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COMPREHENSIVE REVIEW

Hybrids: a new paradigm to treat Alzheimer's disease

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Abstract Alzheimer's disease (AD) is a complex neurodegenerative condition with several target proteins contributing to its etiology. With 35.6 million cases worldwide documented in 2011, AD constitutes a devastating health, political, economic, and social problem for all nations. The cases are expected to increase beyond 107 million in 2050; unless an advanced therapy having a capability to delay the disease progression is developed. The curative paradigm of one-compound one-target that has been followed so far has not reached the desired mark. The research focus moved towards single molecule targeting two or more pathogenic mechanisms involved in neuronal death. Over the last few years, medicinal chemists have been paying attention to the design and synthesis of the hybrid molecules that are comprised of two pharmacophores from well-established chemical scaffolds endowed with requisite biological activities in a single entity. The hybrid-based approach has grown to be a central point in the medicinal chemistry field. Various important pharmacophores used for AD have been combined with selected biologically active molecules to get homo- and heterodimers with improved efficacy with additional supplementary actions. This review summarizes the pathogenesis of AD and various progress in the design of hybrid molecules based on the one-compound-various targets paradigm for AD therapy.

Keywords Acetylcholinesterase inhibitors · Hybrids · Multi-target · Alzheimer's disease · Tacrine · β-Amyloid

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Abbreviations

Aβ	β-Amyloid
ACh	Acetylcholine
AChE	Acetylchoinesterase
AChEIs	Acetylchoinesterase inhibitors
AD	Alzheimer's disease
AGEs	Advanced glycation end products
APP	Amyloid Precursor Protein
BACE	β-Secretase
BBB	Blood–brain barrier
BuChE	Acetylcholinesterase
CB_1	Cannabinoid 1
CBR	Cannabinoid receptors
ChEs	Cholinesterases
CNS	Central nervous system
DNA	Deoxyribonucleic acid
FDA	Food & Drug Administration
MAO	Monoamine oxygenase
MTDL	Multi-target-directed ligand
NFTs	Neurofibrillary tangles
NMDAR	*N*-methyl-D-aspartate receptor
PAS	Peripheral anionic site
PKC	Protein kinase C
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SAR	Structure–activity relationships
SERT	Serotonin transporter
TcAChE	Torpedo californica acetylcholinesterase
THA	Tetrahydroacridine

Introduction

Alzheimer's disease (AD), a dementia-type memory related disorder, mainly affects aged people. It is a neurodegenera-

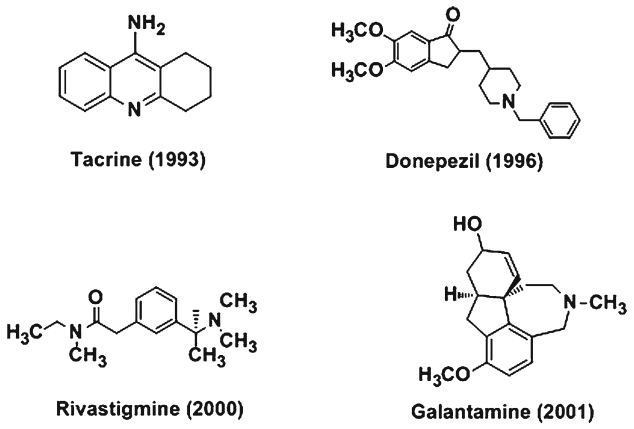


Fig. 1 FDA approved drugs for the treatment of Alzheimer's disease

tive disorder, which is characterized by progressive decline of memory and higher cortical functions that leads to complete degradation of mental and intellectual activities [1,2]. At the pathological level, it is distinguished by the presence of neurotic plaques and neurofibrillary tangles (NFTs) that cause neuronal dysfunction. The socioeconomic outcomes that arise due to AD are shocking due to the noticeable rise in expectancy of life and a remarkable drop in infant mortality mainly in developed countries [3].

Varied efforts have been implemented in the development of drug molecules implicated in the management of AD. In view of this, in 1993, the FDA approved the first drug, tacrine for AD as an AChE inhibitor. After tacrine, some other acetylcholinesterase (AChE) inhibitors including donepezil, rivastigmine, and galantamine were approved by the FDA (Fig. 1). These approved drugs have turned out to be mitigative rather than curative as they only improved the memory and cognitive function, and have little role in preventing or decelerating the progressive neurodegeneration due to involvement of a different hypothesis in AD pathogenesis, such as deposition of abnormal proteins, namely β-amyloid (Aβ) and tau-protein, loss of synapses, oxidative stress, death of nerve cells, etc. [4,5].

Due to the pathological complexity, to date no agent has been proven to be dramatically effective. Thus, AD is the biggest unmet medical need for medical science and research. This review portrays the pathogenesis of AD with promising disease-modifying hybrid molecules, which may represent an important progress towards the treatment of AD [6].

Pathogenesis of AD

Alois Alzheimer, in 1907, described the occurrence of neurotic plaques and NFTs on autopsy in the cerebral cortex of a 56-yr-old woman having dementia [7]. In the late 1970s, the role of acetylcholine in AD was determined. The AChE and butyrylcholinesterase (BuChE) are two enzymes known to hydrolyze the acetylcholine neurotransmitter, and they support the cholinergic hypothesis potentiating the central cholinergic function and improving cognitive function, so as to provide a symptomatic relief from AD [8].

An amyloidal cascade is another characteristic of AD, in which the amyloid precursor protein (APP) mediates cleavage by either β-secretase or α-secretase; the resultant membrane-attached fragments are further sliced by γ-secretase (Fig. 2). The product of α-cleavage trailed by γ-cleavage is greatly soluble and non-amyloidogenic [9]; while amyloid beta (Aβ), formed by β-cleavage proceeded by γ-cleavage, is biochemically insoluble, polymerizes into pathological fibrils and accumulates as aggregated Aβ plaques [10]. The Aβ plaques and oligomers are potent synaptotoxins that block proteosome function, alter intracellular Ca^{2+} levels, restrain mitochondrial activity and activate the inflammatory processes. Moreover, AChE promotes Aβ by a hydrophobic environment, which lies close to the peripheral anionic site (PAS) of AChE, thus accelerating the amyloid fibrils via formation of stable complexes of AChE-Aβ, which further increase the neurotoxicity of the β-amyloid fibrils [11].

Another hallmark of the disease is τ-protein, a key microtubule-associated protein that stabilizes the microtubules. Tau proteins are associated with efficient axonal transport, which is deregulated in AD [12]. β-Amyloid interacts with the signaling pathways regulating the tau protein phosphorylation. The hyperphosphorylated tau (the distorted protein) further affects the threads of tau and leads to the formation of the neurofibrillary tangles (NFTs) in the nerve cell bodies. These NFTs promote the breakdown of microtubules, as a result of which the neuronal transport system collapses, and there is defective biochemical communication between the neurons that ultimately lead to neuronal cell death [13].

Oxidative stress is considered as another major cause that pervades neuronal cell death in age-associated disorders such as AD. In mitochondria, monoamine oxygenase (MAO) endorses the enzymatic oxidation and phosphorylation of biogenic amines by formation of various reactive oxygen species (ROS) and reactive nitrogen species (RNS) that produce functional alterations in lipids, proteins, and DNA. Brain has a high content of Cu^{2+} and Fe^{2+} that potentiate ROS in brain and lead to Aβ neurotoxicity. Aβ also hastens the generation of reactive oxygen species (ROS) and induces oxidative stress in mitochondria [14,15].

Ca^{2+} acts as a vital intracellular messenger in the brain, important for synaptic transmission, neuronal development, and plasticity in neurons by manipulating the cytoskeleton and associated proteins. The long-term, slightly raised cytosolic Ca^{2+} levels and disturbances in Ca^{2+} homeostasis correspond to the neuronal devastation and promote the

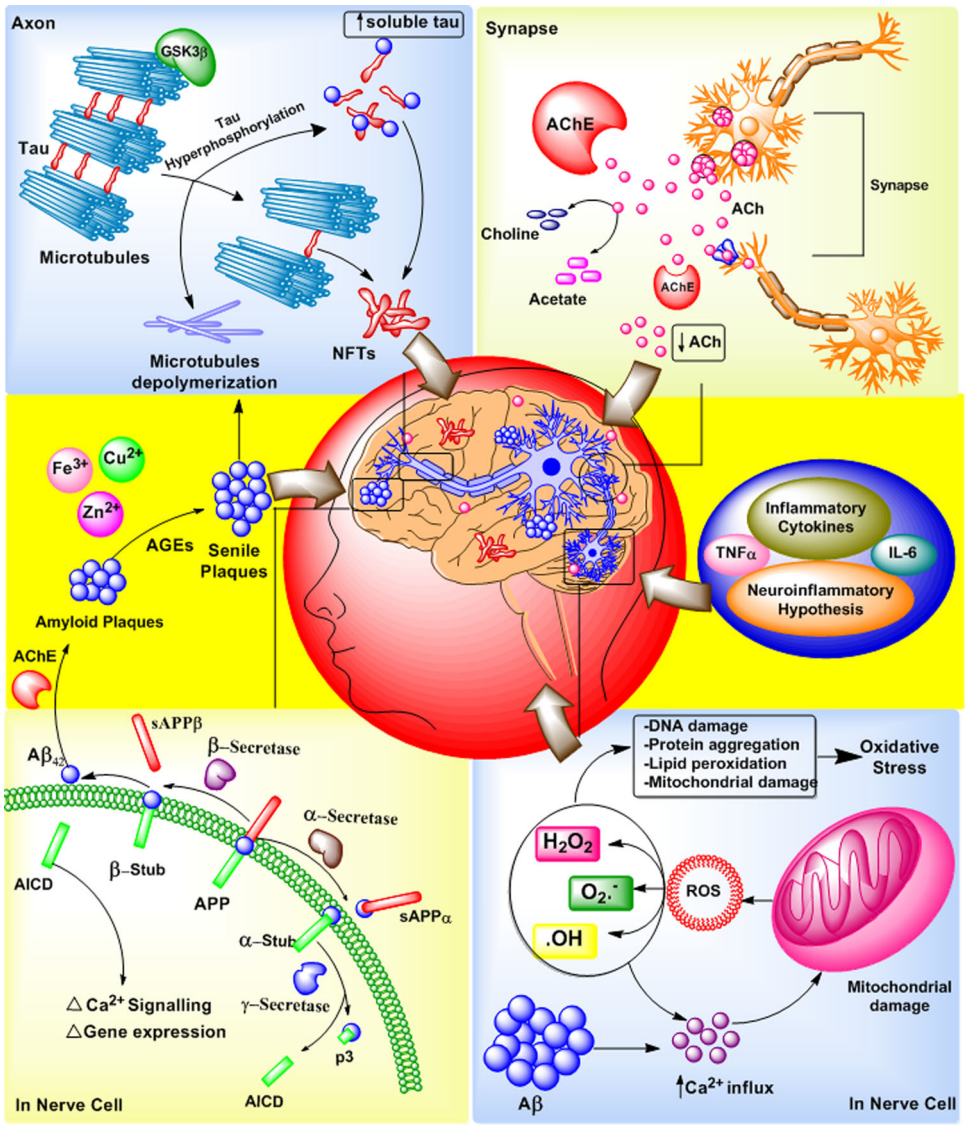


Fig. 2 Pathogenesis of AD

generation of free radicals and ROS [16]. MAO-B is also involved in the pathogenesis of AD and is particularly high near the senile plaques. MAO's catalytic activity produces H_2O_2 within reactive microglia of AD brain tissues, which contributes to the initiation of oxidative stress and the consequent deleterious effects to the cell [17].

Cannabinoid CB_1 receptor antagonists also constitute potential therapeutics for AD like cognitive disorders [18]. CB_1 receptor antagonists increase ACh release in certain areas of the brain comprised of cortical regions and the hippocampus. In addition, muscarinic receptors are involved in the pathogenesis of AD. Furthermore, the "neuroinflammatory hypothesis" affirms that inflammatory processes are critical in promoting degeneration in AD [19]. Advanced glycation end products (AGEs) are also involved in extensive protein cross linking, induction of oxidative stress, and neuronal cell death in AD [20].

