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In silico studies, synthesis and pharmacological evaluation to explore multi-targeted approach for imidazole analogues as potential cholinesterase inhibitors with neuroprotective role for Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with multiple factors associated with its pathogenesis. Our strategy against AD involves design of multi-targeted 2-substituted-4,5-diphenyl-1H-imidazole analogues which can interact and inhibit AChE, thereby, increasing the synaptic availability of ACh, inhibit BuChE, relieve induced oxidative stress and confer a neuroprotective role. Molecular docking was employed to study interactions within the AChE active site. *In silico* ADME study was performed to estimate pharmacokinetic parameters. Based on computational studies, some analogues were synthesized and subjected to pharmacological evaluation involving antioxidant activity, toxicity and memory model studies in animals followed by detailed mechanistic *in vitro* cholinesterase inhibition study. Amongst the series, analogue **13** and **20** are the most promising multi-targeted candidates which can potentially increase memory, decrease free radical levels and protect neurons against cognitive deficit.

1. Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorder mainly disrupting communication within neural circuits vital for memory and other cognitive functions.[1,2] Past two decades have witnessed extensive research dedicated to unravel the molecular, biochemical, and cellular mechanisms of cognitive deficit.[3] Various hypotheses have been proposed in the pathogenesis of AD like Acetylcholine (ACh) imbalance, amyloid beta (Aβ) production and its aggregation, tau hyperphosphorylation, oxidative stress and others including dysfunctional calcium homeostasis, hormonal, inflammatory-immunological causes. Hence, the development of an effectual therapeutics is of extreme importance.[4]

The deficiency of neurotransmitter ACh in the brain is a crucial factor associated with AD pathogenesis. 'Cholinergic hypothesis' states severe failure of cholinergic function in the central nervous system which contributes to cognitive symptoms. Acetylcholinesterase (AChE) or true ChE predominantly hydrolyzes ACh and is found in high concentrations mainly in the cholinergic brain synapses, at neuromuscular junctions and red blood cells. Butyrylcholinesterase (BuChE) or pseudo/plasma ChE is a non-specific type of cholinesterase enzyme that hydrolyzes different types of choline esters and exists ubiquitously throughout the body, most importantly, in the human liver, blood serum, pancreas and the central nervous system.[5] BuChE mostly hydrolyzes ACh in later stages of AD due to increased BuChE activity and decreased AChE activity, so search of BuChE inhibitors for progressed AD patients can be beneficial.[6] However, the most promising approach for symptomatic relief of AD is to inhibit the AChE, which primarily catalyzes the hydrolysis of ACh, thereby increasing synaptic levels of ACh in the brain. Research so far has not led to breakthrough drug candidate or a single pathway that can cure AD. Till date four acetylcholinesterase inhibitors such as tacrine, rivastigmine, galantamine, donepezil and NMDA receptor antagonist memantine are the only US FDA approved drugs for its treatment.[7–9]

A unique structural aspect of AChE is a deep and narrow gorge, about 20 Å long, which penetrates more than halfway and widens out at its base, where the catalytic triad lies.[10] The peripheral anionic site (PAS) is located at the gorge entry lined by highly conserved 14 aromatic amino acid residues.[11] The X-ray crystallo-

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graphic structure of AChE (PDB ID: 1EVE) has revealed the main binding sites i.e. esteratic subsite (catalytic triad) at the bottom of gorge (Ser200, His440, and Glu327); peripheral anionic site (PAS) with Tyr70, Asp72, Tyr121, Trp279 and Tyr334; an anionic substrate (AS) binding site having Trp84, Tyr130, Phe330 and Phe331, oxyanion hole with Gly118, Gly119, Ala201 and an acyl pocket (Phe288, and Phe290).[12]

In the literature, a new non-cholinergic role of AChE has been suggested; the enzyme co-localizes through its peripheral anionic site with the Aβ peptide deposits in the brain of AD patients and promotes Aβ fibrillogenesis by forming stable AChE-Aβ complexes, so inhibition of AChE will deter the fibrillogenesis.[13,14] The accumulation of Aβ in various areas of the brain exhibits its neurotoxic effects through inflammation, calcium dysregulation and activating microglial cells which in turn cause oxidative stress and neuronal death.[15] Due to the connection of various factors in the disease progression, modulation of a single aspect might not be satisfactory to achieve desired effectiveness. This has triggered our curiosity towards the design of multi-targeted analogues which can interact with both the catalytic triad and peripheral binding sites of AChE, thereby, increasing the synaptic availability of ACh, declining the deposition of Aβ, relieving induced oxidative stress and conferring a neuroprotective role.

Literature survey indicates that series developed possessing various heteroaromatic rings like coumarin, benzimidazole, thiazole, piperidine, indanone, spiroindenoquinoxaline, spirodihydropyridines derivatives showed promising acetylcholinesterase inhibitory activity.[16–21] Rahim et al. reported a series of thirty thiazole analogues (**1**) developed using structural-based drug design tool and evaluated their AChE and BuChE inhibitory potential. All analogues exhibited inhibition with an IC_{50} value ranging from 1.59 ± 0.01 to 389.25 ± 1.75 μM with an improvement in memory as well as cognitive functions including lowering of progressive neurodegeneration.[22] Prompted by these findings we replaced the sulphur moiety of thiazoles with nitrogen and explored the imidazole scaffold and developed a series of 2-substituted-4,5-diphenyl-1H-imidazole analogues (**2**) to target multiple factors simultaneously involved in the etiopathogenesis of AD (Fig. 1).

