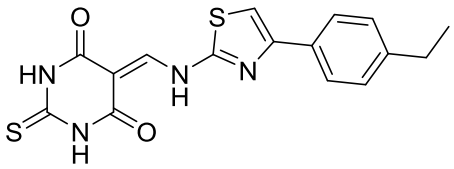
Chapter 6: Summary and Perspective

The work described in this thesis aimed to develop multitargeted small molecules as anti-Alzheimer's agents. It involved the use of *in silico* tools to identify the hits, their synthesis, and evaluation. The structure-based and ligand-based hit(s) identification was performed form 'Zinc15' database molecules. Further, *in vitro* enzyme inhibition studies, compounds afforded ZINC23114578, ZINC21212924, ZINC09613137, and ZINC06455433 as potent MMP-9 inhibitors with IC_{50} values of 81.22±0.23, 122.98±0.15, 242.92±0.12, and 277.61±0.12 nM respectively. The compounds also produced PAS selective AChE inhibition. The hits were accepted as easy BBB permeable, *i.e.*, Pe 10^{-6}, 8.055±0.039, 5.901±0.036, 8.167±0.039, and 4.440±0.041 cm s^{-1} respectively. They had no toxicity at high concentration against human neuroblastoma SH-SY5Y cell line. They had neuroprotective activity against L-glutamate induced excitotoxicity. Finally identified hits as MMP-9 inhibitors afforded π–π stacking interaction(s) with His226, His230, His236, and Tyr248 residues and interacted with Leu188, Tyr245, Pro246, Ala191, and Glu227 amino acids by hydrogen bonding.

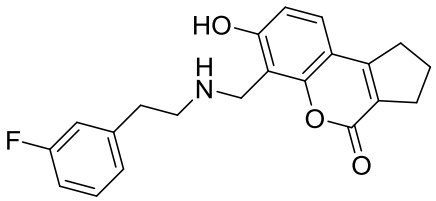
In another study, compounds ZINC20592007, ZINC05354646, ZINC20649934, and ZINC39154782 identified, exhibited PAS selective AChE inhibition with IC_{50} value within nanomolar range, *i.e.*, 482±1.88, 580±1.63, 854±2.65, and 636±1.79 nM respectively. The hits produced significant neuroprotective activity against L-glutamate induced excitotoxicity. The compounds showed high BBB permeability (Pe 10^{-6}), *i.e.*, 5.7±0.20, 9.39±0.36, 5.45±0.79, and 5.29±0.31 respectively and were nontoxic against human neuroblastoma cell. Identified hits as PAS selective AChE inhibitors contributed π–π stacking interaction(s) at PAS site amino acid residues, *i.e.*, Trp286, His287, and

Phe297 and produced hydrogen bond(s) with Tyr72, Asp74, Ser293, Phe295, and Tyr337.



ZINC23114578

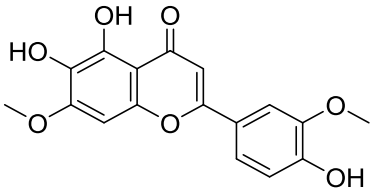
MMP-9, IC_{50}= 81.22±0.23 nM
AChE, IC_{50}= 643.2±0.058 nM
BuChE, IC_{50}= 65026±0.24 nM
Propidium displacement (3 μM)=100%
PAMPA, Pe(10^{-6} cm s^{-1})= 8.055±0.67, CNS+



ZINC20592007

AChE, IC_{50}= 482±1.88 nM
BuChE, IC_{50}= 23954±5.69 nM
Propidium displacement (3 μM)=100%
PAMPA, Pe(10^{-6} cm s^{-1})= 5.7±0.20, CNS+

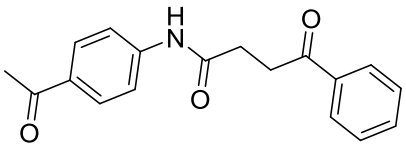
Structure-based pharmacophores integrated with MD simulations afforded ZINC14644839, ZINC00012956, ZINC91332506, and ZINC69775110 as novel TTBK1 inhibitors. Among them, compound ZINC14644839 was found to be a potent inhibitor interacting with Glu77, Gln110, Gly111, Asn113 amino acid residues, similar to TTBK1 inhibitor 2KC. It prevented the formation of NFTs by inhibiting phosphorylation and aggregation of tau protein and PHFs.



ZINC14644839

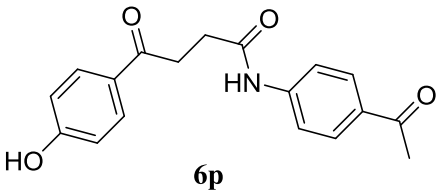
Glide docking score= -10.71 kcal/mole
IFD docking score= -11.29 kcal/mole

4-Oxo-N, 4-diphenylbutanamides were designed, synthesized and evaluated for their biological activities. Compound **6m** of the series was developed as a potential MAO-B inhibitor (IC_{50} value 11.537±0.064 nM). 4-Hydroxy substitution at 4-phenyl ring (compound **6p**) reduced the MAO-B inhibition but enhanced MAO-A and AChE inhibitions.



