

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

Natalia Guzior, Anna Więckowska, Dawid Panek and Barbara Malawska*

Jagiellonian University, Medical College, Chair of Pharmaceutical Chemistry, Department of Physicochemical Drug Analysis, 30-688 Kraków, Medyczna 9, Poland



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 H_3 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 ($A\beta$) 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 $A\beta$ self-aggregation and β -secretase (BACE1) responsible for the synthesis of $A\beta$. 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

*Address correspondence to this author at the Jagiellonian University, Medical College, Chair of Pharmaceutical Chemistry, Department of Physicochemical Drug Analysis, 30-688 Kraków, Medyczna 9, Poland; Tel: +48 12 6205464; Fax: +48 12 6205458; E-mail: mfinalaws@cyf-kr.edu.pl

temporary slowdown of the loss of cognitive functions [11].

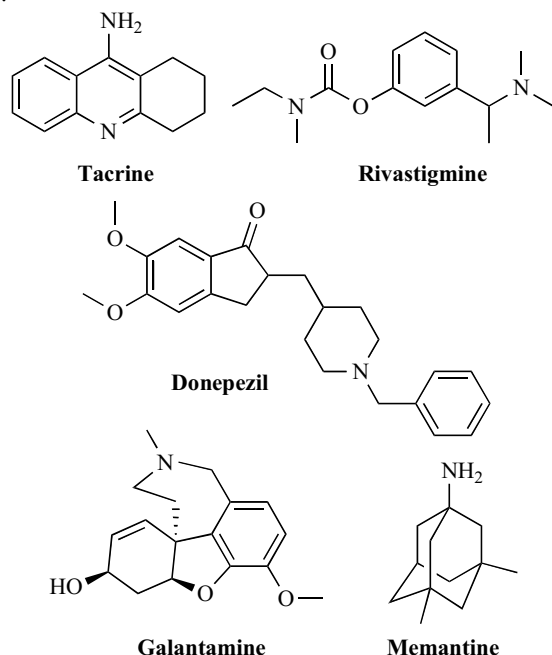


Fig. (1). Drugs approved for the treatment of AD.

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: A β 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 A β , 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 multi-target 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 A β 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 H₃ 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 PPAR γ 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

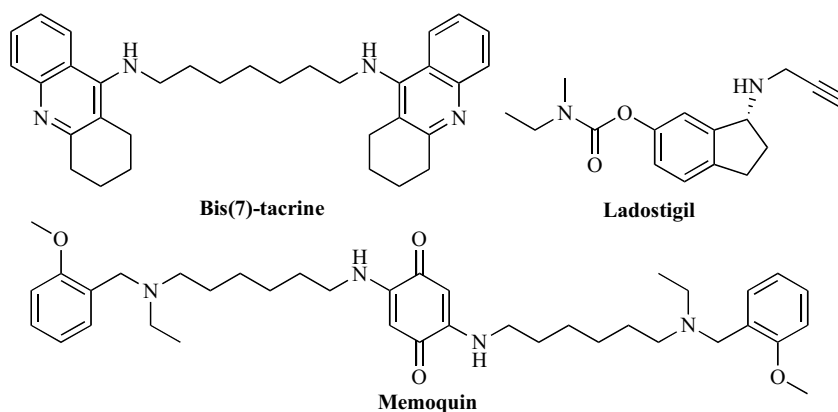


Fig. (2). Structures of selected multifunctional ligands, potential anti-Alzheimer's drugs.

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 A β and neurofibrillary tangles, and therefore they are important in the pathogenesis of AD. AChE was reported to co-localize with A β in neuritic plaques and can enhance the rate of formation of A β fibrils, forming stable complexes with them. Moreover, AChE was suggested to be a pathological chaperone which induces a conformational transition in A β 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 A β aggregation. Thus, cholinesterase inhibitors with A β 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 A β

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 *Menispermum 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 aminoalkyl tether 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 *Ee*AChE inhibitors with IC₅₀ 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 *Eq*BuChE with IC₅₀ values ranging from 21 to 1760 nM. The A β anti-aggregating activity of the novel compounds was estimated in the self-induced A β ₁₋₄₂ and the AChE-induced A β ₁₋₄₀

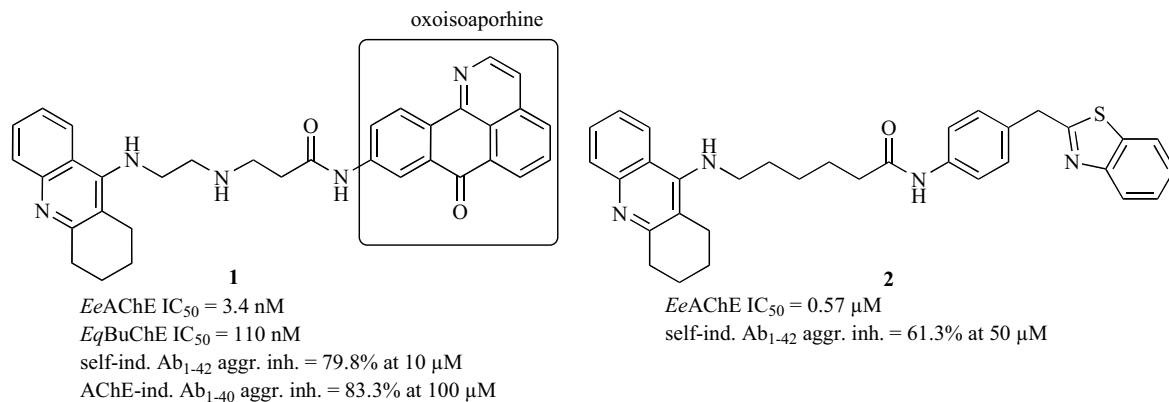


Fig. (3). Tacrine heterodimers as cholinesterase and A β aggregation inhibitors.

thioflavin (ThT) aggregation assays [81]. All the compounds exhibited a high influence on self-induced A β -amyloid aggregation at 10 μ M (35.5 - 85.8%). They also had the ability to inhibit AChE-induced A β_{1-40} aggregation at 100 μ M (60.2 - 89.6%). In summary, among the novel series of oxisoaporphine-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 A β aggregation than the reference, curcumin.

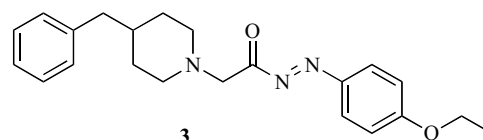
Other multifunctional agents based on the structure of tacrine are tacrine-benzothiazole hybrids [82]. Some benzothiazole derivatives are able to interact with A β peptides and have been used as Alzheimer's brain imaging agents [83], while others possess anti-aggregating and neuroprotective properties [84, 85]. A new series of five hybrids, bearing two pharmacophoric groups: tacrine and a benzothiazole moieties 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 *Ee*AChE inhibitors, with IC₅₀ values in the submicromolar to low micromolar range (0.34 - 1.84 μ M). 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 A β_{1-42} aggregation at 50 μ M ranging from 22.3 to 61.3% with the most active compound **2** (Fig. 3).

Donepezil-Related Compounds

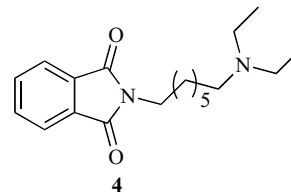
Özer *et al.* [86] designed a new series of compounds based on a donepezil structure, which were expected to inhibit both cholinesterase and A β aggregation. This class of dual-acting compounds was a combination of 4-benzylpiperidine/piperazine and a differently substituted benzene ring connected by an *N*-acylhydrazone 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 μ M for *h*AChE and 48.8-98.8 μ M for *Eq*BuChE, respectively). Compound **3** (Fig. 4), bearing a 4-ethoxybenzyl fragment and 4-benzylpiperidine, was found to be the most potent *h*AChE inhibitor. All compounds were able to inhibit the aggregation of A β_{1-40} and A β_{1-42} , in comparison with the reference substance - rifampicin (69 - 90% at 100 μ M).

Dual-acting compounds with cholinesterase and A β aggregation inhibitory activities were identified in a series of 2-(aminoalkyl)-isoindoline-1,3-dione derivatives [87, 88]. Target compounds were designed from structural fragments using molecular modelling. Two pharmacophoric groups - an isoindoline-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 *Ee*AChE inhibitors, with IC₅₀ values ranging from 0.9 to 19.5 μ M. They were also tested in the modified thioflavin T assay using a smaller peptide containing 11 amino acids instead of the whole A β [89]. The most promising compound was **4** (Fig. 4) (*Ee*AChE IC₅₀ = 1.1 μ M), with a heptamethyl-

ene linker, which inhibits *Ee*AChE (IC₅₀ = 1.1 μ M) and A β fibril formation in 39.4% at 80 μ M.



3
*h*AChE IC₅₀ = 53.1 μ M
*Eq*BuChE IC₅₀ = 67.3 μ M
 self-ind. A β_{1-40} aggr. inh. = 80% at 100 μ M



4
*Ee*AChE IC₅₀ = 1.1 μ M
 self-ind. A β_{25-35} aggr. inh. = 39.4% at 80 μ M

Fig. (4). Donepezil-related derivatives with A β anti-aggregation activity.

Benzotriazinone and Triazafluoranthrone Derivatives

A new series of inhibitors of A β aggregation and AChE/BuChE was identified in two groups of derivatives: benzo[*e*][1,2,4]triazin-7(1*H*)-ones and [1,2,4]-triazino[5,6,1-*j,k*]carbazol-6-ones [90]. A quinonimine moiety presented in both planar triazaheterocyclic systems is a structural element important for blocking the A β 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 triazafluoranthrone (Fig. 5). The majority of the compounds exhibited an A β_{1-40} anti-aggregating activity with IC₅₀ values in the micro- or submicromolar range (A β_{1-40} IC₅₀ = 0.37-65 μ M). In the most cases, triazafluoranthrone 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 *Ee*AChE and *Eq*BuChE, however, among both groups two interesting multifunctional ligands were selected. Compound **5** (Fig. 5), a benzotriazinone derivative with a diaminoalkyl chain and phenyl ring at the end of the linker, displayed balanced biological properties: an inhibition of A β_{1-40} aggregation with IC₅₀ = 1.4 μ M and inhibition of *Ee*AChE and *Eq*BuChE with IC₅₀ values of 1.5 μ M and 1.9 μ M, respectively. Moreover, compound **5** showed A β_{1-42} anti-aggregating activity, which was suggested may result from the ability to disrupt β -sheet interactions. Compound **6** (Fig. 5), a triazafluoranthrone derivative with an octamethylene chain, was found to be a potent inhibitor of A β_{1-40} aggregation with IC₅₀ value of 1.4 μ M and a selective, very potent *Eq*BuChE 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

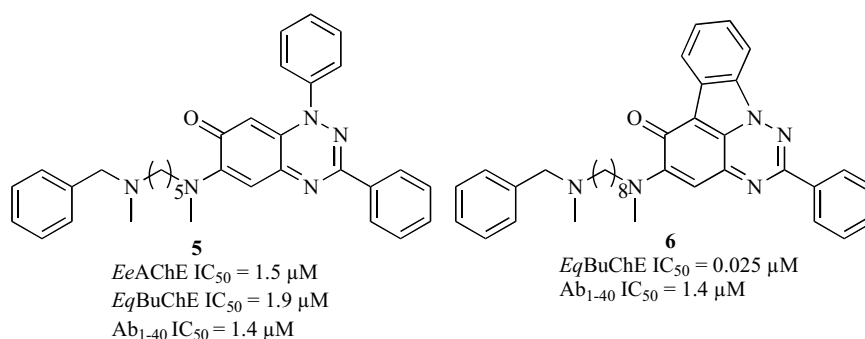


Fig. (5). Benzotriazinones and triazafluoranthenones with AChE/BuChE inhibitory activity and Aβ anti-aggregation activity.

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 IC₅₀ 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 (IC₅₀ = 0.10 μM). Extended biological studies revealed that compound **8** displays a high potency for inhibition of Aβ₁₋₄₀ fibril formation with an IC₅₀ value 5.8 μM. It was proposed that due to the presence of a thiophene moiety, this compound participates in the binding to prefibrils and therefore might prevent or delay the formation of Aβ assemblies.

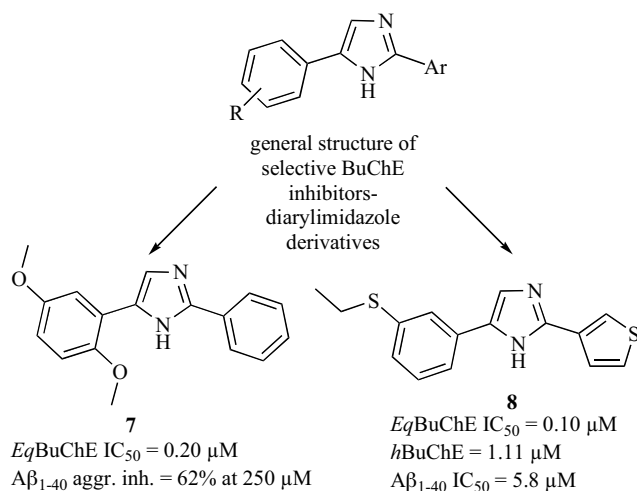


Fig. (6). Diarylimidazole derivatives - selective BuChE and Aβ aggregation inhibitors.

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 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 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 IC₅₀ = 41.0 nM and with a selectivity ratio about 93. All the compounds were tested for their ability to inhibit self-induced Aβ₁₋₄₀ 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 Aβ₁₋₄₀ 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

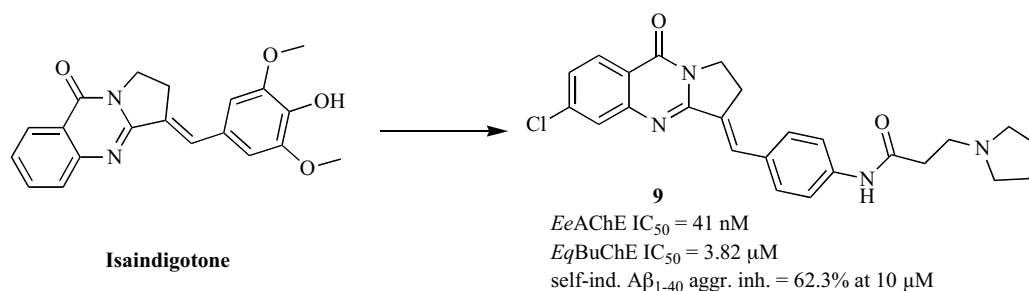


Fig. (7). Isaindigotone derivative as dual cholinesterase and Aβ aggregation inhibitor.

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.

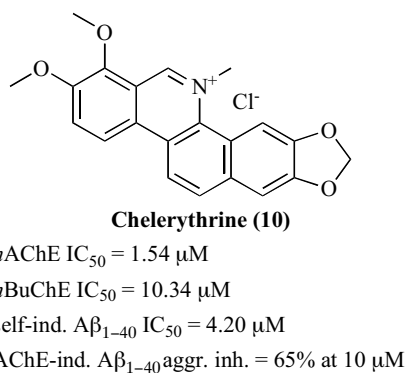


Fig. (8). Structure of multifunctional derivative of isoquinoline - chelerythrine - an inhibitor of *hAChE*, *hBuChE* and Aβ aggregation.

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

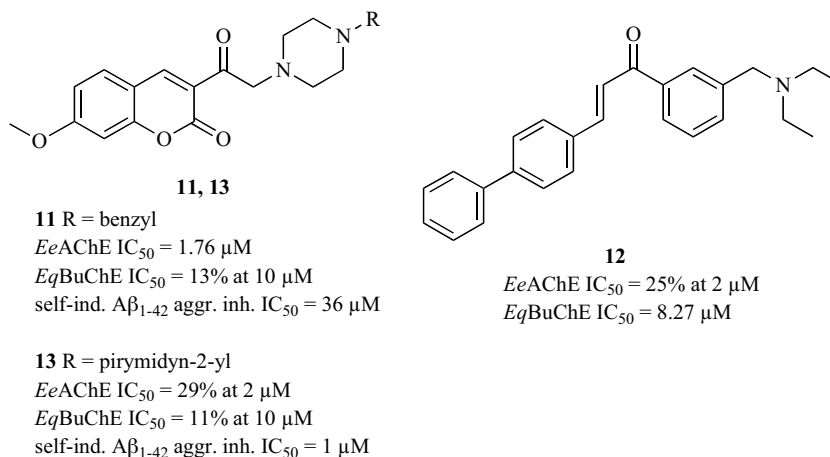


Fig. (9). Multifunctional coumarin and chalcone derivatives with AChE/BuChE and Aβ aggregation inhibitory activity.

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.

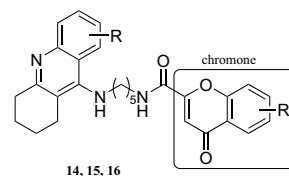
HYBRIDS WITH ACHE/BUChE AND BACE1 INHIBITORY PROPERTIES

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-4*H*-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-4*H*-chromene connected by the linkers of the different lengths (from 7 to 12 carbon atoms). The structures of tacrine and 4-oxo-4*H*-chromene were modified by an introduction of various substituents. In the tacrine unit a chlorine atom was introduced at positions 6, or 6 and 8. The chromene fragment was modified by the introduction of one or two methoxy and hydroxyl groups at positions 5, 6 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 IC_{50} values ranging from 0.175 - 100 nM and 5 - 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 IC_{50} value of 35 pM and compound **15** (Fig. 10) against *hBuChE* with IC_{50} 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 IC_{50} values below 5 μ M. Noteworthy is compound **16** (Fig. 10), which is a potent inhibitor of both cholinesterases and *hBACE1* inhibitor with $IC_{50} = 2.80 \mu$ M. 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.



Compound	R	R'	<i>hAChE</i> IC_{50} [nM]	<i>hBuChE</i> IC_{50} [nM]	<i>hBACE1</i> IC_{50} [μ M]
14	6-Cl	5-OH, 7-OCH ₃	0.035	5.0	-
15	H	6-OCH ₃	0.775	0.038	-
16	H	6-OH	8.0	1.5	2.8

Fig. (10). Tacrine-4-oxo-4*H*-chromene hybrids as AChE, BuChE and BACE1 inhibitors.

