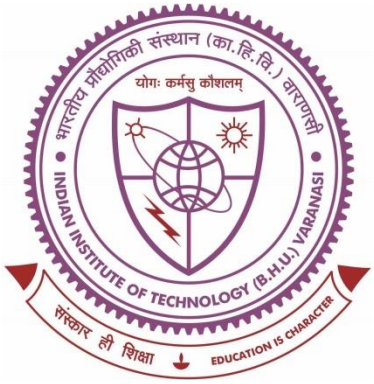
# Structure Activity Relationship Studies on Ferulic Acid Template-Based Novel Molecules to Improve Upon Multifunctional Properties for The Management of Alzheimer's Disease




**Thesis submitted in partial fulfilment for the Award of Degree**

## Doctor of Philosophy

**By**

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## 6 Summary and Conclusions

In this study, we develope a new series of compounds based on the ferulic acid template, aiming to improve the anticholinesterase, antioxidant, and metal chelation properties of the previously reported compounds **EJMC-4e** and **EJMC-10c.** We successfully synthesized various compounds through rigorous medicinal chemistry efforts by introducing multiple functional groups to the ferulic acid structure. These compounds were systematically examined for their effectiveness in inhibiting anticholinesterase activity. Among the synthesized compounds, a range of moderate to strong inhibition of AChE was observed. Compounds **12h**, **12l**, and **12o** notably demonstrated noteworthy inhibitory effects against BChE. The compound **12o** displayed effective inhibition of AChE and BChE enzymes ($IC_{50}$ (μM), AChE, 1.01±0.37 and $IC_{50}$ (μM), BChE, 7.03±0.04). Enzyme kinetics studies further unveil its inhibitory mechanisms. **12o** acts as a mixed inhibitor against both **AChE** and BChE. The findings from the cholinesterase inhibition experiments were corroborated through additional in-silico investigations. Compound **12o** demonstrated a more robust binding affinity with AChE (with a binding energy of -11.5 kcal/mol) in contrast to BChE (with a binding energy of -9.3 kcal/mol). The stability of the most optimal interactions between **12o** and AChE (4EY7) as well as BChE (4BDS) was validated through rigorous molecular dynamics (MD) studies and found that **12o** makes a stable complex with both AChE and BChE. The compound **12o** displayed considerable antioxidant activity and notably $IC_{50}$ value to 6.19 ± 0.33 μM, which represents a remarkable 15-fold decrease compared to the **EJMC-4e** ($IC_{50}$, 94.29 ± 0.19 μM). **12o** effectively displaced propidium iodide from the active site of AChE, showing an efficacy of 21.36 ± 0.14%. The compound **12o** was also evaluated using the PAMPA assay, and the findings from this test indicated the potential of **12o** to cross BBB. Further, **12o** could form a

complex with $Fe^{3+}$ ions, which suggests that it can act as an iron chelator and attenuate excessive iron metal during disease conditions. **12o** exhibits dual inhibitory effect on Aβ aggregation, inhibiting self-induced and metal-induced aggregation. This dual impact might arise from **12o**'s metal-binding, blocking metal-Aβ interaction, or direct Aβ binding, impeding aggregation. At concentrations up to 30 µM, **12o** exhibited no significant cytotoxic effects on PC12 cells. The group treated with $H_2O_2$ alone exhibited a cell viability of merely 46.32%.

In contrast, when treated with **12o** compound at a concentration of 20 µM, cell viability was significantly improved to 79.56% in the PC12 cell line. The compound **12o** regulates the release of ROS and reverses the disturbed mitochondrial membrane potential in human microglial cells. Treatment with compound **12o** notably decreased NF-κB and NLRP3 expression in comparison to LPS+ATP primed cells, indicating its anti-inflammatory and anti-microglial properties. The compound also reduced microglial activation and boosted vimentin levels. These findings highlight compound **12o**'s potential to mitigate neuroinflammation and neurodegeneration. The administration of **12o** reduces mitochondrial and cellular oxidative stress levels within a Drosophila model designed to replicate AD conditions. Compound **12o** exhibited no signs of acute toxicity and hepatotoxicity symptoms at concentrations up to 550 mg/kg when administered to mice. We also investigated the effects of varying doses of **12o** (1 and 5 mg/kg) on alleviating memory deficits induced by scopolamine using the MWM test. Notably, the administration of **12o** at a dosage of 5 mg/kg effectively counteracted scopolamine-induced memory impairment in mice. In *ex-vivo* analyses, **12o** significantly elevated the depleted levels of SOD and CAT. Furthermore, it reduced malondialdehyde (a byproduct of lipid peroxidation), AChE, and BChE in animals treated with scopolamine.

We further extend the SAR and lead to developing a new series of compounds derived from the FA template **EJMC-10c**, explicitly focusing on enhancing anti-cholinesterase, antioxidant, and metal chelation properties. Through meticulous medicinal chemistry efforts, various compounds of FAPIP derivatives were synthesized by introducing diverse functional groups to the ferulic acid structure. Among the synthesized compounds, **24a** demonstrated substantial inhibitory effects on both AChE and BChE, acting as a mixed inhibitor for both enzymes. Molecular dynamics studies validated the stability of interactions between **24a** and AChE/BChE. Additionally, **24a** exhibited potent antioxidant activity, reduced both self and metal-induced Aβ aggregation, and did not induce cytotoxicity in PC12 cells. Furthermore, **24a** demonstrated metal-chelating capabilities, forming a complex with $Fe^{3+}$ ions and suggesting its role in attenuating excessive iron during disease conditions. The PAMPA assay confirmed the efficient penetration of **24a** through the blood-brain barrier, indicating its ability to cross this physiological barrier effectively. Compound **24a** effectively controls the release of reactive oxygen species (ROS) and restores the disrupted mitochondrial membrane potential in human microglial cells. Administration of compound **24a** resulted in a significant reduction in NF-κB and NLRP3 expression compared to cells primed with LPS+ATP, underscoring its anti-inflammatory and anti-microglial properties. Compound **24a** demonstrates the ability to decrease mitochondrial and cellular ROS in the Drosophila model of AD, providing additional evidence of its antioxidant capabilities and reducing oxidative stress. Further, at concentrations up to 550 mg/kg, **24a** displayed no acute or hepatotoxicity symptoms in mice. Moreover, in the Y-Maze test, the administration of **24a** at 5 mg/kg effectively mitigated 6scopolamine-induced memory impairment. *Ex-vivo* analyses highlighted its therapeutic potential by modulating key neurochemical parameters, including AChE, BChE, MDA, SOD,

and CAT. The extensive research findings indicate that compounds **12o** and **24a** demonstrate potential as promising therapeutic agents for AD. These compounds show multifaceted activity by targeting several aspects of the disease, which supports further investigation for developing effective treatment options.