in a parallel artificial membrane permeability assay for blood-brain barrier (PAMPA-BBB) [112], to explore whether they are able to penetrate into the brain. The majority of the tested hybrids showed permeability values, which indicate that they would cross the BBB.

**Huprine Derivatives**

Muñoz-Torrero group [113] developed hybrid compounds consisting of tacrine and huprine fragments connected by an alkyl or alkylamine linker, as potential inhibitors of AChE, BuChE and BACE1. Structural modifications of these hybrids included the type of a linker and substitution in the tacrine moiety, moreover several enantiopure huprinetacrine hybrids were synthesized. All the tested compounds exhibited an excellent or good activity towards investigated targets. They were potent hAChE inhibitors with IC₅₀ values in the subnanomolar to low nanomolar range (0.31 - 9.09 nM). The strongest inhibitory activity towards hAChE was displayed by hybrids with a six carbon atom linker. The elongation of the tether led to a decrease of activity, while the insertion of a methylene group in the linker had a positive effect on the hAChE inhibitory activity. The new compounds were moderately potent inhibitors of hBuChE with IC₅₀ values ranging from 24.6 to 139 nM. Moreover, 6-chlorotacrine-huprine hybrids showed a good inhibitory potency against BACE1 with the most active compound being 17 (Fig. 11) (hBACE1 IC₅₀ = 4.9 μM). These multifunctional compounds also exhibited a good inhibitory activity towards hAChE-induced APβ₁₋₄₀ aggregation at 100 μM, with the most active compound being 18 (Fig. 11) (IC₅₀ = 61.3 μM). The compounds showed a significant inhibitory potency against self-induced Apβ₁₋₄₀ aggregation ranging from 28.1 to 63.7% at 10 μM. Interestingly, this series was also tested for inhibition of prion protein aggregation. All of the described compounds showed the ability to inhibit an AChE-induced PrP-106-126 aggregation (23 - 67% at 100 μM) with the most active compound being 18 (IC₅₀ = 68.7 μM). No significant changes were observed for the enantiopure and racemic heterodimers regarding their biological activity. Brain penetration of these hybrids was predicted in the PAMPA-BBB assay. Results revealed that most of huprine-tacrine hybrids are able to cross the BBB.

The same research group described novel heterodimers of huprine containing rhein as the second fragment which can provide interactions within PAS [114]. Huprine and rhein were connected by an alkyl or arylalkyl chain of a different length (5 to 11 carbon atoms) (Fig. 11). All the synthesized huprine-rhein hybrids displayed an inhibitory activity

| Compound | R | R' | hAChE IC₅₀ [nM] | hBuChE IC₅₀ [nM] | hBACE1 IC₅₀ [μM] | BACE1 IC₅₀ [μM] |
|----------|---|----|----------------|----------------|----------------|----------------|
| 14       | 6-C1 | 5-OH | 0.20 | 0.75 | 0.03 | - |
| 15       | H | 6-OCH₃ | 0.75 | 0.038 | - | - |
| 16       | H | 6-C1 | 1.0 | 1.5 | 2.8 | - |

Fig. (10). Tacrine-4-oxo-4H-chromene hybrids as AChE, BuChE and BACE1 inhibitors.
Fig. (11). Huprine based hybrids - hAChE, hBuChE, BACE1 and Aβ aggregation inhibitors.

Towards both hAChE and hBuChE with IC₅₀ values in the nanomolar range and in the submicromolar to low micromolar range, respectively. Among this series, compound 19 (Fig. 11) displayed the most balanced pharmacological profile with respect to all the tested biological targets. This compound inhibited hAChE with an IC₅₀ of 3.60 nM and it was observed that its levorotatory isomer (−)19 was comparably active to the racemic mixture (hAChE IC₅₀ = 2.39 nM) while its optical isomer (+)19 was over 1000-fold less active. Regarding the BACE1 inhibitory activity, this series of compounds showed a moderate potency with the most active compound being 19 (BACE1 IC₅₀ = 120 nM). It is interesting to note that in this case both enantiomers were stronger inhibitors than the racemic mixture. All the new compounds were potent inhibitors of AChE-induced Aβ₁₋₄₀ aggregation (29.2 - 52.5% at 100 μM) and self-induced Aβ₁₋₄₂ aggregation (32.4 - 43.2% at 10 μM). Huprine-rhein hybrids were also evaluated in the PAMPA-BBB assay to assess their brain permeability. The results indicated that the majority of the compounds could cross the BBB. Additionally, in vivo tests with transgenic APP-PS1 mice were performed for the most promising hybrid (+)19 and (−)19. The results proved their ability to lower the level of hippocampal total soluble Aβ and to increase the level of APP in different stages of the AD model. Thus, this novel huprine-rhein hybrid seems to be an interesting multifunctional ligand for further development as a disease-modifying anti-AD drug.

Fig. (12). Multifunctional pyrimidine derivatives with AChE/BuChE, BACE1 and Aβ inhibitory activity.
Pyrimidine Derivatives

Mohamed et al. [115, 116] designed and synthesized a series of 2,4-disubstituted pyrimidine derivatives. These new multifunctional compounds contain a centrally located pyrimidine moiety substituted at position 2 with various cycloalkylamines, and at position 4 with arylalkylamines. The most promising multifunctional compound selected from this series was 20 (Fig. 12), a dual cholinesterase inhibitor ($h$AChE IC$_{50} = 10 \mu$M, $E_{q}BuChE$ IC$_{50} = 7.6 \mu$M) and $h$AChE-induced A$\beta$$_{1-40}$ aggregation inhibitor (30.8% at 100 $\mu$M). The following generation of pyrimidine derivatives is represented by a series of compounds containing a 1-benzylpiperidin-4-amine derived from donepezil at position 2 with a differently substituted $N$-benzylamine group at position 4 [117]. Synthesized compounds were found to be moderate cholinesterase inhibitors with IC$_{50}$s in the micromolar range. They were also able to inhibit $h$AChE-induced and self-induced A$\beta$$_{1-40}$ aggregation. This series was also found to be BACE1 inhibitors with IC$_{50}$ values ranging from 0.6 $\mu$M to 8.9 $\mu$M and with the most active compound being 21 (Fig. 12). This compound presents an interesting multifunctional profile against all the tested targets.

Benzamide Derivatives

A new series of benzamide derivatives was designed using a structure based approach [118]. The prototype of this series was compound 22 with the benzamide fragment linking two indole moieties (Fig. 13). Molecular modelling revealed that the indole moieties (1-indole, 3-indole) interacted with PAS and CAS of AChE. The indole fragments were replaced by the other moieties i.e. quinoline, isoquinoline and pyrimidine which could interact with both active sites of AChE. Among the new hybrids, compound 23 (Fig. 13) was the most active $h$AChE inhibitor ($K_i = 6.5$ nM) and $h$BuChE inhibitor ($K_i = 55$ nM). The multifunctional compound 23 also displayed the ability to inhibit $h$BACE1 and A$\beta$$_{1-42}$ aggregation with IC$_{50}$ values 85 $\mu$M and 79 $\mu$M, respectively. Compound 24 (Fig. 13), an analogue of compound 23 with a positively charged pyridine ring instead of quinoline, was found to be the most potent inhibitor of BACE1 (IC$_{50} = 0.31$ $\mu$M) with an activity against both cholinesterases in the nanomolar range ($h$AChE $K_i = 81$ nM, $h$BuChE $K_i = 93$ nM). Compound 24 lacked A$\beta$ anti-aggregating activity.

Triazole Derivatives

A new series of tryptoline and tryptamine triazole derivatives was designed and synthesized by Jiaranaikulwanitch et al. [119] as multifunctional agents. In a previous report [120], the authors discovered the structure of tryptoline as responsible for BACE1 inhibition. In the development of new multifunctional ligands the tryptamine moiety was introduced as a bioisostere of tryptoline. The designed structures contained triazolymethyltrypoline and triazolyl-2-amino-propyltryptamine connected with a differently substituted phenyl ring. The compounds were tested for inhibitory activity against $h$BACE1 and for additional activities such as inhibition of A$\beta$ aggregation, metal chelation and antioxidant properties. Generally, tryptamine derivatives showed a higher A$\beta$ anti-aggregating activity than tryptolines, with the exception of the most active compound 25 (Fig. 14) (IC$_{50}$ = 29.86 $\mu$M) which was a tryptoline derivative. It was found that for an A$\beta$ anti-aggregating activity, the optimal length between aromatic terminals is 8-9 Å for tryptolines and 13-14 Å for tryptamine derivatives. Tryptamine derivatives had significantly stronger metal chelating capability which resulted from the ability of a primary amine group of tryptamine to facilitate the formation of a coordination bond.

