Natural Chimeras of Existing Drugs for Alzheimer's Disease: Expanding the Target Landscape

Illham Dhala, Tabassum Khan, Arati Prabhu*

Department of Pharmaceutical Chemistry and Quality Assurance, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Mumbai, Maharashtra, INDIA.

ABSTRACT

Alzheimer's disease (AD) has emerged as a complex, multi-faceted, neurodegenerative disorder. Multiple mechanisms seem to play a part in its pathogenesis, including amyloid beta aggregation, cholinergic deficit, oxidative stress and neuroinflammation. The current FDA-approved anticholinesterase drugs addressing a single mechanism have turned out to be palliative rather than curative. A number of natural medicinal bioactive are known, which have the potential to overcome many of the unaddressed causal mechanisms contributing to AD. They act as excellent antioxidants, metal chelators, anti-inflammatory mediators and are neuroprotectives. Natural products also have the inherent capability of overcoming some of the adverse effects caused by the currently available drugs. Several researchers have worked on the development of chimeric compounds for AD, in which known cholinesterase inhibitors are linked with natural bioactive components. The resulting novel molecular entities formed have been shown to simultaneously modulate multiple targets with higher efficacy and better safety profiles, demonstrated in experimental models. This review presents a compilation and rational analysis of several natural chimeras of known anti-AD drugs, explored to expand the efficacy and safety profile of current FDA-approved anti-Alzheimer's medications.

Key words: Alzheimer disease, Natural bioactives, Cholinesterase inhibitors, Antioxidants, Chimeric compounds, Hepatotoxic.

INTRODUCTION

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disorder which is characterized by an increase in the formation of beta amyloid rich senile plaques and neurofibrillary tangles in the brain.[1,2] The symptoms of AD include progressive memory loss, cognitive decline, psychiatric and behavioral abnormalities and depression.[3] According to the Established populations for Epidemiological study approximately 4,91,000 people of age 65 or older will develop Alzheimer's dementia in the United States in 2020.[4] The average annual incidence in new cases of AD was found to have a rise of about 0.4 % in the age group of 65-74 years, 3.2 % in the age 75-84 years and 7.6 % in the "oldest-old" age group of 85 years and above. Due to the increasing population in the age group of 65 years and older, particularly the oldest-old, the annual number of new cases of AD and other dementias is projected to double by 2050.[4]

The pathogenesis of AD includes low levels of acetylcholine (ACh), formation of β-amyloid plaques, irregularities in tau proteins, oxidative stress and dyshomeostasis of bio metals.[5] Several hypotheses have been proposed for development of AD. These include the cholinergic hypothesis, amyloid hypothesis, tau hypothesis, metal ion hypothesis and excitotoxic hypothesis. According to the cholinergic hypothesis, the cognitive and memory decline in AD is because of the reduction in choline levels, mainly acetylcholine in the brain.[6–9] Protagonists of this hypothesis have successfully developed cholinesterase inhibitors as a means of increasing the levels of acetylcholine in the brain.

The amyloid theory suggests that an enhanced production of β-amyloid peptide and its accumulation in the brain leads to neuronal cell death, which causes the Aβ soluble oligomers to form Aβ aggregates which further results in the formation of fibrils which is harmful to the neurons.[6]

The tau hypothesis indicates oxidative stress as a significant contributing factor in AD. Tau is a microtubule-associated scaffolding protein found in axons. Under pathological conditions, there is an increase of tau hyper phosphorylation which renders the protein aggregation-prone, leading to formation of neurofibrillary tangles and neurodegeneration. Oxidative stress is one of the significant factors causing tau aggregation leading to initiation of aggregation of β-amyloid via tau protein hyper phosphorylation. Therefore, several antioxidants that specifically scavenge oxygen radicals have been found to mitigate AD and prevent disease progression.[10–13]

The metal ion theory indicates that metals, mainly iron, copper and zinc are involved in the pathogenesis of AD.[6,14] It has been found that these metal ions slowly accumulate in AD patients during progression of the disease.[15] The abnormal accumulation of metals in the brains of AD patients is closely linked to the development of Aβ plaques and neurofibrillary tangles (NFT), which are the hallmarks of this disease.[16] The abnormally high concentrations of Cu and Fe in the brain catalyze the production of reactive oxygen species (ROS) resulting in additional oxidative stress contributing to AD pathogenesis.[17,18] Therefore, reducing metal concentration in the brain by chelating them is another appropriate therapeutic strategy in the management of AD.[6]

Glutamate is the principal excitatory neurotransmitter present in the hippocampus and cortex regions of the brai.[19] It mostly binds to the ionotropic receptors known as N-methyl D-aspartate (NMDA) receptors and cause modulation in calcium and sodium influx into neuronal cells.[19] In normal physiological conditions, glutamate binds to the NMDA receptors and depolarization occurs, followed by magnesium ions mediated closing of the cationic channel to prevent the entry of calcium ions in resting stage.[19] In AD, there is excessive activation of NMDA type glutamate receptor in neuronal cells that causes the release of bound magnesium ions and allows the entry of calcium ions in the neuronal cells. The excessive influx of calcium ions hampers the neuronal transmission and damages nerve cells, resulting in neurodegeneration, neuritic injury and cell death.[19]

Currently there are five FDA approved drugs for the treatment of AD, of which Tacrine,[20] Donepezil,[21] Rivastigmine[22] and Galantamine[23] are acetylcholinesterase inhibitors and Memantine[24] is the only approved non-cholinergic NMDA receptor antagonist drug (Figure 1). Tacrine was the first and most potent acetylcholinesterase (AChE) inhibitor approved by the FDA in 1993 but it is rarely used now due to associated hepatotoxicity.[20]

There is an obvious need for the development of multi-target directed ligands for a holistic and more effective treatment of AD. A lot of anti-Alzheimer research is currently being focused on development of multifunctional chimeras that can address all the hypotheses of AD development.

One of the domains which offer invaluable resources to complement the existing single-target anti-Alzheimer drugs is the plant kingdom. Several plant bioactive compounds are known which exhibit a range of functional activities that suggest they could play a remarkable role in overcoming all the causative factors contributing to AD.

Acetylcholinesterase inhibitors like Donepezil, Rivastigmine and Tacrine have been fused or molecularly hybridized with natural bioactive by researchers to form novel compounds with multifunctional activities like antioxidant, anti-inflammatory and metal chelation. These chimeric molecules have been found to modulate an expanded AD target landscape and consequently have an enhanced efficacy profile in comparison to the currently available medications. In this review, we discuss and evaluate the potential of natural chimeras which have been designed by using the hybridization approach using FDA-approved anti-Alzheimer drugs along with other bioactive functional moieties which address the multi-target pathogenesis of AD.
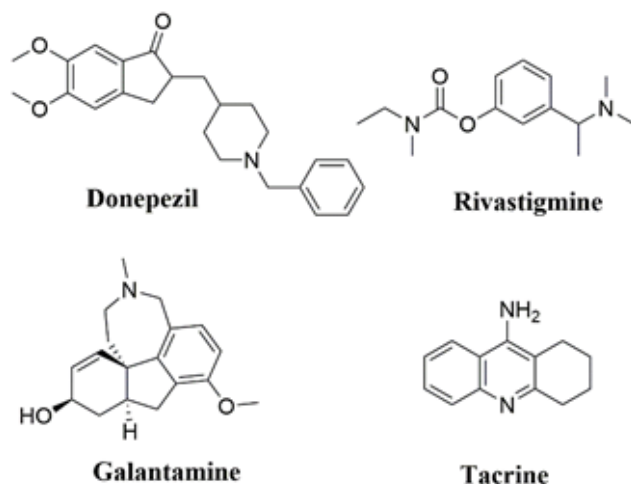

Figure 1: Structures of FDA-approved AChE inhibitor.

MULTI-TARGET DIRECTED LIGANDS (MTDL)

The MTDL design strategy is an approach where a single molecule is designed by incorporating multifunctional ligands that can recognize various targets involved in the disease pathology.[10] Such hybrid compounds which are engineered to simultaneously target various pathways would prove to be more effective in the treatment of AD.[25,26]

Hybrid molecules are entities comprising of two or more structural motifs with distinct pharmacological and biological functions covalently bound within a single molecule.[27,28] The molecular assembly of hybrid molecules consists of more than one pharmacophore, extracted from different bioactive compounds having different mechanisms of action. They retain the ability to interact simultaneously with multiple molecular targets, thereby producing a range of diverse pharmacological responses that would promote multi-factor disease management. Such compounds also have the potential of reducing the risk of drug-drug interactions and simplify pharmacodynamic and pharmacokinetic aspects of drug administration.[11,29]

NATURAL BIOACTIVE COMPOUNDS

Nature is a vast treasure trove of powerful antioxidants and free radical scavengers. A large number of antioxidants are naturally found in many plant sources. These primarily belong to the classes of phenolic acids, flavonoids and vitamins.

Plant phenolics are important human dietary components. Phenolic acids such as Ferulic acid and Caffeic acid are known to exhibit excellent antioxidant activities in addition to bestowing other health benefits. Ferulic acid is also a structural fragment of the wonder drug Curcumin, which itself is a well-established antioxidant and anti-inflammatory agent.

