# 3 Objective rational and plan of work

## 3.1 Objective and rationale

The current treatment protocol for AD provides only symptomatic relief for initial 1-2 years, without addressing elementary pathogenetic factors responsible for the disease(351). Therefore, the disease continues to progress with existing therapies. The development of multifunctional drugs with ability to provide symptomatic treatment along with the modulation of pathogenic factors such as cholinergic pathway, MMPs, Aβ aggregation, NMDA receptors, neuroinflammation and oxidative stress is the one of the major goals of present study. The development of pharmacophore is based on variety of drug design approaches *viz.* hybrid drug design considering donepezil and PQ912, a chemical moiety in phase II of clinical trial, fragment based drug design approaches with different bioactive fragments binding in the receptor cavity and structure based drug design techniques. The molecules obtained by above mentioned *in-silico* techniques were further subjected to different filters like docking, *in-silico* BBB permeability and finally toxicity filter. Four different classes of molecules *viz.* piperazindiones, adamantly, hydroxamates and quinoline/indole analogues were identified for further study. These fully optimized molecules were promoted to synthesis, *in-vitro* enzyme assays and *in-vivo* studies. The potent molecules obtained from *in-vitro* study were further investigated for neuroprotection and toxicity in MC-65 cells along with DPPH based cell free antioxidant assay. The detail rational of design and synthesis of respective series are covered 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, BuChE and MMP-2 inhibition assays.

g) Evaluation of adamantyl analogy on glutamate- and glycineevoked currents in GluN1-1a/GluN2A- and GluN1-1a/GluN2B-containing receptors.

h) Inhibition of currents induced by 100 μM glutamate and 10 μM glycine GluN1-1a/GluN2A containing receptors.

i)Inhibition of currents induced by 100 μM glutamate and 10 μM glycine GluN1-1a/GluN2B containing receptors.

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

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

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

m) AChE-induced $A\beta_{1-42}$ aggregation assay.

n)Antioxidant activity evaluation (DPPH assay).

o)*In-vitro* metal chelation assay.

p) Behavioural studies.

q)Mitochondrial membrane potential determination in brain region.

r)Neurochemical level estimation.

s) Elevated plus-maze, Hole board and Open field tests.

t) Estimation of serotonin, amygdalar monoamines and their metabolites.

u) Flumazenil antagonism on anxiolytic activity in EPM, OFT and hole board.

v) Sedative effect of diazepam and compound **52** in OFT and EPM tests.