Multi-target directed ligand strategy in AD

In light of the various complex mechanisms involved, AD has been known as a multifarious disease with complex pathogenesis. For this reason, the current trend of developing drugs to treat AD based on the reductionist pattern of 'one-molecule-one-target', has now turned out to be palliative rather than curative. Thus, drug combinations acting on different stages of the neurotoxic cascade offer novel horizons for the treatment of AD and other neurodegenerative diseases [21]. In this context, a new drug design approach is emerging, aimed at developing single chemical entities simultaneously modulating numerous targets with higher efficacy and safety profiles, setting a novel paradigm in medicinal chemistry. The 'multi-target-directed ligand' (MTDL) design approach effectively employed the academic as well as industrial echelons in the field of AD and other multifarious diseases [6,22].

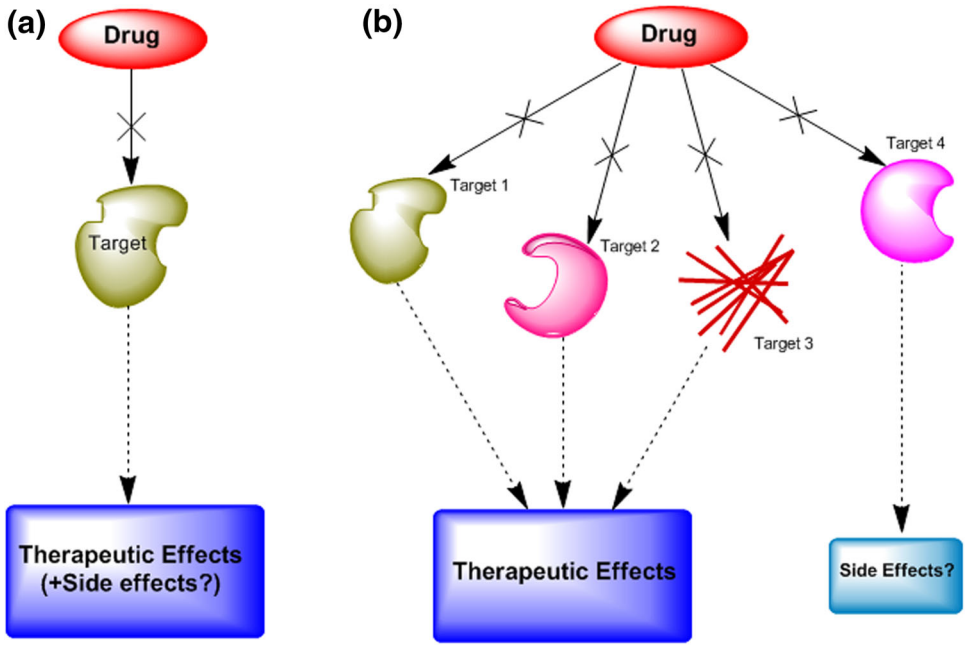


Fig. 3 Pathways leading to the discovery of new medications: **a** target-driven drug discovery approach, that is, one-molecule-one-target paradigm and **b** MTDLs approach to drug discovery

In MTDL design strategy, a single drug molecule can recognize various targets implicated in the disease pathology. Thus, such medication would be greatly efficacious for the treatment of multi-factorial diseases. At the same time, the single molecule could be responsible for side effects as it could also bind to the targets that are not implicated in the disease (Fig. 3).

Henceforth, researchers and various funding agencies are now moving towards the designing of molecules having the ability to simultaneously interact with different targets involved in the same pathology devoid of 'off-target' side effects. The multi-functional compounds can be designed for the management of AD on the basis of the perception. These multifunctional ligands can be designed by targeting the numerous pathways involved in the pathogenesis in AD simultaneously by a single molecule, i.e., the hybrid molecule [23,24]. Hybrid molecules are chemical bodies with two or more structural domains connected covalently in one molecule with distinct pharmacological (and chemical) classes having different biological functions. These molecules have been classified into three different categories on the basis of their mode of action (Fig. 4).

Category 'A' has a single target, in which both chemical entities of the hybrid molecule interact with the single target; i.e., trioxaquines consisting of a trioxane motif covalently linked with an aminoquinoline entity (Fig. 5). The hybrids are very potent in vitro against chloroquine-resistant *P. falciparum* strains (IC_{50} = 5–50 nM) with anti-malarial activity [25]. These hybrids have dual action on the similar target.

Category 'B' is comprised of two chemical entities in the hybrid molecule acting separately on two different and non-related targets. AChE-SERT hybrids, inspired by fluoxetine and rivastigmine, inhibit AChE and serotonin receptors, respectively, and they have been designed to treat AD (Fig. 6). These hybrid compounds were designed by hybridization with the common ethylamine moiety, which acst as a linker [26].

In category 'C', both units of the hybrid act on two related targets at the same time. Quinacrine-imipramine chimers were attained by connecting acridines with imino-dibenzyl entities, which were further explored to inhibit the pathogenic prion protein PrP^{Sc}, an essential element in the pathogenesis of a devastating group of neurodegenerative disorders, by using neuroblastoma cells (ScN2a cells) of scrapie-infected mice. The synergistic antiprion activity of quinacrine and iminodibenzyl-derived hybrids resulted by integrating both recognition elements with a piperazine unit, as the linking element (Fig. 7) [27].

Hybrid molecules consist of a molecular assembly in which distinct pharmacophores of different drugs are present and the pharmacophore of each drug has conserved the potential to interact with its specific sites on the targets, thus making for diverse specific pharmacological responses, which would facilitate the management of multi-factorial diseases. These compounds may be beneficial since they would be expected to lessen the hazard of drug-drug interactions and simplify their pharmacodynamic and pharmacokinetic studies [21].

The polyfunctional agents for neuroprotection, modulation of ACh levels, regulation of calcium homeostasis, anti-apoptotic activity, by combining different properties in one molecule, become a new strategy for the development of novel drugs to treat various multifarious neurodegenerative diseases like AD.

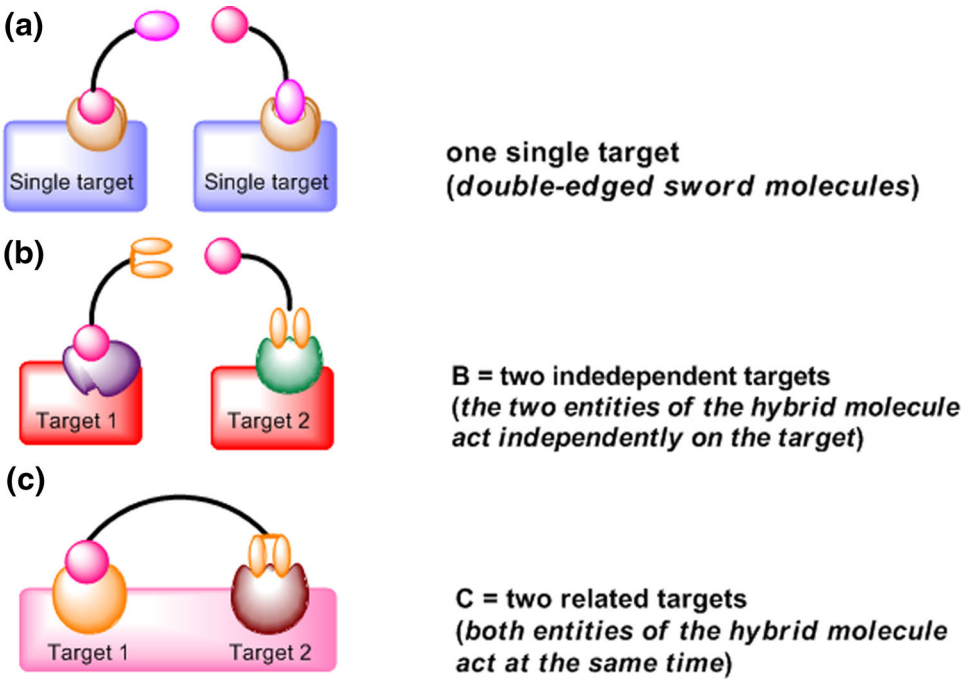


Fig. 4 Schematic representation of three different possible modes of interaction of hybrid molecules

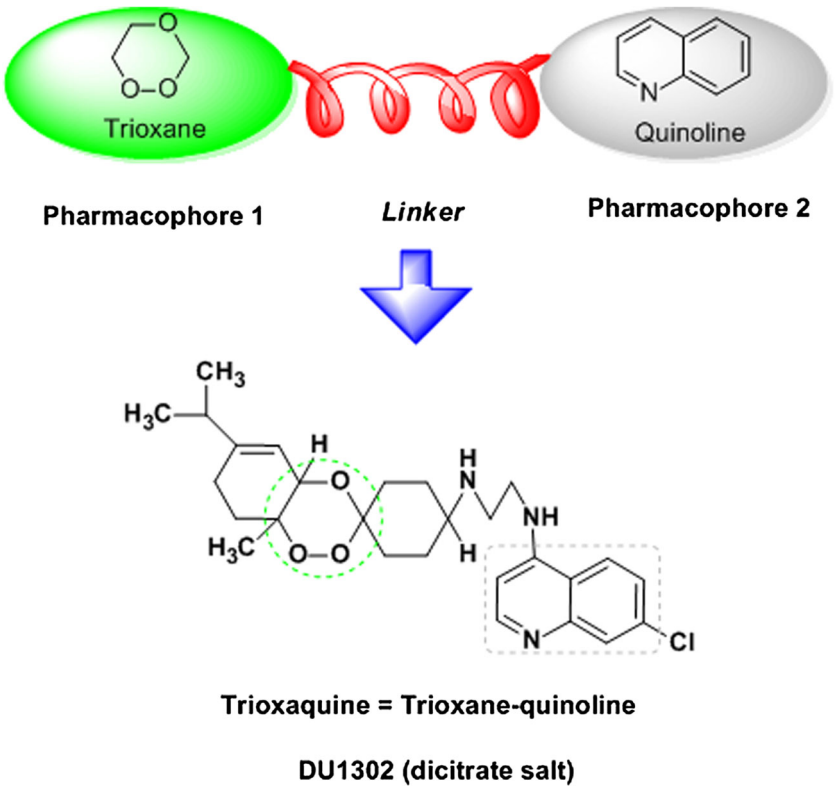


Fig. 5 Schematic representation of anti-malarial trioxaquines and structure of DU1302 (Category A)

Hybrids molecules for the management of AD

Recently, numerous hybrid molecules have been reported for AD, using combined neuroprotective and AChE inhibition properties. Numerous hybrid molecules were designed, synthesized, and evaluated against different targets involved in AD. In the present review, we describe the diverse hybrid molecules designed by exploring pharmacophores of marketed drugs reported in the literature.

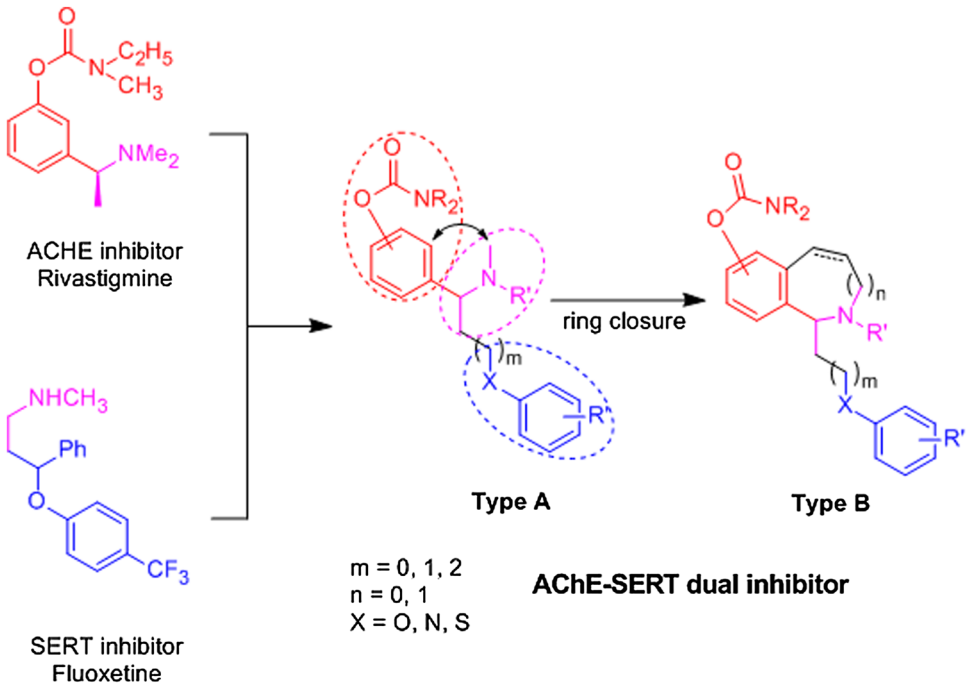


Fig. 6 AChE-SERT dual inhibitor (Category B)

