Novel multitarget-directed tacrine derivatives as potential candidates for the treatment of Alzheimer’s disease

Wen-Yu Wu, Yu-Chen Dai, Nian-Guang Li, Ze-Xi Dong, Ting Gu, Zhi-Hao Shi, Xin Xue, Yu-Ping Tang and Jin-Ao Duan

ABSTRACT
Alzheimer’s disease (AD) is a neurodegenerative disorder, which is complex and progressive; it has not only threatened the health of elderly people, but also burdened the whole social medical and health system. The available therapy for AD is limited and the efficacy remains unsatisfactory. In view of the prevalence and expected increase in the incidence of AD, the design and development of efficacious and safe anti-AD agents has become a hotspot in the field of pharmaceutical research. Due to the multifactorial etiology of AD, the multitarget-directed ligands (MTDLs) approach is promising in search for new drugs for AD. Tacrine, which is the first acetylcholinesterase (AChE) inhibitor, has been selected as the ideal active fragment because of its simple structure, clear activity, and its superiority in the structural modification, thus it could be introduced into the overall molecular skeletons of the multi-target-directed anti-AD agents. In this review, we have summarized the recent advances (2012 to the present) in the chemical modification of tacrine, which could provide the reference for the further study of novel multitarget-directed tacrine derivatives to treat AD.

Introduction
Alzheimer’s disease (AD), which is also called senile dementia of the Alzheimer type (SDAT), was firstly identified by the German psychiatrist and neuropathologist Alois Alzheimer in 1906. The most prominent form of AD is an age-related progressive neurodegenerative brain disorder, resulting in decline in language skills, loss of memory and cognitive functions, and accompanied by behavioral disturbances like aggression and depression. Besides, AD is the fourth leading cause of death in people over 65 years old in the world. According to the data from the European Prevention of Alzheimer’s Dementia (EPAD), AD affected more than 40 million people worldwide and its prevalence is projected to double over the next 20 years. Therefore, AD is considered as the most common neurodegenerative disorder and a major health concern to the societies worldwide.

At present, there is no efficacious treatment available that allows the recovery or even slows the progression of AD and clinical treatments have only palliative effects. Such treatments include acetylcholinesterase inhibitors (AChEIs); tacrine (1), donepezil (2), rivastigmine (3), galantamine (4) and N-methyl-D-aspartic acid (NMDA) receptor antagonist memantine (5) (Figure 1), these inhibitors restore neurotransmitter deficits that are responsible for the symptomatic phase of the disease (cognitive, functional and neuropsychiatric deficits) that appear a decade or more after the onset of the neurodegenerative process. However, due to the pathogenesis of AD is complex, these drugs that modulate such a single target can only enable a palliative treatment instead of curing or preventing the neurodegeneration. At the same time, a large number of biological targets for potential therapeutics have been identified included amyloid β-peptide (Aβ) protein, tau protein, receptors (cholinergic, glutamatergic, serotonergic, dopaminergic, noradrenergic, histaminergic) and enzymes (AChE, BuChE, α, β, and γ-secretase, monoamine oxidase A, monoamine oxidase B). Furthermore, a number of processes such as excitotoxicity, oxidative stress, calcium, and metal dys homeostasis, neuroinflammation and mitochondrial damage have been involved in the pathomechanism of AD, which are considered as promising directions in the search for AD treatment. Thus, drug discovery in AD is gradually moving from the development of molecules with “one-target, one-disease” to the “multi-target-directed ligands” (MTDLs), which is capable to simultaneously address several key pathophysiological processes as described earlier, with the aim of enhancing efficacy or improving safety relative to drugs that address only a single target.

Tacrine was the first approved potent and clinically effective AChE inhibitor by the FDA in 1993 for the treatment of AD. However, due to its hepatotoxicity, this drug was soon withdrawn from the pharmaceutical market in 1998. Because of the good anti-AChE activity, much low molecular weight (MW: 198, lower than other approved AChEIs), potential attenuating Aβ-induced...
neurotoxicity and synthetic accessibility, tacrine once again became a hot research topic and many efforts have been concentrated on the synthesis of tacrine derivatives as MTDLs for AD treatment\textsuperscript{11–13}. Most importantly, the liver toxicity induced by tacrine is closely related to the free primary amine group\textsuperscript{14}–\textsuperscript{16}, so in the multitarget-directed tacrine derivatives design, the amino could be modified by binding other functional fragment, thus greatly reduced the hepatotoxicity of tacrine. In addition, recent studies also demonstrated that homo- and hetero-dimers could improve the biological profile of tacrine and even overcame some of its side effects\textsuperscript{17}. So more and more researches have focused on the combination of the potent AChE inhibitory property of tacrine with additional biological properties for the design of new MTDLs. The most common strategy to design MTDLs is to connect two distinct classes of compounds in one molecule by choosing a proper spacer, and the general structure of multi-target-directed tacrine derivatives (Figure 2) could be represented as follows.

This review mainly summarizes the recent advances (2012 to the present) in the design of multi-target-directed anti-AD agents based on the chemical structure of tacrine, which could provide the reference for the further study of novel multi-target-directed tacrine derivatives for the treatment of AD, the previous research could be seen in the related reviews\textsuperscript{11–13}.

### Structural biology of acetylcholinesterase

Brain atrophy is the most obvious clinical finding in AD in which the levels of acetylcholine (ACh), a neurotransmitter responsible for the conduction of electrical impulses from one nerve cell to another nerve cell, are decreased due to its rapid hydrolysis by cholinesterases (ChE)\textsuperscript{18}. That is the “cholinergic hypothesis” which was initially presented about 30 years ago and provided the first rational approach in the treatment of AD. We now know that ACh could be degraded by two types of cholinesterases including AChE and butyrylcholinesterase (BuChE). Indeed, AD therapy is bolstered mainly by AChEI nowadays.

AChE, which is one of the most crucial enzymes for nerve response and function, catalyzes the hydrolysis of acetylcholines with a relative specificity for ACh. AChE is widely distributed in a variety of nerve muscle tissue in the brain, such as the substantia nigra, hippocampus, caudate nucleus and cerebellum. In 1991, the three-dimensional structure of Torpedo californica AChE (TcAChE) was analysed\textsuperscript{19}. Subsequently, the crystal structure of mouse AChE (mAChE) and human AChE (hAChE) have also been reported\textsuperscript{20,21}. Knowledge of the three-dimensional structure of AChE is essential for understanding its remarkable catalytic efficacy, for rational drug design and for developing new therapeutic approaches. The structures of the catalytic domains of the AChE from such species as Torpedo californica, mouse, and human are quite similar. In all cases, the active site is found at the bottom of a 20 Å long channel leading from the surface of the enzyme, and a group of aromatic side chains forms a bottleneck partway down the channel\textsuperscript{19–21}.

