Chapter 3

Rationale, Objectives and Plan of Work

3.1 Rationale and Objective

AD is an impenetrable neurodegenerative disorder characterized by cognitive decline and memory deficit. Colocalization of acetylcholinesterase with the amyloid β enhances its aggregation into insoluble plaques. Biometals like Fe, Cu and Zn are considered to be involved in the formation of amyloid plaques. Aβ-metal complexes are known inhibitors of Aβ clearance and are involved in the stabilization of the toxic oligomeric state. AChE is the principal cholinesterase in healthy human brains, but a decrease in its activity coupled with an increase of BChE activity has been observed in the cortical regions of the AD brain. Similarly, Genetic mutations in presenilin-1 and 2 can increase the synthesis of $A\beta_{1\text{-}42}$ close to the lipid bilayer, shifting the balance between $A\beta_{40}$ and $A\beta_{42/43}$ in favour of $A\beta_{42/43}$ production. BACE1 triggers the production of Aβ and its activity is predicted to be closely connected with the pathogenic processes that Aβ mediates. Targeted inhibition of BACE1 helps lower Aβ levels since it has been increasingly clear that a pathological amount of Aβ impairs synaptic processes.

Multi-target drugs are designed to achieve high potencies against multiple targets by combining distinct pharmacophores of various drugs. The present AD therapy approach only relieves symptoms for the first one to two years, ignoring the basic pathogenetic mechanisms that underlie the condition. As a result, the disease still advances despite current treatments. One of the main goals of the current work is the development of multifunctional medications that have the ability to treat symptoms as well as modulate pathogenic elements such the cholinergic pathway, MMPs, Aβ aggregation, BACE1, and PDE-9.The pharmacophores were created using a number of different drug design methodologies, including hybrid drug design taking donepezil into account and inclusion of pharmacophoric properties from other AChE, BACE-1 and MMP-2 inhibitors. Along with HTVS-based screening, fragment-based drug design techniques that use various in-house bioactive fragments active against a number of AD targets were also used. The compounds that were obtained using the aforementioned *in-silico* methods

were then put through a variety of filters, including the molecular property filter, PAINS, and the *in-silico* BBB permeability toxicity filter, before being put through docking studies. Following that, the compounds undergo synthesis, *in vitro* enzyme assays, and *in vivo* studies. The compounds that demonstrated potency in the *in vitro* experiments were further examined for neuroprotection and toxicity in the MC-65 and SHSY5Y cell lines. The impact of AD is heavy in India as well as across the globe while the steady rise in life expectancy increases the future burden of AD. Further, the current treatment regimen only provides symptomatic relief without actually treating the cause. Thus, there is urgent need to venture into AD related drug discovery.

The objective is to produce a successful series of novel small compounds that have been well characterised and exhibit robust activity against AChE and BACE-1 with greater selectivity for AChE than for BChE. By using confocal imaging, the compounds should demonstrate suppression of self and AChE induced $A\beta_{1-42}$ aggregation. The chemicals should, at lower doses, have cognitive effects in the in-vivo trials that are comparable to those of the commercial drug (DNP). Additionally, the active compound should not impair general locomotion while enhancing hippocampally reliant working memory. A detailed rationale of design and synthesis of the respective series are furnished in the corresponding chapters.

3.2 Plan of study

It is divided into the following:

a) Pharmacophore development and virtual screening OR fragment identification and its annealing.

b) Drug-likeness, BBB permeability and toxicity filtration.

c) Docking and validation.

d) Docking, molecular dynamics & simulation.

e) Synthesis and Characterization of synthesized derivatives by elemental analysis, FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectrometry.

f) *In-vitro* AChE, BChE, MMP-2, and BACE 1 inhibition assays.

g) *In-vitro* Evaluation of inflammatory markers.

h) *In-vitro* blood-brain barrier permeation assay (PAMPA).

i) Propiduim Iodide displacement assay

j) Cell line based neuroprotection and toxicity studies using MC-65, SH-SY5Y and VERO cell lines.

k) Self and AChE-induced $A\beta_{1\text{-}42}$ aggregation assay.

l) Confocal imaging technique to visualize the aggregation of $A\beta_{1\text{-}42}$

m)Antioxidant activity evaluation (DPPH assay).

n)*In-vitro* metal chelation assay.

o) Behavioural studies:

(i) Scopolamine model: Y-maze test

(ii) $A\beta_{1\text{-}42}$ induced rat model via stereotaxic method: Morris water maze test

p)ROS estimation using DCFHDA method.

q)*In vivo* neurochemical estimation of AChE.

r)*In vivo* neurochemical estimation of ACh.

s)*In vivo* neurochemical estimation of BACE1.

t)Evaluation of pharmacokinetic parameters.