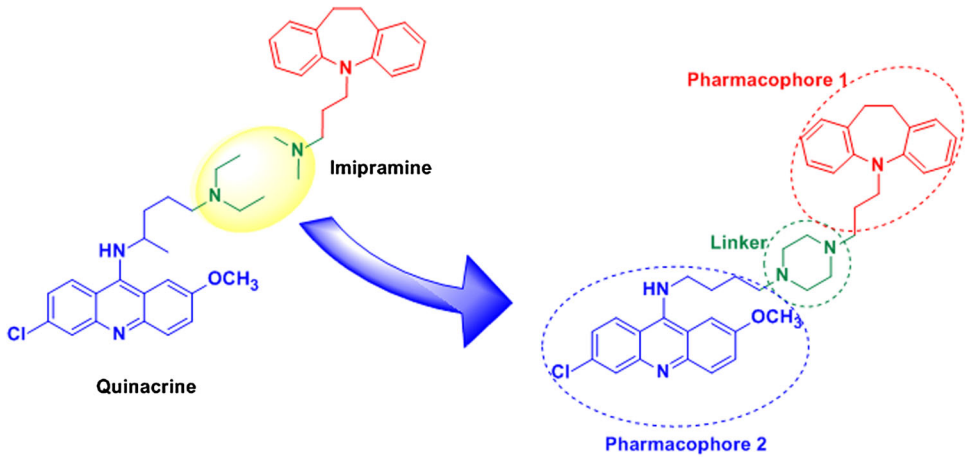


Fig. 7 Hybrid of Quinacrine with Imipramine as an anti-prion agent (Category C)

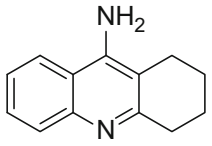
Tacrine hybrids

Tacrine (**Cpd 1**, THA) is the first FDA approved drug for the symptomatic treatment of mild to moderate AD. Although it has some side effects like gastrointestinal disorders and hepatotoxicity, tacrine remains a reference therapeutic agent. Treatment for AD has to be taken life-long, so it was of prior importance to build up safer and more effective AChE inhibitors. Various analogs of tacrine have been synthesized and their structure-activity relationships (SAR) studies were carried out in the last few years.

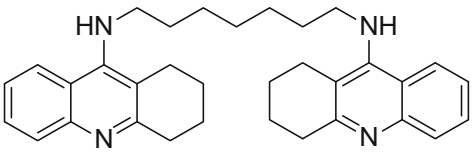
Homodimers hybrids of tacrine

A series of homodimers, consisting of two tacrine moieties linked via alkylene chains of varying lengths, were synthesised by Rodriguez-Franco et al. [28]. These dimeric hybrids have more hydrophobicity as compared to tetrahydroacridine (THA) owed to the alkylene chain linker. Additionally, the alkylene chain simultaneously shows strong interactions with the catalytic anionic site (CAS) as well as the peripheral anionic site (PAS). **Cpd 2**, a dimeric molecule having two tacrine subunits linked by a heptamethylene chain, was found to be more potent than tacrine.

Neuroprotective activities were evaluated in various models, and it was found that bis (7)-tacrine is an NMDAR antagonist (IC_{50} = 0.76 μM), and also repressed AChE-induced AB aggregation (IC_{50} = 41.7 μM). Moreover, pound **2** exhibited BACE-1 inhibitory activity with IC_{50} of 7.5 μM [29]. Tacrine homodimers showed more selectivity for AChE inhibition (IC_{50} = 0.40 nM) than BuChE inhibition (IC_{50} = 390 nM) as compared to tacrine.

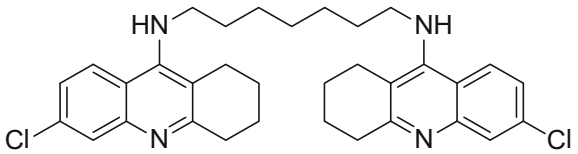


Cpd 1



Cpd 2

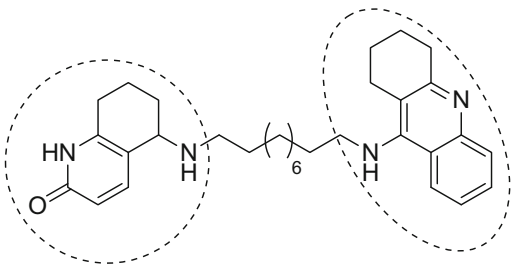
The chloro substitution at the 6th position of tacrine increases AChE inhibitory activity. Hu et al. designed and evaluated numerous derivatives and observed that the heptamethylene-linked bis(6-chloro)-tacrine **Cpd 3** (IC_{50} = 0.07 nM) had more potency (2.9 fold) and more selectivity against AChE (1.7 fold) than the unsubstituted bis(7)-tacrine (**Cpd 2**) (IC_{50} = 1.3 nM) [30].



Cpd 3

Heterodimers hybrids with tacrine

Numerous potent and selective AChE inhibitors have been designed as heterodimers, e.g, compound **Cpd 4**, a heterodimer of tacrine and the 5-amino-5,6,7,8-tetrahydro-2(1*H*)-quinolone fragment from (−)-huperzine A, is almost 13-fold more effective (IC_{50} = 8.8 nM) than (−)-huperzine A and 25-fold more effective than tacrine. It also has tenfold more selectivity against AChE than BuChE (IC_{50} = 81.5 nM) than tacrine [31]. Hybrids of tacrine and donepezil (**Cpd 5,** AChE, IC_{50} = 6.0 nM) are a new family of inhibitors with similar potency as that of donepezil [32].



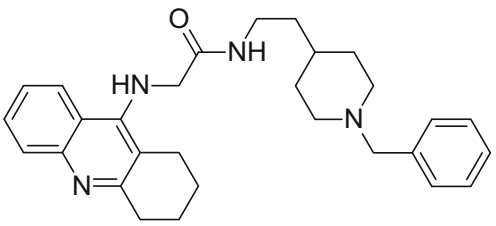
Cpd 4

Tacrine-chromene hybrid

The hybrids of tacrine and 4-oxo-4H-chromene were reported by Fernandez-Bachiller et al. as useful against AD. The tacrine fragment of the hybrid having cholinesterase inhibitory activity and the flavone moiety having free radical scavenging and β-secretase (BACE-1) inhibitory properties, showed more potency than the parent inhibitor tacrine as well as apigenin. The tacrine-chromene hybrid in Fig. 8 showed potent combined inhibition of cholinesterase's and BACE-1, with additional CNS-permeable and antioxidant properties [33].

Tacrine-melatonin hybrids

Amino-substituted melatonin-tacrine hybrids have been reported to have strong AChE inhibitory potential with the ability to scavenge a variety of reactive oxygen species. Among these derivatives, **Cpd 6** showed IC_{50} value of 8 pM for AChE inhibitory activity and is 1000-fold more selective against AChE than BuChE (IC_{50} = 8.0 nM). Intriguingly, **Cpd 6** has also good antioxidant properties, and it is 2.5 times more potent than trolox. Melatonin has also been used as a neuroprotective against Aβ toxicity in microglial cells [34].



Cpd 5

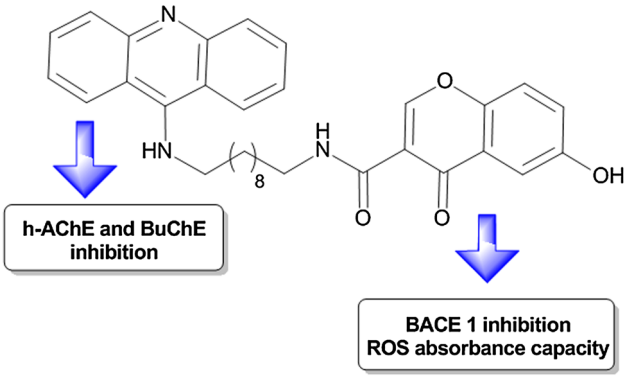
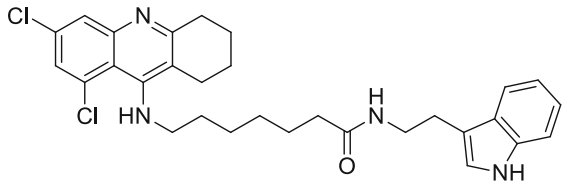


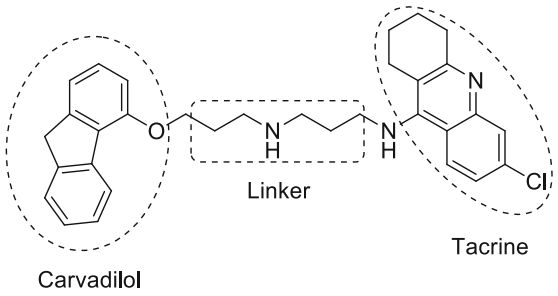
Fig. 8 Tacrine-4-oxo-4*H*-chromene hybrids



Cpd 6

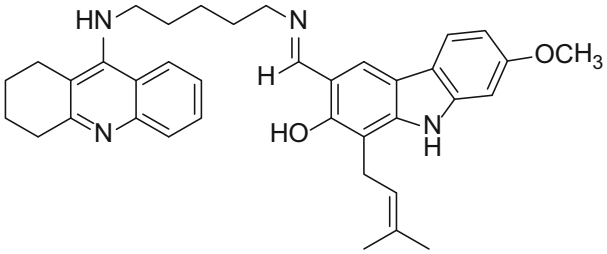
Tacrine-carbazole hybrids

Due to the fact that substituted carbazoles are proficient inhibitors of β-amyloid fibril formation, the carbazole ring of carvedilol, a vasodilating β-blocker, was selected for the design of a new pharmacophore and was linked with the chloro-substituted tetrahydroacridine moiety of the 6-chlorotacrine derivative to give the hybrid named carbacrine (**7**). Carbacrine has good selectivity towards AChE over BuChE. Docking studies of carbacrine revealed that the carbazole and the tetrahydroacridine moieties have good interactions with Trp286 of PAS and Trp86 of the enzymes' catalytic pocket, respectively. Indeed, the three-methylene spacer is needed for the proper interaction with both sites of the enzyme. The ability of **Cpd 7** to counter the reactive oxygen species (ROS) formation was evaluated in human neuronal-like cells (SH-SY5Y), and it was found that it protects the neuronal cells against oxidative stress-induced ROS formation (IC_{50} = 23 μM) efficacy [35,36].

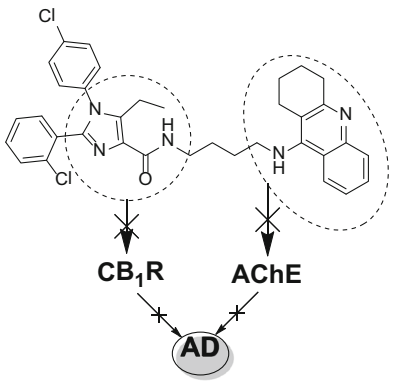


Cpd 7

In this context, Thiratmatrakul et al. reported hybrids of tacrine-carbazole as potential anti-Alzheimer agents with IC_{50} = 0.48 to 1.03 μM for AChE with good inhibition selectivity over butyrylcholinesterase (BuChE). **Cpd 8**, with a 5-methylene spacer, has the highest potency for both AChE inhibitory (IC_{50} = 0.48 μM) and radical scavenging activity, and exhibited an ability to improve both short-term and long-term memory deficits in mice induced by scopolamine. The carbazole moiety directly scavenges the ROS and possesses strong antioxidant actions. Moreover, it also has Aβ aggregation inhibiting capability. Docking studies revealed that the hybrid molecules exhibited multiple binding modes with PAS, CAS and the midgorge AChE enzyme. On the other hand, the tacrine moiety has interactions within the catalytic anionic site with the indole ring of Trp84 and the aromatic ring of Phe330 through π–π interactions. The aromatic nitrogen of tacrine formed a hydrogen-bond with the carbonyl oxygen of His440. The spacer, the alkylenediamine chain, was aligned along the gorge and interacts with the central hydrophobic region (Tyr121 and Tyr334). In addition, this hybrid showed a neuroprotective effect against oxidative stress induced by H_2O_2 and Aβ-1-42 toxicity [37].