Flavonoids, the largest class of naturally occurring polyphenols, including phytochemicals like Apigenin, Genistein and Scutellarin are known for their diverse biological activities, including antioxidant, free-radical scavenger activity as well as anti-inflammatory, neuroprotective, cardioprotective and hepatoprotective effects.[30–33]

Several vitamins and food nutrients, like carotenoids and Vitamins C as well as Vitamin E and its water-soluble analogues like Trolox are well-known antioxidants.[30] The results of the ORAC assay, which measures the antioxidant potential of a compound are commonly referred to as Trolox equivalents as calculated from comparison to a Trolox calibration curve.

However, natural compounds lack AChE inhibitory activity and have several drawbacks like low bioavailability, weak absorption, poor solubility and poor BBB transport which restricts their use as an anti-AD drug.[34]

CHIMERIC CONJUGATES

Several chimeric conjugate series have been developed from natural bioactive constituents, using FDA-approved drugs as starting points. These chimeras of Acetylcholinesterase inhibitors like Donepezil, Rivastigmine or Tacrine and their natural bioactive partners exhibit an expanded efficacy profile and have reduced toxicity.

DONEPEZIL CHIMERAS FOR AD

Donepezil, is a potent, selective, reversible AChE inhibitor which enhances cholinergic activities by increasing ACh levels in the CNS.[35] The clinical data of this single- target drug has shown modest and transient outcomes in treatment of moderate and severe AD patients.[35,36] Donepezil was first approved by the FDA in 1996 and its extended-release form was approved in combination with Memantine in 2014. Although Donepezil is effective in managing the symptoms of AD associated dementia, it does not alter the progression of AD.[37] A lot of effort has been therefore concentrated on designing new multifunctional agents for AD therapy based on rational modification of the Donepezil scaffold.[38]

Donepezil consists of benzyl piperidine and substituted indanone fragments linked via a methylene bridge (Figure 2). The crystal structure of recombinant human acetylcholinesterase in complex with Donepezil is available in the Protein Data Bank (PDB id 4EY7) which has enabled mapping of its binding interactions. The benzyl piperidine fragment is observed interacting with the AChE catalytic site and has been shown to be the crucial pharmacophoric subunit of the molecule.[39] The indanone moiety on the other hand, improves the fit of the molecule within the binding site and binds to the peripheral anionic site of AChE via aromatic stacking interactions. It has been postulated to play a crucial role in inhibiting Aβ aggregation.[39] In order to introduce antioxidant, anti-inflammatory, metal chelating effects into this molecule, the N-benzyl piperidine (pharmacophore subunit) (Figure 2) has been fused with different bioactive fragments,[40] Various functionalities on the benzene ring have also been explored in an attempt to increase the inhibition of AChE.[40,41]

Table 1 summarizes four series of Donepezil-natural compound chimeras *viz.* Feruloyl-Donepezil hybrids, Donepezil-Genistein hybrids, Donepezil-Melatonin hybrids and Donepezil-Trolox hybrids which have shown promising effects on the multiple targets of AD.[34,40–42] Structure-activity relationships have been developed for cholinesterase inhibition, antioxidant, anti-inflammatory, metal chelation, neuroprotective and $A\beta_{1-42}$ amyloid anti-aggregation activities for each of the series.

Donepezil -Ferulic acid chimeras

Ferulic acid, a potent antioxidant and anti-inflammatory agent is a hydroxy cinnamic acid analogue with structural similarity to curcumin. It is an abundant phenolic phytochemical found in plant cell walls. It derives its name from *Ferulac ommunis* or the giant fennel. Ferulic acid is found in high concentrations in bamboo shoots,[43,44] wheat[45] flaxseed[46] and barley grain.[47]
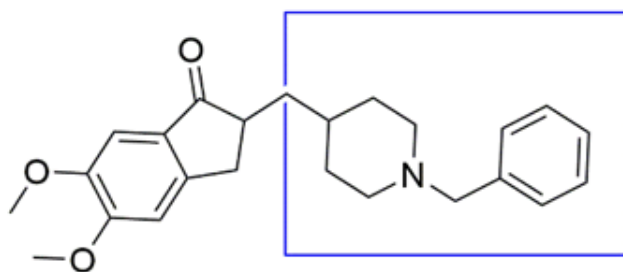

Figure 2: Donepezil with its pharmacophoric unit (boxed).

	Table 1: Donepezil chimeric series and their activities.		
Sr. no.	**Hybrid series**	**Activity profile**	**Ref.**
1.	Donepezil-Ferulic acid hybrids	Anticholinesterase, *in-vitro* direct and indirect antioxidant effects, anti-inflammatory effects, metal chelation, *in-vivo* neuroprotective effects,	40
2.	Genistein-Donepezil hybrids	Anticholinesterase, antioxidant, metal chelation, Aβ anti-aggregating activity, neuroprotective action against Aβ aggregation	34
3.	Donepezil-Melatonin hybrids	Anticholinesterase, antioxidant, Aβ anti-aggregating activity, reduces amyloid burden, improves behavioral activities in mouse models of AD, neuroprotective effects	41
4.	Donepezil-Trolox hybrids	Anticholinesterase, antioxidant, excellent metal chelation and Aβ anti-aggregating activity	42

Like many natural phenols, Ferulic acid shows *in vitro* antioxidant activity and is reactive towards free radicals such as reactive oxygen species. Studies report that it is capable of reverting the formation of amyloid plaques, reduces oxidative stress and has excellent bio-metal chelating ability.[40]

Feruloyl-Donepezil chimeras were created by fusing the benzyl piperidine methyl fragment of Donepezil with Ferulic acid via an ester linkage. Various substitutions on the benzyl fragment were explored.

AChE inhibition

The hybrid molecules were screened for AChE inhibition at a fixed concentration of 30 μM.[40] The SAR studies of the hybrid molecules showed that substitution on the aromatic ring of the N-benzyl piperidine subunit leads to a reduction in the AChE inhibitory activity.[40] Unsubstituted compound 1a (Figure 3) was the most potent AChE inhibitor whereas nitro and bromo substituted compounds designated as 1b and 1c (Figure 3) respectively showed moderate inhibition of AChE. The benzyl piperidine fragment is necessary for interaction with the catalytic site of AChE. Presence of substituents on the benzene ring may negatively impact the binding due to steric hindrance. The crystal structure of Donepezil bound to AChE (PDB id 4EY7) shows that the benzene ring of Donepezil is involved in π-π stacking with Trp84. Electron-withdrawing substituents like nitro and halogens which diminish the electron density in the π-cloud of the substituted ring may decrease the electrostatic repulsion with the π-system of the interacting ring of Trp84, thereby enhancing the π-stacking interaction.[39,40] This may offset the negative effects of steric hindrance and may bestow moderate activity to the nitro and halo-substituted analogues.

Studies reveal that the presence of hydroxyl and methoxy groups on the phenyl propanoid subunit at the other end of the molecules, as was observed in most of the Ferulic acid derivatives leads to retention of
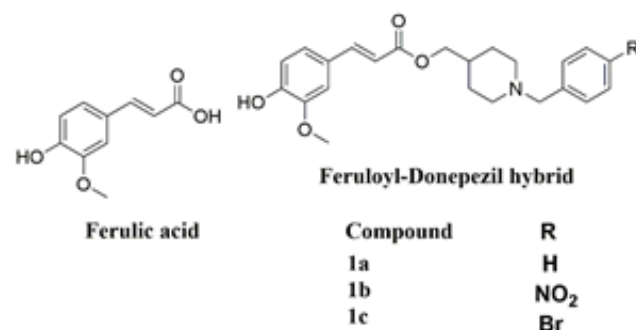

Compound	R
1a	H
1b	NO$_2$
1c	Br

Figure 3: *Left-* **Ferulic acid,** *Right-***Donepezil-Ferulic acid chimeras.**

AChE inhibitory activity.[48] The importance of methoxy substituents has been also confirmed in other studies where coumarin alkylamines matching the structural attributes of Donepezil were designed and developed as AChE inhibitors.

The conjugated aromatic ring system of Ferulic acid may be capable of mimicking the aromatic stacking interactions of the indanone ring system of Donepezil, which may justify the retention of AChE inhibitory activities of the Donepezil-Ferulic acid hybrids.