Most crystallographic studies of AChE involve the electric ray (Torpedo californica) or the mouse homologs\textsuperscript{22}. Of the over 100 AChE structures deposited in the Protein Data Bank, only four human AChE structures of the catalytic core have been solved to date, of which none is in complex with therapeutic drugs used for the treatment of disease. TcAChE contains 537 amino acids and possesses an ellipsoidal shape with dimensions of about 45 Å by 60 Å by 65 Å. The molecule consists of a 12-stranded central β-sheet surrounded by 14α-helices and bears a striking resemblance to several hydrolases\textsuperscript{13,24}. All current kinetic models for TcAChE proposed the existence of at least two substrate-binding sites: the active site, near the bottom of the active-site gorge and the peripheral anionic site (PAS), near its entrance\textsuperscript{25}. The active site composes two subsites, and in the catalytic anionic subsite, it has been proposed that the choline moiety of TcAChE is stabilized principally via a cation–π interaction with Trp84 and also interacts with Glu199 and Phe330\textsuperscript{4}. The esteratic subsite in TcAChE contains a typical serine-hydrolase catalytic triad, Ser200-His440-Glu327. A substantial contribution to ACh binding within the active site also arises from stabilization of the carbonyl oxygen within the oxanion hole composed of Gly118, Gly119, and Ala201, and of the acetyl group in the “acyl-pocket” composed of Trp233, Phe288, Phe290, and Phe331\textsuperscript{26}. The PAS contains three principal amino acids including Trp279, Tyr70, and Asp72, and binding the ligands at the PAS affects catalytic activity, this is established by site-directed mutagenesis and by the binding of inhibitors\textsuperscript{27}. Szegletes et al.\textsuperscript{28} demonstrated that the primary physiologic role of the PAS was to accelerate the hydrolysis of ACh. The gorge of the active site was so deep and broad that it could bind many different substrates and inhibitors. The initial step in the catalytic pathway was substrate bond to the PAS; therefore, the design of new type AChEis should be based on the hypothesis of dual binding mode. The PAS could act as an uncompetitive inhibitor-binding site, the strong binding of inhibitors at
the PAS might hinder the entry of ACh to the enzyme gorge, and such types of inhibitors, which were able to bind to PAS could act as a sterically blocker. Mutagenesis experiments showed that PAS mutations cause AChE inhibitor activity decreased significantly. In addition, PAS was confirmed to have relation with Aβ accumulation induced by AChE; therefore, blocking PAS could help to reduce the formation of amyloid plaques.39

hAChE and TcAChE have a high structural identity (~53%) with a very low root-mean-square deviation (~1 Å). The catalytic triad (Ser203-His447-Glu334) of hAChE is surrounded by three subsites that are important for catalytic activity30: (1) the esteratic subsite or carbonyl-binding site (ES) consists of aromatic residues (Trp86, Tyr133, Tyr337, and Phe338), which bind to the quaternary trimethylammonium moiety of the choline group in the substrate for optimal positioning of the carbonyl carbon in the ester at the acylation site. The catalytic process is facilitated by orientation of the substrate’s carbonyl oxygen toward the oxygen atom hole formed by the hydrogens from Gly120, Gly121, and Ala204; (2) the anionic subsite or acyl pocket (AS) of AChE formed by the side chains of Phe295 and Phe297 is the binding pocket for the acyl group of the substrate or the methyl group of methylphosphonate; (3) the PAS, which plays a significant role in excess substrate concentration kinetics, is located at the rim of the gorge and consists of residues including Tyr72, Asp74, Tyr124, Trp286, and Tyr341.

AChE was reported to colocalize with Aβ in neuritic plaques and could enhance the rate of formation of Aβ fibrils, forming stable complexes with them. Moreover, AChE was suggested to be a pathological chaperone, which induced a conformational transition in Aβ leading to aggregation and fibril formation.31,32 Recent studies have also shown that selective AChE/BuChE inhibitors could reduce Aβ-induced inflammatory processes.33 On the other hand, a number of new evidence have suggested that inhibitors of both cholinesterase enzymes might have beneficial functions in AD. Indeed, in a healthy human brain, BuChE mainly plays a supportive role in the hydrolysis of ACh, while in an AD brain, its role becomes predominant with AD progression as AChE levels decline and BuChE levels rise.34 More importantly, in viv0 experiments showed that brain-targeted BuChE inhibitors not only improved the cognitive performance of aged rats, without the classic adverse effects associated with AChE inhibition, but they also lowered Aβ brain levels in transgenic mice over expressing human mutant amyloid precursor protein (APP) and ameliorated the Aβ-induced cognitive dysfunction in mice.35,36 Furthermore, it has been also demonstrated that both AChE and BuChE play an important role in Aβ-aggregation during the early stages of senile plaque formation. Therefore, AChE and BuChE inhibition have been documented as critical targets for the effective management of AD by an increase in the availability of acetylcholine in the brain regions and decrease in the Aβ deposition.37

In the tacrine–TcAChE complex, the tacrine moiety is stacked against Trp84, with the nitrogen in the ring forming a hydrogen bond with the main chain carbonyl oxygen of His440, its amino nitrogen binds to a water molecule. The Phe330 ring rotates to lie parallel to tacrine, which is sandwiched between the Phe330 and Trp84 rings, this binding mode clearly explains the reason why tacrine has the good inhibitory activity to AChE at the atomic level, and tacrine has been used as a reference to compare the other AChEIs for both clinical efficacy and side effects in the clinical development.48

**Design of multitarget-directed tacrine derivatives**

In recent years, the treatment of AD by multitarget-directed strategy has gradually become the consensus. The design of tacrine inhibitors with dual binding mode from previous studies39–46 lays the foundation for designing multitarget-directed tacrine derivatives at the molecular level. Selecting tacrime as AChE-binding fragment and introducing different types of functional fragments to regulate other important therapeutic target of AD could obtain multifunctional anti-AD drugs, because these multi targeting derivatives have stronger anti-AD activity and less liver toxicity compared with tacrine.17

**Tacrime derivatives with cholinesterase inhibition and Aβ-amyloid antiaggregation properties**

The progressive deposition of Aβ in the brain of AD patients is generally considered to be fundamental to the development of neurodegenerative pathology. The cell toxicity associated with Aβ fibril aggregation provides an explanation for the neuronal cell loss found in AD patients.47 Therefore, Aβ fibril aggregation in the brain is currently another potential target for the treatment of AD.48 Aβ is a 39- to 43-residue peptides generated by the sequential cleaving of the APP by β- and γ-secretases. Aβ (1–40) and Aβ (1–42) are the main isoforms of Aβ peptides. Though the amount of Aβ (1–42) is only 10% of Aβ (1–40), Aβ (1–42) tends to aggregate more rapidly and displays stronger neuronal toxicity than Aβ (1–40). Therefore, the prevention of Aβ (1–42) aggregation attracts much attention. Recent study showed that AChE could also play a key role in accelerating senile Aβ plaques deposition.49,50 It was likely that AChE interacted with Aβ and promoted amyloid fibril formation through a pool of amino acids located in the proximity of PAS.49 Taking into account that the AChE and Aβ aggregation were particularly important targets for inhibition, the structure of tacrine was thus used as a pharmacophoric moiety in the development of MTDLs endowed with an inhibitory activity against cholinesterases and Aβ fibril formation.11