Cpd 8



Cpd 9

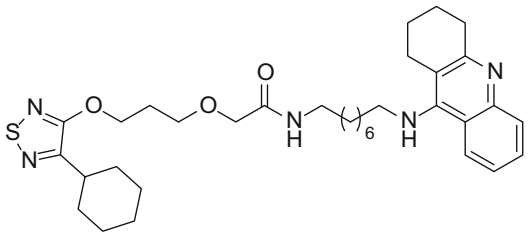
Tacrine–imidazole hybrids

Lange et al. recently targeted Cannabinoid receptors (CBR) along with AChE inhibition. The hybrid molecules were designed by linking tacrine with two known CB_1 antagonists, 1,2-diarylimidazol and 3,4-diarylpyrazoline moieties. The designed **Cpd 9** is a potent AChE inhibitor (IC_{50} = 316 nM) and can block CB_1 receptors with high affinity (K_i = 48.0 nM). Both CB_1 receptor and AChE antagonism were found via a docking study that showed that the *p*-chloro-phenyl moiety plays a vital role in interacting with CB_1R, while the pyrazolone core fits in complement with the anionic site of the Acetylcholinesterase enzyme. Additionally, the core of tacrine interacts with the catalytic site of AChE at the bottom of the gorge [38,39].

Tacrine-xanomeline hybrids

Muscarinic receptors received a lot of attention in the designing of AD drugs as their stimulation lessens Aβ_{42} and tau formation via protein kinase C (PKC) activation, which further encourages the production of soluble amyloid precursor proteins. M_1 receptors can be activated either directly with muscarinic agonists or indirectly with different allosteric agents, which act as ACh enhancers [40].

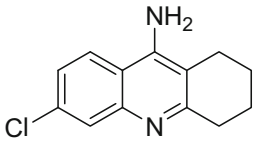
Fang et al. linked the tetrahydroacridine (THA) ring of tacrine with xanomeline, a selective M_1 muscarinic agonist which shows potential in vivo anti-demential activities, with a spacer length of 10–17 atoms. The lead **Cpd 10** of this series (AChE pIC_{50} = 8.21 nM) was more potent than the parent, and it also inhibited BuChE (pIC_{50} = 8.23 nM) with similar binding affinities for the M_1 receptor [41].



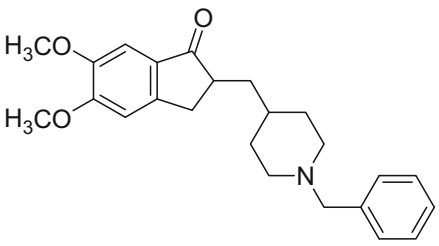
Cpd 10

Tacrine–donepezil hybrids

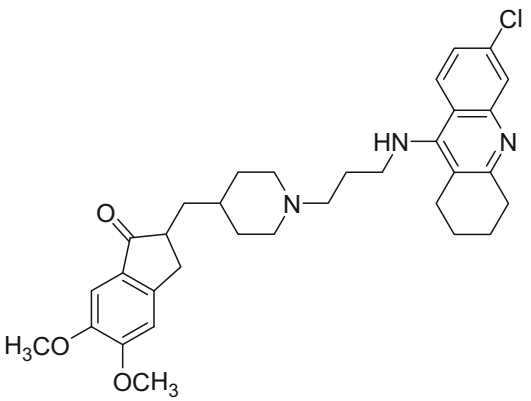
These hybrids were formed by substitution of donepezil (**Cpd 12**) at the amino group of 6-chlorotacrine (**Cpd 11**). **Cpd 13,** a hybrid having dual binding site inhibitory property, was designed based on the binding modes of tacrine and donepezil within *Tc*AChE, by combining tacrine with the 5,6-dimethoxy-2[(4-piperidinyl)-methyl-1-indanone] moiety of donepezil [42]. The indanone ring moiety of donepezil interacts with the PAS and the piperidine moiety interacts with the centre of the gorge. **Cpd 13** was a sub-nanomolar inhibitor (IC_{50} = 0.09 nM) of AChE. It also inhibited BuChE with IC_{50} of 66.3 nM, additionally preventing AChE-induced Aβ aggregation (46.1 %, at 100 μM) via interaction with the peripheral site of AChE [43].



Cpd 11

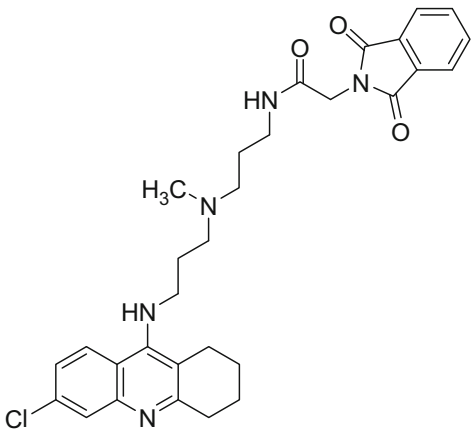


Cpd 12

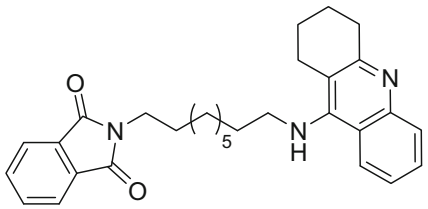


Cpd 13

Similarly designed hybrids (**Cpd 14** and **Cpd 15**) possess an optimum AChE inhibitory potential and also showed significant inhibitory activity against beta-amyloid [44].

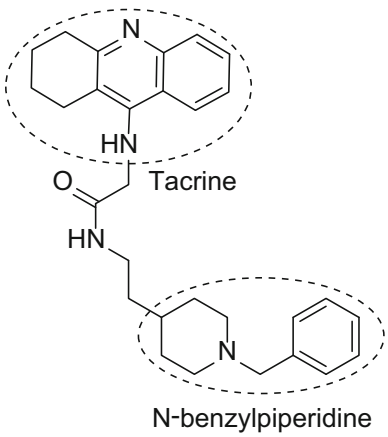


Cpd 14



Cpd 15

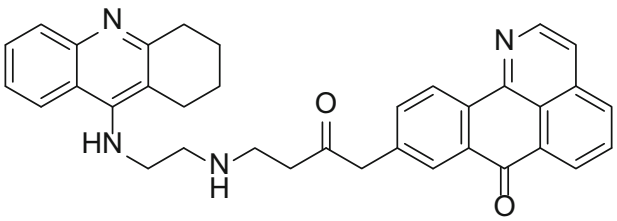
A heterodimer of tacrine and the N-benzylpiperidine moiety of donepezil (**Cpd 16**) was found to be 37-fold more potent AChE inhibitor than tacrine (IC_{50} = 6.0 nM, AChE of rat brain) and 31-fold more selective towards AChE than BuChE. The benzyl moiety of this hybrid displays a π–π stacking with the indole ring of Trp84, the protonated piperidine nitrogen atom interacts with the phenyl ring of Phe330. On the other hand, tacrine interacts via π–π stacking with Trp279 of the PAS. In addition, the N-H of the amide function of the linker was observed to form a hydrogen bond interaction with the oxygen atom of Tyr121 [32].



Cpd 16

Tacrine–oxoisoaporphine hybrids

These hybrids consist of the 1-azabenzanthrone entity with tacrine or its congener, associated by an methylene linker having an amino group at varied positions (**Cpd 17**). These hybrids display good in vitro AChE inhibitory activity (IC_{50} = 3.4 nM), AChE-induced (83.3 %) and self-induced Aβ aggregation (79.8 %). The appropriate binding length for the linker seemed to be six for AChE inhibition [45].



Cpd 17

Tacrine–huperzine hybrids

Badia et al. reported numerous tacrine–huperzine A hybrids [46]. Huprines X and Y, containing a carbobicyclic base of (−)-huperzine A combined with the 4-aminoquinoline moiety of tacrine, displayed inhibitory potential for bovine as well as human AChE, and exhibit intermediate BuChE inhibitory activity. The utmost activity (subnanomolar) was showed by a hybrid, in which the tacrine and (−)-huperzine A moieties were joined with an *N*-methylamine group containing a methylene linker [47] (Fig. 9).

The SAR revealed that the presence of an un-substituted methylene bridge, a three-carbon unsaturated bridge substituted by means of a methyl or ethyl with 4-aminoquinoline base of huprines, is vital to achieve optimal AChEI activity. The chloro, fluoro, and methyl groups at the first and/or third positions increased potency. The 3-chloro substituted huprines (−)-**Cpd 18** (IC_{50} = 1.30 nM) and (−)-**Cpd 19**

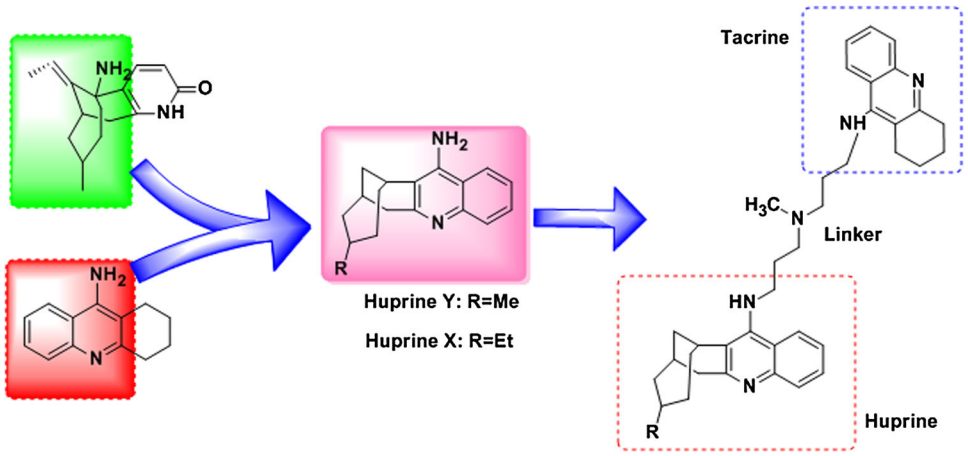
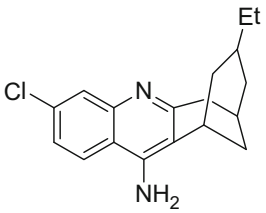
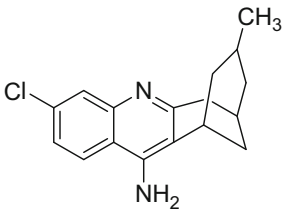


Fig. 9 Design of tacrine-huperzine A hybrids: from classical to dual binding site AChEIs

(IC_{50} = 1.15 nM), came out to be the most potent derivatives, being undoubtedly better than the marketed AChEIs in respect to potency, selectivity, and affinity towards AChE [48,49].

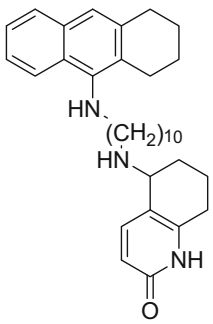


Cpd 18



Cpd 19

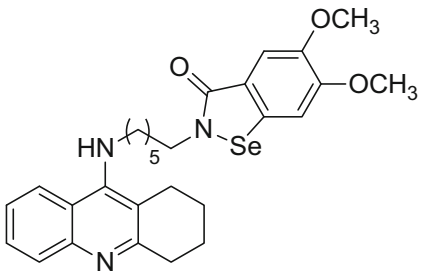
The combination of the structural fragment 5-amino-5,6,7,8-tetrahydro-2(1*H*)-quinolinone of huperzine A in the racemic form with a tacrine unit that was connected with a decamethylene joiner gives **Cpd 20**, which was 13-fold and 25-fold more potent (IC_{50} = 8.8 nM) than (−)-huperzine A and tacrine, respectively. Compound **Cpd 20** was found to be almost 23-fold more selective against AChE than the standard drug tacrine, but was less selective than (−)- huperzine A and bis(7)-tacrine [31].