Antioxidant activity

Oxidative stress plays an important role in inducing and activating multiple cell signaling pathways that result in the formation of toxic substances which further promotes the development of AD.[40,49] Therefore, the designed hybrid series were evaluated for their antioxidant activity by using several *in-vitro* antioxidant methods[40] such as the DPPH assay and the ORAC assay DPPH (1, 1-diphenyl-2-picrylhydrazyl (α, α-diphenyl-β-picrylhydrazyl) is a stable organic nitrogen radical and has a deep purple color.[50,51] The reducing ability of antioxidants towards DPPH is based on the ability of the antioxidant to donate a hydrogen atom to the DPPH radical to form the corresponding colorless hydrazine's while itself getting converted into a radical state. The antioxidant potential is evaluated by electron spin resonance or by monitoring the decrease in absorbance at maximum wavelength at around 520nm.[49-54]

Donepezil-Feruloyl chimeras were screened for their antioxidant activity using DPPH radical scavenging assay at concentrations ranging from 12.5 to 100 μM.[40] Ferulic acid, Isoferulic acid and Trolox were used as standards.[40] Not surprisingly, all Ferulic acid derived hybrids were found to be powerful antioxidant agents and were effective in scavenging free radicals.[40] Based on SAR studies, it was inferred that that the para hydroxy group in Ferulic acid is able to donate a positive charge and therefore leads to radical stabilization. This drives their high ROS scavenging activity. In the meta position, the oxygen atom of the hydroxy group is unable to share the charge and hence the radical scavenging activity is adversely affected.[40] Unsubstituted and nitro substituted compounds i.e. 1a and 1b respectively (Figure 3) counteracted intracellular ROS formation induced by hydrogen peroxide in SH-SY5Y cells in a concentration-dependent manner with a significant decrease of ROS formation at 10, 20 and 40μM whereas bromine substituted compound i.e. 1c (Figure 3) significantly inhibited ROS formation only at 10μM, while showing pro-oxidant effects at higher concentrations.[40]

Anti-inflammatory activity

Suppression of the neuroinflammation process may be a plausible therapeutic intervention for AD. Hybrid compounds were tested by researchers for their *in-vivo* anti-inflammatory activities.[55-57] LPS-induced peritonitis tests were carried out on the Donepezil-Feruloyl hybrids. It was found that the unsubstituted and bromine substituted compound i.e. 1a and 1c respectively (Figure 3) significantly inhibited leukocyte migration in contrast to the control group (vehicle-DMSO+LPS) and Dexamethasone, exhibiting higher anti-inflammatory activity in leukocyte migration test.[40,58-60]

Compounds 1a and 1c (Figure 3) were able to decrease licking duration during the inflammatory process in the formalin-induced anti-nociceptive study, suggesting an anti-inflammatory activity which may be attributed to the indirect inhibition of inflammatory mediator production or the blockage of their receptors directly.[61,62] These compounds did not improve the motor activity in mice models.[63]

Metal chelation

A significant amount of literature proof states that bio metal dyshomeostasis of metal ions such as Cu^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+} in CNS and their interactions with the precursor of amyloid protein (APP) and Aβ precursors lead to AD pathology.[16,64,65] Copper and iron, are usually redox active and are able to generate ROS through the Fenton and Haber-Weiss reaction and facilitate the formation of neurotoxic Aβ oligomers. Metal chelating compounds may serve as an important therapeutic approach in treating AD.[39]

Donepezil-Feruloyl chimeras, either unsubstituted or substituted with nitro and bromine groups (1a, 1b, 1c, Figure 3) were found to selectively chelate bio metals such as Cu^{2+}, Fe^{2+}, but not Fe^{3+} or Zn^{2+}. The shift in absorption spectra after incubation of the compound solutions with metal salts were monitored using UV-Visible spectroscopy at 200-400 nm.[40,66]

Neuroprotective and Aβ$_{1-42}$ anti-aggregation activities

Among different forms of Aβ aggregates, soluble Aβ oligomers (AβO) cause selective synaptic dysfunction and neuronal loss in the cortex and in the hippocampal regions of the brain.[67-70] Among the Donepezil-Feruloyl hybrids, the unsubstituted compound 1a (Figure 3) was found to counteract ROS formation and neuronal death induced by Aβ$_{1-42}$ oligomers in neuronal SH-SY5Y cells.[40,70]

Donepezil-Genistein hybrids

Genistein (4',5,7-trihydroxyisoflavone) found abundantly in soybeans and red clover containing plants like *Trifolium pratense*, is known to possess a wide spectrum of biological effects such as antioxidant and metal chelation as well as neuroprotective effects against Aβ.[71-75] It also shows anti-inflammatory activity which has potential in delaying the progression of a neurodegenerative disease like AD.

In an attempt to supplement this activity profile with AChE inhibition, researchers[34] developed a series of Donepezil-Genistein hybrid molecules and evaluated their potential for AChE inhibition, antioxidant, metal chelation, inhibitory effects on Aβ aggregation and neuroprotective effects in the mouse scopolamine model of memory impairment.[34]

The piperidine ring of the pharmacophoric benzyl piperidine motif of Donepezil was replaced with various lipophilic ring-opened tertiary amines. Hydroxy groups at 4' and 7 positions of Genistein were coupled with various alkyl benzylamines on either side (2a, 2b) as well as simultaneously on both terminal sides (2c).

AChE inhibition

While all modifications gave compounds, which exhibited good inhibition against AChE, the longer, more lipophilic amine 2c showed the highest potency. On the basis of inhibition kinetic analysis and molecular modeling studies, the authors reported that compound 2c showed a mixed-type inhibition, binding to both CAS and PAS of AChE, thereby inducing a strong inhibitory effect.[34]

Antioxidant activity

The ORAC assay method measures the antioxidant scavenging activity against the peroxyl radical, induced by 2, 2'-azobis-(2-amidino-propane) dihydrochloride (AAPH), at 37°C. The peroxyl radical reacts with a fluorescent probe, usually fluorescein or peroxyl radical, induced by reduction in fluorescence that can be evaluated using spectrofluorimetry.[52-54,76] Trolox is used as a reference standard, fluorescein is used as the fluorescent probe[77-79] and the specified ORAC values of the tested potential antioxidants are stated as Trolox equivalents. ORAC assay measures hydrogen atom donating ability of antioxidants. Higher ORAC values indicate good antioxidant capacity. Another assay which has been used to determine Trolox equivalents is the TEAC (Trolox equivalent antioxidant capacity) assay, which is based on scavenging of 2, 20 -azinobis-(3- ethyl benzothiazoline-6- sulfonate) radical anions (ABTS-).[52]

ORAC assay used to determine antioxidant potential of Donepezil-Genistein chimeras[34] showed that compounds where at least one of the Genistein hydroxy groups were left exposed (2a, 2b, Figure 4) had higher antioxidant activity than compound 2c where both the 4' and 7 hydroxy groups were substituted with the lipophilic alkyl benzyl amines. This further reiterates the importance of free hydroxy groups for good radical scavenging and antioxidant ability.

Metal chelation

The chelating ability of Donepezil-Genistein hybrids, 2a, 2b and 2c (Figure 4) for the biologically relevant metal ions Cu^{2+}, Fe^{2+}, Zn^{2+} and Al^{3+}, was studied by researchers[34] and found positive for Cu^2 and Al^{3+} but not for Fe^{2+} and Zn^{2+}.

Neuroprotective and $Aβ_{1-42}$ anti-aggregation activities

The Donepezil-Genistein hybrids 2a, 2b and 2c (Figure 4) exhibited moderate inhibition of self-induced Aβ aggregation, but higher Cu^{2+}-induced Aβ aggregation inhibitory activity in comparison to curcumin.[34] Compound 2c showed significant inhibition of Aβ aggregation, induced disaggregation of Aβ fibrils generated by Cu^{2+}- induced Ab aggregation and reversed cognitive deficit.[34]

Donepezil-Melatonin hybrids

Melatonin, an endogenous neurohormone, has been shown to have a profound effect on the pathogenesis of AD, addressing many of the associated causative factors. It is a powerful antioxidant and free radical scavenger,[80-83] efficiently attenuates hyper phosphorylation of tau,[84-89] modulates the synthesis and maturation of amyloid precursor protein (APP) (41) butyrylcholinesterase (eqBuChE and hBuChE and also
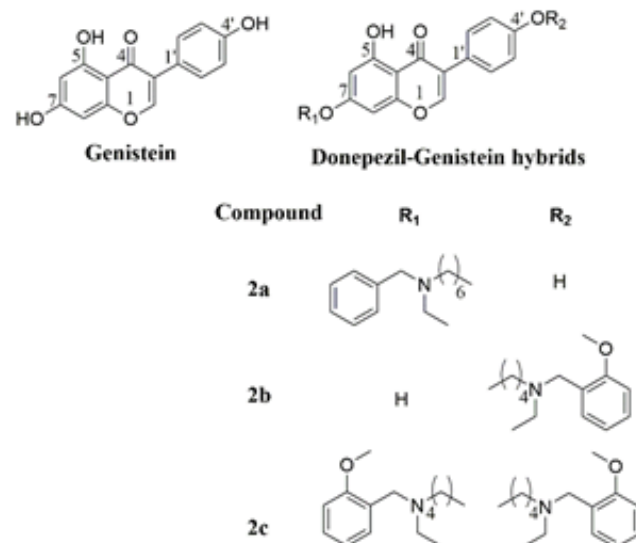

Figure 4: *Left*- Genistein, *Right*-Donepezil-Genistein chimeras.

inhibits the progressive formation of β-sheet and/or amyloid fibrils.[90-92]

Much of the antioxidant effectiveness of Melatonin *in vivo* has been attributed to the cascade of Melatonin antioxidant metabolites produced (Figure 5). In presence of ROS, Melatonin sequentially forms the melatonin radical cation and melatoninyl neutral radical which undergoes rearrangement to cyclic 3-hydroxy melatonin, N(1)-acetyl-N(2)-formyl-5-methoxykynuramine) (AFMK), N(1)-acetyl-5-methoxykynuramine (AMK) as well as 6-hydroxy melatonin via one electron transfer processes.[93]

Each of these products of rearrangement also have potent antioxidant activities.

