Chapter 7

Summary and Conclusions

7.1 Summary and Conclusions

AD is a multifaceted neurodegenerative disorder characterized by cognitive decline and memory deficit. In addition to Cholinergic dysfunction the co-localisation of acetylcholinesterase with amyloid β enhances its aggregation into insoluble plaques. Aβ-metal complexes are known inhibitors of Aβ clearance and are involved in the stabilization of the toxic oligomeric state. Matrix metalloproteinase levels are found to be very low in the healthy human brain, however, injuries and neurodegenerative diseases such as AD are reported to upregulate the levels of MMPs. Aβ oligomer injection into the brain has been shown to increase the MMP activity.

Co-treatment of Aβ oligomers with a non-selective MMP inhibitor, GM6001, via the intracerebroventricular, reduced Aβ oligomers-mediated disruption of the BCSFB. MMP-2 and 9 are found to be involved in pathological cascade of AD, however, the role of MMP-9 in the Aβ based neurotoxicity is not yet clear. AChE is the principal cholinesterase in healthy human brains, but a decrease in its activity coupled with an increase in the butyryl cholinesterase (BChE) activity has been observed in the cortical regions of the AD brain. BChE is expressed at lower levels than that of AChE in both the hippocampus and neocortex (temporal part). It has been shown in several AChE-knockout mice models that BChE can act as a substitute for AChE by hydrolyzing ACh.

The enhanced presence of BChE in Aβ /apolipoprotein E (APOE) complexes compounded with plaques leading to neurodegeneration necessitated the development of BChE inhibitors. Currently approved drugs used in the treatment of AD are cholinesterase inhibitors viz. donepezil (DNP), galantamine, and rivastigmine. A fourth drug memantine, an NMDA antagonist, is used both in monotherapy and as a part of the combination therapy with the AChEI's. A new drug, lecanemab an Aβ targeted monoclonal antibody, has also been approved very recently. Multidisciplinary synthetic strategies are in high demand in the field of current

drug discovery. The design and development of singular chemical entities which can act simultaneously at several molecular targets is a key factor in the multi-target directed ligand (MTDL) drug discovery. With the use of contemporary drug discovery techniques, three different chemical scaffolds were explored in the current study.

In order to create the first series of compounds, a hybrid pharmacophore-based approach was used to identify the pharmacophoric characteristics of the centrally acting cholinesterase inhibitors (such as DNP), metal chelators, and MMP inhibitors. According to the chosen parameters, it was discovered that quinoline derivatives were superior to indole derivatives. Comparing the derivatives substituted at positions 6 and 3, quinolines substituted at position 8 demonstrated greater activity. The compound **41,** substituted at position 8, became the leading molecule out of total series of novel molecules synthesized and fully characterized. The study also showed that compounds containing biphenyl substitution had higher potential for inhibiting MMP-2, AChE, and BChE than the monophenyl analogues. The *in-vitro* inhibition studies i.e., AChE (IC_{50} 4.28±0.15 μM), BChE (IC_{50} 1.32±0.02 μM), MMP-2 (IC_{50} 18.24 ±1.62 nM) of the compound established its potency, while $A\beta_{1-42}$ revealed inhibition of Aβ aggregation. According to *in-vivo* experiments the compound had cognitive effects that were comparable to those of the commercial drug (DNP) at its half of the dose. Additionally, the compound did not impair general locomotion while improving working memory that is dependent on the hippocampal region. The multifactorial mode of action of compound **41** appears to be responsible for its therapeutic potential. The pharmacokinetic studies of the lead compound provided additional evidence that, in addition to the therapeutic activity already mentioned, it also has a respectable ADME profile.

Computational biology and molecular modelling have greatly advanced our understanding of protein-ligand binding. The use of molecular modelling techniques has significantly reduced the cost of drug discovery. PDE9, an enzyme that is highly expressed in both the hippocampal

and cerebral cortex, is specifically involved in the maintenance of cGMP levels through its hydrolysis. A small molecule that inhibits the PDE9 enzyme and subsequently raises the level of cGMP may be promising for the treatment of AD since cGMP is necessary for synaptic plasticity and neuronal transmission. The study covers a number of *in-silico* steps that were carried out to find potential PDE9 inhibitors. From 9Q9, a PDE9 inhibitor complexed with the protein's 3D structure (PDB 6A3N), seven different structure-based pharmacophore models were developed. The hits thus obtained were ranked according to the interactions between proteins and ligands during docking, binding energy calculations, ADMET analysis, and MD simulation. A virtual screening method based on molecular docking was used to find compounds with binding energies greater than 10 kcal/mol. These compounds were then tested for potential clinical candidature using *in-silico* pharmacokinetics and toxicity. Two molecules, **ZINC000001305675** and **ZINC000000377099**, underwent a 100-ns molecular dynamics simulation run and displayed the highest binding affinities of -10.90 and -10.30 Kcal/mol, respectively. Both the hits had excellent physicochemical properties and displayed only minor fluctuations in PL-RMSD and L-RMSF. Thus, **ZINC000001305675** i.e., (2- ((4,8-dimethyl quinazolin-2-yl) amino)-1-methyl quinazolin-4 (1H)-one) and **ZINC000000377099** i.e., (1-methyl-2- ((4-methyl quinazolin-2-yl) amino) quinazolin-4 (1H)-one) may be considered as worthy candidates for *in-vitro* and *in-vivo* studies targeting PDE 9 enzyme.