β-carboline alkaloids possesses a wide range of pharmacological properties relating to a variety of neurological disorders, studies indicated that naturally occurring as well as the chemically synthesized β-carboline analogs exhibited potent AChE inhibitory activity, especially bivalent β-carbolines with IC50 values were in the nanomolar range for AChE inhibition.51,52 For tetrahydro-β-carbolines, these alkaloids occur and accumulate in mammalian tissues, fluids, brain and are able to directly scavenge a variety of reactive oxygen species53–55, making the β-carboline another useful scaffold for AD drug design. Lan et al.56 selected β-carboline to hybridize with tacrine by alkylene linkers to design a series of new hybrids; tacrine was used to inhibit AChE through its binding to the CAS of AChE, while β-carboline was used to interact potentially with the PAS due to its aromatic character. In vitro studies showed compound 6 (Figure 3) presented the greatest ability to inhibit cholinesterase (IC50 = 21.6 nM for eeAChE, 63.2 nM for hAChE, and 39.8 nM for BuChE), more potent than the reference compound tacrine (IC50 = 260 nM for eeAChE). Furthermore, 6 also showed good inhibition against Aβ aggregation (65.8% at 20 μM) relative to that of curcumin (42.4% at 20 μM), and good antioxidant activity (1.57 Trolox eq.). In addition, compound 6 could inhibit Cu2+-induced Aβ aggregation effectively by chelating Cu2+, reduce PC12 cells death induced by oxidative stress and penetrate the blood–brain barrier (BBB).

Based on that benzothiazole derivatives could interact with Aβ peptides and have been used as Alzheimer’s brain imaging agents57, in 2013, Keri et al. synthesized a series of novel multifunctional compounds bearing two pharmacophoric groups: tacrine and a benzothiazole moiety connected by different linkers, containing an amide bond, an alkyl or arylalkyl chain.58 All these new tacrine–benzothiazole hybrids displayed high in vitro activities
with IC_{50} values were in the low micromolar to submicromolar concentration range (0.34–1.84 μM) toward the inhibition of AChE. In addition, the compounds also showed high inhibitory activity against self-induced Aβ_{1–42} aggregation at 50 μM ranging from 22.3% to 61.3%. Among them, compound 7 (Figure 3) presented the moderate inhibitory activity against AChE (IC_{50} = 0.57 μM) compared with the commercial drug tacrine (0.19 μM). Interestingly, it exhibited the highest activity as anti-Aβ_{32} self-aggregation (61.3% at 50 μM) due to the biaryl heterocyclic scaffold of BTA-moiety could recognize and interact with the abnormal β-sheet conformation of the Aβ peptide.

Because of the extensive biological actions, such as protecting neurons from ischemic damage and anti-inflammatory activity of phenylthiazole derivatives\(^5\), Wang et al.\(^8\) designed and synthesized a series of novel multifunctional compounds by conjugating tacrine with 4-phenyl-2-aminothiazole group, through different middle linkers including an alkyl chain and succinamide or glycinate. The inhibitory activities against AChE, BuChE, and Aβ-aggregation as well as a calcium overload blockade effect were studied for these phenylthiazole–tacrine hybrids, and the results showed that these hybrids were potent inhibitors of cholinesterase with pIC_{50} values ranging from 5.78 to 7.14 nM for AChE and from 5.75 to 10.35 nM for BuChE. Most compounds showed good inhibitory potency on Aβ_{1–42} self-aggregation and their inhibitory effects depended on the type of middle linker and substitutions at 4’-position of 4-phenyl-2-aminothiazole. In addition, some hybrids also displayed the Ca\(^{2+}\) overload blockade effect in the primary cultured cortical neurons. Among them, compound 8 (72.0% at 20 μM) (Figure 3) exhibited the highest inhibitory potency against Aβ_{1–42} self-aggregation, higher than the propidium iodide (57.1% at 20 μM) and moderate AChE inhibition (pIC_{50} = 6.80 nM) compared with tacrine (pIC_{50} = 7.19 nM).

Lately, some researches have suggested that electron-rich aromatics would bind to the peripheral binding site, which has been confirmed in a comparison of aromatics with differing electron density\(^60,61\). In order to further explore the anti-Alzheimer potential of the electron-rich aromatics based on MTDLs strategy, Zhang’s group\(^62\) synthesized a series of novel multifunctional compounds by conjugating a tacrine and electron-rich aromatics including benzoates, phenylacetates, cinnamates, and acetylindole. The screening results showed that most of them exhibited a significant ability to inhibit ChEs, certain selectivity for AChE over BuChE and strong potency against self-induced Aβ aggregation. All IC_{50} values of biological activity were at the nanomolar range. Especially, compound 9 (Figure 3) with a trimethoxyl phenylacetate group, showed the most potent inhibition for AChE with its IC_{50} value was 5.63 nM, which was 13 times stronger than tacrine and the highest selectivity (BuChE/AChE = 64.6). Moreover, it also exhibited a potent inhibitory against Aβ aggregation with an IC_{50} value was 51.81 nM. A Lineweaver–Burk plot and molecular modeling analysis showed that compound 9 targeted both the CAS and PAS of ChEs, in addition, the electron density of aromatic ring, which was linked with tacrine through acetyl group, played a significant role in determining the inhibitory activity.

Recent studies indicated that a naphthoquinone linked to a tertiary benzyamine through a proper spacer showed optimal anti-cholinesterase and antiaggregating features\(^63\). Nepovimova et al. proposed quinone as an anti-amyloid privileged motif and they demonstrated that it might interfere with several amyloid proteins, due to its possibility to form favorable hydrogen bond and π-stacking interactions\(^64,65\). These results were also in agreement with the work of Scherzer-Attaliet et al., who described that naphthoquinone-tryptophan hybrids could effectively inhibit Aβ oligomerization both in vitro and in vivo\(^66\). Motivated by these considerations, Bolognesi’s group\(^67\) rationally designed a novel series of hybrids by linking the naphthoquinone moiety to a tacrine scaffold via a methylene spacer. All compounds displayed excellent in vitro AChE inhibitory potencies with IC_{50} values ranged from micromolar to subnanomolar concentrations, and interesting capabilities to block Aβ aggregation. Among them, compound 10 (Figure 3) exhibited outstanding inhibitory activity (IC_{50} = 0.72 nM for hAChE) in complex with AChE by the X-ray analysis. More meaningful was that the compound 10 showed negligible toxicity in immortalized mouse cortical neurons Neuro2A and primary rat cerebellar granule neurons, as well as was able to completely revert the decrease in viability induced by Aβ in Neuro2A.