AChE inhibition

Donepezil-Melatonin hybrids were designed and synthesized[41] and were shown to possess AChE inhibitory activities when measured by a spectrophotometric enzyme-based assay method.[94]

Compounds 3a and 3b (Figure 6) which are direct hybrids of Melatonin and the benzyl piperidine moiety of Donepezil showed less AChE inhibition than Donepezil but similar antioxidant activity to Melatonin. Compound 3c (Figure 6) on the other hand, where the amide bond is attached in reverse order showed good AChE inhibition comparable to Donepezil after optimization of molecule length.

The methoxy indole scaffold of Melatonin possibly acts as an isostere of the methoxy indanone fragment of Donepezil, thereby retaining the hydrophobic and π-stacking binding interactions of this fragment within the acetylcholinesterase active site. It is possible that in the ROS microenvironment as is found in AD, the direct Melatonin hybrids (3a, 3b) undergo a molecular rearrangement similar to Melatonin, resulting in a molecules which have a different shape and relative orientation of the benzyl piperidine and methoxy indole scaffolds, due to which the molecules are unable to optimally bind into the AChE pocket, resulting in only moderate activity. However, incorporating a reverse amide (as in 3c) may inhibit the rearrangement process, thereby retaining a conformation amenable to the AChE pocket resulting in good inhibition. Variation of substituents on the benzene ring of the benzyl piperidine fragment showed that substituents such as methyl group (3b, Figure 6) at ortho position of the benzene ring are more active than those at meta or para position.[41] Linker length between the piperidine and amide group also plays an important role in AChE activity with a two-carbon linker (3c, Figure 6) showing highest AChE inhibition.

Antioxidant activity

The antioxidant potential of Donepezil-Melatonin hybrids was determined via the ORAC assay. It was found that compounds with a methyl substituent on the benzene ring (3b, Figure 6) and an one or two-carbon methylene (3c, Figure 6) linker between the amide and the piperidine ring showed the highest protective capability, radical scavenging and antioxidant profile higher than Melatonin and Trolox.[41]

Metal Chelation

Unsubstituted indole and benzene rings containing Donepezil-melatonin hybrids like compound 3c (Figure 6) were found to chelate bio metals such as
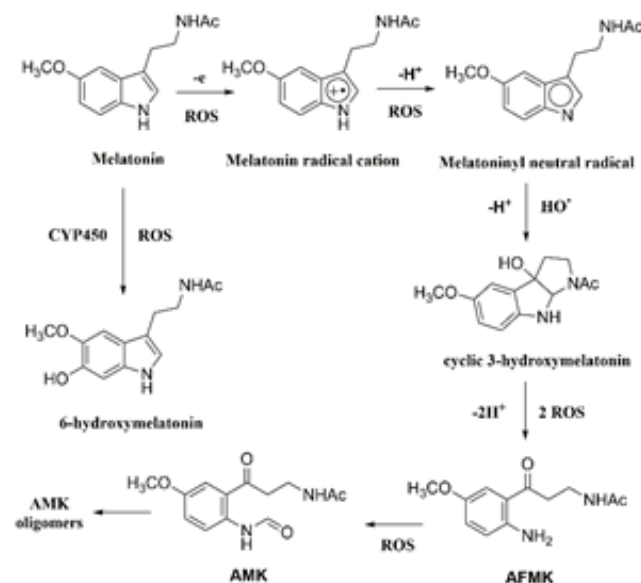

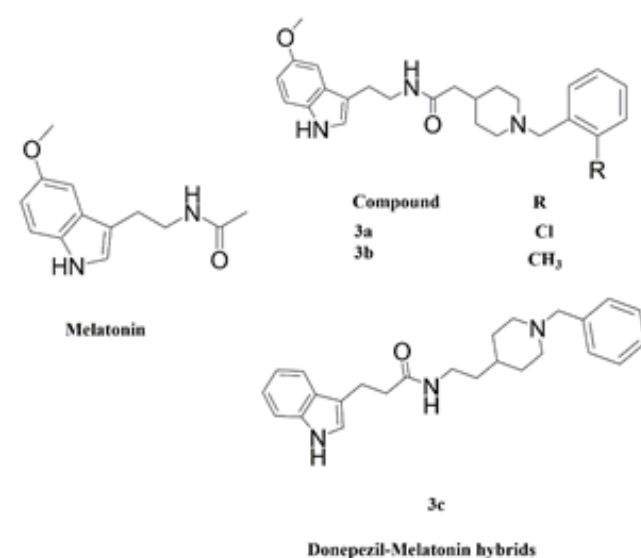**Figure 5: Antioxidant mechanism of action of Melatonin.**

Figure 6: *Left*- Melatonin, *Right*- Donepezil-Melatonin chimeras.

copper, iron and zinc and was evaluated by UV-Vis spectroscopy.[41]

Neuroprotective and Aβ$_{1-42}$ anti-aggregation activities

The Thioflavin-T fluorimetric assay was carried out on all synthesized hybrids using curcumin and resveratrol as reference compounds.[41,95,96] It was observed that electron withdrawing groups like chlorine (3a, Figure 6) on the benzene ring were beneficial and bestowed good Aβ$_{1-42}$ self-induced aggregation inhibitory activity. It was also reported that linker length did not influence the inhibition of Aβ$_{1-42}$ self-induced aggregation.[41]

Donepezil-Trolox hybridsTrolox is a water-soluble analogue of Vitamin E which has strong antioxidant effects, reduces oxidative stress, exhibits neuroprotective effects through ROS scavenging and decreases neurotoxicity caused by Aβ.[42] Donepezil-Trolox hybrids were constructed by joining the Trolox molecule with benzyl piperidine scaffold via an amide-poly-(methylene) linker.

AChE inhibition

It was found that groups such as fluorine (4a, Figure 7) at ortho position of the benzene ring show excellent AChE inhibition in addition to retention of antioxidant, metal chelation and neuroprotective properties. Linker length was also found to play an important role with two-methylene linker showing higher inhibitory activities.[42] It is possible that ortho substitution on the benzene ring as well as the correct linker length bestows the optimum bioactive conformation to the molecule and contributes favorably towards AChE inhibition.

Antioxidant activity

The antioxidant potential of Donepezil-Trolox hybrids was evaluated by carrying out DPPH radical scavenging assay, using Trolox as a reference standard.[42,54] It was reported that all hybrid compounds retained their antioxidant activities in comparison to the reference standard[42] indicating that coupling with benzyl piperidine does not adversely affect the antioxidant capabilities of Trolox.

Along with the DPPH radical scavenging assay, the ORAC assay was done on the Donepezil-Trolox hybrids. The researchers reported that compound 4a (Figure 7) in which fluorine is at ortho position exhibits ORAC value greater than 1, pointing to superior antioxidant activities when compared to Trolox.[42]

Metal chelation

Fluorine-substituted Donepezil-Trolox hybrids like compound 4a (Figure 7) were found to chelate bio metals such as copper, zinc and iron.[35,42] Metal chelation for copper was evaluated by UV-Vis spectrometry using HEPES buffer and investigated at an absorption maximum of 246 nm by determining the isobestic point.[42]

Neuroprotective and Aβ$_{1-42}$ anti-aggregation activities

The Thioflavin T (ThT) fluorescence assay was carried out on all Donepezil-Trolox hybrid series using transmission electron microscopy (TEM).[42,97] It was reported that fluorine substituted compound 4a (Figure 7) can inhibit Aβ$_{1-42}$ fibril formation and copper induced Aβ$_{1-42}$ aggregation effectively.[42] Further compound 4a, when treated against oxidative injuries on PC12 cells was reported to show poor neuroprotective activity in oligomycin-A assay whereas it showed good neuroprotective activity in hydrogen peroxide and rotenone assays.[42,98] The neuroprotective effect was also carried out against LPS-stimulated inflammation on BV$_{-2}$ microglial cells.[42,99] It was reported that compound 4a showed higher inhibition of NO production in LPS-simulated BV$_{-2}$ cells than the reference compounds Donepezil and Trolox. Thus, this compound also showed significant anti-neuroinflammatory activities[42] Inhibiting ROS production can result in suppression of intracellular proinflammatory signals. LPS induces ROS production whereas compound 4a effectively decreases the intracellular ROS accumulation.[42] Compound 4a was reported to be a promising neuroprotective agent based on various assays carried out by researchers. Moreover, this Donepezil-Trolox chimeric compound showed good *in vitro* blood-brain barrier permeation when tested in the parallel artificial membrane permeation assay for BBB (PAMPA-BBB),[42] which is a necessary attribute for potential anti-AD drugs.