Fig. (13). Benzamide derivatives as AChE/BuChE, BACE1 inhibitors.
between tryptamine and iron ions. Furthermore, it was proven that some of the compounds exhibited the antioxidant activity and that 3,4-dihydroxyphenyl derivatives were the most potent. Three compounds, 25, 26 and 27 (Fig. 14), were found to be multifunctional. Compound 26 showed a moderate inhibitory activity against hBACE1 (IC₅₀ = 20.75 μM), an inhibitory effect on Aβ fibril formation (IC₅₀ = 83.23 μM) and also the ability to chelate iron ions (61% at 100 μM). In turn, compounds 25 and 27 exhibited a weaker activity against hBACE1 (inhibition at 25 μM 21.72% and 40.03%, respectively), an inhibitory effect on the Aβ fibril formation (25 IC₅₀ = 29.86 μM, 27 IC₅₀ = 56.39 μM) and moderate metal chelating properties (42.75% and 66.45% at 100 μM, respectively). Interestingly, these two compounds showed an antioxidant activity in the (di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium) (DPPH) free radical scavenging assay [121] with IC₅₀ values of 42.91 μM and 92.70 μM, respectively. Compounds 25, 26, 27 also showed a neuroprotective effect against the neuronal death induced by Aβ1-42, comparable to that provided by curcumin. In summary, the tryptoline and tryptamine triazole derivatives described here represent an interesting multifunctional profile in several in vitro assays.

CHOLINESTERASE AND MAO INHIBITORS

The therapeutic potential of monoamine oxidase inhibitors (MAOIs) in the treatment of AD has been suggested due to their neuroprotective properties beyond their effect on monoaminergic neurotransmission. The neuroprotective effect of MAOIs may result not only from the increased neurotransmission, but also from a reduction in the formation of neurotoxic products. Neurotoxic substances, such as hydrogen peroxide and aldehydes promote the generation of reactive oxygen species (ROS) [122-125]. In recent years, numerous multifunctional ligands with MAO inhibitory activity have been described.

Donepezil-Related Derivatives

The findings reported by Bolea et al. [126] are the most representative. Based on their previous study [127], the novel hybrids were designed by combining a benzylpiperidine fragment of donepezil with compound 28 (Fig. 15), which was one of the most interesting MAOIs previously investigated by this group. Both cores were joined by carbon linkers of the different lengths. The length of the tether that connects these two main structural fragments has
IC50 = 43 nM) and was also found to inhibit AChE and BuChE as well as to be a relevant effect on the inhibition of MAO-A and MAO-B. A three carbon atom spacer was found to be optimal. In their series of new hybrids, compound 29 (Fig. 15) was found to be the most potent MAO inhibitor (rMAO-A IC50 = 5.2 nM, rMAO-B IC50 = 43 nM) and was also found to inhibit AChE and BuChE in the submicromolar range (EeAChE IC50 = 0.35 μM, EqBuChE IC50 = 0.46 μM). The observed activity against BuChE is surprising, due to the fact that donepezil is a weak inhibitor of BuChE and compound 28 lacks activity. Compound 29 also presents significant inhibitory properties against self-induced (32.4% at 100 μM) and AChE-induced (47.8% at 10 μM) Aβ1-42 aggregation. Due to the promising preliminary results obtained from the biological evaluation, compound 29 was further investigated for its additional properties. Recent studies have demonstrated that 29 shows anti-apoptotic and antioxidant properties and also possesses a favourable blood-brain barrier permeability [128]. These results indicate that 29 is a potential multi-target drug candidate for the treatment of AD. This compound has become a scaffold for the novel compounds with modifications at position 2 in the indole moiety [129]. Thus, amide, amine, ester and carboxylic acid groups were introduced. The most potent was the analogue of 29 with a propargylamine instead of an N-propargylamine moiety and with an additional methyl group at the nitrogen atom of indole. This modified compound displays similar activities as compound 29. Furthermore, their research has shown that the propargylamine fragment is necessary to maintain an activity against MAO in this group of compounds. However, a moderate activity against MAO-A was also displayed by compounds with an ester and a hydroxyl group. Among the amines, apart from propargylamine, morfoline is a suitable substituent, as it is found in moclobemide which is a selective MAO-A inhibitor. Combining the N-benzylpiperidine fragment of donepezil with the N-propargylamine moiety by a central pyridine or a 1,8-naphthyridine ring resulted in the next series of multifunctional MAO inhibitors [130]. In a series of naphthyridine derivatives, compound 30 was a very potent AChE inhibitor (EeAChE IC50 = 37 nM) and a moderate, but selective MAO-A inhibitor (rMAO-A IC50 = 41 μM) (Fig. 15). Compound 31, the most potent and selective MAO-A inhibitor (rMAO-A IC50 = 25 μM) with a weaker activity against acetylcholinesterase (EeAChE IC50 = 4 μM) (Fig. 15), was a member of the pyridine series.

**Tacrine-Seleagine Hybrids**

In their continuing search for multifunctional compounds for treating AD Lu et al. [131] designed hybrids of tacrine connected by carbon spacers of different lengths with seleagine, a well-known inhibitor of MAO. The inhibition studies of hMAO-A and hMAO-B showed that these new compounds are effective inhibitors of both enzymes. The MAO inhibitory potency was related to the length of the linker. Compounds with a six to ten carbon linker were potent MAO inhibitors with submicromolar activities, whereas compounds with shorter linkers displayed activities in the micromolar range. All the compounds were potent AChE and BuChE inhibitors (EeAChE IC50 = 14.2 - 456 nM, EqBuChE IC50 = 2.03 - 66.0 nM). Compound 32 (Fig. 16) with a nine carbon atom tether, turned out to be a potent inhibitor of EeAChE (IC50 = 22.6 nM) and EqBuChE (IC50 = 9.37 nM) and a balanced inhibitor of both monoamine oxidases (hMAO-A IC50 = 0.372 μM, hMAO-B IC50 = 0.181 μM).

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Fig. (15). Benzylpiperidine derivatives as MAO inhibitors with anti-cholinesterase activity.
Thus, it is expected that this hybrid could improve cholinergic neurotransmission by AChE and BuChE inhibition and protect neurons by maintaining the activity of selegiline.

Tacrine-Homoisoflavonoid Hybrids

Sun et al. [132] also used tacrine as a pharmacophore for the novel homoisoflavonoids. Derivatives of homoisoflavonoids were chosen because of their known MAO-B inhibitory activity [133]. In a new series, tacrine and homoisoflavonoid fragments were connected using carbon spacers of different lengths. They also had different substituents in the homoisoflavonoid moiety. All the compounds were potent cholinesterase inhibitors with activities in the nanomolar range, and were selective MAO-B inhibitors. These compounds were selected through a structure-based virtual screening. They were 3,5-diaryl pyrazolines substituted by anthracene at position 3 and by a NO2 group at position 5 in pyrazoline (Fig. 16) with a methoxy group at para position in a phenyl ring and a six carbon atom linker, provided the best results for cholinesterase inhibition (EeAChE IC50 = 67.9 nM, EqBuChE IC50 = 33.0 nM) and hMAO-B inhibition (IC50 = 0.401 μM). Moreover, the PAMPA-BBB assay indicated that compound 33 should be able to cross the BBB to target the enzymes in the CNS.