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 huprine-tacrine hybrids were synthesized. All the tested compounds exhibited an excellent or good activity towards investigated targets. They were potent *hAChE* inhibitors with IC_{50} 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 methylamine group in the linker had a positive effect on the *hAChE* inhibitory activity. The new compounds were moderately potent inhibitors of *hBuChE* with IC_{50} 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) ($hBACE1$ $IC_{50} = 4.9 \mu$ M). These multifunctional compounds also exhibited a good inhibitory activity towards *hAChE*-induced $A\beta_{1-40}$ aggregation at 100 μ M, with the most active compound being **18** (Fig. 11) ($IC_{50} = 61.3 \mu$ M). The compounds showed a significant inhibitory potency against self-induced $A\beta_{1-42}$ aggregation ranging from 28.1 to 63.7% at 10 μ M. 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 μ M) with the most active compound being **18** ($IC_{50} = 68.7 \mu$ M). 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

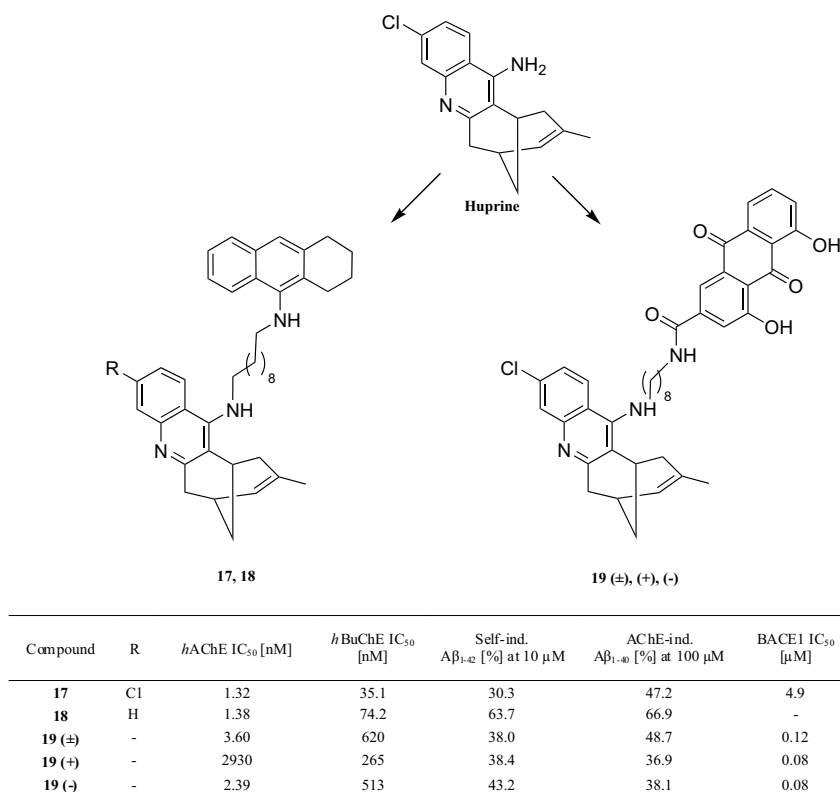


Fig. (11). Huprine based hybrids - *hAChE*, *hBuChE*, BACE1 and Aβ aggregation inhibitors.

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.

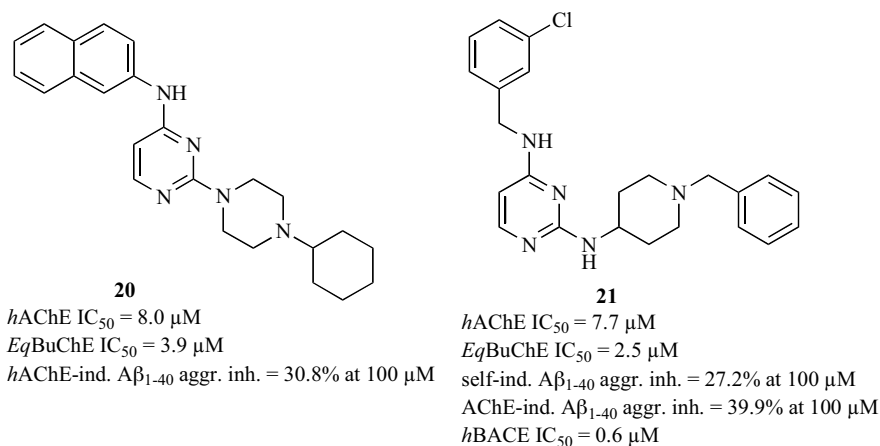


Fig. (12). Multifunctional pyrimidine derivatives with AChE/BuChE, BACE1 and Aβ inhibitory activity.

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 (*hAChE* IC_{50} = 10 μ M, *EqBuChE* IC_{50} = 7.6 μ M) and *hAChE*-induced $A\beta_{1-40}$ aggregation inhibitor (30.8% at 100 μ 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 *hAChE*-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 μ M to 8.9 μ 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 *hAChE* inhibitor (K_i = 6.5 nM) and *hBuChE* inhibitor (K_i = 55 nM). The multifunctional compound **23** also displayed the ability to inhibit *hBACE1* and $A\beta_{1-42}$ aggregation with IC_{50} values 85 μ M and 79 μ 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 μ M) with an activity against both cholinesterases in the nanomolar ranges (*hAChE* K_i = 81 nM, *hBuChE* 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 triazolylmethyltryptoline and triazolyl-2-amino-propyltryptamine connected with a differently substituted phenyl ring. The compounds were tested for inhibitory activity against *hBACE1* 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-aggregation activity than tryptolines, with the exception of the most active compound **25** (Fig. 14) (IC_{50} = 29.86 μ 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

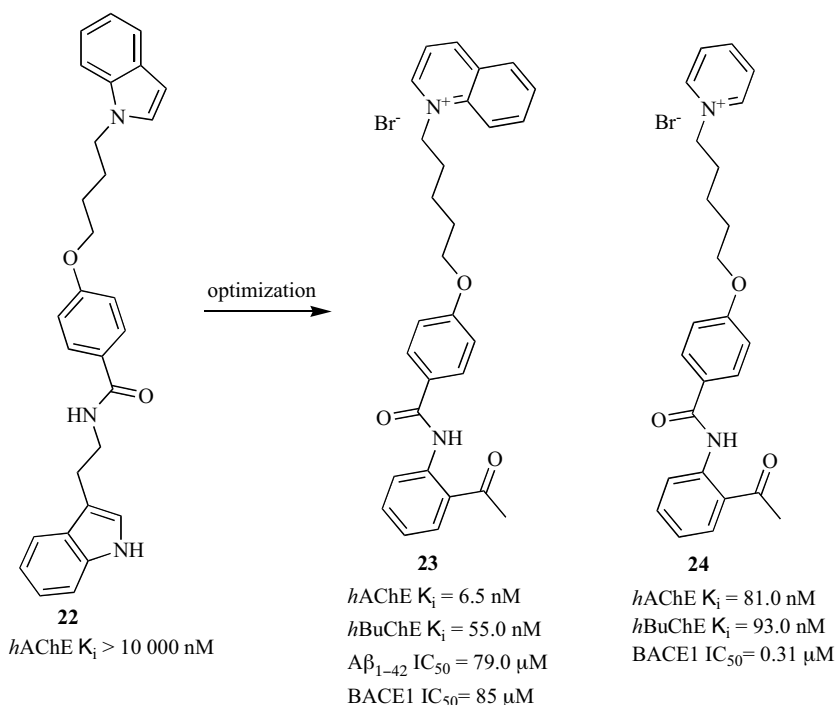


Fig. (13). Benzamide derivatives as AChE/BuChE, BACE1 inhibitors.

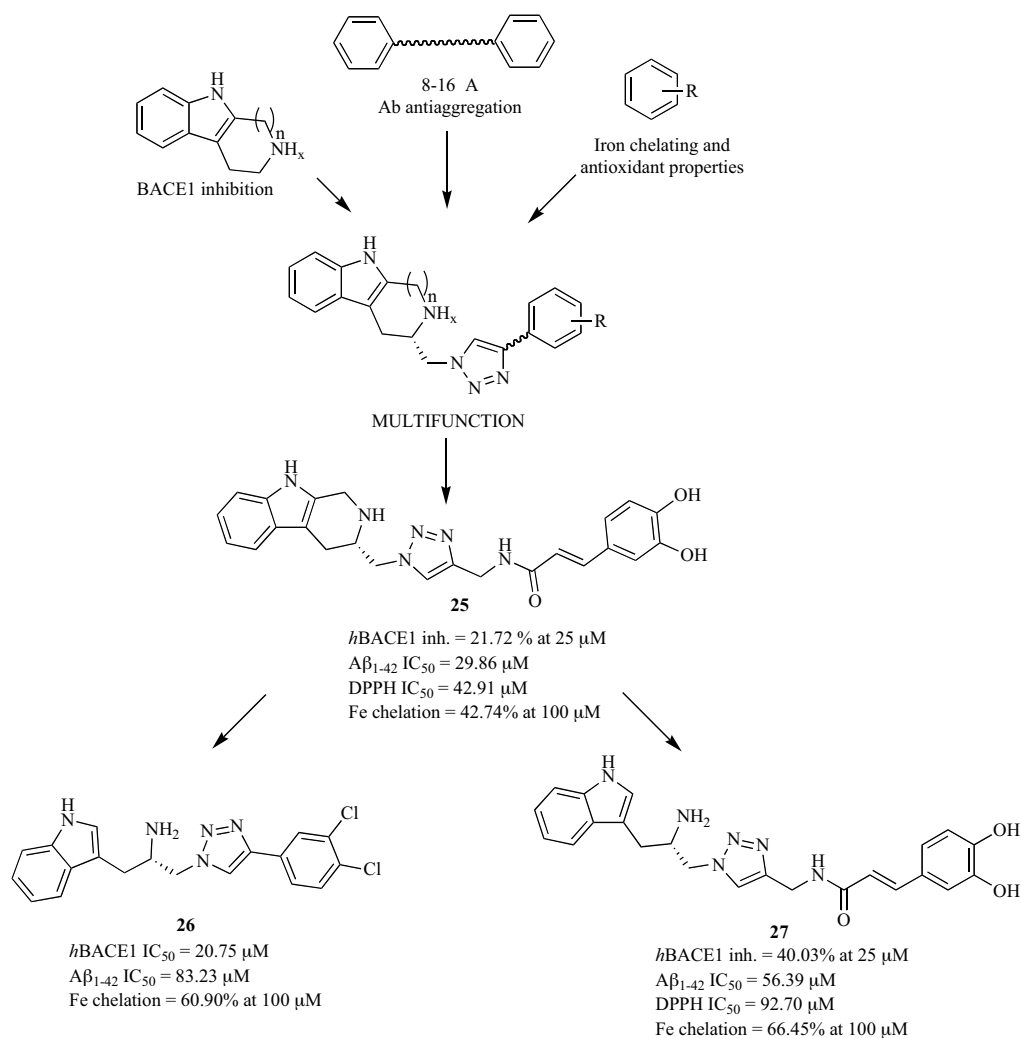


Fig. (14). Tryptoline and tryptamine triazole derivatives - BACE1 and $A\beta$ aggregation inhibitors with antioxidant and metal chelating properties.

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 *h*BACE1 (IC₅₀ = 20.75 μ M), an inhibitory effect on $A\beta_{1-42}$ fibril formation (IC₅₀ = 83.23 μ M) and also the ability to chelate iron ions (61% at 100 μ M). In turn, compounds **25** and **27** exhibited a weaker activity against *h*BACE1 (inhibition at 25 μ M 21.72% and 40.03%, respectively), an inhibitory effect on the $A\beta$ fibril formation (**25** IC₅₀ = 29.86 μ M, **27** IC₅₀ = 56.39 μ M) and moderate metal chelating properties (42.75% and 66.45% at 100 μ M, 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 μ M and 92.70 μ M, respectively. Compounds **25**, **26**, **27** also showed a neuroprotective effect against the neuronal death induced by $A\beta_{1-42}$, 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 an 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

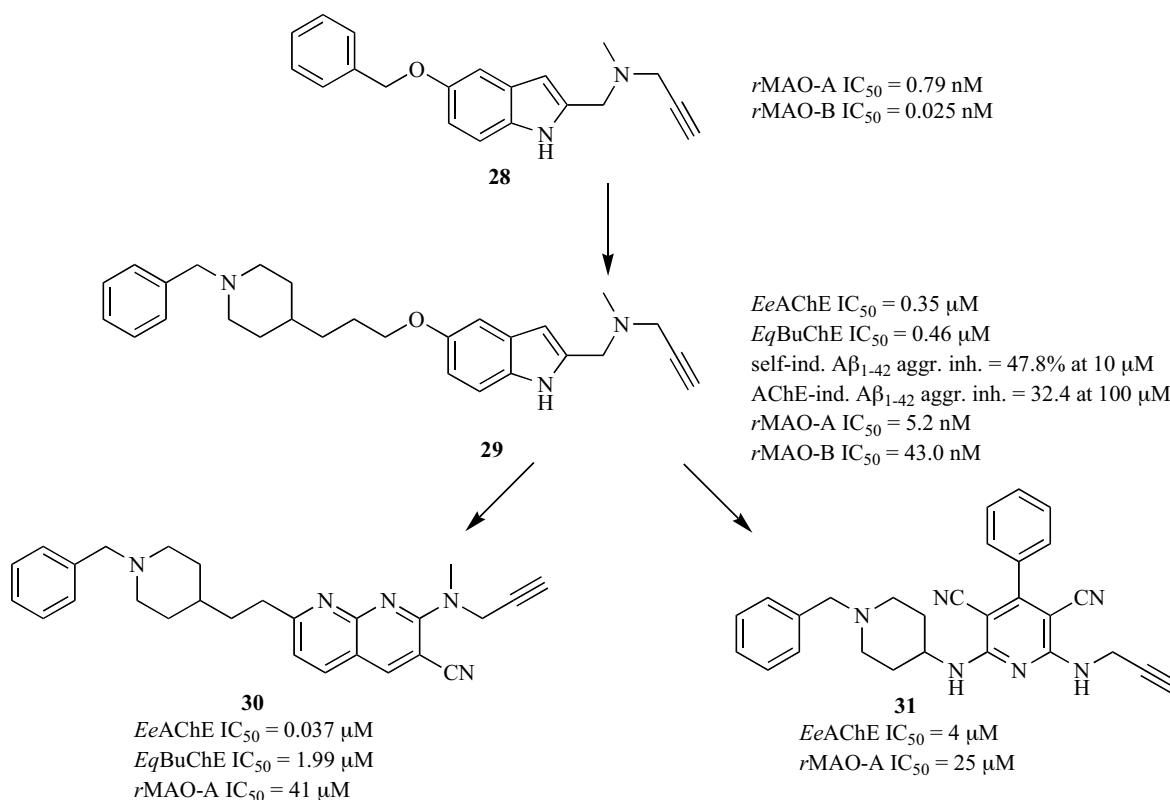


Fig. (15). Benzylpiperidine derivatives as MAO inhibitors with anti-cholinesterase activity.

a relevant effect on the binding to MAO and a weak influence on the inhibition of AChE and BuChE. 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 ($r\text{MAO-A IC}_{50} = 5.2 \text{ nM}$, $r\text{MAO-B IC}_{50} = 43 \text{ nM}$) and was also found to inhibit AChE and BuChE in the submicromolar range ($Ee\text{AChE IC}_{50} = 0.35 \text{ }\mu\text{M}$, $Eq\text{BuChE IC}_{50} = 0.46 \text{ }\mu\text{M}$). 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) $\text{A}\beta_{1-42}$ 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*-methylpropargylamine 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, morpholine 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 ($Ee\text{AChE IC}_{50} = 37 \text{ nM}$) and a moderate, but selective MAO-A inhibitor ($r\text{MAO-A IC}_{50} = 41 \text{ }\mu\text{M}$) (Fig. 15). Compound **31**, the most potent and selective MAO-A inhibitor ($r\text{MAO-A IC}_{50} = 25 \text{ }\mu\text{M}$) with a weaker activity against acetylcholinesterase ($Ee\text{AChE IC}_{50} = 4 \text{ }\mu\text{M}$) (Fig. 15), was a member of the pyridine series.

Tacrine-Selegiline 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 selegiline, a well-known inhibitor of MAO. The inhibition studies of *h*MAO-A and *h*MAO-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 ($Ee\text{AChE IC}_{50} = 14.2 - 456 \text{ nM}$, $Eq\text{BuChE IC}_{50} = 2.03 - 66.0 \text{ nM}$). Compound **32** (Fig. 16) with a nine carbon atom tether, turned out to be a potent inhibitor of *Ee*AChE ($\text{IC}_{50} = 22.6 \text{ nM}$) and *Eq*BuChE ($\text{IC}_{50} = 9.37 \text{ nM}$) and a balanced inhibitor of both monoamine oxidases ($h\text{MAO-A IC}_{50} = 0.372 \text{ }\mu\text{M}$, $h\text{MAO-B IC}_{50} = 0.181 \text{ }\mu\text{M}$).

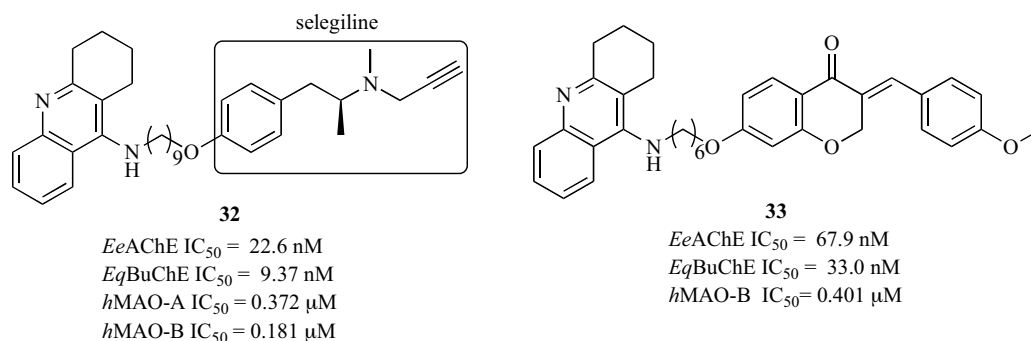


Fig. (16). Tacrine derivatives with MAO and cholinesterase inhibitory activity.

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-Homoisoflavanoid Hybrids

Sun *et al.* [132] also used tacrine as a pharmacophore for the novel homoisoflavanoid hybrids. Derivatives of homoisoflavanoids were chosen because of their known MAO-B inhibitory activity [133]. In a new series, tacrine and homoisoflavanoid fragments were connected using carbon spacers of different lengths. They also had different substituents in the homoisoflavanoid moiety. All the compounds were potent cholinesterase inhibitors with activities in the nanomolar range, and were selective MAO-B inhibitors. Compound **33** (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 3-nitrophenyl at position 5 in pyrazoline (*hMAO-A* K_i = 32.16 nM and *hMAO-B* K_i = 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 K_i values in the nanomolar range. Compound **34** inhibits *hAChE* with K_i = 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 antioxidant

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.

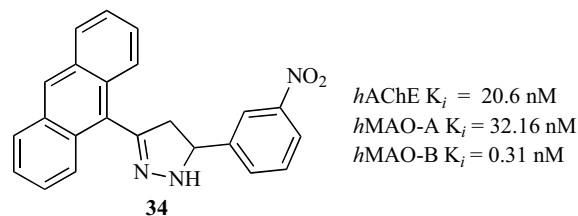


Fig. (17). Pyrazoline derivatives with MAO and AChE inhibitory activity.

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 aggregation 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 *ortho*-methylphenyl ring, was found to be the most active *EeAChE* inhibitor with IC₅₀ = 27 nM. It also displayed good antioxidant activity (ORAC = 4.05 eq. of Trolox) and inhibited self-induced Aβ₁₋₄₂ aggregation with IC₅₀ = 2.73 μM. Other modifications of 9-*O*-substituted berberine derivatives resulted in a new series of berberine-tiophenyl hybrids [143].