All four Donepezil hybrid series (Table 1) have been subjected to pharmacokinetic and pharmacodynamic evaluations *viz in-vitro* BBB permeation assays,[41,42,100] cognition and memory improvement tests,[42,101,102] on specific mouse models and *in-vitro* toxicity profiling.[42]
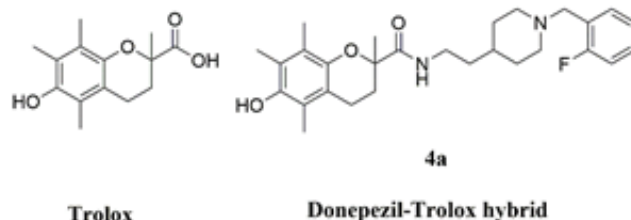

Trolox   **Donepezil-Trolox hybrid**

Figure 7: *Left-* **Trolox,** *Right-* **Donepezil-Trolox chimera.**

It has been reported that all the hybrid molecules have a better safety index as well as permeation rate as compared to the parent compounds.

RIVASTIGMINE CHIMERAS FOR AD

Rivastigmine, is the only FDA approved carbamate AChE inhibitor for the treatment of mild to moderate AD. However, as found in clinical trials, Rivastigmine does not prevent the course of illness but only leads to a modest improvement in cognitive functions.[103] This can be attributed to the multifactorial nature of AD.

The carbamate moiety is the pharmacophore subunit of Rivastigmine which binds to the AChE catalytic site and is responsible for cholinesterase inhibitory activity.

The mechanism of action of Rivastigmine, as revealed by X-ray crystallography studies has been reported[104] to involve carbamoylation of an AChE active-site serine residue, with concomitant formation of leaving group S-3-[1-(dimethylamino)-ethyl]-phenol (NAP) (Figure 8). The carbamoyl moiety is covalently linked to the active-site serine S200 which is part of the catalytic triad and remains attached until it is slowly removed by hydrolysis making Rivastigmine a pseudo-irreversible inhibitor of AChE.[104]

Table 2 summarizes three series of hybrid molecules which have shown promising effects on the multiple targets of AD[103,105,106] viz. Rivastigmine-hydroxy cinnamic acid hybrids, Rivastigmine-Apigenin hybrids and Rivastigmine-scutellarin hybrids.

Multitarget design ligand strategies have been developed where the AChE inhibiting carbamoyl fragment of Rivastigmine is coupled with various bioactive molecules with favorable properties for treatment of AD functioning where they replace the NAP fragment. These molecules have been explored in order to investigate their potential of possessing an expanded activity profile for a more holistic treatment of AD.

Structure-activity relationships have been developed for cholinesterase inhibition, antioxidant, metal chelation, neuroprotective and $A\beta_{1-42}$ amyloid anti-aggregation activities for each of the series.

RIVASTIGMINE-CAFFEIC ACID HYBRIDS

Caffeic acid, an analogue of hydroxy cinnamic acid is found in all plants as it is a key intermediate in the biosynthesis of lignin.[107] It is found in the bark of *Eucalyptus globules*,[108] the herb *Dipsacus asperoides*,[109] the freshwater fern *Salvinia molesta*,[110] the mushroom *Phellinus linteus*[111] and in a variety of herbs such as thyme, sage and, spearmint, Ceylon cinnamon and star anise. Caffeic acid decreases ROS generation and Aβ aggregation, effectively blocks the progression of AD and prevents oxidative damage in neurons.[106]
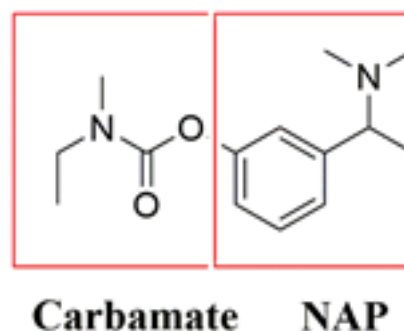

Figure 8: Subunits of Rivastigmine showing the carbamate (pharmacophoric) and NAP.

Table 2: Rivastigmine chimeric series and their activities.

Sr. no.	Hybrid series	Activity profile	Ref.
1.	Rivastigmine-hydroxy cinnamic acid hybrids	Anticholinesterase, antioxidant, metal chelation, Aβ anti-aggregating activity, neuroprotective action against Aβ aggregation	106
2.	Rivastigmine-Apigenin hybrids	Anticholinesterase, antioxidant, metal chelation, Aβ anti-aggregating activity, neuroprotective action against Aβ aggregation	105
3.	Rivastigmine-scutellarin hybrids	Anticholinesterase, antioxidant, metal chelation, Aβ anti-aggregating activity, neuroprotective action against Aβ aggregation	103

Compound	R	n	X
5a	H	0	O
5b	OCH3	0	O
5c	H	1	O
5d	OCH3	1	O
5e	H	2	O
5f	OCH3	2	O
5g	H	0	N
5h	OCH3	0	N

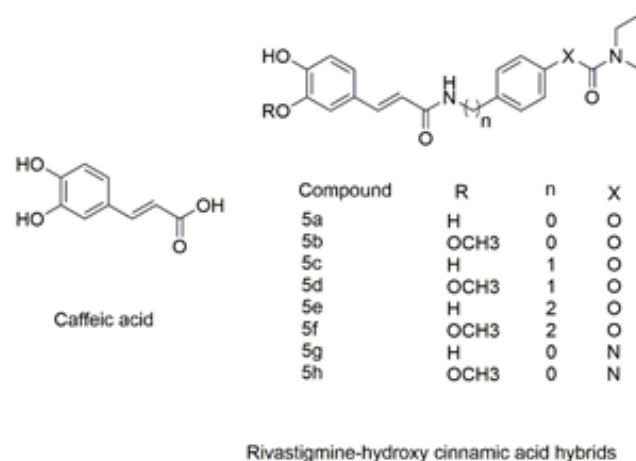Rivastigmine-hydroxy cinnamic acid hybrids

Figure 9: *Left*- Caffeic acid, *Right*- Rivastigmine-Caffeic acid chimeras.

Researchers synthesized two types of Rivastigmine-hydroxy cinnamic acid hybrids - carbamate analogues (5a-5f) and urea analogues (5g, 5h) (Figure 9). Other modifications explored were the ring substituents on the benzene of cinnamic acid (3,4-dihydroxy derivatives – caffeic acid analogues; 4-hydroxy, 3-methoxy derivatives – ferulic acid analogues) and the linker length between the amide and the aromatic ring.

AChE inhibition

All the hybrid compounds 5a-5h exhibited enhanced cholinesterase inhibition activity for both AChE and BuChE as compared to the native drug Rivastigmine.[106] The longer caffeic acid fragments (compared to the NAP fragment) may have the capacity of having additional binding interactions within the AChE pocket which may be responsible for the higher AChE inhibition of the hybrid molecules.

Antioxidant activity

The DPPH assay was carried out to evaluate the antioxidant potential in Rivastigmine-caffeic acid hybrids. The urea analogues with two hydroxy groups attached to the phenyl ring and carbamate analogues (5g) with a one-carbon spacer and two hydroxy groups attached to the phenyl ring (5c) exhibited the highest radical scavenging activities (Figure 9).[106]

Metal chelation

Compounds which are capable of chelating metals like copper, zinc and iron and have the ability to cross BBB would be invaluable in treatment of AD.[112,113] Rivastigmine-caffeic acid hybrid with a two-carbon spacer (5e, Figure 9) was found to have copper chelating properties. It also reduced copper-induced $A\beta$ aggregation and oxidative stress.[106,114] It was surmised by the authors that chelation activity may be due to the presence of the two phenyl hydroxyl groups.[106]

Neuroprotective and $A\beta_{1-42}$ anti-aggregation activities

Glutamate and hydrogen peroxide assays were carried out in order to evaluate neuroprotective effects of Rivastigmine-caffeic acid hybrids. Urea analogues (5g, 5h, Figure 9) exerted good neuroprotective effects in glutamate-induced cell death. Hydroxy-substituted caffeic acid hybrids (5a, 5c, 5e, 5g) potently inhibited the $A\beta_{1-42}$ self-aggregation whereas the methoxy-substituted ferulic acid hybrids (5b, 5d, 5f, 5h) showed moderately potent effects in $A\beta_{1-42}$ self-aggregation.[106]

Rivastigmine-Apigenin hybrids

Apigenin (4',5,7-trihydroxyisoflavone) is a natural flavone, found abundantly in parsley, celery and chamomile. It has been found to possess antioxidant, neuroprotective, anti-inflammatory and anti-$A\beta$ aggregation activities.[105,115] When these moieties are coupled with the carbamoyl portion of Rivastigmine which inactivates AChE, multifunctional ligands targeting the major causative factors of AD are obtained.

