ABSTRACT

Alzheimer's disease (AD) is a multifactorial disorder, and several pathophysiological mechanisms are involved in its progression and development. Current therapeutic regimens only provide symptomatic relief and are unable to halt the disease progression. The new strategy of multi-targeted inhibitors has been adopted recently, which involves targeting multiple enzymes simultaneously with a single molecule. This strategy was built upon the fact that AD is a multifactorial disorder, linked with multiple targets. Thereby, the design and development of multitarget-directed ligands could prove beneficial for successfully mitigating the disease progression rather than providing symptomatic relief only.

In Part-I of the thesis, we have identified the potential hit bearing *N*-benzylpiperidine nucleus as multitarget-directed ligand against acetylcholinesterase (AChE) and β-secretase 1 (BACE-1) using e-pharmacophore models with their cocrystallized ligands donepezil, and F1M, respectively. Several computational tools, such as phase-based screening, virtual screening (VS), docking-post processing (DPP), and molecular mechanics generalized Born surface area (MM-GBSA), were used to screen the top hit SEW06622 (**S_I3a**). A series of *N*-benzylpiperidine analogs were rationally designed by modifying the identified hit to improve its binding to AChE and BACE-1. The modification of 2,3-diOCH_3 functional group of SEW06622 with several electron-withdrawing (EWGs) and electron-donating groups (EDGs) resulted in an increased binding of compounds to peripheral anionic site (PAS) of AChE. Further, it was observed that the modification by reduction of methanimine to methanamine leads to a significant improvement in binding of compounds to the aspartate dyad of BACE-1. This hypothesis behind designed ligands was corroborated by molecular docking and dynamics simulation studies.

The designed series of ligands (**S_I3a–o** and **S_I4a–o**) were synthesized and characterized by spectroscopic techniques (^{1}H NMR, ^{13}C NMR, and MS), and percentage purity was determined by high performance liquid chromatography (HPLC).

The results of *in vitro* assays indicated balanced inhibitory potential against hAChE and hBACE-1 in sub-micromolar ranges by compounds **S_I3i**, **S_I3j**, **S_I4i**, and **S_I4j**. Additionally, **S_I4i** and **S_I4j** showed excellent displacement of propidium iodide (PI) from PAS-AChE, and inhibition of amyloid beta (Aβ) aggregation in self- and AChE-induced experiments. The inhibition of Aβ was also ascertained by the morphological characterization of Aβ aggregates through atomic force microscopy (AFM) study. Compounds **S_I4i** and **S_I4j** were also found to be devoid of neurotoxicity toward SH-SY5Y neuroblastoma cell lines upto the concentration of 20 μM.

The *in vivo* studies in rats showed amelioration of scopolamine-induced cognitive dysfunction in the Y-maze experiment by compounds **S_I4i** and **S_I4j**. The treatment by compounds **S_I4i** and **S_I4j** in *ex vivo* study of hippocampal rat brain homogenates suggested attenuation in brain AChE levels, and favorable alteration of oxidative stress biomarkers (malonaldehyde, MDA; and superoxide dismutase, SOD). Moreover, **S_I4j** improved $A\beta_{1-42}$-induced cognitive impairment in rats by Morris water maze experiment with significant oral absorption characteristics ascertained by pharmacokinetic analysis. The results of *in vivo* experiments also substantiated the results of *in vitro* assays and computational studies.

Based on the above outcomes, Part-II (Series II-V) was designed using a molecular hybridization approach. The results of Series I revealed that substitution of terminally phenyl moiety with EWGs provided better activity compared to EDGs. Thereby, only EWGs were selected for substitution at the terminal phenyl group in Series II-V. Further, the molecules can be extended farther into the catalytic anionic site (CAS) of AChE by increasing length of the linker chain connecting *N*-benzylpiperidine and terminal phenyl moiety.