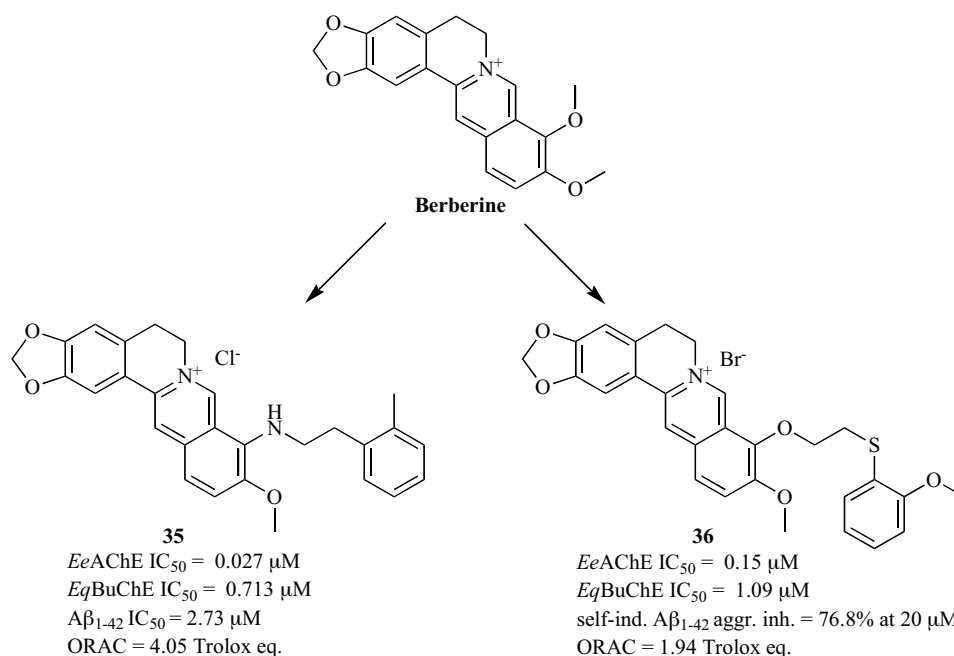


Fig. (18). Multifunctional berberine derivatives with antioxidant activity.

In this series, most of the hybrids demonstrated *EeAChE* 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 Aβ₁₋₄₂ aggregation test (76.8% at 20 μM) (Fig. **18**). It was also found to be a moderate *EeAChE* 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-7*H*-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 *hAChE* in the micromolar and submicromolar range (IC₅₀ = 0.30 - 5.74 μM). 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 μM). The oxidative stress in cells was induced by the mixture of oligomycin-A and rotenone which

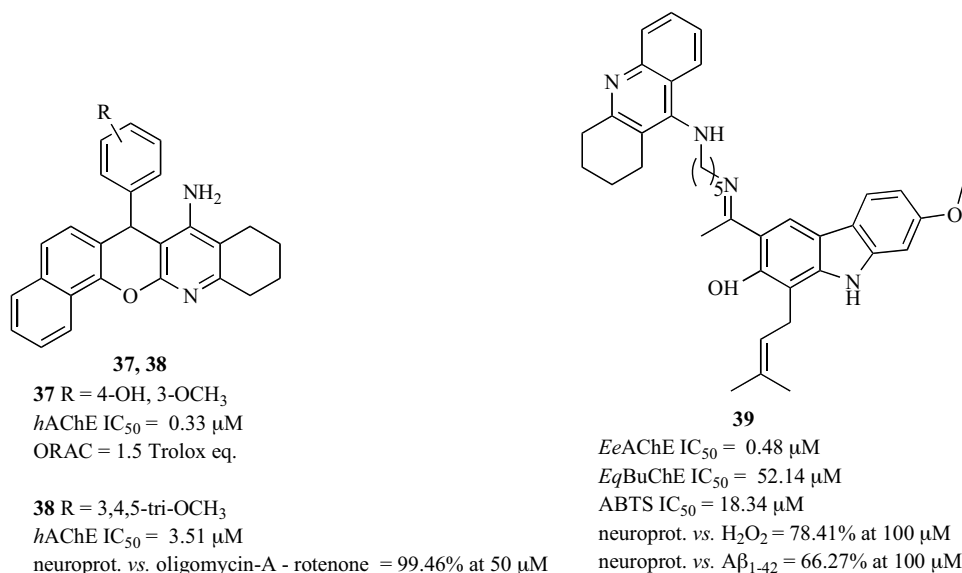


Fig. (19). Tacrine derivatives with the antioxidant properties.

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 Thiramatrakul *et al.* [147]. Synthesized compounds were found to be moderate *EeAChE* 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 ($IC_{50} = 23.67 \mu\text{M}$). The compounds showed higher radical scavenging activity than Trolox ($IC_{50} = 8.34 - 11.24 \mu\text{M}$). Furthermore, they displayed a neuroprotective effect against the oxidative stress induced by H_2O_2 in the neuroblastoma cells and against the toxicity induced by $\text{A}\beta_{1-42}$ peptide in C6 astrogloma cells at $100 \mu\text{M}$. 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.

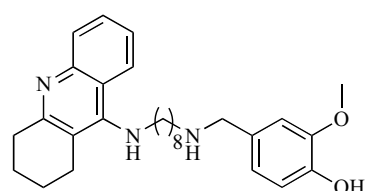
The results reported by Luo *et al.* [150] present hybrids of tacrine connected by an alkyl linker to benzylamine. Methoxy and hydroxyl groups were introduced to benzylamine to investigate their influence on the antioxidant properties. The best results were provided by compounds with at least one hydroxyl 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 *EeAChE* and *EqBuChE* inhibitors in the nanomolar range. The most active compound was found to be **40** (Fig. 20) (*EeAChE* $IC_{50} = 4.55 \text{ nM}$ and *EqBuChE* $IC_{50} = 3.41 \text{ nM}$), which had a hydroxyl group at position 4 and a methoxy group at position 3 in the benzene ring. Compound **40** inhibited self-mediated $\text{A}\beta_{1-42}$ aggregation in 71% at $20 \mu\text{M}$ and was 1.9-fold more potent than Trolox in the ORAC assay.

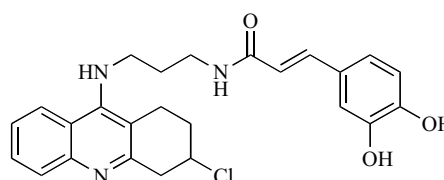
Caffeic acid displays the antioxidative activity and chemopreventive effect against $\text{A}\beta_{1-42}$ [151]. Therefore, Chao *et al.* [152] connected caffeic acid with tacrine by alkyl linkers with the hope of finding cholinesterase inhibitors endowed with the antioxidant properties. Based on their previous studies on ferulic acid - tacrine hybrids, they chose two, three and six carbon atom linkers. To gain selectivity towards *EeAChE* over *EqBuChE* they also prepared compounds with a 6-chlorotetrahydroacridine instead of tacrine. The most active compound was **41** (Fig. 20) with an IC_{50} of $0.3 \mu\text{M}$ against *AChE* and $29.5 \mu\text{M}$ against *BuChE*. This compound showed also an inhibitory effect against self-induced and *AChE*-induced $\text{A}\beta$ aggregation (36.2% at $20 \mu\text{M}$ and 67.7% at $100 \mu\text{M}$, 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 $\text{A}\beta_{1-40}$ aggregation (21.4% at $100 \mu\text{M}$), 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_2O_2 by glutathione [155]. The rates were $123.5 \mu\text{M}/\text{min}$ for the compound **42** and $121.3 \mu\text{M}/\text{min}$ for ebselen. Compound **42** showed similar or better scavenging activities than ebselen

**40**

EeAChE $IC_{50} = 4.55 \text{ nM}$
EqBuChE $IC_{50} = 3.41 \text{ nM}$
 self-ind. $\text{A}\beta_{1-42}$ aggr. inh. = 71% at $20 \mu\text{M}$
 ORAC = 1.9 Trolox eq.

**41**

EeAChE $IC_{50} = 0.3 \mu\text{M}$
EqBuChE $IC_{50} = 29.5 \mu\text{M}$
 self-ind. $\text{A}\beta_{1-40}$ aggr. inh. = 36.2% at $20 \mu\text{M}$
AChE-ind. $\text{A}\beta_{1-40}$ aggr. inh. = 67.7% at $100 \mu\text{M}$
 DPPH $IC_{50} = 4.8 \mu\text{M}$

Fig. (20). Tacrine hybrids - cholinesterase and $\text{A}\beta$ aggregation inhibitors with antioxidant properties.

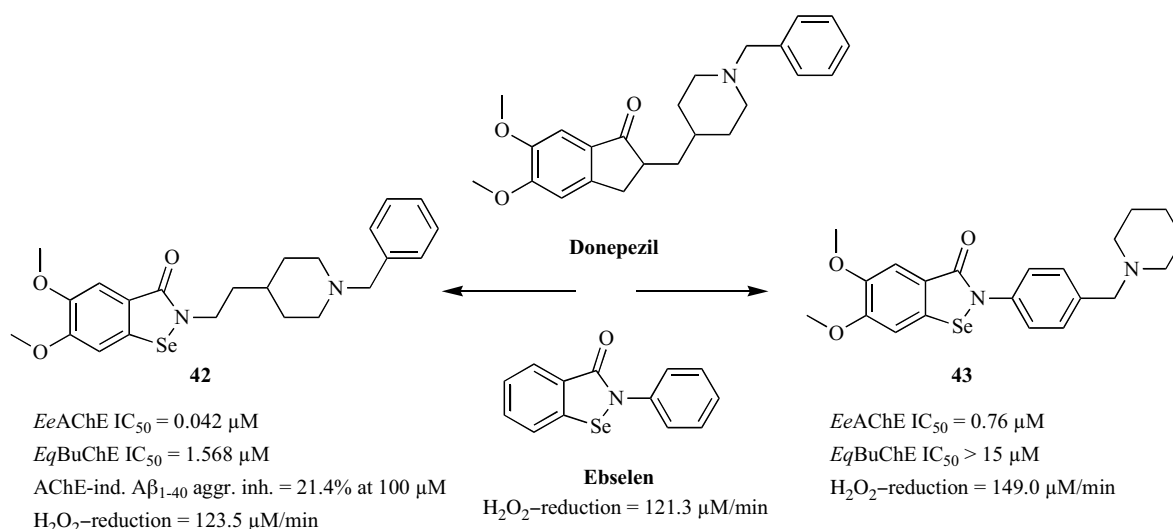


Fig. (21). Donepezil-ebsele hybrid compounds - cholinesterase inhibitors with the antioxidant properties.

on hydrogen peroxide and on peroxyxynitrite 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 *EeAChE* 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 morpholinonydnimine (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 cardiomyocytes (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 cardiomyocytes 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

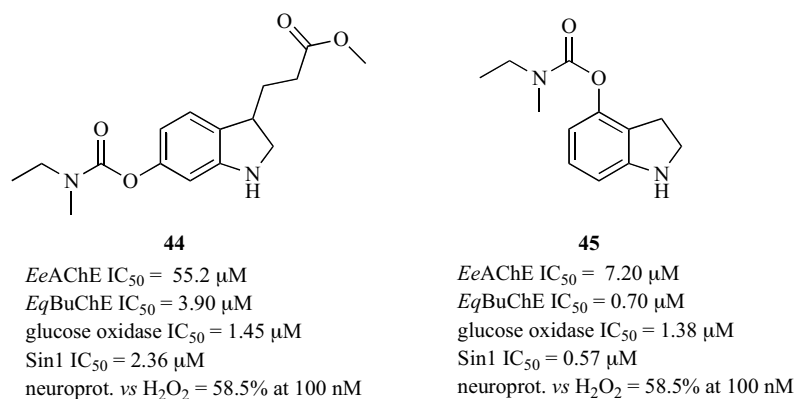


Fig. (22). Carbamate derivatives of indoline with antioxidant properties and anti-cholinesterase activity.

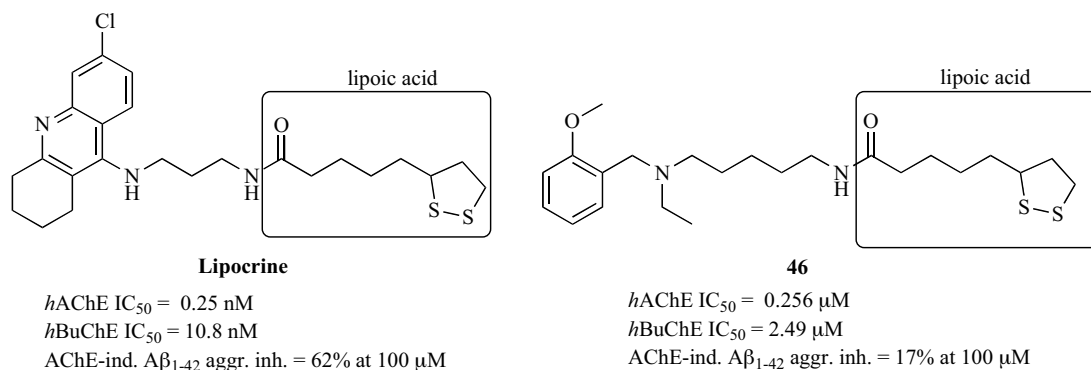


Fig. (23). Lipoic acid derivatives as antioxidants and cholinesterase inhibitors.

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.

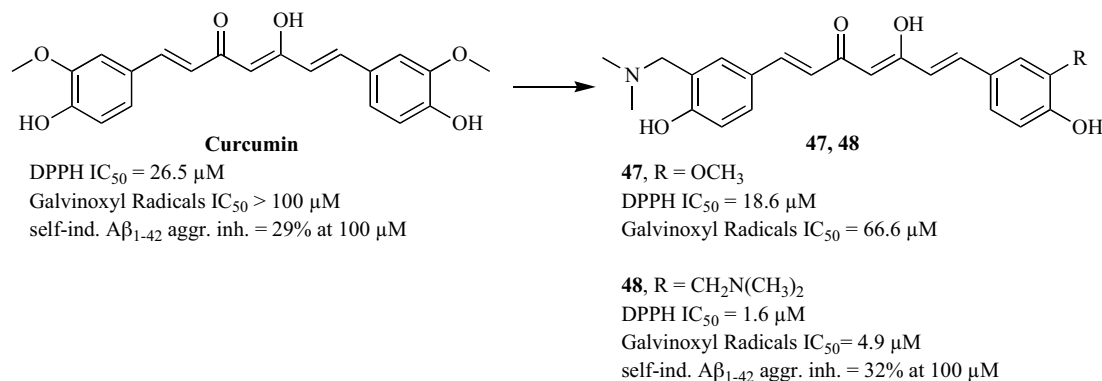


Fig. (24). Curcumin and its dimethylaminomethyl-substituted derivatives as antioxidants.

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 A β and increase its aggregation. An alternative explanation is that an imbalance in the levels of metals may increase production of ROS induced by A β . 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 A β 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 fla-

vonoid scaffold by a four carbon atom linker (Fig. 25). Compound **49** exhibited the most potent AChE activity (IC_{50} = 130 nM), high selectivity for AChE, inhibition of self-induced A β_{1-42} aggregation at 20 μ M (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-A β (A β_{1-42} IC_{50} = 6.5 μ M) and anti-cholinesterase (*Ee*AChE IC_{50} = 0.133 μ M, *Eq*BuChE IC_{50} = 0.558 μ M) 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 A β 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 *Ee*AChE (IC_{50} = 0.092 μ M) and the best anti-aggregation properties (67.8% inhibition at 20 μ M). It also showed moderate *Eq*BuChE inhibition (IC_{50} = 0.234 μ M) and effective chelation of Cu²⁺ and Fe²⁺.

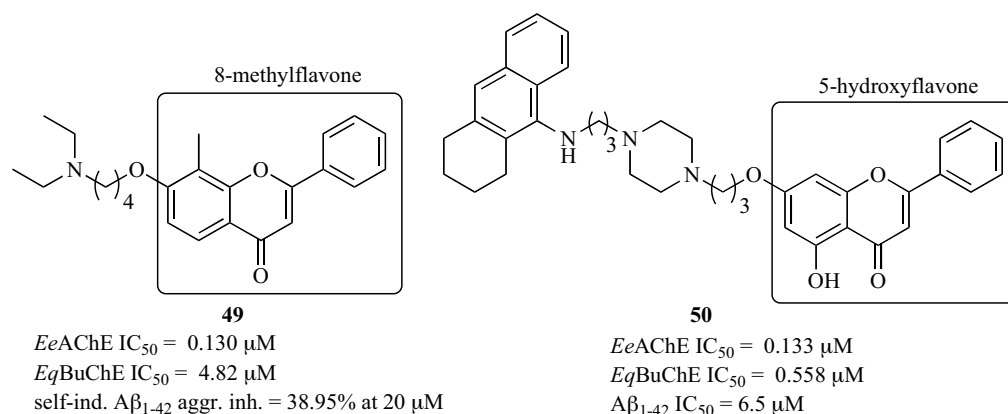


Fig. (25). Flavonoid derivatives as cholinesterase inhibitors with A β anti-aggregating and metal chelating properties.

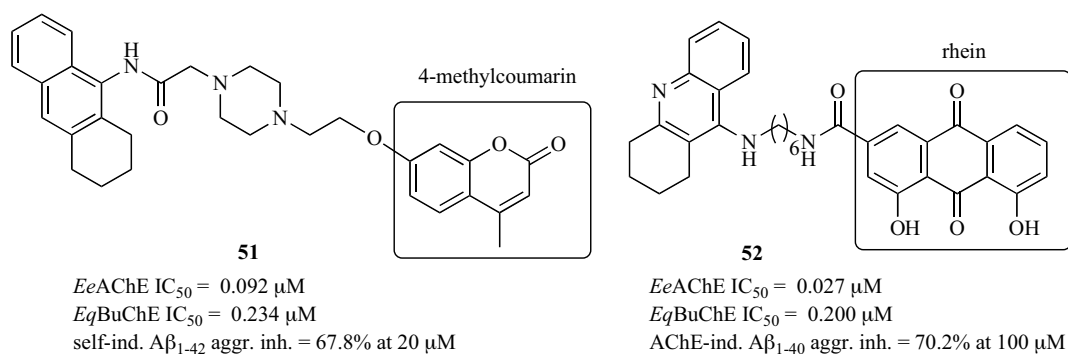


Fig. (26). Coumarin and rhein derivatives as cholinesterase inhibitors with metal chelating properties.