Pyrazoline Derivatives

The first generation of MAOIs was represented by hydrazine derivatives [134]. 2-Pyrazoline can be considered as a cyclic hydrazine moiety and this scaffold, besides a propargyl moiety, is the most common scaffold in MAO inhibitors [135]. Mishra et al. [136] synthesized selective, reversible and very potent (100 times more potent than selegiline) inhibitors of MAO-B. These compounds were selected through a structure-based virtual screening. They were 3,5-diaryl pyrazolines substituted by anthracene at position 3 and by a phenyl ring with different substituents at position 5 in pyrazoline. The most potent was compound 34 (Fig. 17) with a nitrophenyl at position 5 in pyrazoline (hMAO-A Ki = 32.16 nM and hMAO-B Ki = 0.31 nM). In further studies, 3,5-diaryl pyrazolines were examined against hAChE [137]. The majority of the molecules were found to be potent and selective hAChE inhibitors with Ki values in the nanomolar range. Compound 34 inhibits hAChE with Ki = 20.6 nM. The new pyrazolines are very interesting dual-acting compounds due to their balanced effect on MAO-A, MAO-B and AChE.

CHOLINESTERASE INHIBITORS WITH ANTIoxidant PROPERTIES

Oxidative stress is characterized by an imbalance between the production of ROS and their removal by antioxidative mechanisms. Extensive evidence suggests that free radicals may be involved in the pathogenesis of AD because the brain tissues in AD patients are exposed to oxidative stress during the development of the disease. ROS production is due to a variety of sources including mitochondrial abnormalities, disturbances in the level of transition metals and amyloid peptides themselves. Thus, antioxidant therapy in dementia may bring benefits, particularly in the early stage of AD [138, 139]. Searching for cholinesterase inhibitors with additional antioxidant properties is one of the trends in the development of an effective therapy for AD.

Berberine Derivatives

The results presented by Shan et al. [140] outline a series of novel cholinesterase inhibitors with antioxidant activity. In their previous works, they developed a novel class of 9-O-substituted berberine derivatives, which are cholinesterase inhibitors [141, 142]. Berberine was chosen as a scaffold because it inhibits AChE (IC50 = 0.374 μM) and reverses the Aβ-induced memory impairment. To develop a novel series of compounds with multi-target profile they decided to replace the oxygen atom in the 9-O-substituted berberine derivatives with an NH group. This novel series of 9-N-substituted berberine derivatives showed multiple activities including the cholinesterase and self-induced Aβ inhibition, and significant antioxidant properties in the ORAC assay. In this series, berberine was connected with a differently substituted phenyl ring by carbon spacers of different lengths. Among the series, compound 35 (Fig. 18), with an ethylene linker between the berberine and orthomethylphenyl ring, was found to be the most active hEeAChE inhibitor with IC50 = 27 nM. It also displayed good antioxidant activity (ORAC = 4.05 eq. of Trolox) and inhibited self-induced Aβ aggregation with IC50 = 2.73 μM. Other modifications of 9-O-substituted berberine derivatives resulted in a new series of berberine-thiophenyl hybrids [143].
In this series, most of the hybrids demonstrated \( Ee \text{AChE} \) inhibitory activity in the submicromolar range and a moderate to good antioxidant capacity with the ORAC values of 0.47 - 1.94 Trolox equivalents. Compound 36 performed the best results in the ORAC assay (1.94 eq. of Trolox) and in a self-induced A\( \beta_{1-42} \) aggregation test (76.8% at 20 \( \mu \)M) (Fig. 18). It was also found to be a moderate \( Ee \text{AChE} \) inhibitor.

**Tacrine Derivatives**

Tacrine was chosen as a common pharmacophore in the search for multifunctional ligands with the antioxidant properties. Based on their previous studies [144], Maalej et al. [145] developed tacrine analogues bearing a racemic 9,10,11,12-tetrahydro-7H-benzo[7,8]chromeno[2,3-b]quinolin-8-amine heterocyclic structure with a differently substituted benzene ring at position 7 (Fig. 19, compounds 37 and 38). Among the series, the compounds were found to be potent and selective inhibitors of \( h \text{AChE} \) in the micromolar and submicromolar range (IC\( _{50} \) = 0.30 - 5.74 \( \mu \)M). The antioxidant activity of the compounds was evaluated by the ORAC method. Only compound 37, which has a 4-hydroxy-3-methoxyphenyl substituent, displayed an interesting antioxidant activity (1.5-fold more potent than Trolox). Despite the fact that compound 38 did not display the antioxidant activity, it showed good neuroprotective effects against the oxidative stress in cell-based assay (99.46% at 50 \( \mu \)M). The oxidative stress in cells was induced by the mixture of oligomycin-A and rotenone which

![Fig. (18). Multifunctional berberine derivatives with antioxidant activity.](image)

![Fig. (19). Tacrine derivatives with the antioxidant properties.](image)
blocked the mitochondrial electron transport chain. Compound 38 displayed lower hepatotoxicity in the cell based assay than tacrine. The majority of the compounds were also tested in the PAMPA-BBB to explore whether they would be able to penetrate into the brain. All the tested compounds showed the permeability values which indicate that these molecules could cross the BBB by passive diffusion.

Recent studies have shown that carbazol derivatives extracted from root bark of *Clausena harmandiana* have strong antioxidant properties [146]. Carbazol derivatives, heptaphylline and 7-methoxyheptaphylline, were connected with tacrine by a five or three carbon atom chain and reported by Thiratmatrakul *et al.* [147]. Synthesized compounds were found to be moderate *Ee*AChE inhibitors in the low micromolar and submicromolar range, and they displayed very potent antioxidant activity in the ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging assay [148]. The ability to scavenge radicals was displayed as IC$_{50}$ and Trolox was used as a reference (IC$_{50}$ = 23.67 μM). The compounds showed higher radical scavenging activity than Trolox (IC$_{50}$ = 8.34 - 11.24 μM). Furthermore, they displayed a neuroprotective effect against the oxidative stress induced by H$_2$O$_2$ in the neuroblastoma cells and against the toxicity induced by Aβ$_{1-42}$ peptide in C6 astrogliaoma cells at 100 μM. Compound 39 (Fig. 19), bearing 7-methoxyheptaphylline and a five carbon atom spacer, was chosen for *in vivo* studies. The effect of 39 on learning and memory impairment was evaluated using the Morris water maze and Y-maze test [149]. The Morris water maze test is performed to evaluate hippocampal-dependent spatial learning ability which refers to long-term memory, whereas the Y-maze test evaluates immediate spatial working memory, a form of short-term memory. Memory deficits in mice were induced by an anti-cholinergic agent, scopolamine. Behavioural studies indicated that 39 could improve both short- and long-term memory deficits through the enhancement of cholinergic signalling. The presented compounds are promising multifunctional candidates for further development.

The results reported by Luo *et al.* [150] present hybrids of tacrine connected by an alkyl linker to benzylamine. Methoxy and hydroxyl groups were introduced to benzylamine to investigate their influence on the antioxidant properties. The best results were provided by compounds with at least one hydroxyl moiety in a benzene ring. These compounds were shown to be potent antioxidants in the ORAC assay (1.2 - 2.7 equivalents of Trolox) and *Ee*AChE and *Eq*BuChE inhibitors in the nanomolar range. The most active compound was found to be 40 (Fig. 20) (*Ee*AChE IC$_{50}$ = 4.55 nM and *Eq*BuChE IC$_{50}$ = 3.41 nM), which had a hydroxyl group at position 4 and a methoxy group at position 3 in the benzene ring. Compound 40 inhibited self-mediated Aβ$_{1-42}$ aggregation in 71% at 20 μM and was 1.9-fold more potent than Trolox in the ORAC assay.

Caffeic acid displays the antioxidative activity and chemo preventive effect against Aβ$_{1-42}$ [151]. Therefore, Chao *et al.* [152] connected caffeic acid with tacrine by alkyl linkers with the hope of finding cholinesterase inhibitors endowed with the antioxidant properties. Based on their previous studies on ferulic acid - tacrine hybrids, they chose two, three and six carbon atom linkers. To gain selectivity towards *Ee*AChE over *Eq*BuChE they also prepared compounds with a 6-chlorotetrahydroacridine instead of tacrine. The most active compound was 41 (Fig. 20) with an IC$_{50}$ of 0.3 μM against AChE and 29.5 μM against BuChE. This compound showed also an inhibitory effect against self-induced and AChE-induced Aβ aggregation (36.2% at 20 μM and 67.7% at 100 μM, respectively). These new compounds displayed a DPPH radical scavenging activity comparable to that of caffeic acid.

