Design, Synthesis, and Biological Evaluation of Piperic Acid Template Based Naturally Inspired Novel Multifunctional Molecules for the Treatment of Alzheimer's Disease

Thesis submitted in partial fulfilment for the

Award of Degree

Doctor of Philosophy

By

JITENDRA KUMAR

DEPARTMENT OF PHARMACEUTICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI – 221005
INDIA

Roll No: 16161003 **Year: 2024**

Chapter 6: Summary and Conclusion

Based on the **PA** template, we designed and developed a novel series of glycine amide derivatives and related analogs as a part of our approach to develop naturally inspired multifunctional drugs for AD management. The rationale behind the design of the novel molecules is to have improved cholinergic inhibitory activities and an increase in cLogP of **PA** analogs. The introduction of an amide linker followed by an aromatic or substituted aromatic or heterocyclic or substituted piperazine feature leads to significant improvement in the enzyme's inhibition properties. The developed molecules were tested for *in-vitro* AChE and BChE inhibitory activities. Among the tested compound **6j** showed the highest inhibitory activities for AChE. All the compounds were further subjected to BChE inhibitory activity evaluation. From among all the compound, **6j** showed moderate BChE inhibition properties. Enzyme inhibition studies identified compound **6j** as a lead molecule with preferential AChE inhibition (AChE, IC_{50} = 2.13 ± 0.015 μM; BChE, % inhibition activity = 28.19 ± 0.20 %) compared to the parent molecule **PA** (% inhibition of AChE and BChE at 20 μM, 7.14 ± 0.98 % and 5.87±0.76%, respectively).

The molecular docking studies revealed that **6j** could bind to peripheral and catalytic sites and expose the two different binding modes with AChE. The stabilities of the best complexes between **6j** and AChE and BChE were confirmed with the help of MD studies. The calculated physicochemical properties clearly demonstrated the druggable properties of the developed novel molecules. The data from the enzyme kinetic study proved that **6j** caused competitive inhibition of AChE and mixed inhibition of BChE.

In the pH-dependent UV-based complexation study and mass spectrometric analysis, the lead molecule **6j** also exhibited moderate antioxidant activity in the DPPH assay (% radical scavenging activity = 35.41 ± 1.09%) but did exhibit iron-chelation property.

Based on the enzyme inhibition and antioxidant studies, **6j** was selected as a lead molecule for further *in-vitro* and *in-vivo* studies. The results from cell-based toxicity studies showed cytocompatibility of **6j** with SH-SY5Y cells at all of the tested concentrations.

In the acute toxicity studies, **6j** was found to be well-tolerated and non-toxic up to 500 mg/kg oral dose. Compound **6j** exhibited promising *in-vivo* activity upon administration through the oral route in the scopolamine-induced AD model without affecting locomotor activity in the mice.

We have further extended the structure-activity relationship (SAR) studies of this series of molecules in a calibrated manner to improve upon the ChEs inhibition and antioxidant property to identify the novel potent multifunctional molecule. All synthesized compounds were tested for their AChE and BChE inhibitory properties. The *in-vitro* enzyme inhibition studies suggested that the presence of tryptamine moiety could significantly improve the inhibitory activities of these molecules towards the BChE and the AChE. Several compounds from this developed latest series, for example, **9a-9m**, can be considered selective ChE inhibitors because they turned out to be effective inhibitors of BChE. Compound **9m** exhibited the highest activity for the BChE/AChE in the current series. The enzyme kinetic studies demonstrated a mixed inhibitory nature of **9m** on both the AChE and BChE. The lead molecules 9m also tested against DPPH and metal chelation property but the result suggests that the compound, 6j did not show antioxidant and metal chelation property. The PAMPA-BBB assay demonstrated that **9m** could effectively cross BBB and can reach their target located in the brain. The results from cell-based cytotoxicity studies indicated that the compound had no significant effect on cell viability at all the tested concentrations. Compound **9m** showed promising *in-vivo*

activity in the scopolamine-induced Y- maze AD model without affecting locomotor activity in the mice.

These findings suggest that **6j** and **9m** can act as lead molecules to develop naturally-inspired multifunctional molecules to manage Alzheimer's and other neurodegenerative disorders.