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 IC_{50} values it appeared that the most suitable was a six carbon atom tether. Compound **52** (Fig. 26) was a potent AChE inhibitor with $IC_{50} = 27.3$ nM and a potent inhibitor of AChE-induced $A\beta_{1-40}$ aggregation (70.2% at 100 μ M). 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 *Ee*AChE inhibitor with $IC_{50} = 1.8$ nM, and a moderate *Eq*BuChE inhibitor with $IC_{50} = 9.5$ μ M. It also displayed metal chelating activity towards Cu^{2+} , Fe^{2+} and Zn^{2+} . 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 $A\beta$ aggregation, antioxidant properties and metal chelating properties. The indanone fragment was combined with differently substituted benzaldehydes. The strongest ability to inhibit self-mediated $A\beta_{1-42}$ aggregation was provided by compound **54** (Fig. 27) (80.1% at 20 μ M). It also inhibits Cu^{2+} -induced aggregation, disassembles the well-structured $A\beta$ fibrils and chelates metals. Compound **54** also displays a moderate activity against *h*MAO-B ($IC_{50} = 7.50$ μ M) and *h*MAO-A ($IC_{50} = 37.7$ μ M). 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 $A\beta$ 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) (*Ee*AChE $IC_{50} = 0.6$ nM). Compound **55** also inhibited self-induced (45.9%) and AChE-induced (90.6%) $A\beta_{1-42}$ aggregation at 25

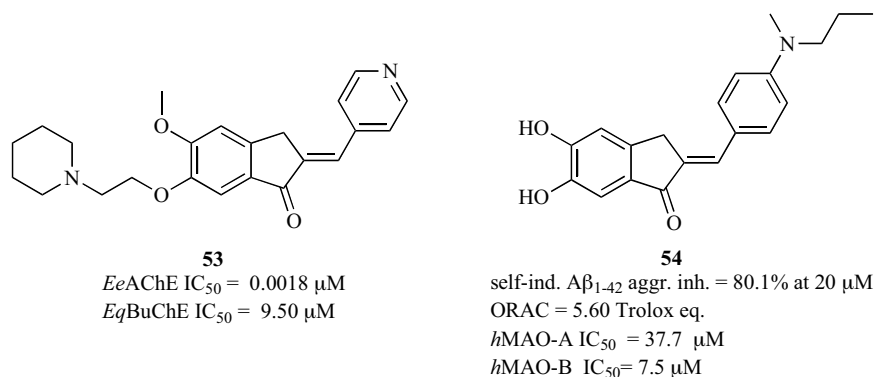


Fig. (27). Indanone derivatives as cholinesterase and monoamine oxidase inhibitors with metal chelating properties.

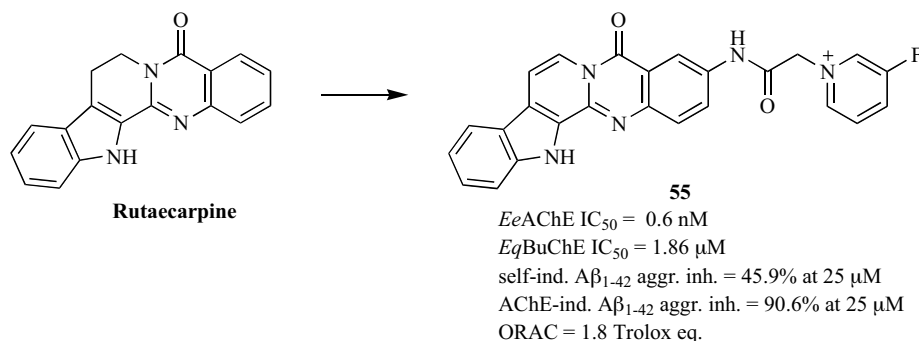


Fig. (28). Rutaecarpine derivative as a multifunctional metal chelator.

μM . This lead compound chelates metals and has better antioxidant properties than Trolox (ORAC at $1 \mu\text{M} = 1.8$).

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 $\text{A}\beta_{1-42}$ (71.65% at $20 \mu\text{M}$) with antioxidant activity (4.12 of Trolox eq. at $1 \mu\text{M}$) [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 $\text{A}\beta$ aggregation (79.50% at $20 \mu\text{M}$) with antioxidant properties (4.72 of Trolox eq. at $1 \mu\text{M}$) (Fig. 29). Moreover, compound **57** chelates metals and inhibits Cu(II) -induced $\text{A}\beta$ aggregation (94.23% at $20 \mu\text{M}$). It also shows inhibitory activity towards cholinesterases and monoamine oxidases in the micromolar range (*EeAChE* $\text{IC}_{50} = 6.27 \mu\text{M}$ and *EqBuChE* $\text{IC}_{50} = 21.25 \mu\text{M}$, *hMAO-A* $\text{IC}_{50} = 7.08 \mu\text{M}$, *hMAO-B* $\text{IC}_{50} = 14.09 \mu\text{M}$) (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 $\text{A}\beta$ self-aggregation

(64.6% at $20 \mu\text{M}$) with neuroprotective activity better than resveratrol and it reduced Cu^{2+} -induced $\text{A}\beta$ 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 contrary 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 IC_{50} 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

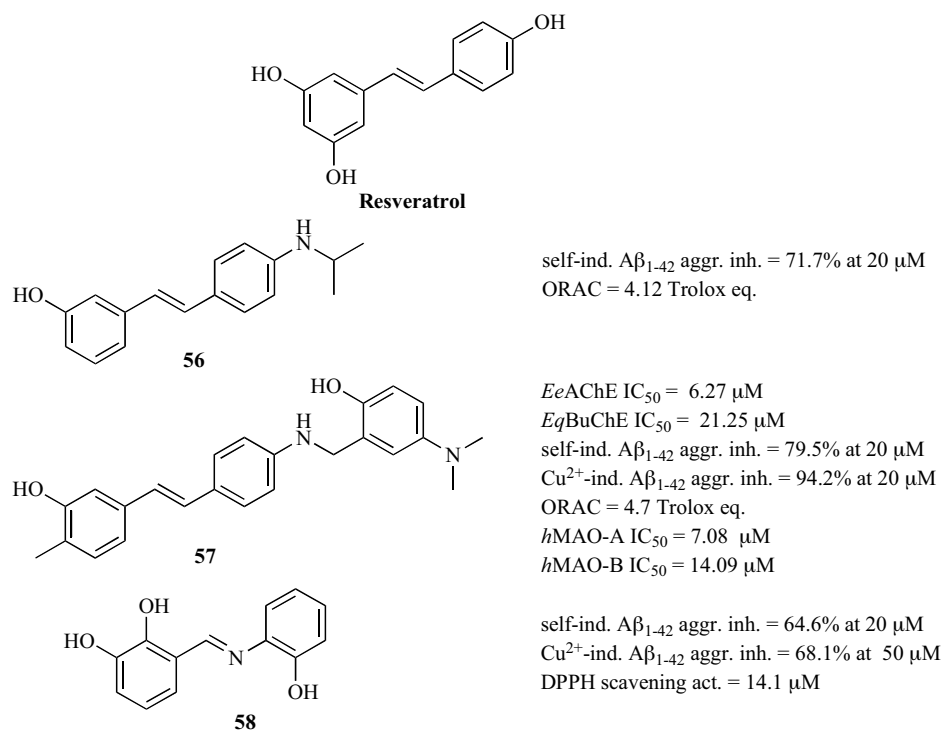


Fig. (29). Resveratrol derivatives as metal-chelating agents with additional biological properties.

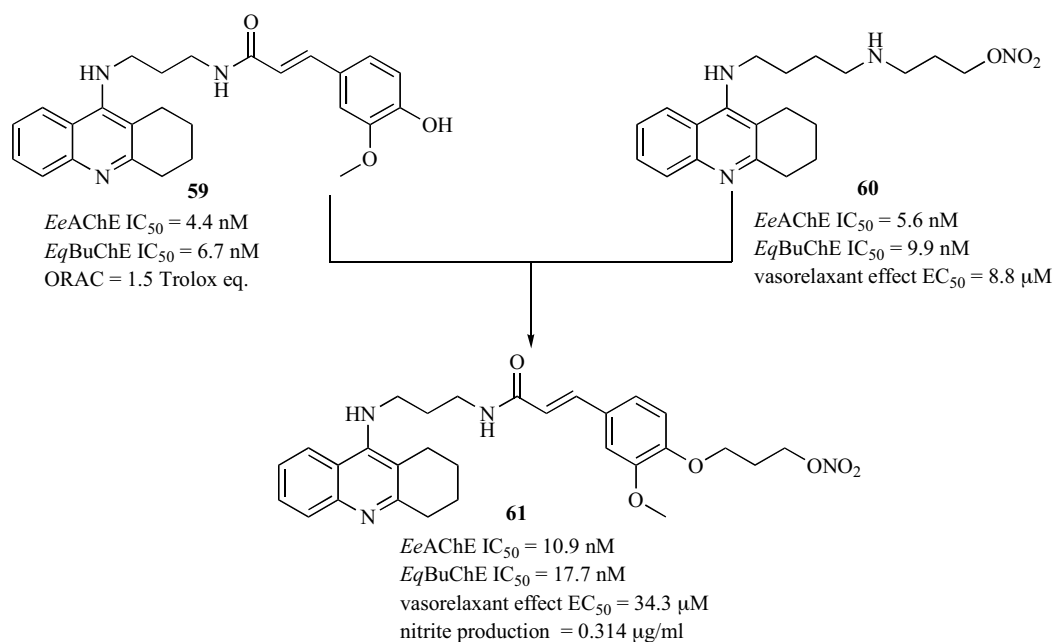


Fig. (30). Tacrine-ferulic acid-NO-donor trihybrid and its precursors.

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 the nitrite 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 *EqBuChE* 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 *EeAChE* and *EqBuChE* (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.

Dibenzofurane Derivatives

NO releasing organic nitrate was also incorporated into the dibenzofurane 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**, *EeAChE* 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.

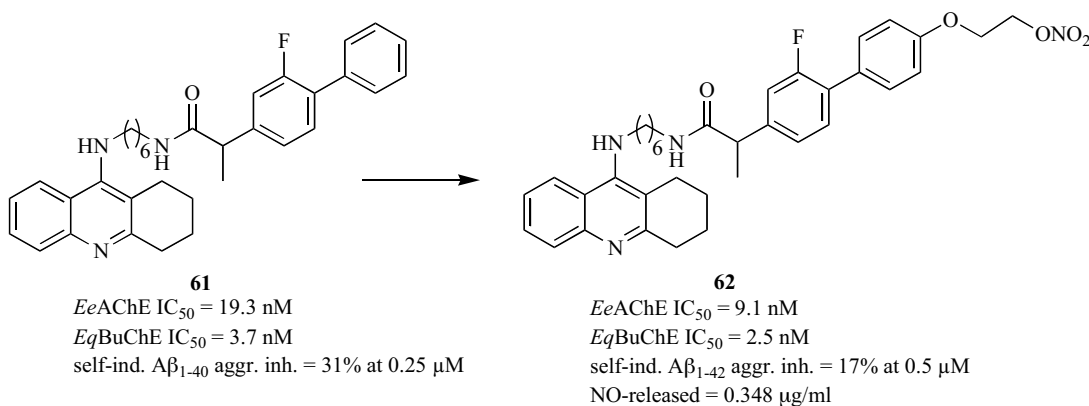


Fig. (31). Tacrine-flurbiprofen hybrid compound and its NO-releasing analogue.

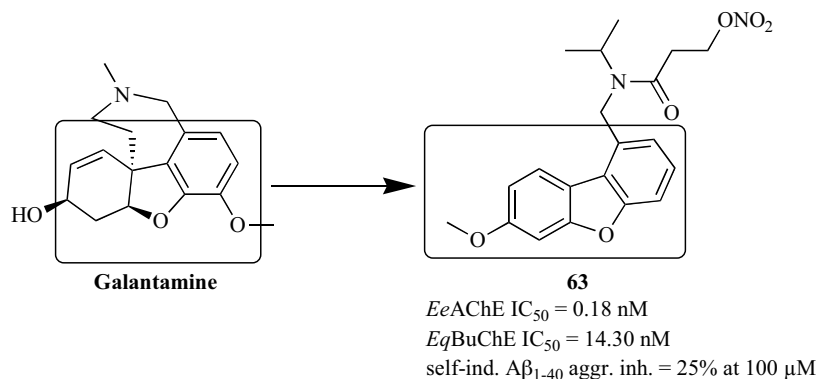


Fig. (32). Galantamine and its simplified analogue with NO-releasing nitrate group.

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 glycineamide 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 pIC₅₀ = 7.14, and which also displayed the most significant Ca²⁺ 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 pIC₅₀ = 6.98 against *EeAChE* and with the lowest pIC₅₀ =

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*-acylaminophenothiazines modified within the amine group. They developed a series of selective BuChE inhibitors with IC₅₀ 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 H₂O₂ 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 Aβ₁₋₄₂ 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).

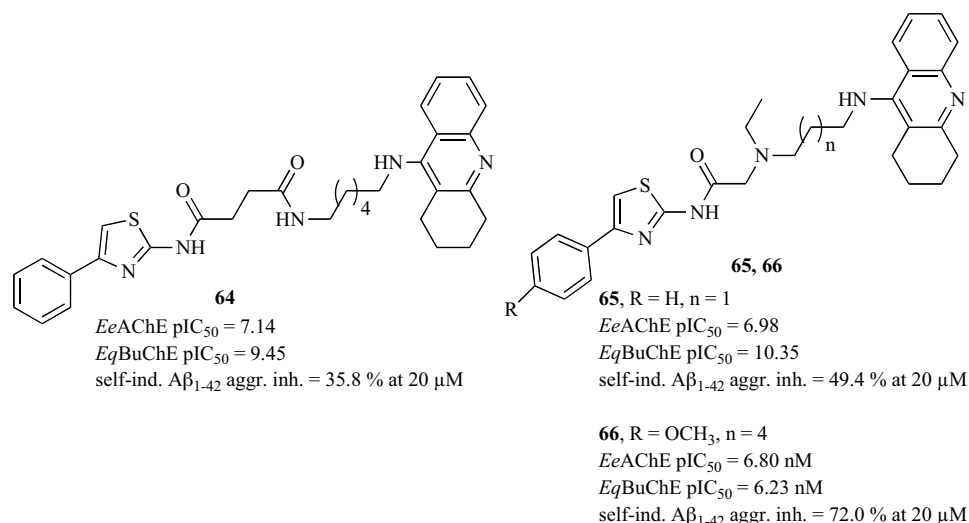


Fig. (33). 4-Phenyl-2-aminothiazole-tacrine hybrids - cholinesterase and Aβ aggregation inhibitors with Ca²⁺ overload blocking activity.

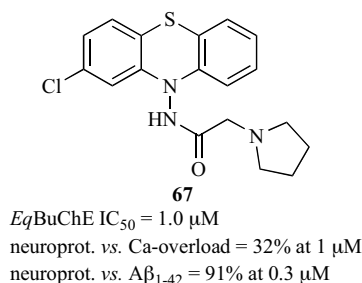


Fig. (34). *N*-acylamiphenothiazine derivative with the neuroprotective activity.

Cystamine-Tacrine Dimer

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* IC₅₀ = 5.04 nM, *hBuChE* IC₅₀ = 4.23 nM) and inhibitory properties in the self-induced Aβ aggregation assay with IC₅₀ = 24.2 μM and in the AChE-induced Aβ aggregation test (52% at 100 μM). Its neuroprotective effect against oxidative stress induced by H₂O₂ was tested on SH-SY5Y cell line. A complete

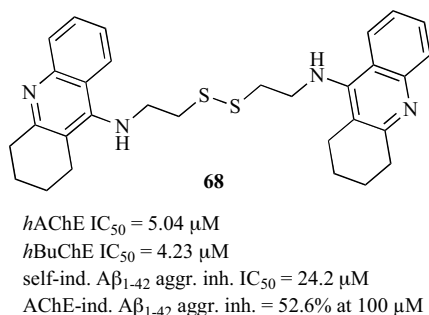


Fig. (35). Cystamine-tacrine dimer.

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 IC₅₀ 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

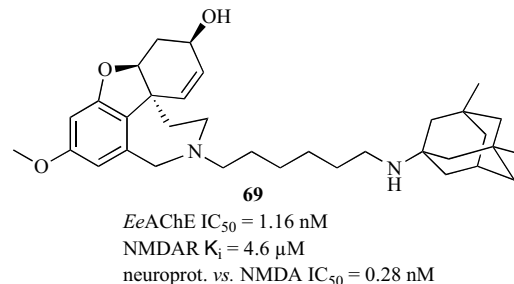


Fig. (36). Memagal - a chimera derivative of galantamine and memantine.

was proven to inhibit NMDA-induced neurotoxicity ($IC_{50} = 0.28 \text{ nM}$) in a cell-based assay.

Novel multifunctional bis- γ -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 γ -carboline fragments of latrepirdine connected by different linkers. Among synthesized 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 *h*AChE ($IC_{50} = 0.692 \text{ }\mu\text{M}$) and *h*BuChE ($IC_{50} = 0.737 \text{ }\mu\text{M}$) and it inhibited $A\beta_{1-42}$ self-aggregation (71% at $50 \text{ }\mu\text{M}$). Compound **70** acted as NMDAR antagonist ($IC_{50} = 12.6 \text{ }\mu\text{M}$ at -100 mV) at recombinant NMDARs but it was less potent than memantine ($IC_{50} = 0.71 \text{ }\mu\text{M}$ 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-arylbenzofuran and *N*-methyl-*N*-benzylamine and the substituent at position 3 of the benzofuran scaffold. The influ-

ence 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 *para* to *meta* resulted in the development of compound **72** (Fig. 38), which was the most active inhibitor of *h*AChE in the series and 180-fold more potent than the parent compound **71** ($IC_{50} = 0.24 \text{ }\mu\text{M}$ vs. $40.7 \text{ }\mu\text{M}$). 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 ($IC_{50} = 3.9 \text{ }\mu\text{M}$). 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 β -amyloid aggregation. Among the $A\beta$ fibril formation inhibitors, compounds **71** and **72** counteracted neurotoxicity induced by $A\beta_{25-35}$ by inhibiting peptide-induced ROS formation and preventing the interaction of the $A\beta_{25-35}$ 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 $0.32 \text{ }\mu\text{M}$). Compounds of the *para* series with amino moieties on the benzoyl group showed weak affinities for CB1 receptors ($K_i = 0.55 - 2.57 \text{ }\mu\text{M}$) and CB2 receptors ($K_i = 0.58 - 1.18 \text{ }\mu\text{M}$). 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 Ellman'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.

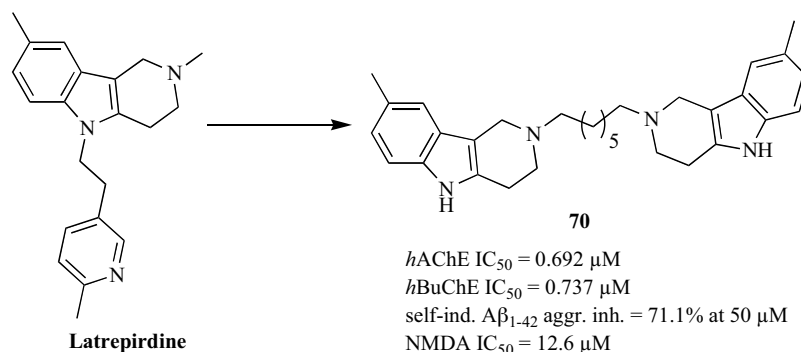


Fig. (37). Latrepirdine-based bivalent ligand with anti-cholinesterase, $A\beta$ anti-aggregation activity and neuroprotective effect.