**Ebselen Derivatives**

Ebselen, which is a glutathione peroxidase (GPx) mimic, has the antioxidant activity which results from catalyzing the reduction of peroxides by glutathione [153]. This and several other pharmacological effects of ebselen, namely an anti-inflammatory activity and inhibition of iron-induced tau phosphorylation, were considered in the design of new MTDLs against AD combining the important pharmacophores of this compound and donepezil [154]. Among a set of 15 derivatives, the authors chose compound 42 (Fig. 21) as a potential lead for their further studies. This compound was not only a potent AChE inhibitor with an IC$_{50}$ value of 42 nM and an effective inhibitor of AChE-induced Aβ$_{1-40}$ aggregation (21.4% at 100 μM), but also displayed antioxidant effects similar to that of ebselen. Its antioxidant properties were evaluated in different assays. GPx-like catalytic activity was tested by measuring the rates of the reduction of H$_2$O$_2$ by glutathione [155]. The rates were 123.5 μM/min for the compound 42 and 121.3 μM/min for ebselen. Compound 42 showed similar or better scavenging activities than ebselen.

![Fig. (20). Tacrine hybrids - cholinesterase and Aβ aggregation inhibitors with antioxidant properties.](image-url)
on hydrogen peroxide and on peroxynitrite and turned out to be a substrate for thioredoxin reductase. Aside from the pharmacological properties of compound 42, its ability to penetrate into the CNS was tested in an in vitro blood-brain barrier model and the results indicated that it could reach the CNS. Finally, the compound did not show any acute toxicity and mortality in mice at doses of up to 2000 mg/kg. The authors further explored the idea by fusing the pharmacophores of ebselen and donepezil in a slightly different manner (43, Fig. 21) [156]. They developed a series of 11 new compounds modified mostly at the donepezil part. Generally, they were weaker ChEs inhibitors with IC50 values ranging from 0.46 μM to 5.66 μM against EcAChE and from 1.97 μM to more than 15 μM against EqBuChE. Their GPx-like activity was preserved as well as their H2O2 scavenging activity.

Indoline Derivatives

The results reported by Yanovsky et al. [157] present derivatives of indoline-3-propionic acid. Indole-3-propionic acid (IPA), the natural compound, was found to be a potent antioxidant against oxidative damage induced by β-amyloid [158]. The further studies showed that a reduced analogue of IPA, indoline-3-propionic acid, was an even more potent antioxidant. Thus, a series of indoline derivatives was developed. These new compounds were substituted at position 3 with propionic acid and its ester analogues, and substituted in the aromatic ring with carbamate moieties at position 4, 6 or 7. Some of the compounds were N-methylated to compare their activity to unsubstituted analogues. To evaluate the importance of propionic acid, they synthesized derivatives unsubstituted at position 3 in the indoline. Among the series, almost all of the compounds were found to be moderate cholinesterase inhibitors with IC50 values in the micromolar and submicromolar range. The antioxidant scavenging ability of the new compounds was tested using two luminol-dependent chemiluminescence-inducing systems [159]. The first system measured the scavenging of H2O2 and OH• generated by glucose oxidase (GO). The second system checked the activity against NO released by morpholinosydnonimine (Sin1). Melatonin, a derivative of IPA, was used as a reference (GO IC50 = 95.8 μM, Sin1 IC50 = 1024 μM). The synthesized compounds were found to be more potent than melatonin in both tests, with IC50 in the test with GO ranging from 70 nM to 24 μM and in the test with Sin1 ranging from 0.57 to 8.5 μM. Among the derivatives of propionic acid, compound 44 displayed the best radical scavenging properties in both tests (Fig. 22). It also significantly reduced apoptosis induced by H2O2 in the H9c2 cardiomiocytes (58.6% at 100 nM). Among the derivatives without propionic acid moieties, compound 45 was found to be the most potent antioxidant (Fig. 22). Compound 45 also decreased the cell death induced by oxidative stress in cardiomiocytes and reduced apoptosis induced by serum deprivation in a primary neuronal cell culture. Serum deprivation induces oxidative stress in cells because of a lack of necessary nutrients and trophic factors.

Lipoic Acid Derivatives

Rosini et al. [160] presented their studies on lipocrine and its analogues. Lipocrine was developed in 2005 as one of the first multifunctional antioxidant and cholinesterase inhibitors [161]. Lipocrine is a hybrid of lipoic acid (a natural occurring antioxidant) and tacrine. In a new series of lipoic acid derivatives, lipoic acid was linked with fragments of rivastigmine and memoquin. Compound 46 (Fig. 23), which is a hybrid of lipoic acid and memoquin, displayed the best multifunctional profile (Fig. 23). It was found to be a potent hAChE inhibitor (IC50 = 256 nM) and a moderate hBuChE inhibitor (IC50 = 2.49 μM). Its activity against intracellular ROS formation was assessed in SH-SY5Y cells after treatment with tert-butyl hydroperoxide, a compound used to induce oxidative damage. Compound 46 showed a significant dose-dependent inhibitory effect on ROS formation in concentrations of 1 to 50 μM. Moreover, it did not display cytotoxicity in SH-SY5Y cells up to the highest concentration. These results indicate that compound 46 functions as a balanced antioxidant and cholinesterase inhibitor. However, its AChE-induced Aβ anti-aggregation activity is poor.

Curcumin Derivatives

Curcumin is structurally related to ferulic and caffeic acids and like they it has chemopreventive, antioxidant and...
Fig. (22). Carbamate derivatives of indoline with antioxidant properties and anti-cholinesterase activity.

Fig. (23). Lipoic acid derivatives as antioxidants and cholinesterase inhibitors.

anti-inflammatory properties which could be very useful in the treatment of AD [162-164]. Unfortunately, its therapeutic application fails due to its poor pharmacokinetics. Fang L. et al. [165] and Fang X. et al. [166] modified the structure of curcumin to improve its physicochemical and pharmacokinetic properties while preserving a neuroprotective effect. To achieve this goal, they kept the heptadiendione bridge chain and one phenolic hydroxyl group unchanged because they are crucial for the neuroprotective effect. At the same time, they replaced the methoxy group with a bulky dimethylaminomethyl substituent creating a steric hindrance to the hydroxyl group, which is the site of metabolism of curcumin (compound 47, Fig. 24). With the replacement of both methoxy groups the stability and the antioxidant activity of the obtained compounds increased. The most active compound 48 (Fig. 24) showed a strong free radical scavenging activity in the DPPH assay (IC50 = 1.6 µM) and towards galvinoxyl radicals (IC50 = 4.9 µM) whereas curcumin IC50 values in these tests were 26.5 µM and >100 µM, respectively. Other modifications, namely the introduction of electron-withdrawing groups (-Cl and -F) and electron-donating groups (-OCH3) did not influence these properties to such extent. Compound 48 was also the most potent inhibitor of Aβ self-induced aggregation among the obtained compounds. Its activity, 32% of inhibition at the concentration of 100 µM in thioflavin T assay, was similar to that of the reference compound, curcumin, which was 29% at the same concentration.

Fig. (24). Curcumin and its dimethylaminomethyl-substituted derivatives as antioxidants.
Multifunctional Metal Chelators

Transition metals such as iron (Fe), copper (Cu) and zinc (Zn) are essential for the proper functioning of antioxidant systems in the cell and play an important catalytic role in many enzymes. In the brains of AD patients, disturbances in the level of biometals were noticed. Biometals are suggested to have two distinct roles in the pathology of AD. The presence of biometals within the amyloid deposits indicates that they may directly interact with Aβ and increase its aggregation. An alternative explanation is that an imbalance in the levels of metals may increase production of ROS induced by Aβ. Thus, the modulation of the level of these biometals in the brain is also a potential therapeutic strategy for treating AD [167-171]. Multifunctional metal chelators may block metal-related oxidative stress and modulate Aβ aggregation.