AChE inhibition

A series of Rivastigmine-Apigenin hybrids were developed[105] and evaluated for AChE inhibition These studies demonstrated the strategic success of capping the Apigenin terminal hydroxy groups with aliphatic carbamates.Compound 6a (Figure 10) showed a four-fold improvement in AChE inhibition as compared to Rivastigmine.[105] The authors attributed this to a significant improvement in the overall binding of the hybrid molecule within the AChE binding cavity. Molecular docking studies of compound 6a within AChE showed it to simultaneously occupy the entire peripheral anionic site, catalytic site and the mid-gorge sites, with both the carbamate fragment as well as the chromone scaffold of apigenin contributing via favorable hydrogen bonding, π-π stacking as well as hydrophobic binding interactions.

Antioxidant activity

The Rivastigmine-Apigenin hybrids were evaluated for antioxidant activity[105] using the ORAC assay. Apigenin is a natural flavone with three hydroxy groups at 4', 5 and 7 positions. The Rivastigmine-Apigenin hybrids with the 4' and 7 hydroxy groups substituted with carbamate functionalities (eg. Compound 6a, Figure 10) exhibited significantly less antioxidant activity than Apigenin, but were equal in potency to the reference standard Trolox. Substituting all three hydroxy groups drastically lowered the antioxidant activity of the molecules.

Metal chelation

The 5-hydroxy and 4-ketone groups of Rivastigmine-Apigenin hybrids have the potential of exhibit metal-chelating properties. Compound 6a (Figure 10) exhibited
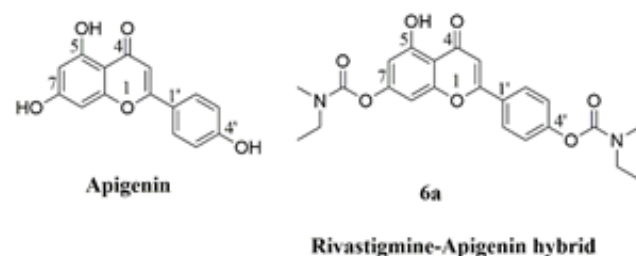

Apigenin

6a

Rivastigmine-Apigenin hybrid

Figure 10: *Left-* Apigenin, *Right-* Rivastigmine-Apigenin chimera.

selective metal chelation for copper and aluminum but not iron and zinc metal ions.[105]

Neuroprotective and Aβ$_{1-42}$ anti-aggregation activities

The effects on Aβ aggregation were evaluated for the Rivastigmine-Apigenin hybrids[105] by performing Thioflavin T (ThT) fluorescence assay using curcumin as a reference standard.[105] Dual carbamates like compound 6a (Figure 10) showed excellent neuroprotective ability as well as inhibition of self-mediated Aβ$_{1-42}$ aggregation, huAChE-induced Aβ$_{1-40}$ aggregation and Cu^{2+}-mediated Aβ$_{1-42}$ aggregation. However, bulky substituents on the hydroxy group at 5-position on the chromone scaffold fragment lowered the anti-aggregation effects.

Rivastigmine-Scutellarin hybrids

Scutellarin (4',5,6-trihydroxy flavone-7-glucuronide) is a flavone first isolated from the Chinese herb *Erigeron breviscapus*.[116] Its broad spectrum of pharmacological activities include free radical scavenging ability, anti-inflammatory efficacy, neuroprotection and ability to inhibit Aβ fibril formation.[117-119] However, poor solubility, weak oral absorption and poor blood-brain barrier (BBB) penetration has restricted the clinical application of scutellarin.[120,121]Researchers[103] synthesized and evaluated a series of Rivastigmine-scutellarin hybrids.

AChE inhibition

It was reported that substitution on the flavonoid nucleus as well as the carbamate capping groups affect cholinesterase inhibition. Methoxy (7a, 7b, Figure 11) or hydroxy (8a, Figure 11) substituted phenyl rings on the scutellarin fragment show better cholinesterase inhibition as compared to Rivastigmine, with the 5-methoxy substitution on the flavonoid nucleus showing higher activity as compared to 5-hydroxyl substitution. The carbamate nitrogen was completely substituted with small alkyl fragments such as methyl, ethyl and isopropyl groups as well as with rings such as morpholine, piperidine and substituted piperazines. Compounds containing N, N-diethyl carbamate side chain (7b) and N-ethyl-methyl carbamate side chain (7a) exhibited strongest inhibition for AChE and BuChE respectively. Hybrid series with carbamate moiety at para position (Figure 11) were also reported to have more potent activity than those at meta position.[103]

Antioxidant activity

SAR studies on Rivastigmine-scutellarin hybrids using ORAC fluorescein assay[97] showed that presence of carbamate side chains on to the flavonoid nucleus decreases the antioxidant potential but they have little influence on the radical scavenging activity on the same nucleus.[103] Compounds with 5-hydroxyl groups (8a, Figure 11) exhibit better antioxidant properties in comparison to 5-methoxy groups (7c, Figure 11). Compound 8a exhibited best antioxidant activity which is 1.3-fold higher than the reference standard Trolox.[103]

Metal chelation

Researchers monitored metal chelation of Rivastigmine-scutellarin hybrids using UV-visible spectroscopy at 322-341 nm and reported that compounds containing N,N-diethyl carbamate side chain (7b, Figure 11) showed excellent metal chelation of bio metals such as copper, aluminum but not iron and zinc.[103] Chelation can be attributed to the 5-hydroxyl and 4-carbonyl groups of the flavonoid nucleus.

Neuroprotective and Aβ$_{1-42}$ anti-aggregation activities

Studies[103] evaluating the neuroprotective capacities of Rivastigmine-scutellarin hybrids against oxidative stress showed that compounds with 5-hydroxyl groups exhibit a higher cell survival ratio as compared to compounds with 5-methoxy group substituents, possibly due to the capability of hydroxy group of capturing the free radicals. The N, N-diethyl carbamate side chain containing compound 8a (Figure 11) exhibited remarkable neuroprotective effects.[103]

SAFE TACRINE CHIMERAS FOR AD

Tacrine, was the first FDA approved anti-AD drug, which had dual inhibitory properties of acetyl cholinesterase and butyl cholinesterase inhibition, but it was withdrawn from the market shortly after FDA approval due to serious hepatotoxicity.[122] Due to the good inhibitory activity, low molecular weight and its ability to reduce Aβ-induced neurotoxicity, Tacrine has been widely investigated by researchers worldwide, especially for the synthesis of safer multi-target
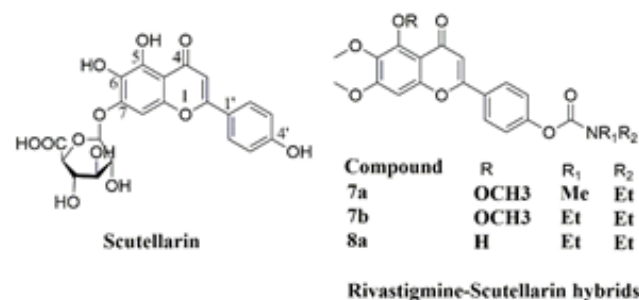

Compound	R	R$_1$	R$_2$
7a	OCH$_3$	Me	Et
7b	OCH$_3$	Et	Et
8a	H	Et	Et

Figure 11: *Left*- Scutellarin, *Right*- Rivastigmine-Scutellarin chimeras.

directed ligands (MTDLs) for AD treatment.[123] Several assays have been used by researchers to monitor the hepatotoxicity of Tacrine analogues such as the MTT colorimetric assay,[124] ALT and AST activity assay method[125] and the Cell Titre Glow assay.[126]

Tacrine hybrids with several fragments, some of them known hepatoprotectives, have been extensively studied in order to identify safer anti-AD molecules without any hepatotoxicity liability.

Table 3 summarizes four series of hybrid molecules which have shown promising effects on the multiple targets of AD[127-130] viz. Tacrine-Silibinin derivatives, Tacrine-Trolox hybrids, Tacrine-Scutellarin compounds and Tacrine-Rhein hybrids.

Tacrine-Silibinin analogues[127]

Silibinin is one of the main components of the silymarin complex, a standardized mixture obtained from the fruits of *Silybum marianum* commonly known as milk thistle. Silibinin has anti-inflammatory[127] and neuroprotective properties[131–133] and has also been used medically for therapeutic intervention in hepatic disorders.[134] A co-drug of Tacrine and Silibinin was developed, linked via a succinimide hexamethylene bridge (compound 9a, Figure 12). This compound was tested for AChE inhibition and neurotoxicity. In addition, it was evaluated for hepatotoxicity in human hepatic stellate cells (HSC). Compound 9a (Figure 12) exhibited potent AChE inhibition and neuroprotective effects. In addition, it almost eliminated the hepatotoxicity of Tacrine.

Tacrine-Trolox analogues[128]

Trolox is a water-soluble analogue of Vitamin E and is known to have strong antioxidant activity.[42] Numerous studies indicate that Trolox can protect liver from the

damage induced by chemical insults both in *in-vivo* and *in-vitro* conditions.

