Design, Synthesis, and Biological Evaluation of Some Heterocyclic Multifunctional Molecular Hybrids for Alzheimer's disease Therapy

Thesis submitted in partial fulfilment for the Award of Degree

DOCTOR OF PHILOSOPHY

By

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Chapter 6

(Summary and Conclusion)

6.1 SUMMARY AND CONCLUSION

A multifaceted pathophysiology is involved in AD and interference of a single target to treat the early and late onset of the disease could no longer be considered a good therapy. To treat such a complex disease a molecular candidate that could hit several targets (MTDL) at the same time is required indeed. To strengthen the proposed concept we have developed three series of 44 small molecules (**SD 1-17, 4a-j,** and **5a-q**) as MTDLs following molecular hybridization and in silico approaches. The 5-phenyl-1,3,4-oxadiazole is a proven scaffold for MTDL for AD. The substituted and non-substituted 5-phenyl-1,3,4-oxadiazole-2-thione moiety was used to design novel series of MTDLs.

After successful synthesis and characterization of the molecules, all the compounds were subjected to various *in vitro* biological evaluations such as hAChE, hBChE, hBACE-1, Aβ-aggregation inhibition, neuroprotective and neurotoxicity estimation, PI-displacement to examine AChE-PAS binding and BBB permeability were determined. Further, most active compounds of the series were tested for *in vivo* behavioral studies *via* Y-maze, elevated plus maze, and Aβ-induced Morris water maze test followed by various *ex vivo* biological studies to determine enzymatic and various oxidative stress biomarker levels in brain homogenate. A detailed brain tissue histopathology was also performed to examine the effects of the compounds under investigation on neuronal density in the cortex and hippocampal region of the brain. A western blot analysis and IHC studies of the animal brain homogenate were also performed in the second part of the thesis to examine the molecular expression of the enzymes after treatment with the most active compounds of the series. The In silico molecular docking studies were also performed to see the binding of the compounds within the active site of the enzymes while molecular dynamics studies were performed check the stability of theligand–

protein docked complex. An in silico ADME parameter was also predicted for the active compounds while DFT calculations were performed to check the stability and reactivity of the compounds.

In the first part of the thesis, a series (**SD 1-17**) containing Mannich base-like molecules of substituted 5-phenyl-1,3,4-oxadiazole-2-thione linked with benzyl piperazines were designed and prepared using a molecular hybridization strategy. Compounds **SD-4** and **SD-6** from the first series have shown multitargeting inhibitory effects in a micromolar to nanomolar range. Both compounds have shown ChE-inhibitory potential comparable to standard donepezil and rivastigmine, while BACE-1 inhibition of both compounds was better than donepezil. Along with good enzymatic inhibitory potential, both compounds have displayed anti-Aβ aggregation, PI displacement, and BBB permeability better than donepezil at their tested concentrations.

The *in vivo* behavioral Y-maze test (scopolamine-induced) and Aβ-induced (AD-phenotypic model) Morris water maze test results suggested that the compound **SD-6** has the capability to ameliorate both cholinergic and Aβ-induced memory and cognitive deficits. The *ex vivo* study of the brain hippocampal region suggested an increase in ACh, SOD, GSH, and catalase levels while a decline in AChE and MDA levels in a dose-dependent manner. However, the histopathological examination of brain slices demonstrated an improvement in neuronal density and DG morphology in **SD-6** treated animals. The *in silico* dockings and MD simulation studies also corroborate our *in vitro* and *in vivo* findings. The Quikprop analysis suggested drug-like properties while MM/GBSA results suggested minimum ΔG_{bind} energy of ligand-protein complexes. Finally, it can be concluded based on our experimental findings, that compound **SD-6** is a lead candidate to be explored more in AD therapy.