The next series of designed compounds included a library of twenty novel N-(2-oxo-2-(4-phenylpiperazinyl) ethyl) and N-(2-(4-benzylpiperazinyl)-2-oxoethyl) sulfonamide derivatives. The compounds were finalised after docking studies against all three targets (AChE, BACE-1 and $A\beta_{1-42}$) and were then synthesized, purified & characterized. The IC_{50} against BACE1, AChE & BChE were then determined. Blood brain barrier (BBB) penetration was estimated using PAMPA assay. The Propidium iodide assay was carried out for establishing AChE specificity. Compound **72** was found to be the most potent followed by

compound **73**. $A\beta_{1-42}$ aggregation inhibition assay followed by confocal imaging was also performed. The lead compound, *N-(2-oxo-2-(4-phenylpiperazin-1-yl) ethyl)-[1,1'-biphenyl]-4-sulfonamide*, exhibited potent inhibitory activities against hAChE (IC_{50} = 0.046±0.003 μM, hBChE IC_{50} = 4.202±0.215 μM), BACE1 (IC_{50} = 0.44±0.071μM) as well as significant amyloid-beta aggregation (10 and 20 μM) inhibition. Compound **73**, N-(2-(4-benzylpiperazin-1-yl)-2-oxoethyl) naphthalene-2-sulfonamide with the benzyl linker in its structure, showed higher potency against BACE1 (IC_{50} = 0.26±0.018 μM compared to compound **72.** However, it was less potent against hAChE (IC_{50} = 0.062±0.004 μM, hBChE = 9.162±0.831 μM). The compounds showed optimal blood-brain barrier permeability (compound **72**, *Pe* = 6.627±0.009 x 10^{-6} cm s^{-1}, compound **73**, *Pe* = 6.656±0.06 x 10^{-6} cm s^{-1} in PAMPA assay and displayed neuro protective properties (40 μM) on SH-SY5Y neuroblastoma cell lines. Compound **72** also showed stable interactions with the protein 4ey7 (AChE) throughout the 100 ns of molecular dynamics simulations carried out in the NPT ensemble (T = 310.15 K) at a constant pressure of 1.01 bar with an average RMSD of 1.84 Å.

It demonstrated excellent displacement of propidium iodide from PAS-AChE and inhibition of Aβ aggregation. Morphological characterization of the Aβ aggregates was carried out through confocal fluorescence microscopy studies.

Even at the highest tested concentration of 60 μM, compound **72** was found to be free of neurotoxicity towards SH-SY5Y neuroblastoma cell lines. The Y-maze experiment evaluated the effects of the compound on scopolamine-induced cognitive dysfunctions in a dose-dependent manner. The fact that the total number of arm entries across all groups remained constant shows that scopolamine had no negative effects on the locomotor function of the animals. The scopolamine-induced model supported spatial and short-term memory improving ability of compound **72**. *Ex vivo* and biochemical analysis of the evaluated compounds revealed a significant inhibition of the brain AChE and a positive change in the biomarkers of oxidative

stress via the DCFHDA studies. Morris water maze experiment was used to measure escape latency time (ELT) and the number of platform crossings over a 90-second span on the final five days of treatment. The prolonged ELT after treatment with $A\beta_{1-42}$ and the gradual decrease in the number of platform crossings in the model group of animals compared to the sham group, indicated learning and memory were impaired.

The outcomes of the *in vivo* tests confirmed those from computational and *in vitro* studies. It is expected that this successful series of novel small molecules displaying potent activity against AChE and BACE-1 would translate into comparable cognitive effects to that of the marketed drug (DNP) upon clinical evaluation after further lead development, if any. Pharmacokinetic assessment studies of the active compound further establish a safe DME profile along with the mentioned therapeutic activity whereas the general locomotor activity remained unhindered. The majority of the evidence points to compound **72** as the most promising multi-target directed ligand and possible "lead" in the fight against AD.