Cpd 20

Tacrine–ebselen hybrids

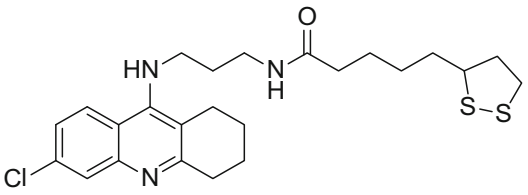
Mao et al. synthesized hybrids of tacrine-ebselen with an alkyl linkage against Alzheimer's disease as multifunctional agents. Ebselen, structurally derived from donepezil, scavenge peroxynitrite and hydrogen peroxide [50]. The inhibition of both AChE and BuChE enzymes were affected by the length of the alkyl linkage. For example, a six-carbon spacer between tacrine and 5,6-dimethoxybenzo[d] [1,2] selenazol-3(2*H*)-one (**Cpd 21**) was found to be most potent AChE and BuChE inhibitor, with IC_{50} values of 2.55 and 2.80 nM, respectively, with effective hydrogen peroxide and peroxynitrite scavenging activities. Molecular modeling studies indicates that **Cpd 21** has favorable interactions with both the catalytic and peripheral anionic sites of the enzyme [51].



Cpd 21

Tacrine–lipoic acid hybrid

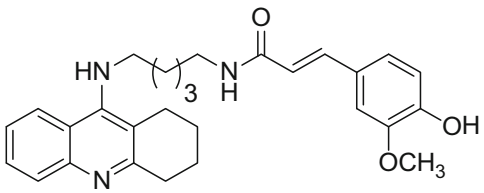
Lipocrine **Cpd 22**, a hybrid of tacrine and lipoic acid, was designed to be neuroprotective and have free radical scavenging properties. This hybrid is the most potent AChE inhibitor with IC_{50} = 0.25 nM, due to strong interaction with the PAS of AChE along with AChE-induced Aβ aggregation inhibition with an IC_{50} of 45 μM. It also reduced ROS production by 51 % at 10 μM [52].



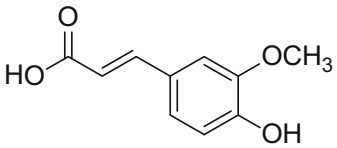
Cpd 22

Tacrine–ferulic acid hybrid

Similarly, **Cpd 23** is a hybrid of tacrine and ferulic acid (**Cpd 24**), a potent natural antioxidant, and is a multi-potent anti-Alzheimer drug candidate [53]. **Cpd 23** reduced toxicity in Aβ_{1-42} exposed rat brains. It has also the ability to inhibit AChE with $IC_{50} = 4.4$ nM, which is ten times more potent than tacrine. Due to its natural antioxidant potential, it also showed good antioxidant properties [54].



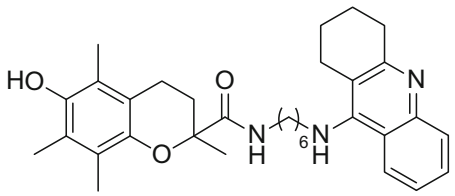
Cpd 23



Cpd 24

Tacrine–trolox hybrids

Recently, the novel multifunctional hybrids of tacrine with trolox have been designed and synthesized against AD with hepatoprotective properties. Most of the hybrids inhibited AChE in the nanomolar range with remarkable antioxidant activity comparable to trolox alone. **Cpd 25** was found to be a potent AChEI ($IC_{50} = 23.5$ nM), BuChEI ($IC_{50} = 20.5$ nM) and is less hepatotoxic than tacrine. **Cpd 25** also exhibited a neuroprotective effect against H_2O_2-induced PC 12 cell injury and had good ability to cross the BBB [55].



Cpd 25

Donepezil based hybrids

Donepezil-AP2238 hybrids

Rizzo et al. reported on the hybrids of AP2238 and donepezil. As depicted in Fig. 10, the phenyl-*N*-methylbenzylamino moiety of AP2238 was united with the indanone core of donepezil, to preserve the π-OH interaction with amino acid Tyr124, by a double bond. The substitution at positions 5 and 6 appeared to be essential to interact with both CAS and PAS of the AChE. SAR indicated that (i) the benzyl group interacts via π–π stacking with the indole ring of Trp86; (ii) the phenyl ring acts as a common spacer establishing a π–π interaction with the phenol of Tyr341, a π-OH interaction with the –OH of Tyr124, and a T-shaped interaction with the side chain of Phe338; (iii) the protonated amino group has an interaction with the phenol ring of Tyr337 via cation-π interaction; the bicyclic aromatic fraction interacts with Trp286 via π–π stacking; and (iv) the carbonyl group formed H-bond interactions with the backbone amide groups of Arg296 and Phe295.

However, the preclinical toxicity and clinical safety profile of the compound is yet to be recognized.This hybrid is a good candidate as an inhibitor of AChE ($IC_{50} = 1.14$ μM) and AChE induced Aβ aggregation [56].

Donepezil–minaprine hybrids

Minaprine (**Cpd 26**, $IC_{50} = 600$ μM), an antidepressant, has very weak AChE inhibitory activity. Contreras et al. developed hybrids combining the N-benzylpiperidine fraction of donepezil (**Cpd 12**) with the 3-amino-6-phenylpyridazine fraction of minaprine (**Cpd 26**) as new AChE inhibitors with enhanced activity. Among all the synthesized derivatives, **Cpd 27** ($IC_{50} = 21$ nM) and **Cpd 28** ($IC_{50} = 10$ nM) were found to be the most potent hybrids, even though they were found to be less selective than donepezil [57]. A docking study revealed that the nitrogen of the protonated piperidine atom formed a π-cation interaction with Phe330 and Trp84

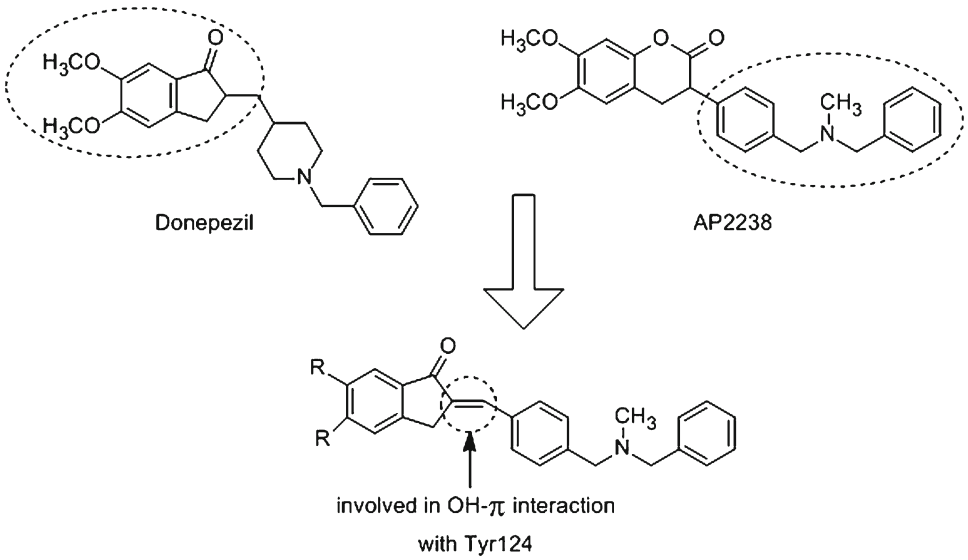
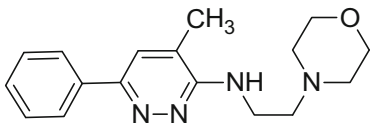


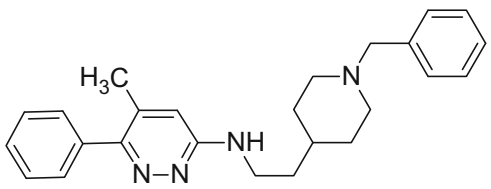
Fig. 10 Hybrid of donepezil and AP2238

amino acid residues, and a water-bridged H-bond interaction with Ser122 and Tyr12, whereas the benzyl group of the aromatic ring showed π–π stacking with the indole ring of Trp84. However, the arylpiridazine unit interacts with PAS (Tyr70 and Trp279) of AChE [58].

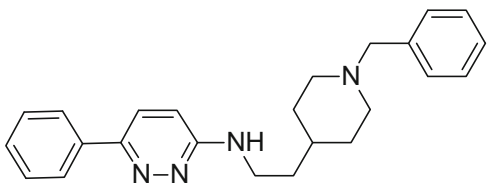


Cpd 26

amine]) was the most potent hMAO A inhibitor with an IC_{50} value of 5.5 ± 1.4 nM and a moderately potent hMAO B inhibitor with an IC_{50} value 150 ± 31 nM. **Cpd 29** also inhibited EeAChE with $IC_{50} = 190 \pm 10$ nM, and eqBuChE ($IC_{50} = 830 \pm 160$ nM) inhibitor. The binding mode of **Cpd 29** confirmed that the indole positioned at the base of the gorge, and the propargylamine scaffold interacted with Trp86 via π–π stacking. The aliphatic alkyl chain of **Cpd 29** was encircled by the phenyl rings of Tyr341, Tyr124 and Phe338. In PAS, the phenyl ring of the compound is stacked between Trp286 and Tyr72 at the edge of the gorge. The molecular modeling studies concluded that **Cpd 29** binds with both the CAS and PAS of AChE [59].



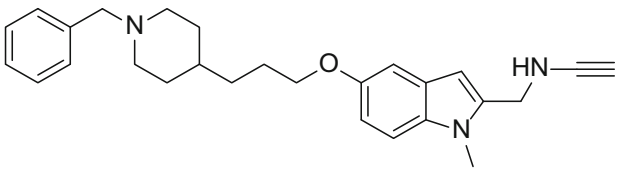
Cpd 27



Cpd 28

Donepezil–indolyl hybrids

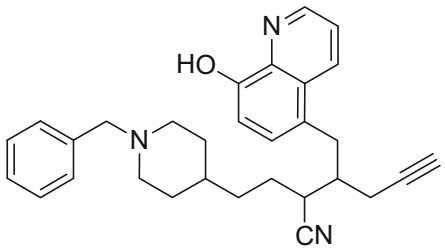
Donepezil–indolyl based hybrids were designed, synthesized, and evaluated for monoamine oxidase (MAO) and cholinesterase (ChE) enzyme inhibition against AD. The hybrid molecules were synthesized by linking the *N*-benzylpiperidine moiety via three methylene carbon chains to the oxygen at C5 of the indole nucleus. Among all those evaluated, **Cpd 29** ([*N*-((5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1*H*-indol-2-yl)methyl)prop-2-yn-1-



Cpd 29

Donepezil–propargylamine–8-hydroxyquinoline (DPH) hybrids

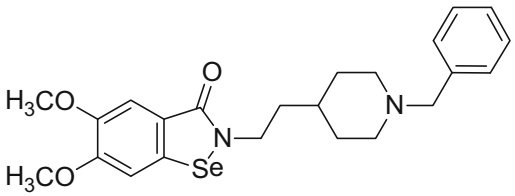
Recently, Wang et al. reported the synthesis, ADMET, toxicity studies, biochemical estimations, and computational studies of new multifunctional hybrids of DPH for the management of AD. The most interesting derivative was racemic α-aminotrile-4-(1-benzylpiperidin-4-yl)-2-(((8-hydroxyquinolin-5-yl)methyl)(prop-2-yn-1-yl)amino) butanenitrile (**Cpd 30**) [MAO A (IC_{50} = 6.2 ± 0.7 μM); MAO B (IC_{50} = 10.2 ± 0.9 μM); AChE (IC_{50} = 1.8 ± 0.1 μM); and BuChE (IC_{50} = 1.6 ± 0.25 μM)], an irreversible MAO A/B inhibitor and mixed-type inhibitor of AChE with additional metal-chelating properties. Docking studies revealed that **Cpd 30** has mixed-type AChE inhibition properties as it interacted with the catalytic as well as peripheral anionic site of AChE. The cyano group play an important role in positioning properly the ligand by H-bond formation with important amino acid residues of the binding sites of AChE, BuChE, and MAO A. Moreover, **Cpd 30** exhibited moderate to good ADMET properties and brain penetration for CNS activity. Thus, **Cpd 30** is the first diasteromeric, α-aminonitrile, that has been identified as a multi-functional compound, which is capable of interacting in two key enzymatic systems involved in AD, and is a new lead compound that deserves further investigation for the potential management of this disease [60].