**Tacrine derivatives with cholinesterase and BACE1 inhibition properties**

At current, the most popular hypothesis is that AD is caused by aggregates of the Aβ, which is generated by cleavage of APP by β-secretase1 (BACE1) follows by γ-secretase, making BACE1 a particularly good drug target for the generation of inhibitors that lower Aβ. The identification and cloning of this enzyme led to better understanding of its physiological function and to the
development of BACE1 inhibitors. Recently, numerous BACE1 inhibitors have been discovered, and a few of them have been tested in the early stage of clinical trials, so the identification of BACE1 as a novel class of type-I transmembrane aspartic protease has been a landmark discovery in AD.

Galdeano et al. designed a series of tacrine–huperzine A hybrids, by combining the 4-aminoquinoline moiety of tacrine with huperzine fragments through an alkyl or alkylamine linker, as potential inhibitors for AChE, BuChE, and BACE1. All the tested compounds were potent hAChE inhibitors with IC50 values in the subnanomolar to low nanomolar range (0.31–9.09 nM). The strongest inhibitory activity towards hAChE was displayed by hybrid with a six carbon atom linker. The elongation of the tether led to a decreased 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 against hBuChE with IC50 values ranging from 24.6 to 139 nM. The 6-chlorotacrine–huprine hybrids showed a good inhibitory potency against BACE1 with the most active compound was 11 (Figure 4) (IC50 = 4.9 μM for hBACE1), these data indicated that the huprine–tacrine heterodimers could be considered as promising lead compounds for AD.

Figure 4. The chemical structures of tacrine derivatives as cholinesterase and BACE1 inhibitors.

Flavonoids, which are ubiquitously present in fruits and vegetables, have attracted much attention in recent years because they could limit the neurodegeneration associated with a variety of neurological disorders. Flavonoids mediate their effects by several routes including their capacity to scavenge neurotoxic species, such as free radicals, and their interactions with important neuronal enzymes, such as BACE1. In 2012, Fernandez-Bachiller et al. choose a flavonoid scaffold derive from 4-oxo-4H-chromene for its antioxidant, BACE1 inhibitory activities as well as its potential interaction with the AChE-PAS due to the aromatic character to connect with tacrine by linkers of different lengths (from 7 to 12 carbon atoms), and modified by an introduction of various substituents for the treatment of AD. The developed compounds showed a stronger inhibitory activity against human AChE and BuChE, with IC50 values were in the nano- and picomolar ranges, being more potent than the parent inhibitor tacrine. Compound 12 (Figure 4) showed potent inhibition activities against human BACE1 (IC50 = 2.8 μM) and cholinesterases (IC50 = 8.0 nM for hAChE, IC50 = 1.5 nM for hBuChE), as well as good antioxidant properties (1.3 Trolox eq.). Thus, it was expected that this hybrid could augment patient cognition (by increasing levels of acetylcholine), protect neurons from oxidative stress (by capturing free radicals), and reduce the formation of senile plaques (from inhibition of BACE-1).

Considering the widened biological profile toward amyloid aggregation from previous research, Zha et al. selected the benzoafuran nucleus (and the modification thereof) as the framework to connect with tacrine by an amido or amino linkage to build new MTDLs for AD. Based on the previous studies have shown that the presence of a properly spaced protonable amino group and aromatic residues could facilitate the interaction with the PAS, and the introduction of an amido group coupled with aromatic planar moieties might enhance the binding properties toward BACE-1, their synthetic hybrids exhibited good inhibitory activities on AChEs and Aβ self-aggregation. In particular, some compounds displayed significant inhibition of hBACE1. Among the hybrids, 13 (Figure 4) showed the most interesting profile as a subnanomolar selective inhibitor of hAChE (IC50 = 0.86 nM for hAChE) and a good inhibitor of both Aβ aggregation (hAChE- and self-induced, 61.3% at 50 μM and 58.4% at 100 μM, respectively) and hBACE-1 activity (IC50 = 1.35 μM). Kinetic studies indicated that 13 acted as a slow, tight-binding, mixed-type inhibitor, while X-ray crystallographic studies highlighted the ability of 13 to induce large-scale structural changes in the active-site gorge of Torpedo californica AChE. In vivo studies confirmed that 13 significantly ameliorated performances of scopolamine-treated ICR mice and did not exhibit significant hepatotoxicity. Thus, 13 could be used for further studies to help understand the structural requirements for an optimal MTDL for AD treatment.

Tacrine derivatives with cholinesterase inhibition and antioxidant properties

Oxidative stress is characterized by an imbalance between the production of reactive oxygen species (ROS) and their removal by antioxidative mechanisms. Recent researches have demonstrated that the antioxidant defense system in elderly people lost its capacity to neutralize oxidative species. In the early stage of the AD, the oxidative stress plays a key role in initiating the aggregation of Aβ and tau protein hyperphosphorylation. So the oxidative stress might be a risk factor for the initiation and progression of AD. Extensive evidences suggested that free radicals might be involved in the pathogenesis of AD, because the brain tissues in patients with AD were 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 might bring benefits, particularly in the early stage of AD. Many evidences proved that antioxidants could attenuate the syndrome of AD and prevent the progression of the disease. Searching for cholinesterase inhibitors with additional antioxidant properties is one of the trends in the development of an effective therapy for AD.

Trolox is a powerful antioxidant agent widely used in biological or biochemical applications to reduce oxidative stress. Numerous reports indicated that trolox could protect liver from the damage induced by chemical insults both in vitro and in vivo. In addition, it showed neuroprotective effect through scavenging ROS and attenuated the neurotoxicity mediated by Aβ and H2O2 on hippocampal neurons. Furthermore, as a lipid-soluble analog of trolox,
vitamin E has been already used to prevent the tacrine-induced hepatotoxicity in clinic and delayed the AD progression in patients with moderately severe AD\textsuperscript{87}. Xie et al.\textsuperscript{88} connected the trolox with tacrine moiety via various methylene chain and obtained a novel series of compounds. Among them, compound 14 (Figure 5) was the most potent inhibitor against AChE (IC\textsubscript{50} = 9.8 nM for eeAChE and 23.5 nM for hAChE), it was also a strong inhibitor against BuChE (IC\textsubscript{50} = 22.2 nM for eqBuChE and 20.5 nM for hBuChE). Fortunately, in\textit{vivo} hepatotoxicity assays indicated that compound 14 did not show signs of hepatotoxicity, which was much safer than tacrine.