In this paper, we discuss computational studies which includes molecular docking within AChE active site and *in silico* ADME studies, synthesis, evaluation of antioxidant activity, toxicity and memory model studies in animals as well as *in vitro* cholinesterase inhibition activity of 2-substituted-4,5-diphenyl-1H-imidazole analogues.

2. Results and discussion

2.1. Molecular docking studies with AChE enzyme

In the current study, docking approach has been utilized to explore the AChE active site, foresee orientation (pose) of the designed ligands in the binding pocket and assess the tightness of target-ligand interactions (scoring) using GOLD (Genetic Optimization for Ligand Docking) program with full range of ligand conformational flexibility and selective rotational flexibility of the receptor.[23,24] The docking protocol was validated by reproduction of binding pose of the co-crystallized ligand donepezil in the enzyme active site (PDB: 1EVE; rmsd: 0.600). To further substantiate the protocol, some reported AChE inhibitors (AChEIs): Physostigmine (IC_{50}0.220 μM), Galantamine (IC_{50} 0.623 μM), Alfuzosin (IC_{50} 0.018 μM), Dyclonine (IC_{50} 0.181 μM), Nefazodone (IC_{50} 1.037 μM), Indanone derivatives (IC_{50} 0.0018 μM) were also docked within the active site.[25–28]

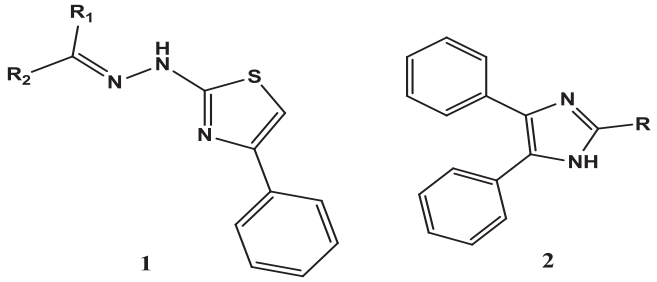

Fig. 1. Substituted thiazole and 2-substituted-4,5-diphenyl-1H-imidazole scaffolds.

The active marketed drug donepezil spans the entire gorge while the docked reported AChEIs occupy bottom and entrance of the gorge. Donepezil exhibited hydrogen-bond interactions through water molecules with the various active site residues such as Tyr70, Asp72, Trp84, Gly117, Tyr121, Ser122, Tyr130 and Ser200. Hence, water molecules play a crucial role in drug receptor interactions.[29] Also; the cyclic ketone group of indanone moiety depicted hydrogen-bond interaction with the Phe288 residue of the acyl pocket. The benzyl ring and indanone ring of donepezil displayed a classical π–π stacking with the indole ring of Trp84 and Trp279 respectively. *N*-Benzyl piperidine fragment displayed π-sigma interaction with aromatic ring of Phe330. Similarly, methoxy group exhibited π-sigma interaction with indole ring of Trp279. π-alkyl interaction of the piperidine nitrogen was observed with Tyr334 residue of the active site. These results indicate positively that the docking protocol was able to acceptably reproduce the crystal pose for co-crystallized donepezil and retain nearly all the interactions that were reported in the literature (Fig. 2). Also, most of the reported AChEIs interacted with the amino acid residues of catalytic triad especially Ser200, His440 and peripheral anionic subsite (PAS) which play a decisive role in enzymatic catalysis and inhibitor binding. Thus, the docked pose of donepezil and reported AChEIs reproduced analogous interactions as mentioned in the literature within AChE active site.[11] From this it can be envisaged that the proposed docking protocol will suitably predict the binding mode of the designed series.

Subsequently, a series of 2-substituted-4,5-diphenyl-1H-imidazole analogues (Table 1) was docked in the AChE active site. The test analogues have reached bottom of the gorge and formed hydrogen bonds through water molecules with similar amino acid residues as observed for donepezil. The docking analysis revealed that imidazole analogues interact with active site primarily through hydrogen bonds and hydrophobic interactions such as pi-pi stacking, pi-pi stacking (T-shaped), pi-sigma, pi-alkyl. Mostly the interactions of ligands were observed with residues present at bottom or middle of the gorge i.e. with the catalytic triad (Ser200) and PAS (Tyr70, Asp72, Tyr121 and Tyr334). Anne Imberty et al. related the distance of hydrogen bonds between a ligand and receptor with the type such as strong, average and weak interactions.[30] Results of imidazole analogues depicted that hydrogen bond interactions were of strong or average nature. In the designed test series, substituents have been varied at the 2-position in the imidazolyl ring system from simple alkyl groups such as methyl, ethyl to phenyl and heteroaryl. Further, the phenyl ring has been substituted with mono-, di-, and tri-substitutions of different functional groups such as nitro, hydroxyl, methoxy, amino and halogens. In case of 2-hydrogen and 2-methyl substituted analogues, interactions were mainly observed through the imidazole ring nitrogen with active site residues Tyr121 and Tyr334. However, on increasing the carbon chain length to ethyl and *n*-propyl showed no contacts. On substituting 2-position of imidazole ring with heteroaryl furan group, depicted interaction with indole ring nitrogen of Trp279 while phenyl ring substitution exhibited interaction with only one water molecule. Interestingly, on substituting the 2-phenyl ring with various functional groups depicted