Cpd 30

Donepezil–ebselen hybrids

A new series of donepezil–ebselen hybrids were synthesized by joining donepezil (**Cpd 12**) and ebselen as multi-functional agents to manage the AD. **Cpd 31** is the most potent AChE inhibitor (IC_{50} = 0.042 μM for *Electrophorus electricus* AChE and 0.097 μM for hAChE) with strong BuChE inhibitory activity (IC_{50} = 1.586 μM). Moreover, it also scavenged H_2O_2 and peroxynitrite with additional glutathione peroxidase-like activity (v_o = 123.5 μM min^{-1}), without any toxicity in mice at a dose of 2000 mg/kg.



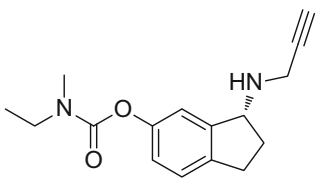
Cpd 31

Molecular docking studies suggested that the carbonyl group on the dimethoxybenzo[d] [1,2] selenazol-3(2*H*)-one of **Cpd 31** binds with PAS as well as with catalytic pocket. The dimethoxy group of the phenyl ring interacted with the Trp279 residue of the PAS, and the piperidine ring that is situated on the aromatic gorge interacted with both the peripheral and catalytic anionic sites [61].

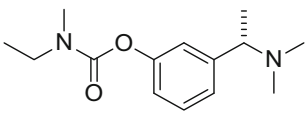
Rivastigmine-based hybrids

Rivastigmine–rasagiline hybrids

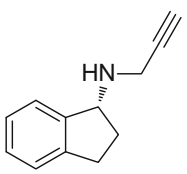
Weinstock et al. designed TV3326 (**Cpd 32**) by combining the phenyl N-ethyl-N-methylcarbamate fraction of rivastigmine (**Cpd 33**) with the *N*-propargyl-(1R)-aminoindane moiety of the rasagiline (**Cpd 34**), an anti-Parkinson drug with a selective irreversible MAO-B inhibitory property [62]. **Cpd 32** possesses good AChE inhibitory action with the addition to monoamine oxidase (MAO-A and MAO-B) inhibitory activity with a better safety profile. Moreover, it also enhances cognition and reduces the oxidative stress with an antidepressant action.



Cpd 32



Cpd 33



Cpd 34

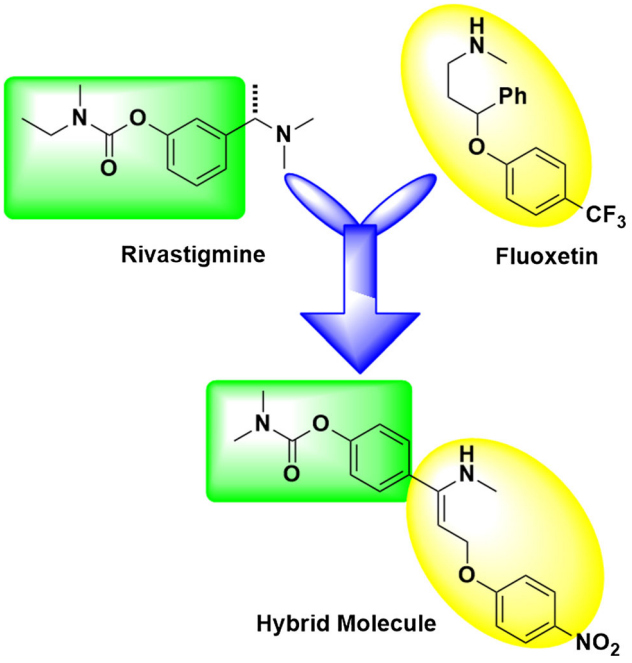


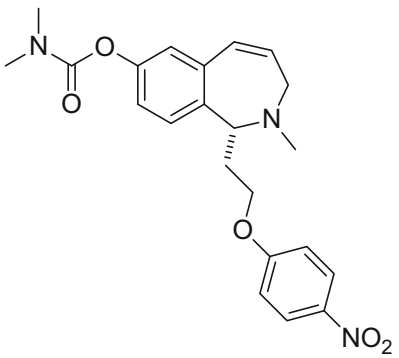
Fig. 11 Rivastigmine–fluoxetin hybrid

Interestingly, **Cpd 32** also increased the processing of APP in human SH-SY5Y and rat PC-12 neuroblastoma cells by modulation of α-secretase pathway [63].

Rivastigmine–fluoxetin hybrids

Depression associated with AD is treated with serotonin transporter (SERT) inhibitors, although these compounds do no have any anti-cholinergic action. Thus, In this context, Kogen et al. designed dual AChE and SERT inhibitors, being valuable for the management of AD with depressive symptoms (Fig. 11).

Among the designed hybrids, **Cpd 35** was designed by using the AChEI rivastigmine **Cpd 33**, and the potent SERT inhibitor fluoxetine as model structures with higher potency as AChEIs (IC_{50} = 101 nM) than rivastigmine (IC_{50} = 11 μM, AChE from mouse brain) and as serotonin transporter inhibitors rather than fluoxetine [64,65].

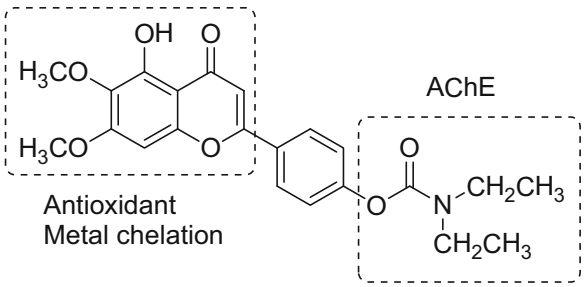


Cpd 35

Rivastigmine–scutellarin hybrids

Recently, Sang et al. developed various rivastigmine-scutellarin hybrids on the basis of multi-target-directed ligand strategy. Among all the synthesized hybrids, **Cpd 36** exhibited dual inhibitory potency on AChE and BuChE (IC_{50} = 0.57 and 22.6 μM, respectively) with good antioxidant activity, with a value 1.3-fold of that of trolox. Additionally, these hybrids have the ability to cross the blood–brain barrier (BBB) in vitro and had significant neuroprotective effects in scopolamine-induced cognitive impairment in mice.

Scutellarin (4′,5,6-trihydroxyflavone-7-glucuronide) possesses a broad range of pharmacological properties related to neurological disorders, such as free radical scavenging effects, metal chelating properties, anti-inflammatory effects, neuroprotective action, and the inhibition of Aβ fibril formation. However, due to low oral absorption and poor blood–brain transport of Scutellarin, it is restricted for its clinical use as an anti-AD drug, which was improved by linking it with rivastigmine. The kinetic characterization of **Cpd 36** showed a mixed-type inhibition, as it binds with both PAS and CAS of AChE [66].

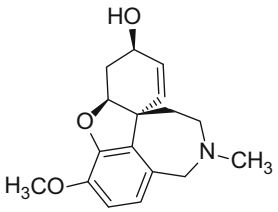


Cpd 36

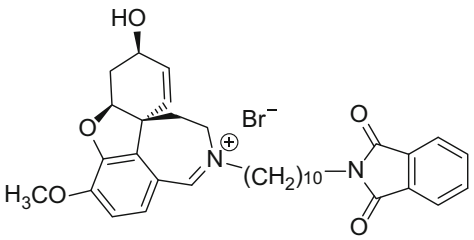
Galantamine based hybrids

Galantamine (**Cpd 37**, IC_{50} = 360 nM) is an alkaloid that can be obtained synthetically or from the flowers and bulbs of *Galanthus caucasicus*. It is an FDA-approved competitive and reversible cholinesterase inhibitor used for AD treatment. Thus, galantamine-based hybrids have been designed to act as dual binding site AChE inhibitors having improved potency. In this view, various neutral and cationic bis-interacting galantamine-based homo- and heterodimers have been developed. Among these, heterodimers **Cpd 38** (IC_{50} = 10 nM) and **Cpd 39** (IC_{50} = 22 nM) are 2−5 fold more potent than tacrine (IC_{50} = 50 nM) and 16−36 fold than galantamine. The iminium function is an essential structural feature for interacting with Trp84 residues of AChE forming cation-π interactions responsible for improv-

ing the inhibitory potency relative to the neutral counterparts. However, the presence of a permanent positive charge is unfavorable for the central action needed in AD treatment [67].



Cpd 37

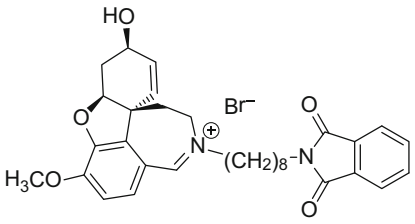


Cpd 39

The galantamine-homodimers **Cpd 40** and **Cpd 41** are superior to the parent compound. Galantamine hetero-hybrid **Cpd 42** also has good AChE inhibition potential with an IC_{50} of 10 nM [68].

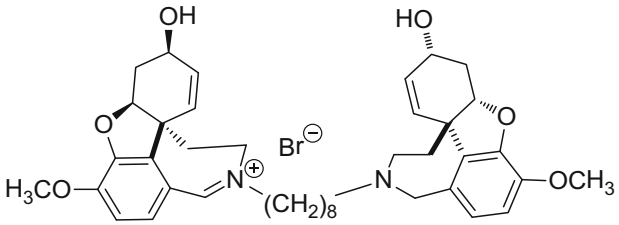
Physostigmine based hybrids

Physostigmine (**Cpd 43**) was reported as an AChEI (IC_{50} = 14 nM) for the management of AD. Various analogs of physostigmine were designed with the purpose of introducing a dual binding site character and improving pharmacological analogs. Recanatini et al. first designed physostigmine analogs by using *N*-methyl-*N*-(3-carbamoyloxyphenyl)methylamino derivatives linked to the tertiary amino nitrogen of physostigmine via an alkoxy linker of variable length [69]. The heterodimer hybrid xanthostigmine (**Cpd 44**) interacts with the PAS of AChE with increased affinity and potency. It displayed remarkable human AChE inhibitory activity with 46-fold more potency and 100-fold more selectivity over physostigmine.

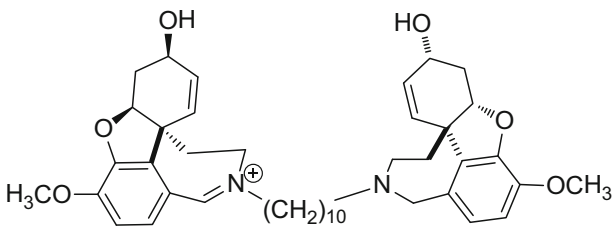


Cpd 38

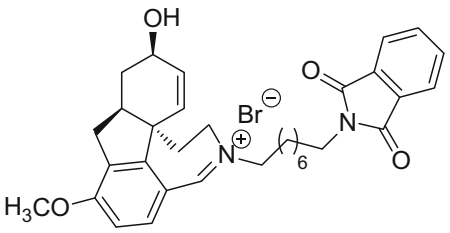
Another physostigmine hybrid, **Cpd 45** with IC_{50} = 0.32 nM, is more potent and selective than physostigmine, but is less selective than xanthostigmine [70].



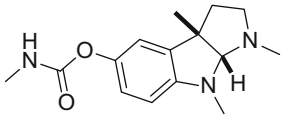
Cpd 40



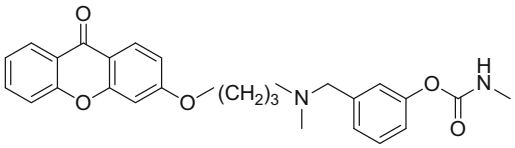
Cpd 41



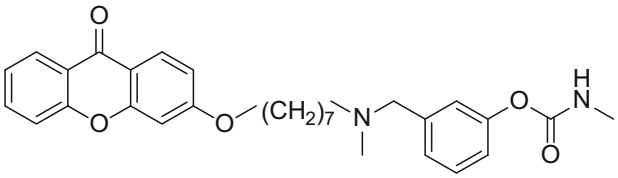
Cpd 42



Cpd 43

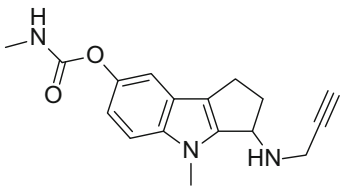


Cpd 44

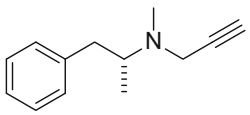


Cpd 45

Fink et al. synthesized the hybrid (**Cpd 46**) by combining a unit of the heteroaromatic N-methylcarbamate of physostigmine with the propargylamine part of the irreversible MAO inhibitor selegiline (**Cpd 47**). The synthesized hybrid was found to be more active than parent compounds [71].