Flavonoid Derivatives

Li et al. developed a novel series of compounds with metal chelating properties [172]. This series contained flavonoid derivatives because of the well-established pharmacological properties of flavonoids. Flavonoids show the antioxidant activity which depends on the ability to inhibit the activity of cyclooxygenase and lipoxygenase and the ability to chelate the transition metals [173-175]. In this new series, a flavonoid scaffold was connected with an amine group (aliphatic or the cyclic tertiary amine) using carbon spacers of different lengths. The most promising compound was 49 which contained a diethylamine group connected to the flavonoid scaffold by a four carbon atom linker (Fig. 25). Compound 49 exhibited the most potent AChE activity (IC50 = 130 nM), high selectivity for AChE, inhibition of self-induced Aβ1-42 aggregation at 20 μM (38.95%) and a Cu2+ and Fe2+ chelating effect. The flavonoid pharmacophore was also hybridized with tacrine. The flavonoid fragment was connected to tacrine by a piperazine-based alkyl spacer, which was able to adopt the appropriate conformation to establish additional interactions within the enzyme. Compound 50 (Fig. 25) had the most balanced multitarget profile. Its anti-Aβ (Aβ1-42 IC50 = 6.5 μM) and anti-cholinesterase (EeAChE IC50 = 0.133 μM, EqBuChE IC50 = 0.558 μM) activity goes in association with its metal chelating effect [176].

Coumarin Derivatives

Xie et al. [177] developed a new tacrine-coumarin hybrid series. Coumarin was selected due to its Aβ anti-aggregation activity and its ability to interact with PAS of AChE. Both scaffolds were connected by a piperazine-based alkyl spacer. A secondary amine group of tacrine in flavonoid-tacrine hybrids was converted into amide moiety, which has the ability to chelate metal ions. Among the target molecules, compound 51 (Fig. 26) showed the highest activity against EeAChE (IC50 = 0.092 μM) and the best anti-aggregation properties (67.8% inhibition at 20 μM). It also showed moderate EqBuChE inhibition (IC50 = 0.234 μM) and effective chelation of Cu2+ and Fe2+.
Rhein Derivatives

Tacrine was also hybridized with rhein, which was mentioned above [178]. Both fragments were connected by alkylene linkers of different lengths to find the optimal spacer. From the IC50 values it appeared that the most suitable was a six carbon atom tether. Compound 52 (Fig. 26) was a potent AChE inhibitor with IC50 = 27.3 nM and a potent inhibitor of AChE-induced Aβ1-40 aggregation (70.2% at 100 μM). Further studies indicated that it acts as a metal chelator.

Indanone Derivatives

The indanone moiety is one of the fragments of donepezil that influences high affinity and selectivity for AChE [179]. Meng et al. [180] decided to combine the indanone moiety with an aromatic ring via a linker with a double bond to enhance the affinity for PAS. Furthermore, additional double bonds may provide a metal chelating activity. A series of indanone derivatives with various amine groups substituted at position 6 in indanone was synthesized. In this series, compound 53 (Fig. 27) was the most potent EeAChE inhibitor with IC50 = 1.8 nM, and a moderate EqBuChE inhibitor with IC50 = 9.5 μM. It also displayed metal chelating activity towards Cu2+, Fe2+ and Zn2+. The structural analogue of 53, with a longer spacer connecting piperidine and indanone, did not show chelating properties. This fact indicates that the distance between the nitrogen of the piperidine ring and the oxygen of the phenolic group played a key role for chelation.

Huang et al. [181] also evaluated a novel series of indanone derivatives with inhibitory potency against MAO and with other multidirectional biological activities, including the inhibition of self-induced Aβ aggregation, antioxidant properties and metal chelating properties. The indanone fragment was combined with differently substituted benzaldehydes. The strongest ability to inhibit self-mediated Aβ1-42 aggregation was provided by compound 54 (Fig. 27) (80.1% at 20 μM). It also inhibits Cu2+ induced aggregation, disassembles the well-structured Aβ fibrils and chelates metals. Compound 54 also displays a moderate activity against hMAO-B (IC50 = 7.50 μM) and hMAO-A (IC50 = 37.7 μM). The hydroxyl group at position 5 in the indanone is critical to the MAO activity. This was confirmed by the introduction of a methoxy group, which resulted in the loss of the activity. This compound was active in the ORAC test and proved to be 5.6-fold more potent than Trolox.

Rutaecarpine Derivatives

He et al. [182] evaluated 7,8-dehydrorutaecarpine derivatives as multifunctional agents. Previously, they reported that compounds with 7,8-dehydrorutaecarpine moiety (a carbazole-based structure) were potent AChE inhibitors [183]. Furthermore, carbazole derivatives were reported as Aβ aggregation inhibitors with the free-radical scavenging effect [184]. Thus, a series of new 3-aminoalkanamido-substituted 7,8-dehydrorutaecarpine derivatives was investigated for their multi-activity. The obtained compounds were demonstrated to be very potent acetylcholinesterase inhibitors with the most active compound being 55 (Fig. 28) (EeAChE IC50 = 0.6 nM). Compound 55 also inhibited self-induced (45.9%) and AChE-induced (90.6%) Aβ1-42 aggregation at 25

Fig. (27). Indanone derivatives as cholinesterase and monoamine oxidase inhibitors with metal chelating properties.

Fig. (28). Rutaecarpine derivative as a multifunctional metal chelator.
μM. This lead compound chelates metals and has better antioxidant properties than Trolox (ORAC at 1 μM = 1.8).

Resveratrol Derivatives

In recent years, resveratrol (3,5,4’-trihydroxystilbene) has been extensively investigated as a cardioprotective, anticancer and anti-aging compound [185-187]. Recently, resveratrol-based compounds were found to have a strong anti-aggregation and antioxidant activity [188, 189]. In a novel series of stilbene derivatives, compound 56 was found to be an inhibitor of self-induced aggregation of Aβ1-42 (71.65% at 20 μM) with antioxidant activity (4.12 of Trolox eq. at 1 μM) [190]. As a continuation of this study, Lu et al. [191] developed a series of compounds with a stilbene fragment combined with differently substituted benzylamine moieties. The most promising of them, compound 57, was a potent inhibitor of self-induced Aβ aggregation (79.50% at 20 μM) with antioxidant properties (4.72 of Trolox eq. at 1 μM) (Fig. 29). Moreover, compound 57 chelates metals and inhibits Cu(II)-induced Aβ aggregation (94.23% at 20 μM). It also shows inhibitory activity towards cholinesterases and monoamine oxidases in the micromolar range (EeAChE IC50 = 6.27 μM and EqBuChE IC50 = 21.25 μM, hMAO-A IC50 = 7.08 μM, hMAO-B IC50 = 14.09 μM) (Fig. 29).

Li et al. reported small molecules bearing the main features of resveratrol and clioquinol [192]. They obtained imine resveratrol analogues with differently substituted hydroxyl groups as bifunctional compounds with a metal chelating and anti-aggregation activity. The most active compounds were also examined for their antioxidant and neuroprotective properties. Among the target compounds, 58 (Fig. 29) was the most interesting. It was an inhibitor of Aβ self-aggregation (64.6% at 20 μM) with neuroprotective activity better than resveratrol and it reduced Cu²⁺-induced Aβ aggregation.

NO-RELEASING COMPOUNDS

Being involved in a variety of physiological and pathophysiological processes, nitric oxide (NO) is regarded as a potential tool in the pharmacotherapy of many disorders, including dementia. NO-releasing drugs may be especially beneficial for the treatment of AD due to its role in the regulation of the cerebral circulation and inflammatory reactions [193, 194].