Carbazoles, naturally occurring phytochemicals, are widely present in many plant species and possess a wide range of biological activities associated with AD. It has been reported that naturally occurring carbazole derivatives were able to directly scavenge a variety of ROS and possessed strong antioxidant activities\textsuperscript{49}. Thiratmatrakul et al.\textsuperscript{90} studied a series of carbazole derivatives extracted from root bark of \textit{Clausena Harmandiana} as an antioxidant and found that 7-methoxyheptaphylline and heptaphylline possessed a strong antioxidant effect \textit{in vitro}. So they combined the tacrine to the active functions of natural antioxidants, 7-methoxyhepta phylline and heptaphylline, as potential multi-functional agents for the treatment of AD. The synthesized compounds showed high inhibitory activity on AChE with IC\textsubscript{50} values ranged from 0.48 to 1.03 nM and exhibited good inhibition selectivity against AChE over BuChE. The compounds showed higher radical-scavenging activity (IC\textsubscript{50} = 8.34–11.24 \mu M) than trolox. Furthermore, they displayed a neuroprotective effect against the oxidative stress induced by H\textsubscript{2}O\textsubscript{2} in the neuroblastoma cells and against the toxicity induced by A\textsubscript{\beta}\textsubscript{1-42} peptide in C6 astroglialoma cells at 100 \mu M. Compound 15 (Figure 5), bearing 7-methoxyhheptaphylline and a five carbon atom spacer, exhibited the highest potency for both radical scavenging and AChE inhibitory activity and could improve both short- and long-term memory deficits through the enhancement of cholinergic signaling in behavioral studies\textsuperscript{91}.

Based on that tacrine–lipoic acid\textsuperscript{92}, tacrine–ferulic acid\textsuperscript{93}, and tacrine–caffeic acid\textsuperscript{94} hybrids showed a good multiple biological profile, displaying AChE inhibition property and antioxidant activity. Digiacomo et al.\textsuperscript{95} synthesized a new class of tacrine derivatives by inserting 1,3-diamino-2-propanol to widen the pharmacological profile of the hybrids, which was applied successfully to a number of aspartyl proteases including BACE1. For example, compound 16 (Figure 5) possessed a good ability to inhibit A\textsubscript{\beta} self-aggregation (53.2\% at 50 \mu M), submicromole AChE (IC\textsubscript{50} = 0.15 \mu M)/BuChE (IC\textsubscript{50} = 0.36 \mu M) inhibition and modest BACE1 inhibition (13.07\% at 10 \mu M). Importantly, compound 16 was also a DPPH radical scavenger (60.87\% at 10 \mu M) and copper chelator, as well as a potent neuroprotective agent against glutamate-induced cell death with low toxicity in HT22 cells. Similarly, Quintanove et al.\textsuperscript{96} reported a series of novel tacrine–cinnamate and tacrine–cinnamylidenene acetate hybrids in 2015. These hybrid compounds all presented high activity against AChE, with IC\textsubscript{50} values were mostly in the sub-micromolar range, and the best inhibitor was 17 (Figure 5) (IC\textsubscript{50} = 0.09 \mu M) with 3,4-dimetoxy substitutions in the cinname unit. Moreover, the compounds with free catechol moieties showed very high antioxidant activity (EC\textsubscript{50} in the low micromolar range), eventually due to their iron-chelating contribution.

5,6,7-Trimethoxyflavone, methylations of the hydroxyl groups of oroxylin A or baicalein,\textsuperscript{97} possesses a wide range of pharmacological properties such as free radical scavenging effect, A\textsubscript{\beta} fibril inhibition effect, anticancer activity, anti-inflammatory activity, and neuroprotective effect\textsuperscript{98}. Based on these, Liao et al.\textsuperscript{99} created a series of novel hybrids that combined 5,6,7-triethoxyflavone with 6-chlorotacrine, although the molecular weight of hybrids were more than 500, the hybrids possessed more complementary biological activities and could cross the BBB. The results showed that the target compounds exhibited good AChE inhibitory potency, high selectivity toward AChE over BuChE, potential antioxidant activity and significant inhibitory potency against self-induced A\textsubscript{\beta}\textsubscript{1-42} aggregation. Among them, compound 18 (Figure 5) showed the strongest inhibitory activity against AChE with its IC\textsubscript{50} value of 12.8 nM was higher than the reference compound 6-chlorotacrine (IC\textsubscript{50} = 78.5) and indicated remarkable inhibition on self-induced A\textsubscript{\beta}\textsubscript{1-42} aggregation with inhibition ratio of 33.8\% at 25 \mu M.

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**Figure 5.** The chemical structures of tacrine derivatives with cholinesterase inhibition and antioxidant properties.
Furthermore, compound 18 acted as an antioxidant, as well as a good neuroprotective agent against H$_2$O$_2$-induced PC12 cell injury determined by the ORAC-FL method (Oxygen Radicals Absorbance Capacity by Fluorescence)$^{100}$. Ebselen, an organoselenium found in 1996, is known as a classic glutathione peroxidase mimic, reacted in a rapid reaction with peroxynitrite$^{101}$, possessed antioxidant activity and anti-inflammatory property and exhibited neuroprotective effect in several preclinical studies$^{102,103}$. Mao et al.$^{104}$ synthesized a series of tacrine–ebselen hybrids as multifunctional anti-AD agents with cholinesterase and antioxidant activities. Most of these compounds were potent inhibitors of AChE and BuChE (with IC$_{50}$ values ranged from 2.55 to 657 nM for AChE and from 2.80 to 38.88 nM for BuChE), they also demonstrated similar hydrogen peroxide and peroxynitrite-scavenging activity as ebselen by horseradish peroxidase and peroxynitrite-scavenging activity assay. Compound 19 (Figure 5) exhibited superior activity and a better balance of AChE and BuChE activities than tacrine (IC$_{50}$ = 2.55 nM for EeAChE, IC$_{50}$ = 2.80 nM for EqBuChE) and showed effective hydrogen peroxide and peroxynitrite scavenger property (neuroprot. vs. H$_2$O$_2$ = 83.7% at 25 μM), indicating that this hybrid was a good multifunctional drug candidate for the treatment of AD.

**Tacrine derivatives with cholinesterase inhibition and NO-releasing properties**

Nitric oxide (NO) is an essential signaling molecule involved in various physiological functions in humans, and the over and under production of NO are responsible for a number of pathological conditions. The biosynthesis of NO by brain neuronal NOS is associated with stroke and chronic neurodegenerative diseases, such as Alzheimer’s, Parkinson’s, and Huntington’s diseases$^{105}$. Increasing evidences suggested that NO, a free radical gas, might be beneficial for the treatment of AD by increasing blood supply and regulating the cerebral circulation. Being involved in a variety of physiological and pathophysiological processes, NO is regarded as a potential tool in the pharmacotherapy of AD, therefore NO-releasing drugs might be especially beneficial for the treatment of AD$^{106}$.

Chen et al.$^{107}$ conjugated a nitrate moiety to the tacrine–ferulic acid hybrid compounds via alkylene side chain in order to obtain new multifunctional AChEIs, which endowed with additional properties at least such as vessel relaxation activity on the basis of previous study$^{105,108}$. All the synthetic NO-donating tacrine–ferulic acid hybrids were potent cholinesterase inhibitors with IC$_{50}$ values ranged from 3.6 nM to 44.3 nM against eAAChE and 1.0 nM to 24.9 nM against eqBuChE. Compound 20 (Figure 6) was one of the most potent inhibitors toward AChE (IC$_{50}$ = 10.9 nM) and BuChE (IC$_{50}$ = 17.1 nM), respectively, and possessed an activity correlated with the NO production (0.314 μg/ml) compared with the reference isosorbide dinitrate in the vascular relaxation assay. In addition, compound 20 was confirmed to have beneficial effects for the short-term learning ability, and could improve memory impairment in the passive avoidance test in the scopolamine-induced cognition impairment animal model, the hepatotoxicity study also proved that 20 was much safer than tacrine. These tests demonstrated that the strategy of NO-donating drugs was rational and beneficial.