Chimeric molecules combining Tacrine with Trolox were synthesized by joining them through various linkers, resulting in identification of compounds with cholinesterase inhibition in nanomolar range as well as strong antioxidant activity. The most potent compound, compound 10a (Figure 13) in addition, also had neuroprotective effects, had good BBB penetration and hepatotoxicity much less than that of Tacrine. In addition, it also displayed neuroprotective effect against hydrogen peroxide induced PC-12 cells and had good penetration ability. Molecular modelling and kinetic studies demonstrated that this compound showed mixed-type enzyme inhibition with potential
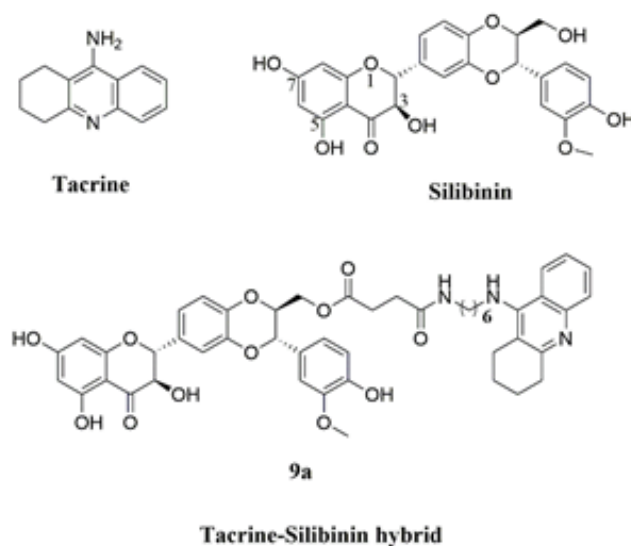
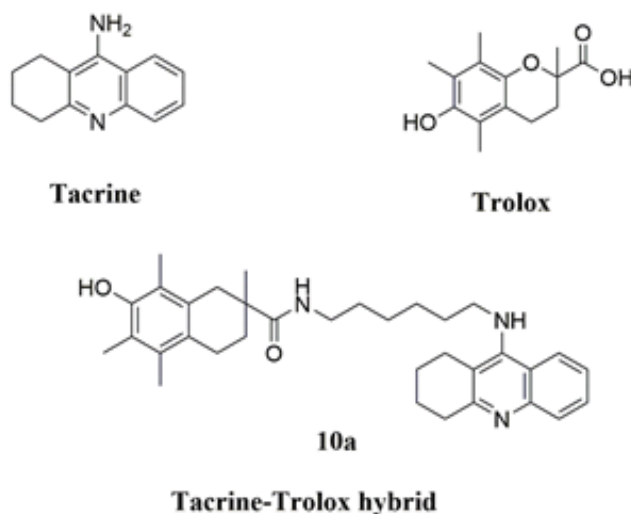**Figure 12:** *Top panel-* Tacrine, Silibinin *Bottom panel-* Tacrine-Silibinin chimera.

Table 3: Tacrine chimeric series and their activities.

Sr. no.	Hybrid series	Activity profile	Ref.
1.	Tacrine-Silibinin	Anticholinesterase, neuroprotective, no hepatotoxicity	127
2.	Tacrine-Trolox	Anticholinesterase antioxidant, neuroprotective, no hepatotoxicity	128
3.	Tacrine-Ferulic acid	Anticholinesterase, partial inhibition of AChE-induced Aβ fibril formation and amyloid self-aggregation, significantly lower hepatotoxicity than Tacrine	129
4.	Tacrine-Rhein	Anticholinesterase, antioxidant, inhibition of AChE-induced Aβ aggregation, metal chelating properties, low hepatotoxicity	130

Figure 13: *Top panel-* Tacrine, *Bottom panel-* Tacrine-Trolox chimera.

for additional binding to the peripheral anionic site of AChE along with the catalytic site.

Tacrine-Ferulic acid analogues[129]

Ferulic acid is a naturally occurring phenolic acid, abundantly found in medicinal plants such as *Triticum aestivum* or *Eucalyptus globules*. Ferulic acid scaffolds and esters have been reported to show strong antioxidant and hepatoprotective activity[135] as well as inhibition of $A\beta_{1-42}$ in the brain when investigated on mice.[136,137]

Novel Tacrine-Ferulic acid chimeric molecules were developed in an attempt to combine the AChE inhibitory properties of Tacrine and the *in-vitro* inhibitory effects on Aβ fibril formation and aggregation reported for the ferulic acid nucleus. The aromatic hydroxy group of Ferulic acid was capped with various substituted benzyl groups. The benzyloxy ferulic acid analogues were coupled to Tacrine via a functionalized amide linkage. The most active hybrid molecule, compound 11a (Figure 14) had multiple methyl substituents on the benzyl ring and showed nanomolar inhibitory potency against AChE, as well as an ability to partially inhibit amyloid self-aggregation.

The authors employed molecular docking studies in order to determine the probable binding mode of the hybrid molecules. The Tacrine fragment was seen to occupy its place at the catalytic site, engaging in stacking interactions with Trp86 and hydrogen bonding interactions with Tyr337 while the Ferulic acid moiety was found to make contact with the peripheral anionic site via hydrogen bonding interactions with Tyr 341 and stacking interactions with Trp286 and Tyr341. In

addition, compound 11a had a better safety profile and showed no significantly hepatotoxicity when tested by the ALT and ASAT activity assay.

Tacrine-Rhein analogues[130]

Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) is the active component of rhubarb plant (*Rheum officinale*), a traditional Chinese herb. It is used to treat chronic liver disease, has hepatoprotective effect, prevents injury in hepatic cells and development of hepatic fibrosis in rats.[138,139]

Researchers developed a series of Tacrine-Rhein hybrids, most of which inhibited AChE in the nanomolar range. Compound 13a (Figure 15) was five-fold more active than Tacrine, inhibited AChE-induced Aβ aggregation, exhibited metal chelating properties and had low hepatotoxicity.[130]

Kinetic evaluation supported by molecular modelling studies revealed the compounds to interact with AChE via a mixed type of enzyme inhibition, with capability of binding to both the active catalytic site and the peripheral anionic sites of AChE.

CONCLUSION

The chimeric strategy for design of multifunctional agents, which combines different biological activities in a single molecule, holds a lot of promise for the development of novel drugs in the treatment of multi-target neurodegenerative diseases like AD. One of the main drawbacks of currently available FDA-approved drugs for the treatment of AD is that they target only a
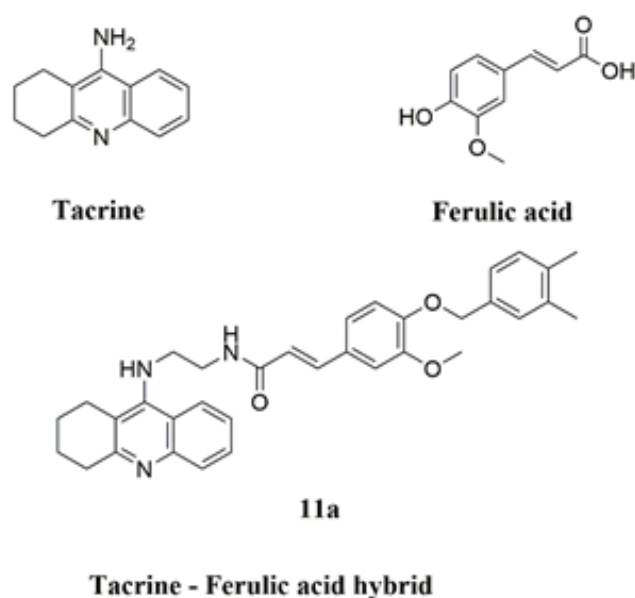

Figure 14: *Top panel-* Tacrine, Ferulic acid, *Bottom panel-* Tacrine-Ferulic acid chimera.
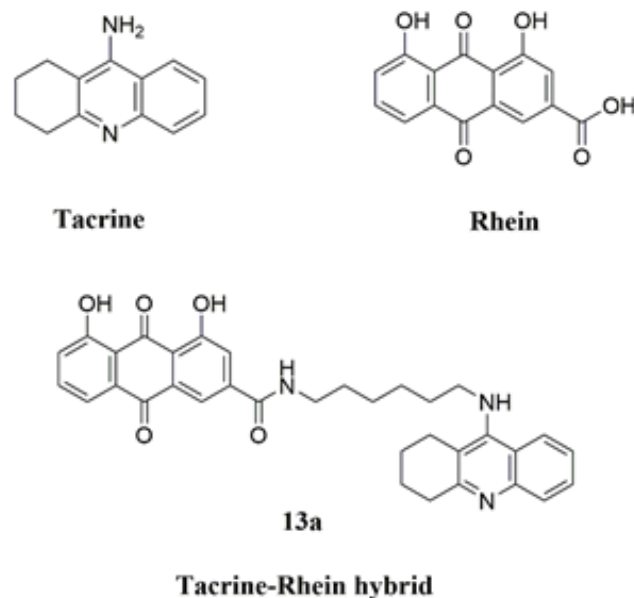

Figure 15: *Top panel-*Tacrine, Rhein, *Bottom panel-* Tacrine-Rhein chimera.

single mechanism in the development of this multifactorial disease. Several natural molecules have a plethora of activities such as antioxidant, anti-inflammatory and neuroprotective properties which are vital for complete cure of patients with AD. Hybridization of these molecules with FDA-approved cholinesterase inhibitors would expand the anti-AD spectrum by creating molecular entities which address most of the causal hypotheses of AD development.