Tacrine-Ferulic Acid Hybrids

In our previous review paper [63], we described tacrine-ferulic acid hybrids (Fig. 30, compound 59) reported by Fang et al. [195]. Aside from their AChE and BuChE inhibitory activities, they displayed moderate to good antioxidant activity in the ORAC assay. Simultaneously, the same group designed and synthesized a series of tacrine hybrids with the NO-donating nitrate and diazeniumdiolate moieties [196]. The developed compounds (Fig. 30, compound 60) displayed a cholinesterase inhibitory potency in the nanomolar range and a moderate blood vessel relaxant activity. In the behavioural studies they significantly improved the scopolamine-induced cognition impairment in rats. In contrast to tacrine, the new derivatives did not cause a serious hepatotoxicity [197]. Lately, the group combined those two ideas and presented tacrine - ferulic acid - NO donor trihybrids (Fig. 30, compound 61) [198]. All the compounds were potent cholinesterase inhibitors with IC50 values ranging from 3.6 nM to 44.3 nM against EeAChE and 1.0 nM to 24.9 nM against EqBuChE. The ability of these compounds to release

Fig. (29). Resveratrol derivatives as metal-chelating agents with additional biological properties.
Nitric oxide was tested in the Griess reaction [199]. For the most active compound 61, the amount of NO produced in the reaction (0.31 μg/mL) was similar to that produced by isosorbide dinitrate (0.38 μg/mL), which was used as a positive control in this test. Production of nitrite was positively correlated with the vascular relaxation activity of the compounds, which was the highest for 61 (EC₅₀ = 34.3 μM). Compound 61 was subjected to the passive avoidance test in the scopolamine-induced cognition impairment animal model. It significantly decreased the number of errors made by mice in the test as well as reduced the transfer latency time, indicating beneficial effects for the short-term learning ability and improving memory impairment. Even though the described compounds did not show the expected antioxidant activity, their cholinesterase inhibitory activity, NO-releasing ability and vasorelaxant effects contributed to significant cognition improving activity. Additionally, compound 61 was much safer in terms of hepatotoxicity than tacrine.

Tacrine-Flurbiprofen Hybrids

Encouraged by the positive results associated with the introduction of NO-donor group, Chen et al. [200, 201] conjugated a nitrate group with the previously obtained tacrine-flurbiprofen hybrid compounds [202]. Derivatives with short linkers (two to four carbon atoms) connecting tacrine with flurbiprofen displayed a comparable or higher than tacrine EeqBuChE inhibitory activity (IC₅₀ = 3.9 nM - 13.9 nM). The majority of the compounds with longer linkers (six or eight carbon atoms) were more potent inhibitors of both EeqACH and EeqBuChE (IC₅₀ = 9.1 - 225.6 nM; IC₅₀ = 0.6 - 3.7 nM respectively) than tacrine. All the trihybrid compounds released NO in amounts comparable to isosorbide mononitrate (0.209 - 0.565 μg/mL vs. 0.412 μg/mL), as shown in the Griess reaction. To evaluate the pharmacological effect resulting from this feature, the selected compounds were tested in ex vivo isolated organs (coronary arteries from rats) using the vascular relaxation assay. In this study, a moderate vasorelaxation effect (21.9% - 31.3%) was observed, which might result from NO generation. In the passive avoidance test, with the scopolamine-induced impaired mice, compound 61 and its NO-releasing analogue 62 (Fig. 31) proved to improve memory impairments, although it seems that this activity was not dependent on the presence of the nitrate group. Finally, compound 62 displayed much lower hepatotoxicity than tacrine and compound 61, indicating the hepatoprotective role of NO.

Dibenzo furane Derivatives

NO releasing organic nitrate was also incorporated into the dibenzo furane and carbazole derivatives reported by Fang et al. [203]. Considering previous studies and the X-ray structure of galantamine co-crystalized with AChE [204], the authors concluded that the aromatic moiety (i.e., the benzene ring) and the nitrogen atom create the crucial interactions responsible for the inhibitory activity of galantamine. Therefore, they designed compounds with a simplified structure of galantamine using dibenzofuran/carbazole as a backbone. The molecular modelling studies showed that the analogues with the azepane ring opened overlap with galantamine and they could effectively block the catalytic site of AChE. This hypothesis was confirmed in in vitro tests where the majority of the new compounds retained AChE inhibitory activity, and two of them were more potent than galantamine. The most active were the analogues with a nitric oxide donor attached to the backbone on a flexible aliphatic chain (i.e., compound 63, EeqAChE IC₅₀ = 0.18 μM) (Fig. 32). Unexpectedly, their NO-releasing activity was rather poor (4.8 - 6.1% of NO released from the nitrate). Compound 63 displayed a moderate Aβ-aggregation inhibitory activity (25% at 100 μM) in the thioflavin T assay but also a dose-dependent neuroprotective effect against Aβ-induced toxicity. Most importantly, compound 63 showed a significant improving effect on spatial memory in rats with scopolamine-induced cognition impairment.
**MULTIFUNCTIONAL COMPOUNDS WITH NEUROPROTECTIVE PROPERTIES**

As previously stated, neurodegeneration is characterized by a progressive loss of the structure and function of neurons. The purpose of neuroprotection is to counteract this process by targeting the most common mechanisms leading to it, like oxidative stress, mitochondrial dysfunction, excitotoxicity, inflammatory changes, iron accumulation, and protein aggregation.

**Aminothiazol Derivatives**

The neuroprotective and anti-inflammatory properties of aminothiazoles [205] were the basis for Wang et al. [206] in designing a series of new potential multifunctional drugs for AD. The structures comprised tacrine and para-substituted 4-phenyl-2-aminothiazole moieties connected by various linkers. The linkers differed in the length (6 - 11 atoms) and structure, including an alkyl chain and succinamide or glycaminamide fragments. An inhibitory activity against AChE, BuChE and Aβ-aggregation was reported for these compounds as well as a calcium overload blockade effect. The most potent inhibitor of EeAChE was compound 64 (Fig. 33) with its pIC\textsubscript{50} = 7.14, and which also displayed the most significant Ca\textsuperscript{2+} overload blockade effect. Unfortunately, its anti-aggregation activity was quite low (35.8% at 20 μM) compared to other compounds in the group. Compound 65 (Fig. 33) deserves attention with its pIC\textsubscript{50} = 6.98 against EeAChE and with the lowest pIC\textsubscript{50} = 10.35 against EqBuChE. Aβ self-aggregation was affected the most by compound 66 (Fig. 33) - 72% at the concentration of 20 μM (57% for propidium iodide).

**Aminophenothiazine Derivatives**

As a continuation of their previous project Gonzalez-Muñoz et al. [207] described a group of analogues of N-acylaminephenothiazines modified within the amine group. They developed a series of selective BuChE inhibitors with IC\textsubscript{50} values in the range from 0.4 μM to 7.1 μM. According to the PAMPA-BBB assay, the compounds could cross the BBB by passive diffusion. Several compounds displayed neuroprotective activity as shown in two toxicity models on human neuroblastoma cell line SH-SY5Y. The first one was a model using hydrogen peroxide, which generates exogenous free radicals, the other one uses the combination of rotenone and oligomycin A for the induction of mitochondrial ROS. Compound 67 (Fig. 34) protected the cells from the damage induced by H\textsubscript{2}O\textsubscript{2} in a dose-dependent manner (54.6% at 1 μM, 57.7% at 3 μM, 79.9% at 10 μM) and it was a moderate free-radical scavenger in rotenone and oligomycin A assay (14.7% of free radical capture vs. 27.8% for Trolox). Compound 67 was evaluated in additional assays. It displayed neuroprotective activity against Aβ\textsubscript{1-42} induced cytotoxicity (91.3% at 0.3 μM) and against okadaic acid (28.3% at 0.3 μM) - a toxin which induces tau phosphorylation and aggregation into neurofibrillary tangles [208]. The compound had also a neuroprotective effect in calcium overload assay (44.5% at 1 μM).
A novel multipotent anti-AD agent was designed by linking two tacrine moieties via cystamine (2,2'-disulfanediyldiethanamine) [209]. Cystamine is known for its antioxidant, cytoprotective and neuroprotective properties [210]. The obtained cystamine-tacrine dimer 68 (Fig. 35) is a structural analogue of bis(7)-tacrine with a disulfide bridge. Compound 68 displayed an inhibitory activity against both cholinesterases in the nanomolar range (hAChE $IC_{50} = 5.04$ nM, hBuChE $IC_{50} = 4.23$ nM) and inhibitory properties in the self-induced Aβ aggregation assay with $IC_{50} = 24.2$ μM and in the AChE-induced Aβ aggregation test (52% at 100 μM). Its neuroprotective effect against oxidative stress induced by H$_2$O$_2$ was tested on SH-SY5Y cell line. A complete protection in this assay was observed at 0.5 μM concentration. A study of a mechanism of neuroprotection revealed that compound 68 acts by activating two anti-apoptotic kinase pathways (kinase 1 and 2, and Akt/protein kinase B).