To search for multifunctional anti-AD agents with good safety, the previously synthesized tacrine–flurbiprofen hybrids were modified into tacrine–flurbiprofen–nitrate trihybrids by Liao team$^{109}$. These compounds displayed higher cholinesterase inhibitory activity relative to the bivalent hybrids with IC$_{50}$ values were in the nanomolar ranges. The results revealed that the length of the alkyl, which was connected to nitrate moiety of the target compounds, could significantly influence the AChE inhibitory activity. When the length increased, the activity decreased and the optimal spacer length was two carbon atoms. Compound 21 (Figure 6) was the most potent trihybrid compound, which released moderate NO (0.348 μg/ml), exerted blood vessel relaxative activity and showed significant Aβ inhibitory effects whereas tacrine and flurbiprofen did not exhibit any Aβ inhibitory activity at the same dose. In addition, compound 21 was active in improving memory impairment in vivo and was much safer than tacrine in the hepatotoxicity study, and the higher safety of 21 qualified that it was more beneficial for the treatment of AD.

Nitroxides are stable free radicals that rapidly cross cell membranes, preempt free-radical formation by oxidizing redoxactive metal ions, and function both as intra- and extracellular SOD mimics. Several studies indicated that modification of cardioprotective agents$^{110}$, PARP-inhibitors$^{111}$, or neuroprotective ebselen$^{112}$ with nitroxides had a beneficial influence on their activity, being supplemented by the nitroxide’s “in status nascend acting” antioxidants and radical scavengers. With these concepts in mind, Kalai et al.$^{113}$ combined nitroxide and/or nitroxide precursors (amines

![Figure 6. The chemical structures of tacrine derivatives with cholinesterase inhibition and NO-releasing properties.](image-url)
and hydroxylamines) with tacrine via methylene or piperazine space. The synthetic compounds were tested for their hydroxyl radical and peroxyl radical-scavenging ability, acetylcholinesterase inhibitor activity, and protection against Aβ-induced cytotoxicity. Compounds 22 and 23 (Figure 6) were found to be the most efficient AChEIs and radical scavengers, and they exhibited remarkable cell protection toward Aβ-induced toxicity, providing direction for further development of additional candidates with dual functionality (anti Alzheimer’s and antioxidant).

**Tacrine derivatives with cholinesterase inhibition and neuroprotective properties**

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, NMDA receptor, synaptic plasticity, mitochondrial dysfunction, excitotoxicity, inflammatory changes, iron accumulation, and protein aggregation.

In 2012, Minarini et al. designed a novel multipotent anti-AD agent by linking two tacrine moieties via cystamine (2,2'-disulfane-diyldiethanamine) for its important activities as antioxidant, cyto-, and neuroprotective agent. In particular, systemic administration of cystamine was reported to diminish neural toxicity associated with different toxins and to protect against neurodegeneration. The obtained cystamine–tacrine compound 24 (Figure 7) displayed an inhibitory activity against both AChEs with its IC50 values were in the nanomolar range (IC50 = 5.04 nM for hAChE, IC50 = 4.23 nM for hBuChE), and inhibitory properties against the self-induced Aβ aggregation assay with its IC50 value was 24.2 μM. It also exerted a neuroprotective activity on SH-SYSY cell line against H2O2-induced oxidative injury. Fortunately, 24 endowed with a lower toxicity in comparison to bis(7)tacrine.

The long-term potentiation (LTP) in hippocampus has been widely considered as a key cellular mechanism underlying learning and memory, and chemical entities containing mercapto group, such as dithiothreitol (DTT), glutathione (GSH) and N-acetyl cysteine (NAC) were confirmed to facilitate the induction of LTP in the normal rats and even reversed the LTP impairment in aged rats. Therefore, Wang et al. choose the mercapto groups to cooperate with tacrine in a single molecular for novel multifunctional compounds as anti-AD potential drugs. These mercapto–tacrine derivatives displayed a synergistic pharmacological profile of LTP enhancement, cholinesterase inhibition, neuroprotection and less hepatotoxicity. Moreover, they could improve the memory and cognitive impairment efficiently with fewer side effects and diminish the oxidative damage caused by free radicals. Compound 25 (Figure 7), in which the mercapto group was covered with the acetyl group, exhibited the neuroprotective effect mostly in a concentration-dependent manner, with the maximal effect at 30 μM (56.5%), emerging as promising molecule for the therapy of age-related neurodegenerative diseases. Soon after that, Santos’s group also synthesized a set of mercaptotacrine derivatives by conjugation of a tacrine moiety with an S-allylcysteine (garlic constituent) or S-propargylcysteine moiety, aimed at improving the cholinergic system and neuroprotective capacity, and the most promising result was achieved by compounds 26 (Figure 7) for the AChE inhibition (IC50 = 0.3 μM) and 27 (Figure 7) for the remarkable prevention of superoxide production and Aβ-induced cellular toxicity.

A codrug of the natural product silibinin and the AChEI tacrine was synthesized by Chen et al. These compounds showed potent AChE and BuChE inhibitory activities, while the toxicities of the co-drugs were markedly reduced in comparison to that of tacrine. Moreover, using a neuronal cell line (HT-22), a neuroprotective effect against glutamate-induced toxicity could be observed that the co-drugs showed no hepatotoxicity and no induction of the cytochrome P450 system. In a scopolamine-induced cognitive impairment model, the codrugs were as potent as tacrine in reversing memory dysfunction, being superior to the physical mixture of tacrine and silibinin. The obtained silibinin–tacrine codrug 28 (Figure 7) exhibited pronounced pro-cognitive effects just like tacrine, but lacked tacrine’s therapy-limiting hepatotoxic effects completely both in vitro and in vivo.

**Tacrine derivatives with cholinesterase inhibition and metal-chelating properties**

Recent studies indicated another hypothesis, called metal hypothesis, might also contribute to AD pathologyn. It was observed that the level of metal ions such as iron (Fe), copper (Cu), and zinc (Zn) in AD patients was 3–7 folds higher than that of healthy individuals. The abnormal accumulation of metals was able to accelerate the formation of Aβ aggregates and neurofibrillary tangles (NFTs), which promoted inflammation and activated neurotoxic pathway, leading to dysfunction and death of brain cells. In addition, the redox-active ions like Cu2+ and Fe2+ were involved in the production of ROS, and oxidative stress was also critical for Aβ neurotoxicity. Thus, the modulation of the level of these biometals in the
brain is a potential therapeutic strategy for treating AD\textsuperscript{123,124}, and multifunctional metal chelators might block metal-related oxidative stress as well as modulate Aβ aggregation.