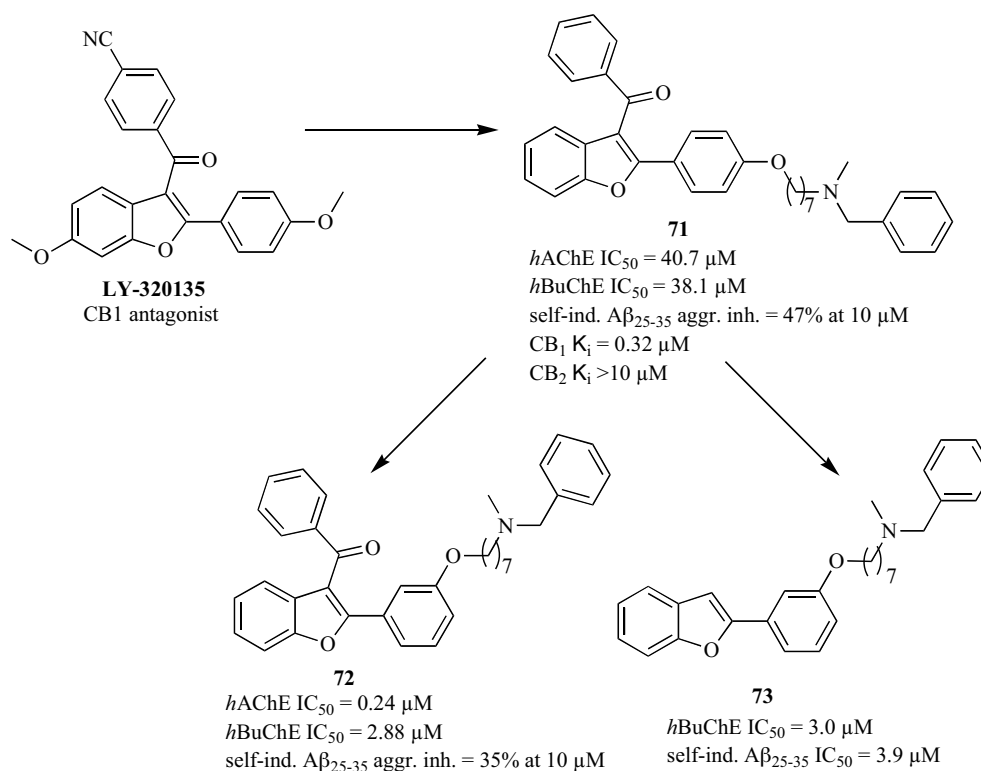
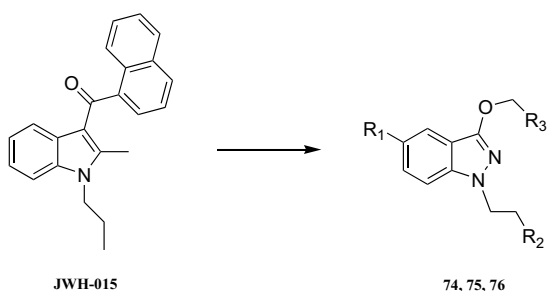


Fig. (38). LY320135 - CB1 receptor antagonist and MTDLs against AD, targeting AChE, BuChE, amyloid-β formation and cannabinoid receptors.



Compound	R ₁	R ₂	R ₃	<i>hBuChE</i> IC ₅₀ [μM]	ORAC [Trolox eq.]	CB ₁ K _i [μM]	CB ₂ K _i [μM]
74	H	-N-CH(CH ₃) ₂	4-methoxyphenyl	2.28	1	> 40	7.7
75	H	piperidine	2-naphthyl	1.4	0.5	1.4	2
76	NH ₂	piperidine	2-naphthyl	1.5	0.8	> 40	2

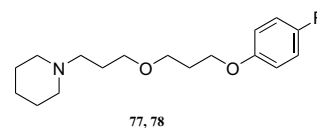
Fig. (39). Indole-based CB2 receptor agonist JWH-015 and indazole-based compounds CB2 receptor agonists with BuChE inhibitory activity and antioxidant properties.

DUAL-ACTING COMPOUNDS TARGETING HISTAMINERGIC SYSTEM

The histamine H₃ receptor (H₃R) belongs to the class of G protein-coupled receptors (GPRs), 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, mem-

ory 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 (*K_i* 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 *hH₃*R potency also showed the highest anti-cholinesterase activity. Two of the most interesting multifunctional compounds (**77** and **78**) (Fig. 40) displayed high affinity for *hH₃*R (**77** *K_i* = 3.48 nM, **78** *K_i* = 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	R	<i>EeAChE</i> IC ₅₀ [μM]	<i>EqBuChE</i> IC ₅₀ [μM]	<i>hH₃</i> R K _i [nM]
77	Cl	7.91	4.97	3.48
78	C(CH ₃) ₃	12.03	1.28	7.74

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 *h*H₃ 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 piperidinyloxyphenyl 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 *h*H₃ receptors in a wide range. Compound **79** (Fig. 41) showed high binding affinity at *h*H₃R ($K_i = 17.5$ nM) but displayed a moderate inhibitory activity towards *Ee*AChE (IC₅₀ = 6.77 μM) and *Eq*BuChE (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 *h*H₃ receptor affinities, they were ranging from the micromoles to nanomoles for the most active compound **80** ($K_i = 76.2$ nM). Moreover, all of the tested compounds showed a very good selectivity profile with regard to the *h*H₃ receptor over all the other *h*H 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 *h*H₃R, with a balanced potency against both targets.

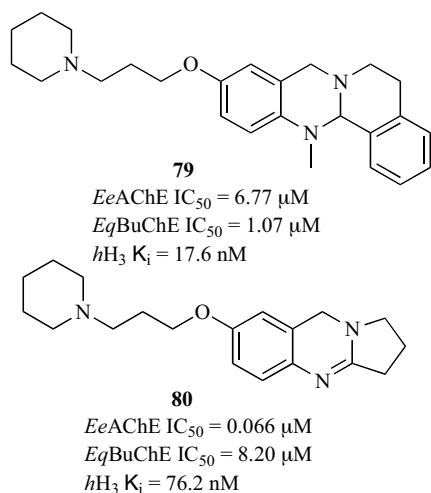


Fig. (41). Tri- and tetracyclic derivatives acting as dual AChE inhibitors and *h*H₃ receptor antagonists.

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 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.

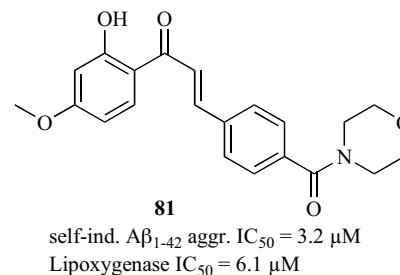


Fig. (42). An isoliquiritigenin derivative with activity against 5-LO and Aβ aggregation.

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, butyrylcholinesterase, 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 isaindigotone, 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
A β	= 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
<i>Ee</i> AChE	= Acetylcholinesterase from <i>Electrophorus electricus</i>
<i>Eq</i> BuChE	= 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
H ₃ R	= Histamine H ₃ receptor
<i>h</i> AChE	= Human acetylcholinesterase
<i>h</i> BACE1	= Human β -secretase
<i>h</i> BuChE	= Human butyrylcholinesterase

<i>h</i> H ₃ R	= Human histamine H ₃ receptor
<i>h</i> MAO-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	= <i>N</i> -methyl- <i>D</i> -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
PPAR γ	= Peroxisome proliferator-activated receptor γ
<i>r</i> AChE	= Acetylcholinesterase from rat brain
<i>r</i> MAO-A/B	= Monoamine oxidase A/B from rat brain
ROS	= Reactive oxygen species
SAR	= Structure-activity relationship
Sin1	= Morpholinolonydnonimine
ThT	= Thioflavin

CONFLICT OF INTEREST

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

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REFERENCES

- Querfurth, H.W.; LaFerla, F.M. Alzheimer's disease. *N. Engl. J. Med.*, **2010**, *362*, 329-344.
- Huang, Y.; Mucke, L. Alzheimer Mechanisms and Therapeutic Strategies. *Cell*, **2012**, *148*, 1204-1222.
- Reitz, C.; Brayne, C.; Mayeux, R. Epidemiology of Alzheimer disease. *Nat. Rev. Neurol.*, **2011**, *7*, 137-152.
- Reitz, C.; Mayeux, R. Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochem. Pharmacol.*, **2014**, *88*, 640-651.
- Bartus, R.; Dean, R.; Beer, B.; Lippa, A. The cholinergic hypothesis of geriatric memory dysfunction. *Science*, **1982**, *217*, 408-414.
- Terry, A.V. Jr.; Buccafusco, J.J. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. *J. Pharmacol. Exp. Ther.*, **2003**, *306*, 821-827.
- Marksteiner, J.; Schmidt, R. Treatment strategies in Alzheimer's disease with a focus on early pharmacological interventions. *Drugs Aging*, **2004**, *21*, 415-426.
- Meunier, J.; Ieni, J.; Maurice, T. The anti-amnesic and neuroprotective effects of donepezil against amyloid β_{25-35} peptide-induced toxicity in mice involve an interaction with the sigma1 receptor. *Br. J. Pharmacol.*, **2006**, *149*, 998-1012.
- Wilkinson, D. G.; Francis, P. T.; Schwam, E.; Payne-Parrish, J. Cholinesterase inhibitors used in the treatment of Alzheimer's dis-

- ease: the relationship between pharmacological effects and clinical efficacy. *Drugs Aging*, **2004**, *21*, 453-478.
- [10] Rodda, J.; Carter, J. Cholinesterase inhibitors and memantine for symptomatic treatment of dementia. *BMJ*, **2012**, *344*, e2986.
- [11] van Marum, R. J. Current and future therapy in Alzheimer's disease. *Fundam. Clin. Pharmacol.*, **2008**, *22*, 265-274.
- [12] Martinez, A. *Emerging Drugs and Targets for Alzheimer's Disease: Volume 1: Beta-Amyloid, Tau Protein and Glucose Metabolism*; RSC: Cambridge, UK, **2010**.
- [13] Martinez, A. *Emerging Drugs and Targets for Alzheimer's Disease: Volume 2: Neuronal Plasticity*. RSC: Cambridge, UK, **2010**.
- [14] Grill, J. D.; Cummings, J. L. Current therapeutic targets for the treatment of Alzheimer's disease. *Expert Rev. Neurother.*, **2010**, *10*, 711-728.
- [15] Hardy, J.; Allsop, D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol. Sci.*, **1991**, *12*, 383-388.
- [16] Frisardi, V.; Solfrizzi, V.; Imbimbo, P.B.; Capurso, C.; D'Introno, A.; Colacicco, A.M.; Vendemiale, G.; Seripa, D.; Pilotto, A.; Capurso, A.; Panza, F. Towards disease-modifying treatment of Alzheimer's disease: drugs targeting beta-amyloid. *Curr. Alzheimer Res.*, **2010**, *7*, 40-55.
- [17] Rampa, A.; Gobbi, S.; Belluti, F.; Bisi, A. Emerging targets in neurodegeneration: new opportunities for Alzheimer's disease treatment? *Curr. Top. Med. Chem.*, **2013**, *13*, 1879-1904.
- [18] Caraci, F.; Bosco, P.; Leggio, G.M.; Malaguamera, M.; Drago, F.; Bucolo, C.; Salomone, S. Clinical pharmacology of novel anti-Alzheimer disease modifying medications. *Curr. Top. Med. Chem.*, **2013**, *13*, 1853-1863.
- [19] Bennett, D.A.; Yu, L.; De Jager, P.L. Building a pipeline to discover and validate novel therapeutic targets and lead compounds for Alzheimer's disease. *Biochem. Pharmacol.*, **2014**, *88*, 617-630.
- [20] Morphy, R.; Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.*, **2005**, *48*, 6523-6543.
- [21] Morphy, R.; Rankovic, Z. Designing multiple ligands - medicinal chemistry strategies and challenges. *Curr. Pharm. Des.*, **2009**, *15*, 587-600.
- [22] Espinoza-Fonseca, L.M. The benefits of the multi-target approach in drug design and discovery. *Bioorg. Med. Chem.*, **2006**, *14*, 896-897.
- [23] Muller-Schiffmann, A.; Sticht, H.; Korth, C. Hybrid compounds: from simple combinations to nanomachines. *BioDrugs*, **2012**, *26*, 21-31.
- [24] Rapposelli, S.; Balsamo, A. In: *Multifunctional drugs: new chimeras in medicinal chemistry*; Rapposelli, S., Ed.; Transworld Research Network: Kerala, **2010**, pp. 1-17.
- [25] Nielsch, U.; Schafer, S.; Wild, H.; Busch, A. One target-multiple indications: a call for an integrated common mechanisms strategy. *Drug Discov. Today*, **2007**, *12*, 1025-1031.
- [26] Speck-Planche, A.; Luan, F.; Cordeiro, M.N. Role of ligand-based drug design methodologies toward the discovery of new anti-Alzheimer agents: futures perspectives in Fragment-Based Ligand Design. *Curr. Med. Chem.*, **2012**, *19*, 1635-1645.
- [27] Costantino, L.; Barlocco, D. Designed multiple ligands: basic research vs clinical outcomes. *Curr. Med. Chem.*, **2012**, *19*, 3353-3387.
- [28] Anand, S.; VandeVrede, L.; Luo, J.; Thatcher, G.R. J. In: *Multifunctional drugs: new chimeras in medicinal chemistry*; Rapposelli, S., Ed.; Transworld Research Network: Kerala, **2010**, pp. 65-97.
- [29] Cavalli, A.; Bolognesi, M.L. Multitarget drugs for the treatment of Alzheimer's disease. In *Polypharmacology in drug discovery*; Peters, J. U., Ed.; Wiley: **2012**, 441-448.
- [30] Iqbal, K.; Grundke-Iqbal, I. Alzheimer's disease, a multifactorial disorder seeking multitherapies. *Alzheimers Dement.*, **2010**, *6*, 420-424.
- [31] Bolognesi, M.L.; Matera, R.; Minarini, A.; Rosini, M.; Melchiorre, C. Alzheimer's disease: new approaches to drug discovery. *Curr. Opin. Chem. Biol.*, **2009**, *13*, 303-308.
- [32] Calza, L.; Baldassarro, V.A.; Giuliani, A.; Lorenzini, L.; Fernandez, M.; Mangano, C.; Sivilia, S.; Alessandri, M.; Gusciglio, M.; Torricella, R.; Giardino, L. From the multifactorial nature of Alzheimer's disease to multitarget therapy: the contribution of the translational approach. *Curr. Top. Med. Chem.*, **2013**, *13*, 1843-1852.
- [33] Decker, M. Recent advances in the development of hybrid molecules/ designed multiple compounds with anti-amnesic properties. *Mini Rev. Med. Chem.*, **2007**, *7*, 221-229.
- [34] Musial, A.; Bajda, M.; Malawska, B. Recent developments in cholinesterases inhibitors for Alzheimer's disease treatment. *Curr. Med. Chem.*, **2007**, *14*, 2654-2679.
- [35] Tumiatti, V.; Minarini, A.; Bolognesi, M.L.; Milelli, A.; Rosini, M.; Melchiorre, C. Tacrine derivatives and Alzheimer's disease. *Curr. Med. Chem.*, **2010**, *17*, 1825-1838.
- [36] Cavalli, A.; Bolognesi, M.L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.*, **2008**, *51*, 347-372.
- [37] Minarini, A.; Milelli, A.; Simoni, E.; Rosini, M.; Bolognesi, M.L.; Marchetti, C.; Tumiatti, V. Multifunctional tacrine derivatives in Alzheimer's disease. *Curr. Top. Med. Chem.*, **2013**, *13*, 1771-1786.
- [38] Viayna, E.; Sabate, R.; Muñoz-Torrero, D. Dual inhibitors of beta-amyloid aggregation and acetylcholinesterase as multi-target anti-Alzheimer drug candidates. *Curr. Top. Med. Chem.*, **2013**, *13*, 1820-1842.
- [39] Leon, R.; Garcia, A.G.; Marco-Contelles, J. Recent advances in the multitarget-directed ligands approach for the treatment of Alzheimer's disease. *Med. Res. Rev.*, **2013**, *33*, 139-189.
- [40] Fernandez-Bachiller, M.I.; Perez, C.; Campillo, N.E.; Paez, J.A.; Gonzalez-Munoz, G.C.; Usan, P.; Garcia-Palomo, E.; Lopez, M.G.; Villarroja, M.; Garcia, A.G.; Martinez, A.; Rodriguez-Franco, M.I. Tacrine-melatonin hybrids as multifunctional agents for Alzheimer's disease, with cholinergic, antioxidant, and neuro-protective properties. *ChemMedChem*, **2009**, *4*, 828-841.
- [41] Samadi, A.; Valderas, C.; de los Ríos, C.; Bastida, A.; Chioua, M.; González-Lafuente, L.; Colmena, I.; Gandía, L.; Romero, A.; del Barrio, L.; Martín-de-Saavedra, M.D.; López, M.G.; Villarroja, M.; Marco-Contelles, J. Cholinergic and neuroprotective drugs for the treatment of Alzheimer and neuronal vascular diseases. II. Synthesis, biological assessment, and molecular modelling of new tacrine analogues from highly substituted 2-aminopyridine-3-carbonitriles. *Bioorg. Med. Chem.*, **2011**, *19*, 122-133.
- [42] León, R.; Ríos, C. d. I.; Marco-Contelles, J.; Huertas, O.; Barril, X.; Javier Luque, F.; López, M. G.; García, A. G.; Villarroja, M. New tacrine-dihydropyridine hybrids that inhibit acetylcholinesterase, calcium entry, and exhibit neuroprotection properties. *Bioorg. Med. Chem.*, **2008**, *16*, 7759-7769.
- [43] Tomassoli, I.; Ismaili, L.; Pudlo, M.; de los Ríos, C.; Soriano, E.; Colmena, I.; Gandía, L.; Rivas, L.; Samadi, A.; Marco-Contelles, J. Synthesis, biological assessment and molecular modeling of new dihydroquinoline-3-carboxamides and dihydroquinoline-3-carbohydrazide derivatives as cholinesterase inhibitors, and Ca channel antagonists. *Eur. J. Med. Chem.*, **2011**, *46*, 1-10.
- [44] Lange, J.H.; Coolen, H.K.; van der Neut, Martina A.W.; Borst, A.J.; Stork, B.; Verwee, P.C.; Kruse, C.G. Design, synthesis, biological properties, and molecular modeling investigations of novel tacrine derivatives with a combination of acetylcholinesterase inhibition and cannabinoid CB1 receptor antagonism. *J. Med. Chem.*, **2010**, *53*, 1338-1346.
- [45] Piazzini, L.; Cavalli, A.; Colizzi, F.; Belluti, F.; Bartolini, M.; Mancini, F.; Recanatini, M.; Andrisano, V.; Rampa, A. Multi-target-directed coumarin derivatives: hAChE and BACE1 inhibitors as potential anti-Alzheimer compounds. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 423-426.
- [46] Zhu, Y.; Xiao, K.; Ma, L.; Xiong, B.; Fu, Y.; Yu, H.; Wang, W.; Wang, X.; Hu, D.; Peng, H.; Li, J.; Gong, Q.; Chai, Q.; Tang, X.; Zhang, H.; Li, J.; Shen, J. Design, synthesis and biological evaluation of novel dual inhibitors of acetylcholinesterase and β -secretase. *Bioorg. Med. Chem.*, **2009**, *17*, 1600-1613.
- [47] Bertoni, S.; Ballabeni, V.; Flammini, L.; Saccani, F.; Domenichini, G.; Morini, G.; Comini, M.; Rivara, M.; Barocelli, E. *In vitro* and *in vivo* pharmacological analysis of imidazole-free histamine H₃ receptor antagonists: promising results for a brain-penetrating H₃ blocker with weak anticholinesterase activity. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **2008**, *378*, 335-343.
- [48] Incerti, M.; Flammini, L.; Saccani, F.; Morini, G.; Comini, M.; Coruzzi, M.; Barocelli, E.; Ballabeni, V.; Bertoni, S. Dual-Acting Drugs: an *in vitro* Study of Nonimidazole Histamine H₃ Receptor Antagonists Combining Anticholinesterase Activity. *ChemMedChem*, **2010**, *5*, 1143-1149.
- [49] Rook, Y.; Schmidtke, K.; Gaube, F.; Schepmann, D.; Wü nsch, B.; Heilmann, J.; Lehmann, J.; Winckler, T. Bivalent β -carbolines as potential multitarget anti-Alzheimer agents. *J. Med. Chem.*, **2010**, *53*, 3611-3617.
- [50] Rosini, M.; Simoni, E.; Bartolini, M.; Cavalli, A.; Ceccarini, L.; Pascu, N.; McClymont, D.W.; Tarozzi, A.; Bolognesi, M.L.; Minarini, A. Inhibition of acetylcholinesterase, β -amyloid aggregation, and NMDA receptors in Alzheimer's disease: a promising direction for the multi-target-directed ligands gold rush. *J. Med. Chem.*, **2008**, *51*, 4381-4384.