**NMDA RECEPTOR ANTAGONISTS**

A combination of the AChE inhibitor and N-methyl-D-aspartate receptor (NMDAR) antagonist (memantine) is now a standard treatment of AD. Memantine has a neuroprotective effect resulting from the inhibition of an excessive calcium influx induced by chronic overstimulation of the NMDA receptor. Simoni et al. [211] reported a series of compounds - chimeras of galantamine and memantine connected via different linkers. AChE inhibitory activity of the compounds was dependent on the interactions with both CAS and PAS of the enzyme, therefore the length and the kind of the linker was crucial. The most favourable were six to eight-methylene spacers connecting the nitrogen of galantamine and the nitrogen of memantine. The most active compound with $IC_{50}$ of 0.52 nM had an additional methyl group at the nitrogen atom of memantine. Unfortunately, this modification had a detrimental effect on the affinity for NMDAR. None of the obtained compounds was as potent NMDAR antagonists as memantine ($K_i = 1.16$ μM) but a few of them had comparable $K_i$ values. The most interesting compound with a balanced activity against both targets was compound 69 (Fig. 36) called memagal, which additionally...
was proven to inhibit NMDA-induced neurotoxicity (IC\textsubscript{50} = 0.28 nM) in a cell-based assay.

Novel multifunctional bis-\(\gamma\)-carboline derivatives endowed with a cholinesterase inhibitory activity, A\(\beta\) anti-aggregating properties and a neuroprotective effect caused by NMDAR antagonism were reported by Rossini et al. [212]. These compounds have been designed as latrepirdine-based dimers. Latrepirdine (Dimebon) was used as the anti-histamine drug and it has been investigated as a potential anti-AD agent due to its inhibitory activity towards BuChE, AChE and NMDAR [213]. New compounds contained two \(\gamma\)-carboline fragments of latrepirdine connected by different linkers. Among syntehesized derivatives the most promising was compound 70 (Fig. 37), which displayed an interesting multifunctional profile. It was a non-selective cholinesterase inhibitor with a moderate activity against hAChE (IC\textsubscript{50} = 0.692 \(\mu\)M) and hBuChE (IC\textsubscript{50} = 0.737 \(\mu\)M) and it inhibited A\(\beta\)\textsubscript{1-42} self-aggregation (71% at 50 \(\mu\)M). Compound 70 acted as NMDAR antagonist (IC\textsubscript{50} = 12.6 \(\mu\)M at -100 mV) at recombinant NMDARs but it was less potent than memantine (IC\textsubscript{50} = 0.71 \(\mu\)M at -100 mV). Moreover, compound 70 showed neuroprotective effect in a low-serum cell stress model [214] by enhancement of the survival of cortical neurons at 100 nM.

**Cannabinoid Receptor Ligands With Cholinesterase Inhibitory Activity**

The cannabinoid system has recently been gaining more attention due to its involvement in anti-inflammatory, neuroprotective and anti-amnesic actions [215, 216].

**Benzofuran Derivatives**

Rizzo et al. [217] reported a series of novel compounds endowed with activity towards cannabinoid receptors. The authors assumed possible interactions with the receptors based on the structural similarity of the newly obtained compounds to the high affinity CB1 antagonist/inverse agonist (LY320135) (Fig. 38), which is built on a benzofuran scaffold. The work presented the optimization of compound 71 (Fig. 38), which was reported as an MTD lead with AChE inhibitory activity, A\(\beta\) anti-aggregation properties and neuroprotective effect in SH-SY5Y cells. Major modifications include the length and the position of the linker connecting 2-arylfuran and N-methyl-N-benzylamine and the substituent at position 3 of the benzofuran scaffold. The influence of these modifications on the inhibitory activity against AChE and BuChE, A\(\beta\) fibril formation and neuroprotective activity was verified. The major improvement was achieved in terms of cholinesterase inhibition. Changing the position of the heptyloxy-N-methyl-N-benzylamine substituent from \textit{para} to \textit{meta} resulted in the development of compound 72 (Fig. 38), which was the most active inhibitor of hAChE in the series and 180-fold more potent than the parent compound 71 (IC\textsubscript{50} = 0.24 \(\mu\)M vs. 40.7 \(\mu\)M). Regarding A\(\beta\) fibril formation compound 73 (Fig. 38), an analogue of 71 lacking the substituent at position 3 of the benzofuran was the most active (IC\textsubscript{50} = 3.9 \(\mu\)M). The bulkier the substituent at this position, the worse the activity. Compound 72 as well as derivatives with a 1-naphthyl or 3-methoxy substituent in the benzofuran moiety retained the activity of compound 71, whereas the rest of the series were only weak inhibitors of \(\beta\)-amyloid aggregation. Among the A\(\beta\) fibril formation inhibitors, compounds 71 and 72 counteracted neurotoxicity induced by A\(\beta\)\textsubscript{25-35} by inhibiting peptide-induced ROS formation and preventing the interaction of the A\(\beta\)\textsubscript{25-35} peptide with the cell membrane of SH-SY5Y cells. All the introduced modifications adversely affected the affinity for CB1 receptors compared with compound 71 (\(K_i\) value of 32 \(\mu\)M). Compounds of the \textit{para} series with amino moieties on the benzoyl group showed weak affinities for CB1 receptors (\(K_i\) = 0.55 - 2.57 \(\mu\)M) and CB2 receptors (\(K_i\) = 0.58 - 1.18 \(\mu\)M). Functional studies of these compounds were not conducted.

**Indazole Derivatives**

González-Naranjo et al. [218] found that some of the indole-based cannabinoid agonists such as JWH-015 (Fig. 39) inhibit AChE in Eillman’s test. This discovery prompted them to search for new multi-target directed ligands, CB2 agonists with cholinesterase inhibitory activity. They chose an indazole scaffold as an indole bioisostere and applied structural modifications at positions 1, 3 and 5 according to the molecular modelling studies. Most of the compounds obtained showed micromolar affinities for cannabinoid receptors, some of them being selective towards CB2 receptor and three of them (74, 75, 76) (Fig. 39) proved to be CB2 receptor agonists. Compounds 74, 75 and 76 were also among the most potent selective BuChE inhibitors in this group and exhibited moderate antioxidant properties in the ORAC test. Therefore, these compounds were identified as the most interesting for further investigation.

![Fig. (37). Latrepirdine-based bivalent ligand with anti-cholinesterase, A\(\beta\) anti-aggregation activity and neuroprotective effect.](image-url)
DUAL-ACTING COMPOUNDS TARGETING HISTAMINERGIC SYSTEM

The histamine H3 receptor (H3R) belongs to the class of G protein-coupled receptors (GPCRs), and as a presynaptic autoreceptor in the brain, inhibits the release of histamine and is involved in the modulation of the release of other neurotransmitters. In the CNS, the histamine H3 receptors are localized mainly in the regions involved in important physiological processes including cognition, agitation, anxiety, pain, food intake and body temperature regulation [219-221]. H3 antagonists/inverse agonists have been reported to improve cognitive function, spatial orientation, attention, memory and learning in a variety of in vivo models [222-224]. The fusion of AChE inhibitors and histamine H3 receptor antagonists in a single molecule might improve cognitive functions in AD.