In 2014, Li et al. combined tacrine and a rhein scaffold for its metal-chelating ability as novel multifunctional potent AD drug\textsuperscript{125}. Most compounds inhibited AChE in the nanomolar range in vitro effectively. Among them, compound 29 (Figure 8) was one of the most potent inhibitors and was 5-fold more active than tacrine toward AChE, it also showed a moderate BuChE inhibition with IC\textsubscript{50} value was 200 nM. In the inhibition of the AChE-induced Aβ aggregation assay, compound 29 (70.2% at 100 μM) showed the greatest inhibitory activity. Moreover, in metal-chelating effect assay, compound 29 could effectively chelate Cu\textsuperscript{2+} and Fe\textsuperscript{2+}, and thereby could serve as a metal chelator in treating AD.

PBT2 (an 8-hydroxyquinoline derivative) was selected by Fernandezbachiller et al.\textsuperscript{126} to combine with tacrine for its metal-chelating, neuroprotective, and antioxidant properties to synthesize tacrine-PBT2 hybrids as potential candidates for the treatment of AD. These new tacrine-PBT2 hybrids displayed potent inhibitory activities against human AChE and BuChE with their IC\textsubscript{50} values were in the nano- and subnanomolar ranges, which were more potent than the parent fragment tacrine. They could diminish Aβ aggregation promoted by AChE as well as show better antioxidant properties than trolox due to the interaction with the PAS of AChE and Cu\textsuperscript{2+} complexing properties. In human neuroblastoma cells, these hybrids also showed protective properties against damage caused by mitochondrial free radicals. Compound 30 (Figure 8) as a representative hybrid was further confirmed that it controlled several pathological processes at the cellular and neuronal level and might have significant beneficial effects in neurodegenerative diseases\textsuperscript{127}.

Presently, several natural compounds bearing pyridin-2(1H)-ones (2-pyridones) structure have emerged with numerous pharmacological activities, such as AChEIs, antioxidant and antitumour\textsuperscript{128–130}, while many synthetic or semisynthetic clinical drugs also encapsule those nontoxic moieties\textsuperscript{131}. Therefore, Chand et al.\textsuperscript{132} conjugated 2-hydroxybenzoyl-2-pyridone (HBP) with tacrine through different alkyl spacers and evaluated them as AChEIs, antioxidants and biometal chelators to combat AD. The results showed that all of them were dual-binding site AChE inhibitors with activity ranged in submicromolar (IC\textsubscript{50} = 0.57–0.78 μM), which were comparable to the parent tacrine, and had good 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (EC\textsubscript{50} = 204–249 μM) conferred by the HBP moiety. The higher anti-AChE activity of compound 31 (IC\textsubscript{50} = 0.57 μM) (Figure 8) confirmed the possible better fitting of this compound between CAS and PAS as well as its capacity for H-bonding by molecular-modeling studies. The chelating capacity evaluation proved that the HBP moiety of this biometal acted as a moderate/good chelator, being able to form complexes with β-phenol-keto coordination mode.

Recent studies have demonstrated that hydroxyl-substituted coumarins could act as effective redox-active metal chelators, free radical scavengers and powerful chain-breaking antioxidants\textsuperscript{133,134}, and coumarin derivatives in view of their marked implication in the improvement of cognitive functions of patients with neurodegenerative disease attracted people’s attention\textsuperscript{135}. Hrabinova et al.\textsuperscript{136} synthesized a series of hybrids consist of a tacrine unit and a 7-hydroxycoumarin moiety substitutes at the 4-position, linked by alkylenediamine or alkylen polyamine tethers of different lengths via an amide functionality. Most compounds inhibited hAChE/hBuChE at nanomolar concentrations, being more potent than tacrine. The thioflavin T assay showed that these tacrine-coumarin hybrids were able to interfere with Aβ\textsubscript{1-40} aggregation in a concentration-dependent manner. The ABTS assay and EPR spectroscopy indicated that they could suppress the formation of reactive hydroxyl radicals, by the chelation of free copper (II) ions due to the carbonyl oxygen and imino nitrogen atoms of the spacer were coordinated to the copper (II) ion, and confirmed the reduction of Cu(II) to Cu(I), which were further proved by in vitro DNA damage protection experiments. Among them, compound 32 (Figure 8) containing along with the AChE inhibitory segment, also an antioxidant moiety capable of alleviating metal (copper)-induced oxidative stress, might be of importance in the treatment of Alzheimer’s disease.

**Other types of multitarget-directed tacrine derivatives**

**Tacrine derivatives with cholinesterase and MAO inhibition properties**

Monoamine oxidases (MAOs) receive increasing attention in recent years due to their roles in the treatment of AD. MAOs are flavin adenine dinucleotide (FAD)-containing enzymes and localize in the outer mitochondrial membrane in various cells including nerve terminals, liver, intestinal mucosa and other tissues\textsuperscript{137}, which are responsible for the oxidative deamination of neurotransmitters.

![Figure 8. The chemical structures of tacrine derivatives as cholinesterase inhibitors with metal-chelating properties.](image-url)
Based on their substrate and inhibitor specificities, MAOs exist in two distinct enzymatic isofoms, MAO-A and MAO-B. Selective inhibitors of MAO-A have been shown to be effective antidepressants and anti-anxiety agents in the clinic, whereas MAO-B inhibitors are useful in several neurodegenerative disorders such as Parkinson’s disease, AD, Huntington chorea and amyotrophic lateral sclerosis. Evidence showed that high expression levels of MAO-B in neuronal tissue could result in an increase in the level of H2O2 and oxidative free radicals, which played a major role in the etiology of AD. Therefore, MAO-B inhibitors are considered to be potential candidates for anti-Alzheimer drugs.

Among the MAO inhibitors, selegiline, which is an irreversible and selective MAO-B inhibitor, acts as a neuroprotective agent in cellular and animal models of AD, so Lu et al. designed tacrine hybrids that were connected by carbon spacers of different lengths with selegiline. Results showed that all the compounds were potent AChE and BuChE inhibitors (IC50 = 2.03–66.0 nM for eeAChE, IC50 = 32.0 nM for eqBuChE) and were effective inhibitors of both hMAO-A and hMAO-B enzymes in the micromolar range. Compound 33 (Figure 9) with a nine carbon atom tether, turned out to be a potent inhibitor of eeAChE (IC50 = 22.6 nM) and eqBuChE (IC50 = 9.37 nM) and a balanced inhibitor of both monoamine oxidases (IC50 = 0.372 μM for hMAO-A, IC50 = 0.181 μM for hMAO-B). Additional experiments on 33 indicated that it was a mixed-type inhibitor of AChE and an irreversible inhibitor of hMAO-B, which could enhance patient cognition by increasing levels of acetylcholine and protect neurons by keeping the activities of selegiline.