6m

MAO-B, IC_{50}= 11.537±0.064 nM
MAO-A, IC_{50}= 76.986±0.071 nM
AChE, IC_{50}= 1.221±0.47 µM
BuChE, IC_{50}= 49.93±0.829 µM
Pe(10^{-6}cm s^{-1})= 9.63±0.23, CNS+

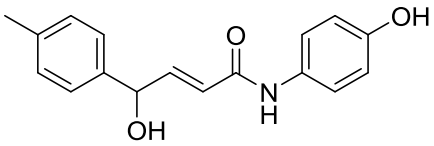


6p

MAO-B, IC_{50}= 25.194±0.017 nM
MAO-A, IC_{50}= 37.212±0.4 nM
AChE, IC_{50}= 0.691±0.43 µM
BuChE, IC_{50}= 58.75±1.25 µM
Pe(10^{-6}cm s^{-1})= 11.35±0.28, CNS+

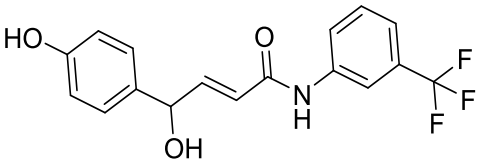
Electron withdrawing groups (*e.g.,* CF_3, COMe) at *meta*-position of N-phenyl was prominent for the potent activity as compared to *para*-substitution in 4-oxo-N, 4-diphenylbutanamides**.** Further, substitution at *para*-hydroxy in 4-phenyl moiety of E-N-aryl-4-hydroxy-4-phenyl but-2-enamides showed better inhibition activity than other groups (*i.e*., H, CH_3, Cl, and OMe) due to the formation of another hydrogen bond with MAO-B.

(E)-N-Aryl-4-hydroxy-4-phenylbut-2-enamides were also designed by modification of 4-oxo-butanamide of 4-oxo-N, 4-diphenylbutanamides by 4-hydroxy-butanamide. Compounds with better *in silico* docking results and calculated physiochemical descriptors were synthesized as racemate and *in vitro* screening was performed. Compounds **7k**, **7u**, and **7v** (IC_{50} values 3.178±0.02, 3.863±0.17, and 5.270±0.13 nM respectively) provided potent MAO-B inhibition comparable to pargyline. Compound **7p** containing 6-quinolinyl amide showed potent MMP-9 inhibition activity (IC_{50} value 167.2±0.074 nM) along with MAO-B and AChE inhibition. *Para*-hydroxy substitution at 4-phenyl moiety of series and 3-CF_3 substitution at N-phenyl moiety provided potent MAO-B as well as AChE inhibitors.



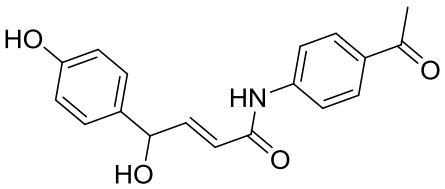
7k

MAO-B, IC_{50}= 3.178±0.02 nM
MAO-A, IC_{50}= 6.222±0.19 nM
AChE, IC_{50}= 0.952±0.26 µM
BuChE, IC_{50}= 32.363±0.21µM
Pe(10^{-6}cm s^{-1}) =4.19±0.14, CNS+



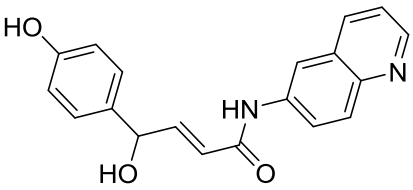
7u

MAO-B, IC_{50}= 3.863±0.17 nM
MAO-A, IC_{50}= 11.229±0.04 nM
AChE, IC_{50}= 0.734±0.20 µM
BuChE, IC_{50}= 22.863±0.36 µM
Pe(10^{-6}cm s^{-1}) = 5.81±0.39, CNS+



7v

MAO-B, IC_{50}= 5.270±0.13 nM
MAO-A, IC_{50}= 23.662±0.07 nM
AChE, IC_{50}= 0.989±0.15 µM
BuChE, IC_{50}= 31.408±0.39 µM
Pe(10^{-6}cm s^{-1}) =2.20±0.048±0.39, CNS+



7p

MAO-B, IC_{50}= 6.953±0.072 nM
MAO-A, IC_{50}= 28.725±0.053 nM
MMP-9, IC_{50}= 167.2±0.074 nM
AChE, IC_{50}= 1.525±0.11 µM
BuChE, IC_{50}= 20.782±0.21 µM
Pe(10^{-6}cm s^{-1}) = 3.77±0.4, CNS+

The etiology of AD is not clear/complex until now. However, deficiency of acetylcholine (neurotransmitter), accumulation of Aβ, tau-phosphorylation, and oxidative stress are the active players of AD. Therefore, the multifactorial nature of AD demands multi-target-directed ligand (MTDLs), by which a single ligand acts on different targets or pathways involved in the disease. The therapy may also diminish the drug-drug interactions (if any) caused by combination therapy. The multitargeted drugable small molecule seems to hold the future to control AD.