AD involves multiple etiological factors, which results in malfunctioning of the brain through several separate but integrated molecular pathways. In this work, we urge the recognition of the multifunctional nature of the disease and paradigm shift of drug development from single to multiple AD targets using a hybrid and bioisosteric approach for the treatment of this life-threatening neurological disorder.

In the Part-I, the thesis deals with designing of several ferulic acids tethered 1,3,4-oxadiazole hybrids as multitarget-directed ligands by molecular hybridization approach. The designed series of ligands (Series I, **5a–o** and Series II, **6a–o**) were synthesized and characterized by spectroscopic (^{1}H NMR, ^{13}C NMR, and MS) and chromatographic (HPLC) analysis. The results of *in-vitro* assays indicated balanced inhibitory potential against hAChE and hBACE-1 in nanomolar ranges by compounds **6j**, **6k**, **6l**, **6m**, **6n**, and **6o.** Additionally, compounds **6j** and **6k** exhibited excellent displacement of propidium iodide from PAS-AChE, brain permeability, inhibition of Aβ aggregation in self- and AChE-induced experiments and neuroprotective effects towards SH-SY5Y neuroblastoma cell lines against Aβ-induced oxidative stress.

Compounds **6j** and **6k** further subjected to *in-vivo* study on rat models showed amelioration of scopolamine-induced cognitive dysfunction in the Y-maze experiment. The *ex-vivo* study on rat brain homogenates suggested that compounds **6j** and **6k** had attenuated the brain AChE levels, and favored the alteration of oxidative stress biomarkers MDA and SOD respectively. Moreover, compound **6j** showed an improved $A\beta_{1-42}$-induced cognitive impairment in rats using Morris water maze experiment owing to significant oral absorption, which was ascertained by pharmacokinetic analysis. The *in-silico* molecular docking studies of compounds **6j** and **6k** also confirmed consensual binding interactions with the active site residues of AChE and BACE-1, which corroborated our claims and found complementary to *in-vitro* and *in-vivo* studies.

In part II, Series III and IV were designed on the basis of the outcomes of series I and II, where molecular hybridization and bioisosteric replacement approaches were utilized. The designing of III and IV series was based on the fusion of pyridyl, piperazine, and 1,3,4 oxadiazole pharmacophoric groups. The pyridine ring was selected on the basis of available literature on Verubecestat, which has the 2-pyridyl ring and reported for proper interaction with aspartate dyad residues of BACE-1. Further, piperazines and 1,3,4-oxadiazoles groups were selected as critical pharmacophoric moieties based on our previous studies and experiments. The designed ligands (Series III and IV) were synthesized, characterized, and evaluated for their *in-vitro* cholineesterases and BACE-1 inhibitory activities. Among the tested compounds, **12n**, **12o**, **13j**, **13k**, **13n**, and **13o** exhibited balanced and significant inhibition of hAChE, hBChE, and hBACE-1 in the nanomolar concentration range. Compounds **13n** and **13o** showed excellent BBB permeability in PAMPA-BBB model, displaced propidium iodide (PI) significantly from PAS-AChE in PI displacement assay, prominently disassembled Aβ aggregates in self- and AChE-induced thioflavin T assay, which was further validated by visualizing the topographical maps of Aβ in presence or absence of inhibitor using AFM study. Compounds **13n** and **13o** also elicited neuroprotective effect on SH-SY5Y neuroblastoma cell lines against Aβ-induced oxidative stress.

Further, the compounds **13n** and **13o** subjected to *in-vivo* studies, which revealed a dose-dependent amelioration of scopolamine-induced cognitive dysfunction in the Y-maze and Aβ-induced rat models. The *ex-vivo* analysis of hippocampal rat brain homogenates asserted remarkable antioxidant potential of compounds **13n** and **13o.** Whereas, the lead compound **13o** also showed amelioration in cognitive dysfunction by both the mechanisms, i.e., scopolamine-induced cholinergic deficit and Aβ aggregation. In summary, the potential lead molecule **13o** showed better antioxidant activity than

standard donepezil along with improved learning and memory by modulating various pathways involved in AD. The pharmacokinetic study also revealed that compound **13o** has promising oral absorption characteristics in the rats.

The molecular docking and dynamics simulation studies were further affirmed the significant and stable binding interactions of lead compounds **13o** with PAS and CAS of AChE, and catalytic dyad (Asp32 and Asp228) of BACE-1. Overall results signified that compound **13o** had promising values for the treatment of AD.

6.1 Scope and future directions

The AD contains diversified pathophysiological mechanisms and pathways. Currently available therapeutic regimens give only symptomatic relief and unable to halt the disease progression. The multitargeted approach was invoked owing to the complex and multifaceted nature of AD, which interacts simultaneously with multiple targets to halt disease progression.

Herein, we have identified potential multifunctional molecular hybrids for the treatment of AD by the molecular hybridization approach. This study establishes ferulic acid, 1,3,4-oxadizole, and 2-pyridylpiperazine moieties are important pharmacophores that can be utilized further for designing of multifunctional leads for the treatment of AD. Overall results emphasized compounds **6j**, **6k**, **13n**, and **13o** as potential multifunctional lead candidates with promising inhibitory activities against cholinesterases (AChE and BChE), BACE-1 enzyme, Aβ aggregation and oxidative stress.

The complexity of the brain and related disorders present an enormous challenge before researchers to tackle. Therefore, rigorous preclinical evaluation in transgenic animal models required to be performed for processing these leads into further clinical trials.