The rational design of chimeric molecules for multiple-targeted clinical use presents its own set of challenges such as achieving a balanced overall activity profile, excluding undesired off-target effects and toxicity while retaining good drug-like properties in the designed molecules. Rational drug design strategies, especially structure-based *in*

silico techniques and binding interaction mapping of parent molecules can be used to identify molecular determinants for focused activities.

The crystallographic structure of AChE reveals that it has a narrow 20 Å gorge, containing two binding sites: the catalytic active site (CAS) at the bottom and the peripheral anionic site (PAS) near the entrance of the gorge. Binding to the CAS is crucial to inhibition of the AChE enzyme. Study of the active site of AChE complexes bound to relatively larger-sized inhibitors like Donepezil and Rivastigmine has helped to identify the pharmacophoric units of these molecules, which make contact with the catalytic active site of AChE. Retaining these fragments viz benzyl piperidine fragment of Donepezil and carbamoyl fragment of Rivastigmine and replacing the

Hybrid series	Cmp	AChEi	DPPH	ORAC	% inhibition of Ab aggregation			Ref.
					Self-induced	Metal-induced	AChE-induced	
Donepezil-Ferulic acid	1a	0.46[*]	49.41[a]	-	-	-	-	40
	1b	16.74[*]	46.66[a]	-	-	-	-	
	1c	> 30[*]	45.38[a]	-	-	-	-	
Donepezil-Genistein	2a	4.98[*]	-	1.2	34.3	72.1	18.7	34
	2b	15.9[*]	-	1.1	24.6	71.9	22	
	2c	0.14[*]	-	0.3	35	77.8	36.2	
Donepezil-Melatonin	3a	16.8[*]	-	1.3	60.5	-	-	41
	3b	2.2[*]	-	3.38	40.5	-	-	
	3c	0.193[*]	-	3.28	56.3	-	-	
Donepezil-Trolox	4a	0.31[*]	43.33[b]	1.62	56.3	63.9	-	42
Rivastigmine-hydroxy cinnamic acid	5a	16.75[#]	37[b]	-	73.2	-	-	106
	5b	24.62[#]	19.5[b]	-	13.2	-	-	
	5c	23.3[#]	69.7[b]	-	77.6	-	-	
	5d	34.28[#]	17.9[b]	-	3.2	-	-	
	5e	23.99[#]	51.7[b]	-	85.3	-	-	
	5f	70.66[#]	18.1[b]	-	2.2	-	-	
	5g	16.4[#]	64.7[b]	-	82.5	-	-	
	5h	14.93[#]	24.1[b]	-	2.2	-	-	
Rivastigmine-Apigenin	6a	4.7[*]	-	1.3	77.9	78.9	73.6	105
Rivastigmine-scutellarin	7a	1.54[*]	-	0.34	-	-	-	103
	7b	0.34[*]	-	0.36	-	-	-	
	8a	0.57[*]	-	1.3	-	-	-	
Tacrine-Silibinin	9a	0.054[*]	-	-	-	-	-	127
Tacrine-Trolox	10a	9.8[*]	-	-	-	-	-	128
Tacrine-Ferulic acid	11a	37[*]	-	-	65.49	-	-	129
Tacrine-Rhein	13a	27.3[*]	-	-	-	-	70.2	130

* IC_{50} (µM)
# % AChE Inhibition at 5 µM
[a] EC_{50} (µM)
[b] %

non-pharmacophoric parts with various natural bioactive fragments which possess antioxidant, anti-inflammatory, metal chelation and neuroprotective activities has helped bring in other AD-relevant biological activities in these AChE inhibitors. These non-pharmacophoric fragments make contact with the peripheral anionic site of the AChE active site and have been optimized to improve the overall binding interactions of the molecules within the AChE binding pocket. Many of the resultant hybrid dual-site binders show mixed type of enzyme inhibition and have been found to have better AChE binding potencies than the parent molecule in addition to having multiple biological functionalities.

Biochemical experiments have reported that AChE facilitates amyloid fibril formation by interacting through the PAS of the enzyme, forming stable AChE-Aβ complexes, which are more toxic than single Aβ peptides. Therefore, dual-site inhibitors that interact with both CAS and PAS appear to be a very effective therapeutic approach, since they can enhance cognition and slow the rate of Aβ-related neurodegeneration.

Tacrine, on the other hand is a relatively small-sized compound and presents opportunities for retaining the entire molecule, while coupling it with bioactive fragments like Silibinin, Trolox, Ferulic acid and Rhein via customized linkers of optimal length. The hybridization approach also allows the introduction of moieties which can counteract the hepatoxicity of this potent but toxic molecule. Molecules with known hepatoprotective properties have been successfully coupled with Tacrine to develop potentially safe, multifunctional hybrid molecules for the holistic treatment of AD.

In this review we have compiled several chimeric series of multifunctional molecules and rationalized their structure activity relationships in depth. The experimental biological data has been tabulated in Table 4. There is currently limited clinical evidence for these chimeric molecules. Nevertheless, multiple insights have been drawn from these studies, which can be used concomitantly to give focused direction to the development of novel, broad-spectrum, effective and safe molecules which can cause complete cure of Alzheimer's disease.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AAPH: 2'-azobis-(2-amidinopropane) dihydrochloride; **ABTS·:** 2, 20-azinobis- (3-ethyl benzothiazoline-6-sulfonate) radical ions; **AChE:** Acetylcholinesterase; **AD:** Alzheimer's disease; **AFMK:** N (1)-acetyl-N (2)-formyl-5-methoxy kynuramine; **ALT:** Alanine transaminase; **AMK:** N (1)-acetyl-5-methoxy kynuramine; **APP:** Amyloid Precursor Protein; **AST:** Aspartate transaminase; **Aβ:** β-amyloid protein peptides; **AβO:** Aβ Oligomers; **BBB:** Blood Brain Barrier; **BuChE:** Butyrylcholinesterase; **CAS:** Catalytic active site; **CNS:** Central nervous system; **DMSO:** Dimethyl sulfoxide; **DPPH:** 1,1-diphenyl-2-picryl hydrazyl (α, α-diphenyl-β-picraylhydrazyl); **HEPES:** 4-(2-hydroxy ethyl)-1-piperazine ethane sulfonic acid; **HSC:** Human Hepatic Stellate Cells; **HuAChE:** Human Acetylcholinesterase; **LPS:** Lipopolysaccharide; **MTDL:** Multi-Target Directed Ligands; **MTT:** 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; **NAP:** S-3- [1-(dimethyl amino) ethyl] phenol; **NFT:** Neurofibrillary tangles; **NMDA:** N-Methyl-D-Aspartate; **ORAC:** Oxygen Radical Absorbance Capacity; **PAS:** Peripheral anionic site; **PDB:** Protein data bank; **ROS:** Reactive Oxygen Species; **TEAC:** Trolox Equivalent Antioxidant Capacity; **TEM:** Transmission Electron microscopy; **ThT:** Thioflavin-T; **Trp:** Tryptophan; **Tyr:** Tyrosine.
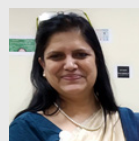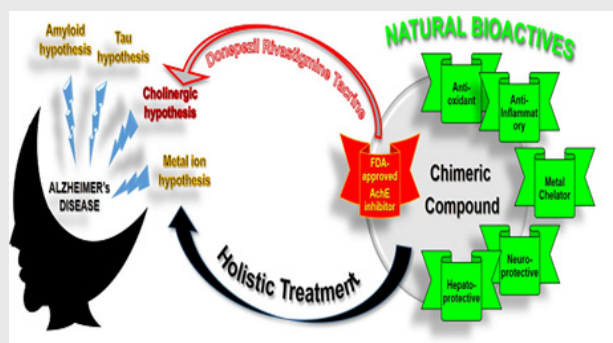
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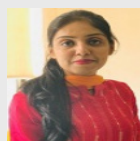
PICTORIAL ABSTRACT

About Authors

Ms. Illham Dhala, Department of Pharmaceutical Chemistry and Quality Assurance, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Mumbai, India.

Dr. Tabassum Khan, Department of Pharmaceutical Chemistry and Quality Assurance, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Mumbai, India.

Dr. Arati Prabhu, Department of Pharmaceutical Chemistry and Quality Assurance, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Mumbai, India.

SUMMARY

- The current FDA-approved single mechanism-targeting anticholinesterase drugs developed for treatment of Alzheimer's disease (AD) have turned out to be palliative rather than curative.
- Many natural medicinal bioactives have the potential to overcome the unaddressed causal mechanisms contributing to AD.
- The first part of the review covers several natural product chimeras of the FDA-approved anticholinesterase inhibitors, donepezil and rivastigmine, developed as efficacious multifunctional anti-AD agents.
- The second part of the review focuses on development of tacrine based natural chimeras, designed with the intention of mitigating tacrine-induced hepatotoxicity.