A series of hH3 receptor antagonists with AChE/BuChE inhibitory activity has been recently reported [225]. Non-imidazole diether derivatives exhibited a good affinity for cloned human histamine H3 receptors (Ki values in the range of 3 to 51 nM). Most of the compounds displayed a moderate or weak EeAChE inhibitory activity and moderate EqBuChE inhibitory activity. Regarding AChE/BuChE activity vs. hH3 receptor affinity, compounds with the highest hH3R potency also showed the highest anti-cholinesterase activity. Two of the most interesting multifunctional compounds (77 and 78) (Fig. 40) displayed high affinity for hH3R (77 Ki = 3.48 nM, 78 Ki = 7.74 nM) and an inhibitory potency against both enzymes (77 EeAChE IC50 = 7.91 μM and EqBuChE IC50 = 4.97 μM). The results of docking studies showed that these compounds could act as dual-binding site inhibitors, interacting with both the CAS and PAS of AChE.

| Compound | R1 | R2 | R3 | AChE IC50 [μM] | ORAC [Trolox eq] | CB1 Ki [μM] | CB2 Ki [μM] |
|----------|----|----|----|---------------|----------------|-------------|-------------|
| 74       | H  | N-CH(CH3)2 | 4-methoxyphenyl | 2.28 | 1              | 40          | 2.7         |
| 75       | H  | piperidine   | 2-naphthyl      | 1.6  | 0.5            | 1.4         | 2           |
| 76       | NH2 | piperidine   | 2-naphthyl      | 1.5  | 0.8            | > 40        | 2           |

Fig. (40). Dual-acting diether derivatives of homopiperidine with histamine H3 receptor antagonistic and anticholinesterase activity.
Recently, Darras et al. [226] presented two novel series of tri- and tetracyclic nitrogen-bridgehead compounds acting as dual AChE inhibitors and hH3 receptor antagonists. The target compounds were designed by connecting a tri- and tetracyclic fragment endowed with an inhibitory activity against cholinesterases with an amine moiety [227]. The amine moiety was based on the piperidinylpropoxyphenyl pharmacophore, which is characteristic for H3 receptor antagonists (Fig. 41). New tetracyclic hybrids were moderate, non-selective AChE/BuChE inhibitors with IC50 values in the submicromolar to micromolar range. The compounds displayed affinity for hH3 receptors in a wide range. Compound 79 (Fig. 41) showed high binding affinity at hH3R (Ki = 17.5 nM) but displayed a moderate inhibitory activity towards EeAChE (IC50 = 6.77 μM) and EqBuChE (IC50 = 1.07 μM). The tricyclic hybrids represent moderate or potent AChE inhibitors, with activities ranging from 8.91 μM to 0.067 μM for compound 80 (Fig. 41). Their activity towards BuChE was in the micromolar range. Regarding, the hH3 receptor affinities, they were ranging from the micromoles to nanomoles for the most active compound 80 (Ki = 76.2 nM). Moreover, all of the tested compounds showed a very good selectivity profile with regard to the hH3 receptor over all the other hH receptor subtypes (H1, H2 and H4). The most promising compound is the tricyclic hybrid 80, which is a reversible and competitive AChE inhibitor and an antagonist hH3R, with a balanced potency against both targets.

![Fig. (41). Tri- and tetracyclic derivatives acting as dual AChE inhibitors and hH3 receptor antagonists.](image)

**5-Lipoxygenase Inhibitors**

It has been proven that 5-lipoxygenase (5-LO) is connected with Aβ aggregation [228, 229] and its inhibition can reduce the formation of amyloid plaques in the brain [230]. Moreover, 5-LO is involved in an inflammatory processes. Chen et al. [231] reported a series of an isoliquiritigenin (4,2′,4′-trihydroxychalcone, ISL) derivatives as new 5-LO and Aβ aggregation inhibitors. Although the activity of the obtained compounds was not very diverse, certain structure-activity relationships may be noted. The most potent inhibitors contained a six-membered cyclic amine (N-methylpiperazine, piperidine and morpholine) substituent at one of the phenyl rings. Compound 81 (Fig. 42) was found to be one of the most potent inhibitors with balanced activity against both 5-LO and Aβ self-induced aggregation tested in the thioflavin T assay (IC50 = 6.1 μM, IC50 = 3.2 μM, respectively). Its ability to inhibit β-sheet aggregation and fibril formation was also confirmed in a circular dichroism spectroscopy assay and an electron microscopy assay.

![Fig. (42). An isoliquiritigenin derivative with activity against 5-LO and Aβ aggregation.](image)

**SUMMARY**

The limited efficacy of the current AD therapy has led to the development of many different approaches in searching for new drug candidates. Among them, MTDLs strategy enables researchers to obtain compounds endowed with advantageous properties resulting from possible interactions with more than one target involved in the pathogenesis of AD. This review collects and presents novel compounds described during the last three years, which were classified by the biological targets and chemical structure. The most common biological targets used for the development of MTDLs for AD are: acetylcholinesterase, butrylcholinesterase, β-secretase, β-amyloid and monoamine oxidases. There is also a large number of compounds which display the antioxidant, metal chelating, neuroprotective and NO-releasing activity.

The majority of the research is focused on modifications of the existing drugs or already known structures with specific biological activity. Tacrine is among the most popular pharmacophores used for the design of MTDLs since it is very active cholinesterase inhibitor. There is also a number of hybrid compounds containing fragments of donepezil, galantamine or memantine. It is worth noting that there is a large group of hybrid compounds containing a structural fragments derived from natural sources. They are naturally occurring alkaloids, flavonoids or other natural products like isaimudigotone, chelerythrine, chalcone, coumarin, huprine, curcumin, rhein, berberine and resveratrol derivatives. Application of these less popular pharmacophores carries a greater risk of obtaining compounds with poor biological activity. On the other hand, this approach may indicate new directions for the development of new anti-AD drugs.

Development of an effective drug for AD has proven to be very difficult. Even though there is a great number of very interesting compounds with diverse pharmacological profile in preclinical studies, the majority of them fail the clinical trials. The results of the analysis published by Cumming et al. [232] showed that during the period between 2002 and 2012 there were 413 AD clinical trials performed and only
one compound (memantine) was advanced to the FDA and approved for marketing. Currently, 108 clinical trials for AD therapies are being conducted, and only 14 agents reached phase 3. Ladostigil, the multifunctional agent which failed phase 3 clinical trials as AD drug, is currently investigated as potential agent for Mild Cognitive Impairment. The statistics indicate that there is no simple way of searching for AD therapy and the presented multi-target directed ligand approach gives hope for further development and for finding new and effective therapy for AD.

**ABBREVIATIONS**

5-LO = 5-lipoxygenase  
ABTS = 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); (2,2-diphenyl-1-picrylhydrazyl)  
ACh = Acetylcholine  
AChE = Acetylcholinesterase  
AD = Alzheimer’s disease  
ADHD = Attention deficit hyperactivity disorder  
AFM = Atomic force microscopy  
AGE = Advanced glycation endproduct  
APP = Amyloid precursor protein  
Aβ = β-amyloid peptide  
BACE1 = β-secretase, β-site APP-cleaving enzyme 1  
BBB = Blood brain barrier  
BTA = Benzothiazole  
BuChE = Butyrylcholinesterase  
CAS = Catalytic active site  
CB = Cannabinoid  
CNS = Central nervous system  
DPPH = Di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium  
EeAChE = Acetylcholinesterase from Electrophorus electricus  
EqBuChE = Butyrylcholinesterase from equine serum  
FDA = Food and Drug Administration  
FRET = Fluorescence resonance energy transfer  
GABA = γ-aminobutyric acid  
GO = Glucose oxidase  
GPSRs = G protein-coupled receptors  
GPx = Glutathione peroxidase  
H = Histamine  
H3R = Histamine H3 receptor  
hAChE = Human acetylcholinesterase  
hBACE1 = Human β-secretase  
hBuChE = Human butyrylcholinesterase  
hH3R = Human histamine H3 receptor  
hMAO-A/B = Human monoamine oxidase A/B  
I = Inhibitor  
IPA = Indole-3-propionic acid  
MAO-A/B = Monoamine oxidase A/B  
MTDL = Multi-target-directed ligand  
NFTs = Neurofibrillary tangles  
NMDA = N-methyl-D-aspartate  
NO = Nitric oxide  
NSAIDs = Nonsteroidal anti-inflammatory drugs  
ORAC-FL = Oxygen radical absorbance capacity fluorescein assay  
PAMPA = Parallel artificial membrane penetration assay  
PAS = Peripheral anionic site  
PPARγ = Peroxisome proliferator-activated receptor γ  
rAChE = Acetylcholinesterase from rat brain  
rMAO-A/B = Monoamine oxidase A/B from rat brain  
ROS = Reactive oxygen species  
SAR = Structure-activity relationship  
Sin1 = Morpholinosydnonimine  
ThT = Thioflavin

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflict of interest.

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