- [51] Cappelli, A.; Gallelli, A.; Manini, M.; Anzini, M.; Mennuni, L.; Makovec, F.; Menziani, M.C.; Alcaro, S.; Ortuso, F.; Vomero, S. Further studies on the interaction of the 5-hydroxytryptamine₃ (5-HT₃) receptor with arylpiperazine ligands. Development of a new 5-HT₃ receptor ligand showing potent acetylcholinesterase inhibitory properties. *J. Med. Chem.*, **2005**, *48*, 3564-3575.
- [52] Toda, N.; Kaneko, T.; Kogen, H. Development of an efficient therapeutic agent for Alzheimer's disease: design and synthesis of dual inhibitors of acetylcholinesterase and serotonin transporter. *Chem. Pharm. Bull.*, **2010**, *58*, 273-287.
- [53] Fernández-Bachiller, M.I.; Pérez, C.; González-Munoz, G.C.; Conde, S.; López, M.G.; Villarroya, M.; García, A.G.; Rodríguez-Franco, M.I. Novel tacrine-8-hydroxyquinoline hybrids as multifunctional agents for the treatment of Alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and copper-complexing properties. *J. Med. Chem.*, **2010**, *53*, 4927-4937.
- [54] Huang, W.; Lv, D.; Yu, H.; Sheng, R.; Kim, S. C.; Wu, P.; Luo, K.; Li, J.; Hu, Y. Dual-target-directed 1, 3-diphenylurea derivatives: BACE 1 inhibitor and metal chelator against Alzheimer's disease. *Bioorg. Med. Chem.*, **2010**, *18*, 5610-5615.
- [55] Avramovich-Tirosh, Y.; Amit, T.; Bar-Am, O.; Zheng, H.; Fridkin, M.; Youdim, M.B. Therapeutic targets and potential of the novel brain-permeable multifunctional iron chelator-monoamine oxidase inhibitor drug, M-30, for the treatment of Alzheimer's disease. *J. Neurochem.*, **2007**, *100*, 490-502.
- [56] Zheng, H.; Youdim, M.B.; Fridkin, M. Site-activated multifunctional chelator with acetylcholinesterase and neuroprotective - neurorestorative moieties for Alzheimer's therapy. *J. Med. Chem.*, **2009**, *52*, 4095-4098.
- [57] Hieke, M.; Ness, J.; Steri, R.; Dittrich, M.; Greiner, C.; Werz, O.; Baumann, K.; Schubert-Zsilavec, M.; Weggen, S.; Zettl, H. Design, synthesis, and biological evaluation of a novel class of γ -secretase modulators with PPAR γ activity. *J. Med. Chem.*, **2010**, *53*, 4691-4700.
- [58] Li, W.; Mak, M.; Jiang, H.; Wang, Q.; Pang, Y.; Chen, K.; Han, Y. Novel anti-Alzheimer's dimer Bis(7)-cognitin: cellular and molecular mechanisms of neuroprotection through multiple targets. *Neurotherapeutics*, **2009**, *6*, 187-201.
- [59] Weinreb, O.; Amit, T.; Bar-Am, O.; Youdim, M.B. Ladostigil: a novel multimodal neuroprotective drug with cholinesterase and brain-selective monoamine oxidase inhibitory activities for Alzheimer's disease treatment. *Curr. Drug Targets*, **2012**, *13*, 483-494.
- [60] Capurro, V.; Busquet, P.; Lopes, J.P.; Bertorelli, R.; Tarozzo, G.; Bolognesi, M.L.; Piomelli, D.; Reggiani, A.; Cavalli, A. Pharmacological characterization of memoquin, a multi-target compound for the treatment of Alzheimer's disease. *PLoS One*, **2013**, *8*, e56870.
- [61] Carreiras, M.C.; Mendes, E.; Perry, M.J.; Francisco, A.P.; Marco-Contelles, J. The multifactorial nature of Alzheimer's disease for developing potential therapeutics. *Curr. Top. Med. Chem.*, **2013**, *13*, 1745-1770.
- [62] Agis-Torres, A.; Solhuber, M.; Fernandez, M.; Sanchez-Montero, J.M. Multi-target-directed ligands and other therapeutic strategies in the search of a real solution for Alzheimer's disease. *Curr. Neuropharmacol.*, **2014**, *12*, 2-36.
- [63] Bajda, M.; Guzior, N.; Ignasik, M.; Malawska, B. Multi-target-directed ligands in Alzheimer's disease treatment. *Curr. Med. Chem.*, **2011**, *18*, 4949-4975.
- [64] Lane, R.M.; Potkin, S.G.; Enz, A. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. *Int. J. Neuropsychopharmacol.*, **2006**, *9*, 101-124.
- [65] Darvesh, S.; Hopkins, D.A.; Geula, C. Neurobiology of butyrylcholinesterase. *Nat. Rev. Neurosci.*, **2003**, *4*, 131-138.
- [66] Mesulam, M.M.; Guillozet, A.; Shaw, P.; Levey, A.; Duysen, E.G.; Lockridge, O. Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine. *Neuroscience*, **2002**, *110*, 627-639.
- [67] Reid, G.A.; Chilukuri, N.; Darvesh, S. Butyrylcholinesterase and the cholinergic system. *Neuroscience*, **2013**, *234*, 53-68.
- [68] Greig, N.H.; Utsuki, T.; Ingram, D.K.; Wang, Y.; Pepeu, G.; Scali, C.; Yu, Q.S.; Mameczarz, J.; Holloway, H.W.; Giordano, T.; Chen, D.; Furukawa, K.; Sambamurti, K.; Brossi, A.; Lahiri, D.K. Selective butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer β -amyloid peptide in rodent. *Proc. Natl. Acad. Sci. U. S. A.*, **2005**, *102*, 17213-17218.
- [69] Diamant, S.; Podoly, E.; Friedler, A.; Ligumsky, H.; Livnah, O.; Soreq, H. Butyrylcholinesterase attenuates amyloid fibril formation *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.*, **2006**, *103*, 8628-8633.
- [70] Podoly, E.; Shalev, D.E.; Shenhar-Tsarfaty, S.; Bennett, E.R.; Ben Assayag, E.; Wilgus, H.; Livnah, O.; Soreq, H. The butyrylcholinesterase K variant confers structurally derived risks for Alzheimer pathology. *J. Biol. Chem.*, **2009**, *284*, 17170-17179.
- [71] Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. beta-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. *Biochem. Pharmacol.*, **2003**, *65*, 407-416.
- [72] Dinamarca, M.C.; Sagal, J.P.; Quintanilla, R.A.; Godoy, J.A.; Arzola, M.S.; Inestrosa, N.C. Amyloid-beta-Acetylcholinesterase complexes potentiate neurodegenerative changes induced by the Abeta peptide. Implications for the pathogenesis of Alzheimer's disease. *Mol. Neurodegener.*, **2010**, *5*, 4-1326-5-4.
- [73] Giacobini, E. Cholinesterase inhibitors: new roles and therapeutic alternatives. *Pharmacol. Res.*, **2004**, *50*, 433-440.
- [74] Pepeu, G.; Giovannini, M.G. Cholinesterase inhibitors and beyond. *Curr. Alzheimer Res.*, **2009**, *6*, 86-96.
- [75] Darreh-Shori, T.; Soininen, H. Effects of cholinesterase inhibitors on the activities and protein levels of cholinesterases in the cerebrospinal fluid of patients with Alzheimer's disease: a review of recent clinical studies. *Curr. Alzheimer Res.*, **2010**, *7*, 67-73.
- [76] Romero, A.; Cacabelos, R.; Oset-Gasque, M.J.; Samadi, A.; Marco-Contelles, J. Novel tacrine-related drugs as potential candidates for the treatment of Alzheimer's disease. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 1916-1922.
- [77] Tang, H.; Zhao, L.Z.; Zhao, H.T.; Huang, S.L.; Zhong, S.M.; Qin, J.K.; Chen, Z.F.; Huang, Z.S.; Liang, H. Hybrids of oxisoaporphine-tacrine congeners: novel acetylcholinesterase and acetylcholinesterase-induced β -amyloid aggregation inhibitors. *Eur. J. Med. Chem.*, **2011**, *46*, 4970-4979.
- [78] Tang, H.; Wei, Y.B.; Zhang, C.; Ning, F.X.; Qiao, W.; Huang, S.L.; Ma, L.; Huang, Z.S.; Gu, L.Q. Synthesis, biological evaluation and molecular modeling of oxisoaporphine and oxoaporphine derivatives as new dual inhibitors of acetylcholinesterase/butyrylcholinesterase. *Eur. J. Med. Chem.*, **2009**, *44*, 2523-2532.
- [79] Tang, H.; Ning, F.X.; Wei, Y.B.; Huang, S.L.; Huang, Z.S.; Chan, A.S.; Gu, L.Q. Derivatives of oxisoaporphine alkaloids: a novel class of selective acetylcholinesterase inhibitors. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 3765-3768.
- [80] Ellman, G.L.; Courtney, K.D.; Andres jr., V.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **1961**, *7*, 88-95.
- [81] LeVine, H. 3rd Thioflavine T interaction with synthetic Alzheimer's disease β -amyloid peptides: detection of amyloid aggregation in solution. *Protein Sci.*, **1993**, *2*, 404-410.
- [82] Keri, R.S.; Quintanova, C.; Marques, S.M.; Esteves, A.R.; Cardoso, S.M.; Santos, M.A. Design, synthesis and neuroprotective evaluation of novel tacrine-benzothiazole hybrids as multi-targeted compounds against Alzheimer's disease. *Bioorg. Med. Chem.*, **2013**, *21*, 4559-4569.
- [83] Leuma Yona, R.; Mazeris, S.; Faller, P.; Gras, E. Thioflavin derivatives as markers for amyloid-beta fibrils: insights into structural features important for high-affinity binding. *ChemMedChem*, **2008**, *3*, 63-66.
- [84] Choi, M.M.; Kim, E.A.; Hahn, H.G.; Nam, K.D.; Yang, S.J.; Choi, S.Y.; Kim, T.U.; Cho, S.W.; Huh, J.W. Protective effect of benzothiazole derivative KHG21834 on amyloid β -induced neurotoxicity in PC12 cells and cortical and mesencephalic neurons. *Toxicology*, **2007**, *239*, 156-166.
- [85] Soto-Ortega, D.D.; Murphy, B.P.; Gonzalez-Velasquez, F.J.; Wilson, K.A.; Xie, F.; Wang, Q.; Moss, M.A. Inhibition of amyloid- β aggregation by coumarin analogs can be manipulated by functionalization of the aromatic center. *Bioorg. Med. Chem.*, **2011**, *19*, 2596-2602.
- [86] Ozturan Ozer, E.; Tan, O.U.; Ozadali, K.; Kucukkilinc, T.; Balkan, A.; Ucar, G. Synthesis, molecular modeling and evaluation of novel N'-2-(4-benzylpiperidin-1-yl)acylhydrazones derivatives as dual inhibitors for cholinesterases and A β aggregation. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 440-443.
- [87] Ignasik, M.; Bajda, M.; Guzior, N.; Prinz, M.; Holzgrabe, U.; Malawska, B. Design, synthesis and evaluation of novel 2-(aminoalkyl)-isoindoline-1,3-dione derivatives as dual-binding site acetylcholinesterase inhibitors. *Arch. Pharm. (Weinheim)*, **2012**, *345*, 509-516.
- [88] Bajda, M.; Wieckowska, A.; Hebda, M.; Guzior, N.; Sotriffer, C.A.; Malawska, B. Structure-based search for new inhibitors of cholinesterases. *Int. J. Mol. Sci.*, **2013**, *14*, 5608-5632.
- [89] Alptuzun, V.; Prinz, M.; Horr, V.; Scheiber, J.; Radacki, K.; Fallarero, A.; Vuorela, P.; Engels, B.; Braunschweig, H.; Erciyas, E.; Holzgrabe, U. Interaction of (benzylidene-hydrazono)-1,4-dihydropyridines with β -amyloid, acetylcholine, and butyrylcholinesterases. *Bioorg. Med. Chem.*, **2010**, *18*, 2049-2059.

- [90] Catto, M.; Berezin, A.A.; Lo Re, D.; Loizou, G.; Demetriades, M.; De Stradis, A.; Campagna, F.; Koutentis, P.A.; Carotti, A. Design, synthesis and biological evaluation of benzo[c][1,2,4]triazin-7(1H)-one and [1,2,4]-triazino[5,6,1-jk]carbazol-6-one derivatives as dual inhibitors of beta-amyloid aggregation and acetyl/butyryl cholinesterase. *Eur. J. Med. Chem.*, **2012**, *58*, 84-97.
- [91] Convertino, M.; Pellarin, R.; Catto, M.; Carotti, A.; Cafilisch, A. 9, 10-Antraquinone hinders β -aggregation: how does a small molecule interfere with $A\beta$ -peptide amyloid fibrillation? *Protein Sci.*, **2009**, *18*, 792-800.
- [92] Karlsson, D.; Fallarero, A.; Brunhofer, G.; Guzik, P.; Prinz, M.; Holzgrabe, U.; Erker, T.; Vuorela, P. Identification and characterization of diarylimidazoles as hybrid inhibitors of butyrylcholinesterase and amyloid beta fibril formation. *Eur. J. Pharm. Sci.*, **2012**, *45*, 169-183.
- [93] Wu, X.; Qin, G.; Cheung, K.K.; Cheng, K.F. New alkaloids from *Isatis indigotica*. *Tetrahedron*, **1997**, *53*, 13323-13328.
- [94] Pan, L.; Tan, J.H.; Hou, J.Q.; Huang, S.L.; Gu, L.Q.; Huang, Z.S. Design, synthesis and evaluation of isaindigotone derivatives as acetylcholinesterase and butyrylcholinesterase inhibitors. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 3790-3793.
- [95] Yan, J.W.; Li, Y.P.; Ye, W.J.; Chen, S.B.; Hou, J.Q.; Tan, J.H.; Ou, T.M.; Li, D.; Gu, L.Q.; Huang, Z.S. Design, synthesis and evaluation of isaindigotone derivatives as dual inhibitors for acetylcholinesterase and amyloid beta aggregation. *Bioorg. Med. Chem.*, **2012**, *20*, 2527-2534.
- [96] Huang, L.; Su, T.; Li, X. Natural products as sources of new lead compounds for the treatment of Alzheimer's disease. *Curr. Top. Med. Chem.*, **2013**, *13*, 1864-1878.
- [97] Brunhofer, G.; Fallarero, A.; Karlsson, D.; Batista-Gonzalez, A.; Shinde, P.; Gopi Mohan, C.; Vuorela, P. Exploration of natural compounds as sources of new bifunctional scaffolds targeting cholinesterases and beta amyloid aggregation: the case of chelerythrine. *Bioorg. Med. Chem.*, **2012**, *20*, 6669-6679.
- [98] Anand, P.; Singh, B.; Singh, N. A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease. *Bioorg. Med. Chem.*, **2012**, *20*, 1175-1180.
- [99] Hasan, A.; Khan, K.M.; Sher, M.; Maharvi, G.M.; Nawaz, S.A.; Choudhary, M.I.; Atta-ur-Rahman; Supuran, C.T. Synthesis and inhibitory potential towards acetylcholinesterase, butyrylcholinesterase and lipoxygenase of some variably substituted chalcones. *J. Enzyme Inhib. Med. Chem.*, **2005**, *20*, 41-47.
- [100] Bag, S.; Ghosh, S.; Tulsan, R.; Sood, A.; Zhou, W.; Schifone, C.; Foster, M.; LeVine, H., 3rd; Torok, B.; Torok, M. Design, synthesis and biological activity of multifunctional alpha,beta-unsaturated carbonyl scaffolds for Alzheimer's disease. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 2614-2618.
- [101] LeVine, H. 3rd Biotin-avidin interaction-based screening assay for Alzheimer's β -peptide oligomer inhibitors. *Anal. Biochem.*, **2006**, *356*, 265-272.
- [102] Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A.K.; Zhang, X.C.; Tang, J. Structure of the protease domain of memapsin 2 (β -secretase) complexed with inhibitor. *Science*, **2000**, *290*, 150-153.
- [103] Venugopal, C.; Demos, C.M.; Rao, K.S.; Pappolla, M.A.; Sambamurti, K. Beta-secretase: structure, function, and evolution. *CNS Neurol. Disord. Drug Targets*, **2008**, *7*, 278-294.
- [104] Klaver, D.W.; Wilce, M.C.; Cui, H.; Hung, A.C.; Gasperini, R.; Foa, L.; Small, D.H. Is BACE1 a suitable therapeutic target for the treatment of Alzheimer's disease? Current strategies and future directions. *Biol. Chem.*, **2010**, *391*, 849-859.
- [105] Luo, X.; Yan, R. Inhibition of BACE1 for therapeutic use in Alzheimer's disease. *Int. J. Clin. Exp. Pathol.*, **2010**, *3*, 618-628.
- [106] Bjorklund, C.; Oscarson, S.; Benkestock, K.; Borkakoti, N.; Jansson, K.; Lindberg, J.; Vrang, L.; Hallberg, A.; Rosenquist, A.; Samuelsson, B. Design and synthesis of potent and selective BACE-1 inhibitors. *J. Med. Chem.*, **2010**, *53*, 1458-1464.
- [107] Ghosh, A.K.; Brindisi, M.; Tang, J. Developing β -secretase inhibitors for treatment of Alzheimer's disease. *J. Neurochem.*, **2012**, *120* Suppl. 1, 71-83.
- [108] Fernandez-Bachiller, M.I.; Perez, C.; Monjas, L.; Rademann, J.; Rodriguez-Franco, M.I. New tacrine-4-oxo-4H-chromene hybrids as multifunctional agents for the treatment of Alzheimer's disease, with cholinergic, antioxidant, and β -amyloid-reducing properties. *J. Med. Chem.*, **2012**, *55*, 1303-1317.
- [109] Kennedy, M.E.; Wang, W.; Song, L.; Lee, J.; Zhang, L.; Wong, G.; Wang, L.; Parker, E. Measuring human β -secretase (BACE1) activity using homogeneous time-resolved fluorescence. *Anal. Biochem.*, **2003**, *319*, 49-55.
- [110] Mancini, F.; De Simone, A.; Andrisano, V. Beta-secretase as a target for Alzheimer's disease drug discovery: an overview of *in vitro* methods for characterization of inhibitors. *Anal. Bioanal. Chem.*, **2011**, *400*, 1979-1996.
- [111] Davalos, A.; Gomez-Cordoves, C.; Bartolome, B. Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. *J. Agric. Food Chem.*, **2004**, *52*, 48-54.
- [112] Di, L.; Kerns, E.H.; Fan, K.; McConnell, O.J.; Carter, G.T. High throughput artificial membrane permeability assay for blood-brain barrier. *Eur. J. Med. Chem.*, **2003**, *38*, 223-232.
- [113] Galdeano, C.; Viayna, E.; Sola, I.; Formosa, X.; Camps, P.; Badia, A.; Clos, M.V.; Relat, J.; Ratiá, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Salmona, M.; Minguillon, C.; Gonzalez-Munoz, G.C.; Rodriguez-Franco, M.I.; Bidon-Chanal, A.; Luque, F.J.; Munoz-Torrero, D. Huprine-tacrine heterodimers as anti-amyloidogenic compounds of potential interest against Alzheimer's and prion diseases. *J. Med. Chem.*, **2012**, *55*, 661-669.
- [114] Viayna, E.; Sola, I.; Bartolini, M.; De Simone, A.; Tapia-Rojas, C.; Serrano, F.G.; Sabate, R.; Juarez-Jimenez, J.; Perez, B.; Luque, F.J.; Andrisano, V.; Clos, M.V.; Inestrosa, N.C.; Munoz-Torrero, D. Synthesis and multitarget biological profiling of a novel family of rhein derivatives as disease-modifying anti-Alzheimer agents. *J. Med. Chem.*, **2014**, *57*, 2549-2567.
- [115] Mohamed, T.; Zhao, X.; Habib, L.K.; Yang, J.; Rao, P.P. Design, synthesis and structure-activity relationship (SAR) studies of 2,4-disubstituted pyrimidine derivatives: dual activity as cholinesterase and $A\beta$ -aggregation inhibitors. *Bioorg. Med. Chem.*, **2011**, *19*, 2269-2281.
- [116] Mohamed, T.; Yeung, J.C.; Rao, P.P. Development of 2-substituted-N-(naphth-1-ylmethyl) and N-benzhydrylpyrimidin-4-amines as dual cholinesterase and $A\beta$ -aggregation inhibitors: Synthesis and biological evaluation. *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 5881-5887.
- [117] Mohamed, T.; Yeung, J.C.; Vasefi, M.S.; Beazely, M.A.; Rao, P.P. Development and evaluation of multifunctional agents for potential treatment of Alzheimer's disease: application to a pyrimidine-2,4-diamine template. *Bioorg. Med. Chem. Lett.*, **2012**, *22*, 4707-4712.
- [118] Peng, D.Y.; Sun, Q.; Zhu, X.L.; Lin, H.Y.; Chen, Q.; Yu, N.X.; Yang, W.C.; Yang, G.F. Design, synthesis, and bioevaluation of benzamides: novel acetylcholinesterase inhibitors with multifunctions on butylcholinesterase, Abeta aggregation, and β -secretase. *Bioorg. Med. Chem.*, **2012**, *20*, 6739-6750.
- [119] Jiaranaikulwanitch, J.; Govitrapong, P.; Fokin, V.V.; Vajragupta, O. From BACE1 inhibitor to multifunctionality of tryptoline and tryptamine triazole derivatives for Alzheimer's disease. *Molecules*, **2012**, *17*, 8312-8333.
- [120] Jiaranaikulwanitch, J.; Boonyarat, C.; Fokin, V.V.; Vajragupta, O. Triazolyl tryptoline derivatives as β -secretase inhibitors. *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 6572-6576.
- [121] Sharma, O.P.; Bhat, T.K. DPPH antioxidant assay revisited. *Food Chem.*, **2009**, *113*, 1202-1205.
- [122] Weinreb, O.; Mandel, S.; Bar-Am, O.; Amit, T. Iron-chelating backbone coupled with monoamine oxidase inhibitory moiety as novel pluripotential therapeutic agents for Alzheimer's disease: a tribute to Moussa Youdim. *J. Neural Transm.*, **2011**, *118*, 479-492.
- [123] Song, M.S.; Matveychuk, D.; MacKenzie, E.M.; Duchcherer, M.; Mousseau, D.D.; Baker, G.B. An update on amine oxidase inhibitors: multifaceted drugs. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2013**, *44*, 118-124.
- [124] Zheng, H.; Fridkin, M.; Youdim, M. From single target to multitarget/network therapeutics in Alzheimer's therapy. *Pharmaceuticals (Basel)*, **2014**, *7*, 113-135.
- [125] Bolea, I.; Gella, A.; Unzeta, M. Propargylamine-derived multitarget-directed ligands: fighting Alzheimer's disease with monoamine oxidase inhibitors. *J. Neural Transm.*, **2013**, *120*, 893-902.
- [126] Bolea, I.; Juárez-Jiménez, J.; de, I.R.; Chioua, M.; Poupiana, R.; Luque, F.J.; Unzeta, M.; Marco-Contelles, J.; Samadi, A. Synthesis, biological evaluation, and molecular modeling of donepezil and N-[(5-(benzyloxy)-1-methyl-1H-indol-2-yl)methyl]-N-methylprop-2-yn-1-amine hybrids as new multipotent cholinesterase/monoamine oxidase inhibitors for the treatment of Alzheimer's disease. *J. Med. Chem.*, **2011**, *54*, 8251-8270.
- [127] Perez, V.; Marco, J.L.; Fernandez-Alvarez, E.; Unzeta, M. Relevance of benzyloxy group in 2-indolyl methylamines in the selective MAO-B inhibition. *Br. J. Pharmacol.*, **1999**, *127*, 869-876.
- [128] Bolea, I.; Gella, A.; Monjas, L.; Perez, C.; Rodriguez-Franco, M.I.; Marco-Contelles, J.; Samadi, A.; Unzeta, M. Multipotent, permeable drug ASS234 inhibits Abeta aggregation, possesses antioxidant properties and protects from $A\beta$ -induced apoptosis *in vitro*. *Curr. Alzheimer Res.*, **2013**, *10*, 797-808.