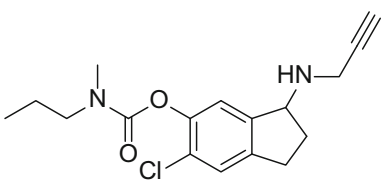
Cpd 46



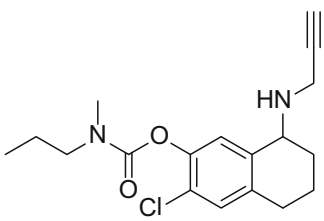
Cpd 47

Carbamate based hybrids

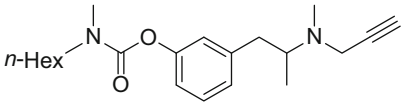
Sterling et al. developed a strategy to design the dual targeted AChE and MAO inhibitors. The carbamate-rasagiline derivatives **Cpd 48–50** were found to have good to moderate AChE and MAO-B inhibitory activities [72]. Coumarin-carbamate hybrid (**Cpd 51**) displayed good inhibitory activity against human and rat AChE (IC_{50} = 5.7 nM and IC_{50} = 1.5 nM, respectively) supported by molecular modeling studies [73].



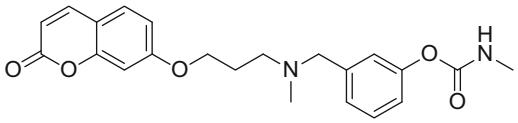
Cpd 48



Cpd 49



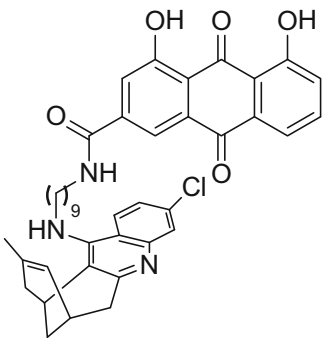
Cpd 50



Cpd 51

Rhein–huprine hybrids

Viayna et al. synthesized novel rhein-huprine hybrid molecules to strike numerous targets for AD. The hybrids consisted of a rhein and a huprine Y system linked through penta- to undecamethylene or 1,4-phenylene-bis(methylene) linkers. All compounds were biologically evaluated against AChE, BChE, BACE-1, dual Aβ42 and tau antiaggregating activity in *Escherichia coli* cells and found to be potent. Among the synthesized compounds, **Cpd 52** emerged as a disease-modifying anti-Alzheimer drug candidate having hAChE (IC_{50} = 2.39 nM), hBChE (IC_{50} = 513 nM), BACE-1 (IC_{50} = 80 nM) along with 43 % Aβ42 aggregation at 10 μM. The in vivo studies of **Cpd 52** showed that it inhibits 47 % of Aβ42 aggregation and 34 % tau aggregation. Moreover, it also prevents the Aβ-induced loss of synaptic proteins and central Aβ lowering in APP-PS_1 mice in vivo. A docking study found that it has the ability to bind simultaneously at both CAS and PAS of the enzyme [74].

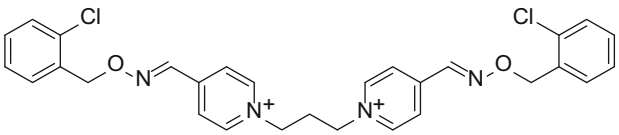


Cpd 52

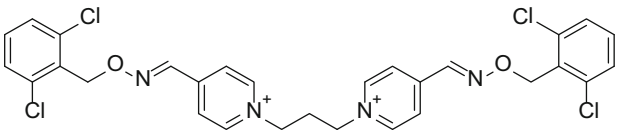
Miscellaneous

Obidoxime–oximes hybrids

A number of hybrid obidoxime-oximes molecules have been reported to possess AChE inhibitory activity. These molecules were composed of obidoxime derivatives and pyridinium oxime ether. However, it was found that the monopyridinium and the bispyridinum hybrids displayed low micromolar AChE inhibitory activity. These hybrids have the dual binding capability, which was designed to allow binding to both the active site and the peripheral site. The highest inhibitory activity observed was for **Cpd 53** (IC_{50} = 0.58 μM)**,** with a 2-chloro group and **Cpd 54** (IC_{50} = 0.62 μM) with a 2,6-dichloro substituent in the phenyl ring. Despite acting an active and peripheral site, they were also involved in the inhibition of the self-assembly and formation of amyloid fibrils [75–77].



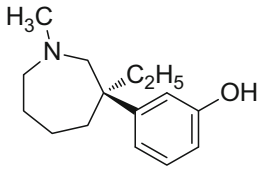
Cpd 53



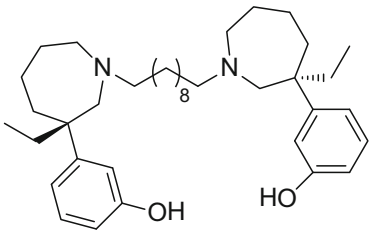
Cpd 54

Bis-(−)-nor-meptazinol hybrid

Xie et al. reported the dual binding site hybrids for AChE inhibition in 2008. They developed the hybrids of meptazinol (**Cpd 55**), a racemic opoid analgesic with low addiction liability. The bis-(−)-nor-meptazinol hybrids, in which the two meptazinol rings were linked by a nonamethylene spacer, were investigated as novel AChE inhibitors with additional β-amyloid peptide aggregation inhibitory activity. **Cpd 56** emerged as the lead compound possessing high inhibitory activity against AChE (IC_{50} = 9.5 nM) and was found to be 1.8-fold more selective for AChE than BuChE (IC_{50} = 17.0 nM). The crystal structure of AChE available with **Cpd 56** confirmed its dual interaction with the catalytic site as well as the peripheral anionic site of AChE, simultaneously [78,79].



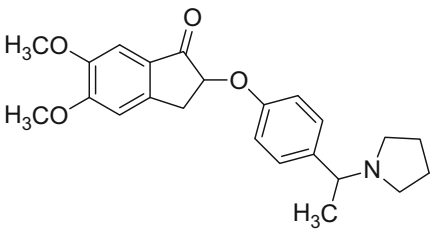
Cpd 55



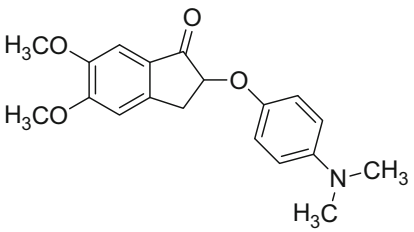
Cpd 56

2-Phenoxy-indan-1-one hybrids

These are dimeric structures consisting of two pharmacophoric moieties, i.e,. 5,6-dimethoxy-indan-1-one derived from donepezil and dialkyl-benzylamine derived from rivastigmine. These hybrids have the ability to interact with central and peripheral binding sites of AChE and, thus, are able to prevent catalytic and non-catalytic actions of the enzyme [80]. **Cpd 57** and **Cpd 58** exhibited the highest activity among all the designed compounds.



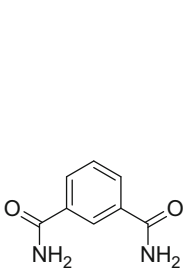
Cpd 57



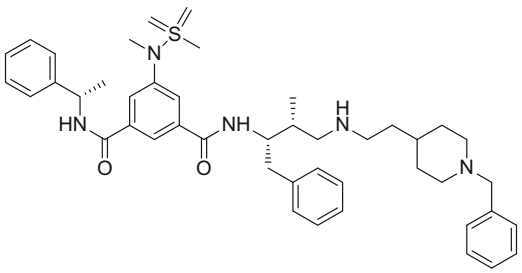
Cpd 58

N-benzylpiperidine hybrids

In 2009, isophthalamide (**Cpd 59**), a broadly used pharmacophore as a BACE-1 inhibitor, was coupled with donepezil to achieve dual action against AChE and BACE-1 simultaneously. Docking studies predicted that interactions could be possible involving the *N*-benzylpiperidine moiety and the catalytic anionic site of AChE, and the isophthalamide moiety at the PAS. **Cpd 60** showed potency against AChE and BACE-1 with IC_{50} of 1.83 and 0.567 mM, respectively. It also exhibited the Aβ formation inhibitory effect (IC_{50} = 98.7 nM) after it was evaluated in a cell-based attempt using HEK293 cells transfected by human βAPP695wt.



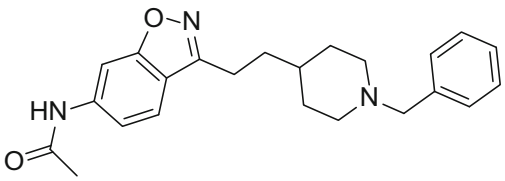
Cpd 59



Cpd 60

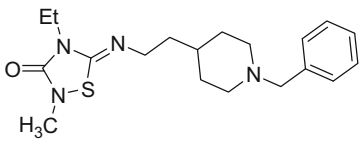
Various groups designed and synthesized the compounds, which chemically resembled donepezil by using bioisosters of its indanone ring system. In this context, Villalobos et al. designed and synthesized N-benzylpiperidine-based hybrids (**Cpd 61**) having a benzisoxazole system [81]. **Cpd 61** displayed potent AChE inhibitory activity in the low nanomolar range (IC_{50} = 2.8 nM), which is 2.9-fold more than the donepezil along with high selectivity for AChE over BuChE. Despite its good activity, **Cpd 61** is metabolically unstable due to hydrolysis of its acetamido group to anilino metabolites, which was less potent. With a view to improve the stability, Villalobos et al. synthesized several more derivatives by protecting the *N*-acetyl functionality using five- or six-membered ring heterocyclic rings.

Among these derivatives, Icopezil (CP118954, **Cpd 62**) came out as an extremely potent AChEI (IC_{50} = 0.33 nM) with an improved pharmacokinetic profile in vivo (>10,000-fold more potent against AChE than BuChE) [82].



Cpd 61

Martinez et al. developed a series of compounds in which the 1,2,4-thiadiazolidinone ring system was used rather than the indanone. Among all the synthesized compounds, **Cpd 63** was found to be equipotent with tacrine but 3.5-fold less potent than donepezil, and to have dual binding site ability against AChE (IC_{50} = 14 nM). It also showed high selectivity (610-fold more potent) towards AChE over BuChE inhibition. Molecular dynamic studies were carried out to explore the binding pattern of **Cpd 63** with TcAChE and showed that it perfectly interacts with the PAS of the AChE with dual binding site quality [83].



Cpd 63

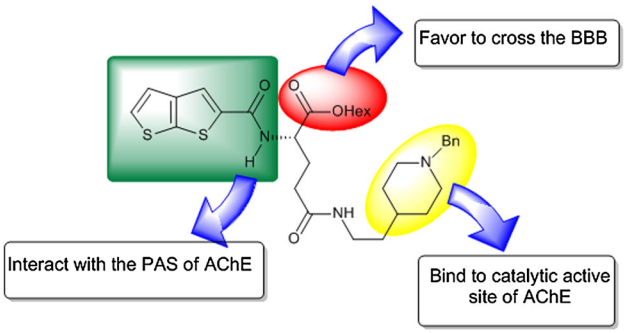
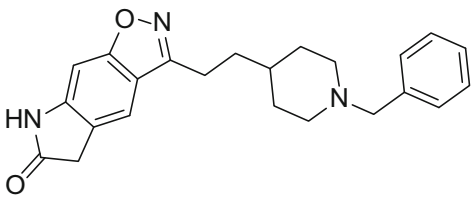


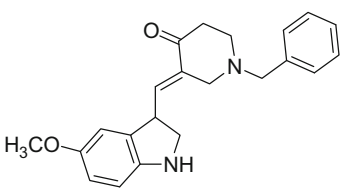
Fig. 12 *N*-benzylpiperidine based Hybrid interaction pattern with AChE

Andreani et al. developed the synthesis of a hybrid of benzylpiperidine and indole or pyrrole ring. Benzylpiperidine-indole hybrids docked in the enzyme were in various orientations with respect to donepezil. It was observed that the indole moiety of **Cpd 64** was not correctly oriented and the compound did not properly interact with the Trp279 residue of PAS of AChE, thus having low inhibitory activity compared to donepezil (IC_{50} = 6000 nM) [84].