The second part of the thesis includes the e-pharmacophore-based design and synthesis of II (**4a-j**) and III (**5a-q**) series of compounds. The II series compounds involved a piperazine bioisosteric substation of the piperidine ring present in both donepezil and F1M. The different substituted piperazines were incorporated at the 3-NH of the oxadiazole-2-thione ring via a single alkyl carbon atom that yielded Mannich base-like molecules. From the *in vitro* hChE and BACE-1 inhibition data of II series of compounds, it was observed that the presence of phenyl piperazines instead of benzyl piperazine is favorable for the *in vitro* enzymatic inhibitory activity. While the most promising compounds emerge from the III series **(5a-q)** with promising multitargeting inhibitory potential. Both compounds **5d** and **5f** have shown comparable hAChE & hBChE to that of the donepezil and rivastigmine respectively while the hBACE-1 and Aβ aggregation inhibition of both compounds were better than donepezil. Both the compounds have also shown significant PI displacement, mixed-type non-competitive ChE inhibition, excellent BBB permeability, and were devoid of neurotoxic liabilities.

Both compounds **5d** and **5f,** when tested on scopolamine and Aβ-induced AD mice models suggested a significant reversal of learning and memory functions. Further, *ex vivo* examinations of the brain hippocampal and cortex suggested a comparable reduction in AChE, MDA, ROS, and NO levels while the reduction of TNFα and IL-6 mRNA levels in scopolamine-treated animals suggested anti-neuroinflammatory properties of the compounds. The reduced molecular expression of the Aβ, APP, tau, and BACE-1 levels in western blot analysis indicated the multifunctional properties of the compounds. The immunohistochemistry data also suggested multifunctional properties of both compounds by showing reduced BACE-1 and Aβ expression levels. The histopathology examination of Aβ treated animal's brain also suggested improvement in neuron population. The *in-silico* studies also corroborated our *in-vitro*

and *in-vivo* findings. Overall, it can be concluded that compounds **5d** and **5f** have proven multitargeting capabilities that need to be explored more in designing potent MTDL for AD therapy.

6.2 Scope and future directions

A multifaceted pathophysiology is involved in the development and progression of AD. Currently, available therapeutic regimens for AD treatment only provide symptomatic relief but don't halt its progression. The multitarget-directed ligand approach has already proven proof of concept to deal with the multifaceted nature of AD by interacting with multiple targets at the same time which will lead to halt the progression of the disease.

Herein, we have identified potential lead candidates for the treatment of AD following molecular hybridization approaches and computational e-pharmacophoric approaches. The outcomes of *in vitro* and *in vivo* studies support the accuracy of our MTDL hypothesis. The present work strongly establishes 5-phenyl-1,3,4-oxadizole-2-thiol and *N*-benzylpiperidine moieties as pharmacophoric features that could be utilized further to design a multitargeted lead compound with increased potency for the treatment of AD. Overall results emphasized that **SD-6**, **5d,** and **5f** compounds have multitargeted potential lead candidates with promising inhibitory activities against cholinesterases (AChE and BChE), BACE-1, Aβ, and oxidative stress.

Brain diseases like AD put an enormous challenge to scientists and researchers to tackle the disease effectively owing to the multifaceted nature involved in its development and progression. A rigorous preclinical investigation on transgenic animal models could be initiated with our identified leads. These preclinical studies will help in delivering a promising candidate that would not only provide symptomatic relief but will indeed halt the progression of the disease. More toxicity and biological experiments on our

compounds could be performed to process our compounds for clinical trials are required.

The replacement of Sp2 with Sp3 hybridized molecular attributes on our compounds could further be studied to optimize its better BBB permeability and stable CNS circulation. The designed molecular hybrids could also be coupled with compounds (mannitol and borneol) that could open BBB temporarily for compounds to get partitioned across it, in response to high osmotic pressure caused by these moieties.

Our compounds could also be tested as P-glycoprotein (P-gp) inhibitors to investigate the distribution of the compounds in the CSF as P-gp may efflux the compounds from intracellular to extracellular part which may lead to compound decrease in brain concentration.