- [129] Bautista-Aguilera, O.M.; Esteban, G.; Bolea, I.; Nikolic, K.; Agbaba, D.; Moraleda, I.; Iriepa, I.; Samadi, A.; Soriano, E.; Unzeta, M.; Marco-Contelles, J. Design, synthesis, pharmacological evaluation, QSAR analysis, molecular modeling and ADMET of novel donepezil-indolyl hybrids as multipotent cholinesterase/monoamine oxidase inhibitors for the potential treatment of Alzheimer's disease. *Eur. J. Med. Chem.*, **2014**, *75*, 82-95.
- [130] Samadi, A.; de los Rios, C.; Bolea, I.; Chioua, M.; Iriepa, I.; Moraleda, I.; Bartolini, M.; Andrisano, V.; Galvez, E.; Valderas, C.; Unzeta, M.; Marco-Contelles, J. Multipotent MAO and cholinesterase inhibitors for the treatment of Alzheimer's disease: synthesis, pharmacological analysis and molecular modeling of heterocyclic substituted alkyl and cycloalkyl propargyl amine. *Eur. J. Med. Chem.*, **2012**, *52*, 251-262.
- [131] Lu, C.; Zhou, Q.; Yan, J.; Du, Z.; Huang, L.; Li, X. A novel series of tacrine-selegiline hybrids with cholinesterase and monoamine oxidase inhibition activities for the treatment of Alzheimer's disease. *Eur. J. Med. Chem.*, **2013**, *62*, 745-753.
- [132] Sun, Y.; Chen, J.; Chen, X.; Huang, L.; Li, X. Inhibition of cholinesterase and monoamine oxidase-B activity by Tacrine-Homoisoflavonoid hybrids. *Bioorg. Med. Chem.* **2013**, *21*, 7406-7417.
- [133] Desideri, N.; Bolasco, A.; Fioravanti, R.; Monaco, L. P.; Orallo, F.; Yanez, M.; Ortuso, F.; Alcaro, S. Homoisoflavonoids: natural scaffolds with potent and selective monoamine oxidase-B inhibition properties. *J. Med. Chem.*, **2011**, *54*, 2155-2164.
- [134] Karuppasamy, M.; Mahapatra, M.; Yabanoglu, S.; Ucar, G.; Sinha, B.N.; Basu, A.; Mishra, N.; Sharon, A.; Kulandaivelu, U.; Jayaprakash, V. Development of selective and reversible pyrazoline based MAO-A inhibitors: Synthesis, biological evaluation and docking studies. *Bioorg. Med. Chem.*, **2010**, *18*, 1875-1881.
- [135] Gokhan-Kelekci, N.; Yabanoglu, S.; Kupeli, E.; Salgin, U.; Ozgen, O.; Ucar, G.; Yesilada, E.; Kendi, E.; Yesilada, A.; Bilgin, A.A. A new therapeutic approach in Alzheimer disease: some novel pyrazole derivatives as dual MAO-B inhibitors and antiinflammatory analgesics. *Bioorg. Med. Chem.*, **2007**, *15*, 5775-5786.
- [136] Mishra, N.; Sasmal, D. Development of selective and reversible pyrazoline based MAO-B inhibitors: virtual screening, synthesis and biological evaluation. *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 1969-1973.
- [137] Mishra, N.; Sasmal, D. Additional acetyl cholinesterase inhibitory property of diaryl pyrazoline derivatives. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 702-705.
- [138] Feng, Y.; Wang, X. Antioxidant therapies for Alzheimer's disease. *Oxid. Med. Cell Longev.*, **2012**, *2012*, 472932.
- [139] Padurariu, M.; Ciobica, A.; Lefter, R.; Serban, I.L.; Stefanescu, C.; Chirita, R. The oxidative stress hypothesis in Alzheimer's disease. *Psychiatr. Damb.*, **2013**, *25*, 401-409.
- [140] Shan, W.J.; Huang, L.; Zhou, Q.; Meng, F.C.; Li, X.S. Synthesis, biological evaluation of 9-N-substituted berberine derivatives as multi-functional agents of antioxidant, inhibitors of acetylcholinesterase, butyrylcholinesterase and amyloid- β aggregation. *Eur. J. Med. Chem.*, **2011**, *46*, 5885-5893.
- [141] Huang, L.; Luo, Z.; He, F.; Lu, J.; Li, X. Synthesis and biological evaluation of a new series of berberine derivatives as dual inhibitors of acetylcholinesterase and butyrylcholinesterase. *Bioorg. Med. Chem.*, **2010**, *18*, 4475-4484.
- [142] Huang, L.; Shi, A.; He, F.; Li, X. Synthesis, biological evaluation, and molecular modeling of berberine derivatives as potent acetylcholinesterase inhibitors. *Bioorg. Med. Chem.*, **2010**, *18*, 1244-1251.
- [143] Su, T.; Xie, S.; Wei, H.; Yan, J.; Huang, L.; Li, X. Synthesis and biological evaluation of berberine-thiophenyl hybrids as multi-functional agents: Inhibition of acetylcholinesterase, butyrylcholinesterase, and A β aggregation and antioxidant activity. *Bioorg. Med. Chem.*, **2013**, *21*, 5830-5840.
- [144] Maalej, E.; Chabchoub, F.; Samadi, A.; de los Rios, C.; Perona, A.; Morreale, A.; Marco-Contelles, J. Synthesis, biological assessment and molecular modeling of 14-aryl-10,11,12,14-tetrahydro-9H-benzo[5,6]chromeno[2,3-b]quinolin-13-amines. *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 2384-2388.
- [145] Maalej, E.; Chabchoub, F.; Oset-Gasque, M.J.; Esquivias-Perez, M.; Gonzalez, M.P.; Monjas, L.; Perez, C.; de los Rios, C.; Rodriguez-Franco, M.I.; Iriepa, I.; Moraleda, I.; Chioua, M.; Romero, A.; Marco-Contelles, J.; Samadi, A. Synthesis, biological assessment, and molecular modeling of racemic 7-aryl-9,10,11,12-tetrahydro-7H-benzo[7,8]chromeno[2,3-b]quinolin-8-amines as potential drugs for the treatment of Alzheimer's disease. *Eur. J. Med. Chem.*, **2012**, *54*, 750-763.
- [146] Songsiang, U.; Thongthoom, T.; Boonyarat, C.; Yenjai, C. Claurailas A-D, cytotoxic carbazole alkaloids from the roots of *Clausena harmandiana*. *J. Nat. Prod.*, **2011**, *74*, 208-212.
- [147] Thiratrakul, S.; Yenjai, C.; Waiwut, P.; Vajragupta, O.; Reubroycharoen, P.; Tohda, M.; Boonyarat, C. Synthesis, biological evaluation and molecular modeling study of novel tacrine-carbazole hybrids as potential multifunctional agents for the treatment of Alzheimer's disease. *Eur. J. Med. Chem.*, **2014**, *75*, 21-30.
- [148] Miller, N.J.; Rice-Evans, C.A. Factors influencing the antioxidant activity determined by the ABTS+ radical cation assay. *Free Radic. Res.*, **1997**, *26*, 195-199.
- [149] Sharma, S.; Rakoczy, S.; Brown-Borg, H. Assessment of spatial memory in mice. *Life Sci.*, **2010**, *87*, 521-536.
- [150] Luo, W.; Li, Y.P.; He, Y.; Huang, S.L.; Li, D.; Gu, L.Q.; Huang, Z.S. Synthesis and evaluation of heterobivalent tacrine derivatives as potential multi-functional anti-Alzheimer agents. *Eur. J. Med. Chem.*, **2011**, *46*, 2609-2616.
- [151] Sul, D.; Kim, H.S.; Lee, D.; Joo, S.S.; Hwang, K.W.; Park, S.Y. Protective effect of caffeic acid against beta-amyloid-induced neurotoxicity by the inhibition of calcium influx and tau phosphorylation. *Life Sci.*, **2009**, *84*, 257-262.
- [152] Chao, X.; He, X.; Yang, Y.; Zhou, X.; Jin, M.; Liu, S.; Cheng, Z.; Liu, P.; Wang, Y.; Yu, J.; Tan, Y.; Huang, Y.; Qin, J.; Rapposelli, S.; Pi, R. Design, synthesis and pharmacological evaluation of novel tacrine-caffeic acid hybrids as multi-targeted compounds against Alzheimer's disease. *Bioorg. Med. Chem. Lett.*, **2012**, *22*, 6498-6502.
- [153] Zhao, R.; Holmgren, A. A novel antioxidant mechanism of ebbselen involving ebbselen diselenide, a substrate of mammalian thioredoxin and thioredoxin reductase. *J. Biol. Chem.*, **2002**, *277*, 39456-39462.
- [154] Luo, Z.; Sheng, J.; Sun, Y.; Lu, C.; Yan, J.; Liu, A.; Luo, H.B.; Huang, L.; Li, X. Synthesis and evaluation of multi-target-directed ligands against Alzheimer's disease based on the fusion of donepezil and ebbselen. *J. Med. Chem.*, **2013**, *56*, 9089-9099.
- [155] Wilson, S.R.; Zucker, P.A.; Huang, R.R.C.; Spector, A. Development of synthetic compounds with glutathione peroxidase activity. *J. Am. Chem. Soc.*, **1989**, *111*, 5936-5939.
- [156] Luo, Z.; Liang, L.; Sheng, J.; Pang, Y.; Li, J.; Huang, L.; Li, X. Synthesis and biological evaluation of a new series of ebbselen derivatives as glutathione peroxidase (GPx) mimics and cholinesterase inhibitors against Alzheimer's disease. *Bioorg. Med. Chem.*, **2014**, *22*, 1355-1361.
- [157] Yanovsky, I.; Finkin-Groner, E.; Zaikin, A.; Lerman, L.; Shalom, H.; Zeeli, S.; Weill, T.; Ginsburg, I.; Nudelman, A.; Weinstock, M. Carbamate derivatives of indolines as cholinesterase inhibitors and antioxidants for the treatment of Alzheimer's disease. *J. Med. Chem.*, **2012**, *55*, 10700-10715.
- [158] Chyan, Y.J.; Poeggeler, B.; Omar, R.A.; Chain, D.G.; Frangione, B.; Ghiso, J.; Pappolla, M.A. Potent neuroprotective properties against the Alzheimer β -amyloid by an endogenous melatonin-related indole structure, indole-3-propionic acid. *J. Biol. Chem.*, **1999**, *274*, 21937-21942.
- [159] Ginsburg, I.; Sadovnic, M.; Oron, M.; Kohen, R. Novel chemiluminescence-inducing cocktails, part I: the role in light emission of combinations of luminal with SIN-1, selenite, albumin, glucose oxidase and Co²⁺. *Inflammopharmacology*, **2004**, *12*, 289-303.
- [160] Rosini, M.; Simoni, E.; Bartolini, M.; Tarozzi, A.; Matera, R.; Milelli, A.; Hrelia, P.; Andrisano, V.; Bolognesi, M.L.; Melchiorre, C. Exploiting the lipoic acid structure in the search for novel multi-target ligands against Alzheimer's disease. *Eur. J. Med. Chem.*, **2011**, *46*, 5435-5442.
- [161] Rosini, M.; Andrisano, V.; Bartolini, M.; Bolognesi, M.L.; Hrelia, P.; Minarini, A.; Tarozzi, A.; Melchiorre, C. Rational approach to discover multipotent anti-Alzheimer drugs. *J. Med. Chem.*, **2005**, *48*, 360-363.
- [162] Chin, D.; Huebbe, P.; Pallauf, K.; Rimbach, G. Neuroprotective properties of curcumin in Alzheimer's disease - merits and limitations. *Curr. Med. Chem.*, **2013**, *20*, 3955-3985.
- [163] Park, S.; Kim, H.; Cho, E.; Kwon, B.; Phark, S.; Hwang, K.; Sul, D. Curcumin protected PC12 cells against beta-amyloid-induced toxicity through the inhibition of oxidative damage and tau hyperphosphorylation. *Food Chem. Toxicol.*, **2008**, *46*, 2881-2887.
- [164] Thapa, A.; Vernon, B.C.; De la Peña, K.; Soliz, G.; Moreno, H.A.; López, G.P.; Chi, E.Y. Membrane-mediated neuroprotection by curcumin from amyloid- β -peptide-induced toxicity. *Langmuir*, **2013**, *29*, 11713-11723.
- [165] Fang, L.; Gou, S.; Liu, X.; Cao, F.; Cheng, L. Design, synthesis and anti-Alzheimer properties of dimethylaminomethyl-substituted curcumin derivatives. *Bioorg. Med. Chem. Lett.*, **2014**, *24*, 40-43.