Initially, a series of compounds were designing using an open-chain linker (methylenehydrazine-1-carboxamide, Series **II**, **S_{II}9a–h**) to connect *N*-benzylpiperidine and a terminal phenyl group. However, the presence of an imine functionality in ligands (Series II) makes them susceptible to acidic hydrolysis. Thereby, to circumvent this pitfall, and to further improve the inhibitory potential against the targets, the *N*-benzylpiperidine nucleus was connected with another multitargeted pharmacophore, i.e., 1,3,4-oxadiazole using an —NH (Series III, **S_{III}10a–h**), an —$NHCH_2$ (Series IV, **S_{IV}14a–h**), or without (series V, **S_V17a–h**) linkers.

The designed ligands (Series II-V) were synthesized, characterized, and biologically evaluated. Among the tested compounds, **S_{III}10f**, **S_{III}10g**, **S_{IV}14f**, **S_{IV}14g**, **S_{IV}14h**, and **S_V17e** exhibited balanced and significant inhibition of hAChE, hBChE, and hBACE-1 in nanomolar concentration range. The enzyme kinetics study of **S_{III}10g** on hAChE and hBChE elicited a mixed-type of inhibition. Hybrids **S_{III}10g** and **S_{IV}14f** significantly displaced PI from PAS-hAChE along with excellent blood-brain barrier (BBB) permeability in parallel artificial membrane permeation assay (PAMPA). Moreover, **S_{III}10g** and **S_{IV}14f** prominently disassembled Aβ aggregation in self- and AChE-induced thioflavin T assay. The morphological characterization of incubated samples of Aβ aggregates in the presence or absence of an inhibitor confirmed the anti-Aβ aggregatory activity of **S_{III}10g**. Compounds **S_{III}10g** and **S_{IV}14f** were also found to be devoid of neurotoxic liabilities against SH-SY5Y neuroblastoma cell lines in MTT assay at the tested concentrations of 10–80 μM.

Furthermore, compounds **S_{III}10g** and **S_{IV}14f** revealed dose-dependent amelioration of scopolamine-induced cognitive dysfunctions in rats assessed by the Y-maze test. The *ex vivo* studies of hippocampal rat brain homogenates asserted AChE inhibitory and remarkable antioxidant potential of compounds **S_{III}10g** and **S_{IV}14f**. Compound **S_{III}10g** exhibited better efficacy than donepezil at the tested doses on Aβ-induced AD-phenotypic model by Morris water maze test. Interestingly, the lead compound **S_{III}10g**

showed amelioration of cognitive dysfunction by both mechanisms, i.e., scopolamine-induced (cholinergic deficit) and Aβ-induced (Aβ aggregation). In summary, the potential lead **S_{III}10g** has shown improvement in the learning and memory of rats by modulating multiple pathways involved in AD. The Western blot and immunohistochemical analysis of the hippocampal region of rat brains confirmed the lowered Aβ and BACE-1 protein expressions by **S_{III}10g**. The preliminary pharmacokinetic investigation in rats revealed promising oral absorption characteristics of **S_{III}10g**.

The molecular docking and dynamics simulation studies further affirm the significant and stable noncovalent binding interactions of lead compounds **S_{III}10g** and **S_{IV}14f** with PAS and CAS of AChE, and catalytic dyad (Asp32 and Asp228) of BACE-1. The results thus corroborated our hypothesis that elongation of the chain length with suitable placement of the 1,3,4-oxadiazole tucked between the *N*-benzylpiperidine core and terminal phenyl group would significantly enhance inhibitory potential against target enzymes. Overall results signified compound **S_{III}10g** as the promising lead candidate for the treatment of AD. Also, these outcomes have accomplished *N*-benzylpiperidine and 1,3,4-oxadiazole as propitious scaffold for the development multitargeted ligands against AD.

The complexity of the brain and related disorders presents an enormous challenge before researchers to tackle. Therefore, rigorous preclinical evaluation in transgenic animal models need to be performed for successfully establishing these lead candidates in halting the progression of AD rather than providing only symptomatic relief. With more advanced toxicity and molecular biology experiments, these promising candidates could be processed for further clinical trials.