Cpd 62

In 2009, new hybrids were designed by three joined pharmacophoric groups with an *L*-glutamic moiety as a appropriate biocompatible linker: (a) a ω-situated *N*-benzylpiperidine moiety capable of interacting with CAS of AChE, and (b) an *N*-protecting group proficiently binding with the peripheral anionic site (PAS) of AChE enzyme, thus able to inhibit Aβ-aggregation. Lastly, (c) the presence of a lipophilic -hexyl ester supported the crossing through BBB. the hybrid in Fig. 12, showed good inhibitory activity against AChE



Cpd 64

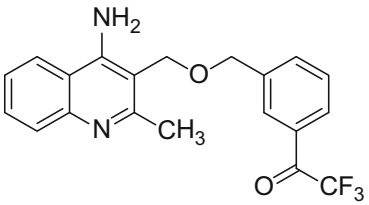
(IC_{50} = 300 nM) as well as having anti-Aβ-aggregation activity. It also depicted neuroprotection against free radicals in mitochondrial with low toxicity and the ability to penetrate into the CNS [85,86].

Tacrine-*m*-(trimethylammonio)trifluoroacetophenone hybrids

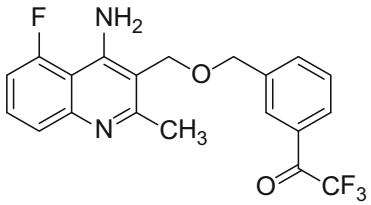
New high affinity selective AChEIs hybrids were developed by combining the trifluoroacetophenone moiety of the transition state analog, AChEI-TMTFA with 4-aminoquinoline moiety of tacrine through a suitable linker. The tacrine unit has the ability to bind with the Trp84 ring via π–π stacking and TMTFA binding to Ser200 of the AChE enzyme. Several hybrids, such as **Cpd 65-68** with IC_{50} = 3, 3.8, 5.1, 3 nM, respectively, exhibited an interesting AChE inhibitory activity [87].

Biflavonoid hybrids

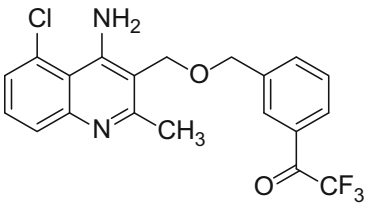
Kang et al. examined neuroprotective effects of biflavonoids obtained from a natural source on amyloid β-peptide-induced and oxidative stress-induced cell death in neuronal cells and suggested that amentoflavone **(Cpd 69)** and ginkgetin **(Cpd 70)**, homodimers of flavone, exhibited strong neuroprotective effects in ischemic stroke and AD [88].



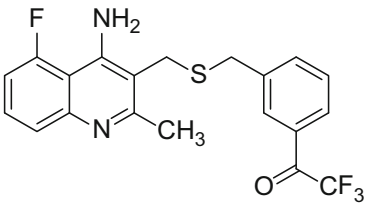
Cpd 65



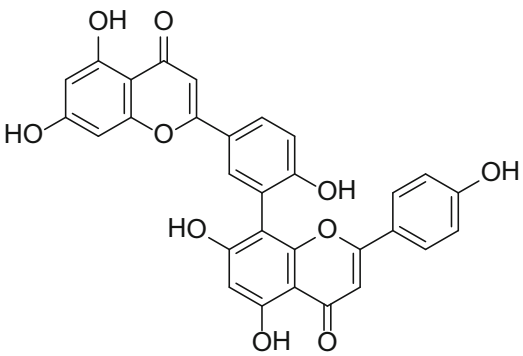
Cpd 66



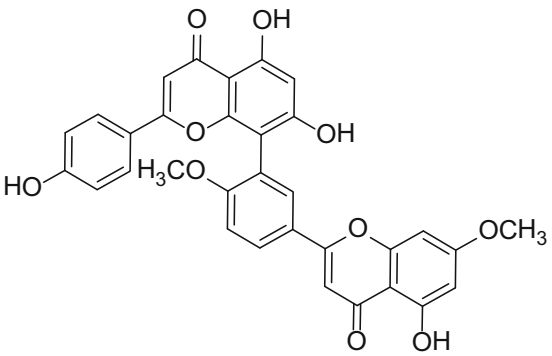
Cpd 67



Cpd 68



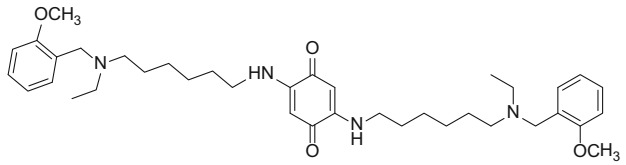
Cpd 69



Cpd 70

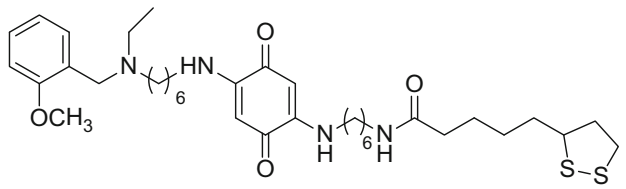
Memoquin based hybrids

Cavalli et al. reported on the memoquin (**Cpd 71**) based hybrids [89]. Memoquine has a polyamine core, as an anticholinesterase property ($IC_{50} = 1.55$ nM) with the addition of muscarinic antagonism, Aβ anti-aggregating effects, and antioxidant activities. The 1,4-benzoquinone fragment in this hybrid is derived from coenzyme Q10 (CoQ10) and is responsible for antioxidant properties [90]. Memoquin has the ability to inhibit AChE-induced $A\beta_{1-40}$ aggregation ($IC_{50} = 28.3$ μM) and self-induced $A\beta_{1-42}$ aggregation ($IC_{50} = 5.93$ μM). It also reduced τ-hyperphosphorylation, Aβ expression, and accumulation [91].



Cpd 71

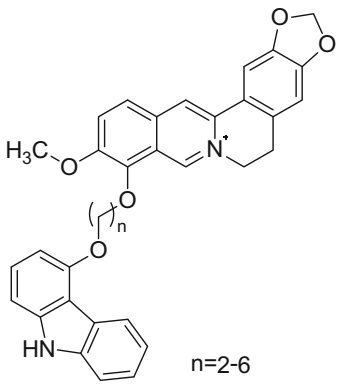
Cpd 72 was developed with good AChE ($IC_{50} = 0.1\mu$M) and Aβ self-aggregation inhibitory activity (45.4 % at 10 μM) with it being 50-fold more selective against AChE than BuChE. The lipoyl moiety from lipoic acid and the benzoquinone moiety from CoQ10 in **Cpd 72** is responsible for its antioxidant property. **Cpd 72** reduced ROS production (50 % at 10 μM) in its oxidized form [92].



Cpd 72

Berberine based hybrids

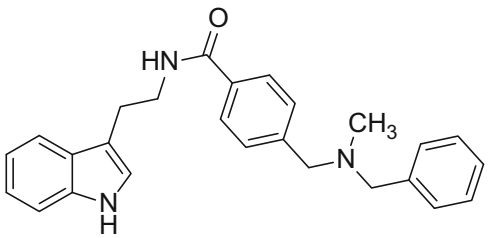
The berberine–carbazole hybrids were reported as being the potent AChE inhibitors with IC_{50} values in the submicromolar range (0.85–2.10 μM). The binding pattern was expected that the aromatic ring of the carbazole scaffold would bind to the catalytic center of the acetylcholinesterase enzyme via cation-π interaction and the berberine moiety interacted with PAS of AChE. The SAR depicted that variation at the length of the alkylene chain affected the AChE inhibitory potency. Four methylene groups as linkers between the carbazole and berberine were found to be the best inhibitors among others. A variety of derivatives like **Cpd 73** with good AChE inhibitory potential have been synthesized with a methylene bridge varying from 2 to 6, for the management of AD [93].



Cpd 73

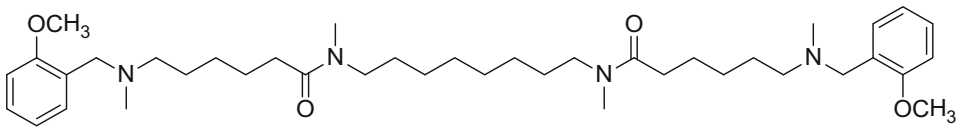
Melatonin-*N*,*N*-dibenzyl(*N*-methyl)amine hybrids

In 2014, Lopez-Iglesias et al. reported a novel class of melatonin–*N*,*N*-dibenzyl(*N*-methyl)amine hybrids, showing a polyfunctional profile including neuroprotective, cholinergic, and antioxidant properties. Melatonin has been reported for various activities, such as an antioxidant, with improvement of mitochondrial energy metabolism, to decrease the neurofibrillary tangles and Amyloid-β proteins. The second fragment of the hybrid, *N*,*N*-dibenzyl(*N*-methyl)amine, present in the well-known AChE inhibitor AP2238, has strong interaction with the catalytic site of AChE. **Cpd 74** inhibits hAChE ($IC_{50} = 1.09 \pm 0.1$ μM), hBuChE ($IC_{50} = 3.70 \pm 0.1$ μM), and it exhibits oxygen radical absorbance capacity (ORAC) (3.2 ± 0.03 trolox equivalent) [94].



Cpd 74

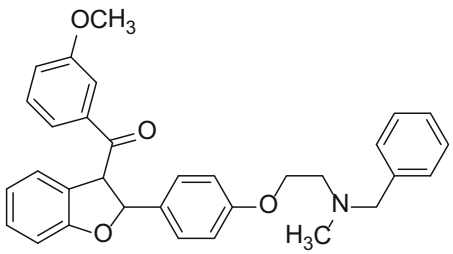
Caproctamine **Cpd 75** is another polyamine-based dual AChE inhibitor with $IC_{50} = 0.17$ μM also blocking the muscarinic (M_2) receptor ($K_b = 0.66$ μM) [95].



Cpd 75

The caproctamine backbone has the ability to fold in such a manner that the *o*-methoxybenzylamine units (distance 10–16Å), reached near the Trp279 (PAS) and Trp84 (CAS), of AChE, respectively, whereas the lipophilic chain formed different hydrophobic interactions with numerous aromatic residues. Despite its action as an AChE inhibitor and a competitive muscarinic (M_2) receptor antagonist, it also has the potential to prevent the AChE-mediated Aβ aggregation [96].

Cpd 76 was developed as a novel BuChE inhibitor, and has the capability to interact with the PAS of AChE enzyme [97]. **Cpd 76** is the hybrid of SKF-64346, a benzofuran derivative and *N*-methyl-*N*-benzylamine, via a heptyloxy chain having good Aβ-fibril formation inhibitory and AChE inhibitory properties. It also showed BuChE inhibitory activity with a IC_{50} value of 1.82 μM and was 5.7-fold more selective towards BuChE over AChE (IC_{50} = 10.5 μM). This hybrid also has the capability to prevent the Aβ self-aggregation with IC_{50} = 13 μM) and have a distinct neuroprotective effect against Aβ_{25-35}peptide-induced neurotoxicity with a neuroprotective effect of 63 % at 30 μM [98].



Cpd 76

Conclusion

For multifarious complex diseases, the paradigm has been shifted from 'one molecule-one target' to multi-target hybrid molecules or poly-functional agents that could achieve a stage of effectiveness not manageable by the earlier paradigm. However, the 'one molecule-one target' paradigm has motivated drug discovery in the pharmaceutical industries for the previous few decades, but the multi-factorial origin of complex diseases makes this perception irrational because single target drugs are ineffective to hit different pathways involved in pathogenesis of these diseases. Consequently, it can be anticipated that poly-pharmacological approaches are the need of the hour for an effective treatment of complex disorders. Among such complex diseases, Alzheimer's disease needs the attention of researchers for developing rational and effective therapy by using these approaches.

Over the last decade more attention has been given to the design and synthesis of hybrid compounds for the treatment of Alzheimer's disease. Using a hybrid-based strategy, two bioactive pharmacophores have been comprehensively united to attain homo- and heterodimers endowed with an improved biological profile together with high affinity, increased potency, and along with additional complementary actions, which makes them potential drug candidates for the treatment of AD. However, some problems still need to be considered and more studies should be intended for the designing of hybrid compounds bearing in mind other off-target side effects. Thus, a number of disease-modifying hybrids could hopefully grow to have next generation potential therapeutics in the treatment of Alzheimer's disease.

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