- [166] Fang, X.; Fang, L.; Gou, S.; Cheng, L. Design and synthesis of dimethylaminomethyl-substituted curcumin derivatives/analogues: Potent antitumor and antioxidant activity, improved stability and aqueous solubility compared with curcumin. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 1297-1301.
- [167] Bush, A.I.; Tanzi, R.E. Therapeutics for Alzheimer's disease based on the metal hypothesis. *Neurotherapeutics*, **2008**, *5*, 421-432.
- [168] Zhao, Y.; Zhao, B. Oxidative stress and the pathogenesis of Alzheimer's disease. *Oxid. Med. Cell Longev.*, **2013**, *2013*, 316523.
- [169] Rivera-Mancía, S.; Pérez-Neri, I.; Ríos, C.; Tristán-López, L.; Rivera-Espinosa, L.; Montes, S. The transition metals copper and iron in neurodegenerative diseases. *Chem. Biol. Interact.*, **2010**, *186*, 184-199.
- [170] Budimir, A. Metal ions, Alzheimer's disease and chelation therapy. *Acta Pharm.*, **2011**, *61*, 1-14.
- [171] Li, X.; Jankovic, J.; Le, W. Iron chelation and neuroprotection in neurodegenerative diseases. *J. Neural Transm.*, **2011**, *118*, 473-477.
- [172] Li, R.; Wang, X.; Hu, X.; Kong, L. Design, synthesis and evaluation of flavonoid derivatives as potential multifunctional acetylcholinesterase inhibitors against Alzheimer's disease. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 2636-2641.
- [173] Schroeter, H.; Spencer, J.; Rice-Evans, C.; Williams, R. Flavonoids protect neurons from oxidized low-density-lipoprotein-induced apoptosis involving c-Jun N-terminal kinase (JNK), c-Jun and caspase-3. *Biochem. J.*, **2001**, *358*, 547-557.
- [174] Kim, H.; Park, B.; Lee, K.; Choi, C.Y.; Jang, S.S.; Kim, Y.; Lee, S. Effects of naturally occurring compounds on fibril formation and oxidative stress of β -amyloid. *J. Agric. Food Chem.*, **2005**, *53*, 8537-8541.
- [175] Sugihara, N.; Arakawa, T.; Ohnishi, M.; Furuno, K. Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with alpha-linolenic acid. *Free Radic. Biol. Med.*, **1999**, *27*, 1313-1323.
- [176] Li, S.; Wang, X.; Xie, S.; Jiang, N.; Wang, K.D.; Yao, H.; Sun, H.; Kong, L. Multifunctional tacrine-flavonoid hybrids with cholinergic, β -amyloid-reducing, and metal chelating properties for the treatment of Alzheimer's disease. *Eur. J. Med. Chem.*, **2013**, *69*, 632-646.
- [177] Xie, S.; Wang, X.; Li, J.; Yang, L.; Kong, L. Design, synthesis and evaluation of novel tacrine-coumarin hybrids as multifunctional cholinesterase inhibitors against Alzheimer's disease. *Eur. J. Med. Chem.*, **2013**, *64*, 540-553.
- [178] Li, S.; Jiang, N.; Xie, S.; Wang, K.D.; Wang, X.; Kong, L. Design, synthesis and evaluation of novel tacrine-rhein hybrids as multifunctional agents for the treatment of Alzheimer's disease. *Org. Biomol. Chem.*, **2014**, *12*, 801-814.
- [179] Kryger, G.; Silman, I.; Sussman, J.L. Structure of acetylcholinesterase complexed with E2020 (Aricept): implications for the design of new anti-Alzheimer drugs. *Structure*, **1999**, *7*, 297-307.
- [180] Meng, F.; Mao, F.; Shan, W.; Qin, F.; Huang, L.; Li, X. Design, synthesis, and evaluation of indanone derivatives as acetylcholinesterase inhibitors and metal-chelating agents. *Bioorg. Med. Chem. Lett.*, **2012**, *22*, 4462-4466.
- [181] Huang, L.; Lu, C.; Sun, Y.; Mao, F.; Luo, Z.; Su, T.; Jiang, H.; Shan, W.; Li, X. Multitarget-directed benzylideneindanone derivatives: anti- β -amyloid ($A\beta$) aggregation, antioxidant, metal chelation, and monoamine oxidase B (MAO-B) inhibition properties against Alzheimer's disease. *J. Med. Chem.*, **2012**, *55*, 8483-8492.
- [182] He, Y.; Yao, P.; Chen, S.; Huang, Z.; Huang, S.; Tan, J.; Li, D.; Gu, L.; Huang, Z. Synthesis and evaluation of 7, 8-dehydrorutaecarpine derivatives as potential multifunctional agents for the treatment of Alzheimer's disease. *Eur. J. Med. Chem.*, **2013**, *63*, 299-312.
- [183] Wang, B.; Mai, Y.; Li, Y.; Hou, J.; Huang, S.; Ou, T.; Tan, J.; An, L.; Li, D.; Gu, L. Synthesis and evaluation of novel rutaecarpine derivatives and related alkaloids derivatives as selective acetylcholinesterase inhibitors. *Eur. J. Med. Chem.*, **2010**, *45*, 1415-1423.
- [184] Yang, W.; Wong, Y.; Ng, O.T.; Bai, L.; Kwong, D.W.; Ke, Y.; Jiang, Z.; Li, H.; Yung, K.K.; Wong, M.S. Inhibition of Beta-Amyloid Peptide Aggregation by Multifunctional Carbazole-Based Fluorophores. *Angew. Chem. Int. Ed. Engl.*, **2012**, *51*, 1804-1810.
- [185] Richard, T.; Pawlus, A.D.; Iglésias, M.; Pedrot, E.; Waffo-Teguo, P.; Mérillon, J.; Monti, J. Neuroprotective properties of resveratrol and derivatives. *Ann. N. Y. Acad. Sci.*, **2011**, *1215*, 103-108.
- [186] Baur, J.A.; Sinclair, D.A. Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat. Rev. Drug Discov.*, **2006**, *5*, 493-506.
- [187] Yao, R.; Lu, X.; Guan, Q.; Zheng, L.; Lu, X.; Ruan, B. Synthesis and biological evaluation of some novel resveratrol amide derivatives as potential anti-tumor agents. *Eur. J. Med. Chem.*, **2013**, *62*, 222-231.
- [188] Sharma, M.; Gupta, Y. Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. *Life Sci.*, **2002**, *71*, 2489-2498.
- [189] Jang, J.; Surh, Y. Protective effect of resveratrol on β -amyloid-induced oxidative PC12 cell death. *Free Radic. Biol. Med.*, **2003**, *34*, 1100-1110.
- [190] Lu, C.; Guo, Y.; Li, J.; Yao, M.; Liao, Q.; Xie, Z.; Li, X. Design, synthesis, and evaluation of resveratrol derivatives as $A\beta_{1-42}$ aggregation inhibitors, antioxidants, and neuroprotective agents. *Bioorg. Med. Chem. Lett.*, **2012**, *22*, 7683-7687.
- [191] Lu, C.; Guo, Y.; Yan, J.; Luo, Z.; Luo, H.; Yan, M.; Huang, L.; Li, X. Design, synthesis, and evaluation of multitarget-directed resveratrol derivatives for the treatment of Alzheimer's disease. *J. Med. Chem.*, **2013**, *56*, 5843-5859.
- [192] Li, S.; Wang, X.; Kong, L. Design, synthesis and biological evaluation of imine resveratrol derivatives as multi-targeted agents against Alzheimer's disease. *Eur. J. Med. Chem.*, **2014**, *71*, 36-45.
- [193] Cooke, J.P.; Dzau, V.J. Nitric oxide synthase: role in the genesis of vascular disease. *Annu. Rev. Med.*, **1997**, *48*, 489-509.
- [194] Faraci, F.M. Role of endothelium-derived relaxing factor in cerebral circulation: large arteries vs. microcirculation. *Am. J. Physiol.*, **1991**, *261*, H1038-H1042.
- [195] Fang, L.; Kraus, B.; Lehmann, J.; Heilmann, J.; Zhang, Y.; Decker, M. Design and synthesis of tacrine-ferulic acid hybrids as multipotent anti-Alzheimer drug candidates. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 2905-2909.
- [196] Fang, L.; Appenroth, D.; Decker, M.; Kiehnopf, M.; Roegler, C.; Deufel, T.; Fleck, C.; Peng, S.; Zhang, Y.; Lehmann, J. Synthesis and biological evaluation of NO-donor-tacrine hybrids as hepatoprotective anti-Alzheimer drug candidates. *J. Med. Chem.*, **2008**, *51*, 713-716.
- [197] Fang, L.; Appenroth, D.; Decker, M.; Kiehnopf, M.; Lupp, A.; Peng, S.; Fleck, C.; Zhang, Y.; Lehmann, J. NO-donating tacrine hybrid compounds improve scopolamine-induced cognition impairment and show less hepatotoxicity. *J. Med. Chem.*, **2008**, *51*, 7666-7669.
- [198] Chen, Y.; Sun, J.; Fang, L.; Liu, M.; Peng, S.; Liao, H.; Lehmann, J.; Zhang, Y. Tacrine-ferulic acid-nitric oxide (NO) donor trihybrids as potent, multifunctional acetyl- and butyrylcholinesterase inhibitors. *J. Med. Chem.*, **2012**, *55*, 4309-4321.
- [199] Sorba, G.; Medana, C.; Fruttero, R.; Cena, C.; Di Stilo, A.; Galli, U.; Gasco, A. Water soluble furoxan derivatives as NO prodrugs. *J. Med. Chem.*, **1997**, *40*, 463-469.
- [200] Chen, Y.; Sun, J.; Huang, Z.; Liao, H.; Peng, S.; Lehmann, J.; Zhang, Y. NO-donating tacrine derivatives as potential butyrylcholinesterase inhibitors with vasorelaxation activity. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 3162-3165.
- [201] Chen, Y.; Sun, J.; Huang, Z.; Liao, H.; Peng, S.; Lehmann, J.; Zhang, Y. Design, synthesis and evaluation of tacrine-flurbiprofen-nitrate trihybrids as novel anti-Alzheimer's disease agents. *Bioorg. Med. Chem.*, **2013**, *21*, 2462-2470.
- [202] Chen, Y.; Sun, J.; Peng, S.; Liao, H.; Zhang, Y.; Lehmann, J. Tacrine-flurbiprofen hybrids as multifunctional drug candidates for the treatment of Alzheimer's disease. *Arch. Pharm. (Weinheim)*, **2013**, *346*, 865-871.
- [203] Fang, L.; Fang, X.; Gou, S.; Lupp, A.; Lenhardt, I.; Sun, Y.; Huang, Z.; Chen, Y.; Zhang, Y.; Fleck, C. Design, synthesis and biological evaluation of D-ring opened galantamine analogs as multifunctional anti-Alzheimer agents. *Eur. J. Med. Chem.*, **2014**, *76*, 376-386.
- [204] Greenblatt, H.; Kryger, G.; Lewis, T.; Silman, I.; Sussman, J. Structure of acetylcholinesterase complexed with (-)-galanthamine at 2.3 Å resolution. *FEBS Lett.*, **1999**, *463*, 321-326.
- [205] Zhang, W.; Ruan, J.; Wu, P.; Jiang, F.; Zhang, L.N.; Fang, W.; Chen, X.; Wang, Y.; Cao, B.; Chen, G. Design, synthesis, and cytoprotective effect of 2-aminothiazole analogues as potent poly (ADP-ribose) polymerase-1 inhibitors. *J. Med. Chem.*, **2009**, *52*, 718-725.
- [206] Wang, Y.; Wang, F.; Yu, J.P.; Jiang, F.C.; Guan, X.L.; Wang, C.M.; Li, L.; Cao, H.; Li, M.X.; Chen, J.G. Novel multipotent phenylthiazole-tacrine hybrids for the inhibition of cholinesterase activity, β -amyloid aggregation and Ca^{2+} overload. *Bioorg. Med. Chem.*, **2012**, *20*, 6513-6522.
- [207] González-Muñoz, G.C.; Arce, M.P.; López, B.; Pérez, C.; Romero, A.; del Barrio, L.; Martín-de-Saavedra, M.D.; Egea, J.; León, R.;

- Villarroya, M.; López, M.G.; García, A.G.; Conde, S.; Rodríguez-Franco, M.I. *N*-Acylaminophenothiazines: Neuroprotective agents displaying multifunctional activities for a potential treatment of Alzheimer's disease. *Eur. J. Med. Chem.*, **2011**, *46*, 2224-2235.
- [208] Zhang, Z.; Simpkins, J.W. An okadaic acid-induced model of tauopathy and cognitive deficiency. *Brain Res.*, **2010**, *1359*, 233-246.
- [209] Minarini, A.; Milelli, A.; Tumiatti, V.; Rosini, M.; Simoni, E.; Bolognesi, M.; Andrisano, V.; Bartolini, M.; Motori, E.; Angeloni, C. Cystamine-tacrine dimer: a new multi-target-directed ligand as potential therapeutic agent for Alzheimer's disease treatment. *Neuropharmacology*, **2012**, *62*, 997-1003.
- [210] Wood, P.L.; Khan, M.A.; Moskal, J.R. Cellular thiol pools are responsible for sequestration of cytotoxic reactive aldehydes: central role of free cysteine and cysteamine. *Brain Res.*, **2007**, *1158*, 158-163.
- [211] Simoni, E.; Daniele, S.; Bottegoni, G.; Pizzirani, D.; Trincavelli, M.L.; Goldoni, L.; Tarozzo, G.; Reggiani, A.; Martini, C.; Piomelli, D. Combining galantamine and memantine in multitargeted, new chemical entities potentially useful in Alzheimer's disease. *J. Med. Chem.*, **2012**, *55*, 9708-9721.
- [212] Rosini, M.; Simoni, E.; Bartolini, M.; Soriano, E.; Marco-Contelles, J.; Andrisano, V.; Monti, B.; Windisch, M.; Hutter-Paier, B.; McClymont, D.W. The bivalent ligand approach as a tool for improving the *in vitro* anti-Alzheimer multitarget profile of dimebon. *ChemMedChem*, **2013**, *8*, 1276-1281.
- [213] Bachurin, S.; Bukatina, E.; Lermontova, N.; Tkachenko, S.; Afanasiev, A.; Grigoriev, V.; Grigorieva, L.; Ivanov, Y.; Sablin, S.; Zefirov, N. Antihistamine agent Dimebon as a novel neuroprotector and a cognition enhancer. *Ann. N. Y. Acad. Sci.*, **2001**, *939*, 425-435.
- [214] Hartbauer, M.; Hutter-Paier, B.; Windisch, M. Effects of Cerebrolysin on the outgrowth and protection of processes of cultured brain neurons. *J. Neural Transm.*, **2001**, *108*, 581-592.
- [215] Bisogno, T.; Di Marzo, V. The role of the endocannabinoid system in Alzheimer's disease: facts and hypotheses. *Curr. Pharm. Des.*, **2008**, *14*, 2299-2305.
- [216] Gowran, A.; Noonan, J.; Campbell, V.A. The multiplicity of action of cannabinoids: implications for treating neurodegeneration. *CNS Neurosci. Ther.*, **2011**, *17*, 637-644.
- [217] Rizzo, S.; Tarozzi, A.; Bartolini, M.; Da Costa, G.; Bisi, A.; Gobbi, S.; Belluti, F.; Ligresti, A.; Allarà, M.; Monti, J. 2-Arylbenzofuran-based molecules as multipotent Alzheimer's disease modifying agents. *Eur. J. Med. Chem.*, **2012**, *58*, 519-532.
- [218] González-Naranjo, P.; Pérez-Macias, N.; Campillo, N.E.; Pérez, C.; Arán, V.J.; Girón, R.; Sánchez-Robles, E.; Martín, M.I.; Gómez-Cañas, M.; García-Arencibia, M. Cannabinoid agonists showing BuChE inhibition as potential therapeutic agents for Alzheimer's disease. *Eur. J. Med. Chem.*, **2014**, *73*, 56-72.
- [219] Berlin, M.; Boyce, C.W.; de Lera Ruiz, M. Histamine H₃ receptor as a drug discovery target. *J. Med. Chem.*, **2011**, *54*, 26-53.
- [220] Gemkow, M.J.; Davenport, A.J.; Harich, S.; Ellenbroek, B.A.; Cesura, A.; Hallett, D. The histamine H₃ receptor as a therapeutic drug target for CNS disorders. *Drug Discov. Today*, **2009**, *14*, 509-515.
- [221] Singh, M.R.; Jadhav, H. Histamine H₃ receptor function and ligands: recent developments. *Mini Rev. Med. Chem.*, **2013**, *13*, 47-57.
- [222] Łażewska, D.; Kieć-Kononowicz, K. Recent advances in histamine H₃ receptor antagonists/inverse agonists. *Expert Opin. Ther. Pat.*, **2010**, *20*, 1147-1169.
- [223] Petroianu, G.; Arafat, K.; Sasse, B.; Stark, H. Multiple enzyme inhibitions by histamine H₃ receptor antagonists as potential pro-cognitive agents. *Pharmazie*, **2006**, *61*, 179-182.
- [224] Esbenshade, T.A.; Fox, G.B.; Cowart, M.D. Histamine H₃ receptor antagonists: preclinical promise for treating obesity and cognitive disorders. *Mol. Interv.*, **2006**, *6*, 77-88, 59.
- [225] Bajda, M.; Kuder, K.J.; Łażewska, D.; Kieć-Kononowicz, K.; Więckowska, A.; Ignasik, M.; Guzior, N.; Jończyk, J.; Malawska, B. Dual-acting diether derivatives of piperidine and homopiperidine with histamine H₃ receptor antagonistic and anticholinesterase activity. *Arch. Pharm. (Weinheim)*, **2012**, *345*, 591-597.
- [226] Darras, F.H.; Pockes, S.; Huang, G.; Wehle, S.; Strasser, A.; Wittmann, H.; Nimczick, M.; Sottriffer, C.A.; Decker, M. Synthesis, biological evaluation, and computational studies of tri- and tetracyclic nitrogen-bridgehead compounds as potent dual-acting AChE inhibitors and hH₃ receptor antagonists. *ACS Chem. Neurosci.*, **2014**, *5*, 225-242.
- [227] Darras, F.H.; Kling, B.; Heilmann, J.; Decker, M. Neuroprotective tri- and tetracyclic BChE inhibitors releasing reversible inhibitors upon carbamate transfer. *ACS Med. Chem. Lett.*, **2012**, *3*, 914-919.
- [228] Puccio, S.; Chu, J.; Pratico, D. Involvement of 5-lipoxygenase in the corticosteroid-dependent amyloid beta formation: *in vitro* and *in vivo* evidence. *PLoS One*, **2011**, *6*, e15163.
- [229] Chu, J.; Praticò, D. Pharmacologic Blockade of 5-Lipoxygenase Improves the Amyloidotic Phenotype of an Alzheimer's Disease Transgenic Mouse Model: Involvement of γ -Secretase. *Am. J. Pathol.*, **2011**, *178*, 1762-1769.
- [230] Firuzi, O.; Zhuo, J.; Chinnici, C.M.; Wisniewski, T.; Pratico, D. 5-Lipoxygenase gene disruption reduces amyloid- β pathology in a mouse model of Alzheimer's disease. *FASEB J.*, **2008**, *22*, 1169-1178.
- [231] Chen, Y.P.; Zhang, Z.Y.; Li, Y.P.; Li, D.; Huang, S.L.; Gu, L.Q.; Xu, J.; Huang, Z.S. Syntheses and evaluation of novel isoliquiritigenin derivatives as potential dual inhibitors for amyloid-beta aggregation and 5-lipoxygenase. *Eur. J. Med. Chem.*, **2013**, *66*, 22-31.
- [232] Cummings, J.L.; Morstorf, T.; Zhong, K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimer's Res. Ther.*, **2014**, *6*, 37.