Sun et al. chose homoisoflavonoid as the pharmacophore for its known MAO-B inhibitory activity to design the novel multi-target-directed tacrine derivatives. Evaluating as inhibitors of cholinesterase and MAOs, all the compounds were potent cholinesterase inhibitors with activities in the nanomolar range and were selective MAO-B inhibitors. Compound 34 (Figure 9) with a six carbon linker between tacrine and (E)-7-hydroxy-3-(4-methoxybenzylidene) chroman-4-one, provided the best results for cholinesterase inhibition (IC50 = 67.9 nM for eeAChE, IC50 = 33.0 nM for eqBuChE) better than tacrine (IC50 = 111.0 nM for eeAChE) and preferable hMAO-B inhibition (IC50 = 0.401 μM). The PAMPA-BBB assay indicated that compound 34 could cross the BBB to target the enzymes in the CNS.

Xie et al. selected piperazine side-armed alkane spacers of different lengths to connect tacrine and coumarin for that the piperazine side-armed alkane spacers could be lodged in the narrow enzymatic cavity, which allowed the compounds simultaneously binding to the PAS, CAS and mid-gorge sites of AChE. The coumarin scaffold was used to inhibit the MAO-B and interacted with the PAS of AChE due to its aromatic character. Most of these tacrine-coumarin hybrids showed potent inhibitory activity toward AChE and BuChE and clearly selective inhibition for MAO-B. Among these compounds, 35 (Figure 9) exhibited strong inhibitory activity for AChE (IC50 = 33.63 nM for eeAChE, IC50 = 16.11 nM for hAChE) and BuChE (IC50 = 80.72 nM for eqBuChE, IC50 = 112.72 nM for hBuChE), and the competitive and highest inhibitory activity against hMAO-B (IC50 = 0.24 μM) from covering the substrate and entrance cavities of MAO-B. Moreover, 35 could penetrate the CNS and showed low cell toxicity.

**Figure 9.** The chemical structures of tacrine derivatives with cholinesterase and MAO inhibitory activities.

**Figure 10.** The chemical structures of tacrine derivatives as cholinesterase and tau-aggregation inhibitors.

**Tacrine derivatives with cholinesterase inhibition and tau antiaggregating properties**

Tau proteins are microtubule-associated proteins that are abundant in neurons in the central nervous system, the main physiological function of tau proteins is to interact with tubulin to stabilize microtubules and promote tubulin assembly into microtubules. Evidences showed that hyperphosphorylation of tau appeared to be an early and central event in tau related neurodegeneration including AD, which was involved in the pathogenesis of AD. Phosphorylation of tau is regulated by a host of kinases, for example, PKN, a serine/threonine kinase. When PKN is activated, it phosphorylates tau, resulting in the self-assembly of tangles of paired helical filaments and straight filaments, and eventually disrupting the microtubule organization, leading to collapse of the neuron’s transport system. Thus, inhibition of abnormal hyperphosphorylation of tau offers a promising therapeutic target for AD and related tauopathies.

Di Pietro et al. obtained eight methylene-linked 1,2,3,4-tetrahydrobenzo[h][1,6]naphthyridine-6-chlorotacrine hybrids, by optimization of an essentially inactive 3,4-dihydro-2H-pyran[3,2-c]quinoline carboxylic ester derivative as PAS-binding motif by double O–NH bio-isosteric replacement, molecular hybridization with the CAS inhibitor 6-chlorotacrine and molecular dynamics-driven optimization of the length of the linker. Further biochemical profiling of hybrids have shown that these eight hybrids were potent inhibitors of hAChE/HBuChE and moderately potent Aβ42 and tau antiaggregating agents, with IC50 values were in the submicromolar and low micromolar range, respectively. The in vitro studies using an artificial membrane model showed these hybrids had good brain permeability. Compound 36 (Figure 10), with the most potent inhibition against hAChE, exhibited IC50 of...
2.06 nM, which was better than the reference compound 6-chlorotacrine ($IC_{50} = 5.90 \text{nM}$), as well as possessed the most potent and balance with self-ind $\mathrm{A}\beta_{1-42}$ antiaggregating (77.5% at 10 $\text{M}$) and tau antiaggregating (68.7% at 10 $\text{M}$) activities.

Phenothiazine, the key pharmacophore of rember (methylene blue, MB), could prevent tau filament formation and regenerate cognition, which is considered to be a bright and promising AD drug structure targeting tau protein $^{149}$. A series of tacrine–pheno-thiazine hybrids via an alkylenediamine-type spacer were designed, according to the principle of multitarget drugs by Hui et al. $^{150}$ with the help of computational chemistry software and molegro virtual docker. Among them, 37 (Figure 10) was found to be the most potent compound with its $IC_{50}$ was 89 nM. Meanwhile, 37 markedly prevented tau hyperphosphorylation induced by okadaic acid in N2$\alpha$ cell with 39.5% inhibition when tested at $10^{-5}$ M and could interact with $\mathrm{A}\beta$ fibril (fA$\beta$) using surface plasmon resonance, and the data of $K_D$ was $5.51 \times 10^{-8}$ M.

Conclusions

The more recent insights about the pathophysiology of AD have proven a complex interconnected variety of deleterious events, with multiple pathways involving in its etiology and a variety of factors that are associated with the installation, progress and severity of AD. Therefore, the development of effective drugs for AD becomes more and more difficult. Even though there is a great number of very interesting compounds with diverse pharmacological profile in preclinical studies, the majority of them fail in the clinical trials. The results of the analysis published by Cumming et al. $^{151}$ is advanced to the FDA and approved for marketing. Currently, 108 clinical trials for AD therapies are being conducted, and only 14 agents reach phase 3. Ladostigil, the multifunctional agent, which fails 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 MTDLs approach that enabled researchers to obtain compounds endowed with advantageous properties, resulting from possible interactions with more than one target involved in the pathogenesis of AD, gives hope for further development of new and effective therapy for AD.

Last decades, efforts have been employed in the design and search for new biological chemical entities capable to direct simultaneously with different molecular targets, which were involved in the pathogenesis of AD, and many new significantly active and promise molecules have been discovered. Tacrine, which is the first AChE inhibitor, has been selected as the ideal active fragment because of its simple structure, clear activity and its superiority in the structural modification, and thus, it could be introduced into the overall molecular skeletons of the multitarget-directed anti-AD agents. This review presents a series of novel multitarget-directed tacrine derivatives up to now, which are classified by the biological targets and chemical structure. They new drug prototype candidates have been identified and they are the starting point of a revolutionary new way of thinking drug design, and some drug candidates are under preclinical evaluation. Despite none of these MTDL drug candidates have reached clinical phase of development, we hope that in the next few years the system biology approach could effectively contribute to the medicinal chemists in the discovery of innovative chemical entities, which are able to understand and effectively modify the course of this devastating neurodegenerative disorder.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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