3.1. Rationale and Objectives

Regardless of the substantial advancement in the high-throughput selection techniques and the enormous exertions for drug discovery, numbers of efficacious drugs for AD have not increased considerably since the last decade. Existing treatment comprises of drugs that typically target the augmentation of cognitive functions or the management of neurological symptoms involving neurotransmitter mechanisms. The researchers have made astonishing strides in developing AChE inhibitors like donepezil, rivastigmine, and galantamine to treat AD. The new strategy of multifunctional inhibitors has been adopted recently, which involves targeting multiple AD pathways and enzymes simultaneously. This strategy was built upon the fact that AD is a multifactorial disorder, linked with multiple targets. Thereby, the design and development of multifunctional ligands could prove beneficial for successfully mitigating the disease progression rather than providing symptomatic relief only.

The AChE inhibition remains to be the most promising target for ameliorating cognitive dysfunction in the AD. BACE-1 inhibition is another important target with a significant role in the cleavage of APP, generation of Aβ aggregates, and the formation of senile plaques. Further, as Aβ is linked with oxidative damage, molecules with BACE-1 inhibitory potential could also possess significant antioxidant effect [Mezeiova et al. 2018].

The thesis work has been carried out in two parts.

3.1.1 Designing of Part-I (Series I and II) ligands

The ferulic acid is an isolated constituent from *Ferula foetida*, and has been reported to possess several therapeutic benefits such as antioxidant, anti-inflammatory, free radical scavenging activity, and anti-Aβ aggregator [Nabavi et al. 2015, Sgarbossa et al. 2015]. Ferulic acid is considered to be an important pharmacophoric moiety due to its higher

therapeutic benefits and lower toxic effects. However, its clinical effect has been circumscribed due to lower oral bioavailability and poor BBB permeability. Therefore, the development of novel ferulic acid-based derivatives with multifunctional activities is encouraged.

In Part-I of the thesis work, ferulic acid-based multifunctional hybrids were designed by tethering it with 1,3,4-oxadiazole pharmacophoric moiety. Several scaffolds of ferulic acid-based hybrids were reported in the recent past to possess significant anticholinesterase and antioxidant activities [Benchekroun et al. 2015, Fang et al. 2008, Li et al. 2017]. Our recently designed 1,3,4-oxadiazole hybrids, compound A, B, and C, displayed potential AChE, BChE, and BACE-1 inhibition [Mishra et al. 2019, Sharma et al. 2019b, Tripathi et al. 2019a]. Moreover, these compounds possessed appreciable PAS-AChE binding and remarkable anti-Aβ aggregatory potential. The 1,3,4-oxadiazole ring achieves the best requisite orientation in the active pocket of target enzymes due to its planer conformation and H-bond acceptor abilities. Thereby, 1,3,4-oxadiazole was selected as basic pharmacophoric moiety to be connected with ferulic acid. The adopted design strategy is depicted in Figure 3.1.

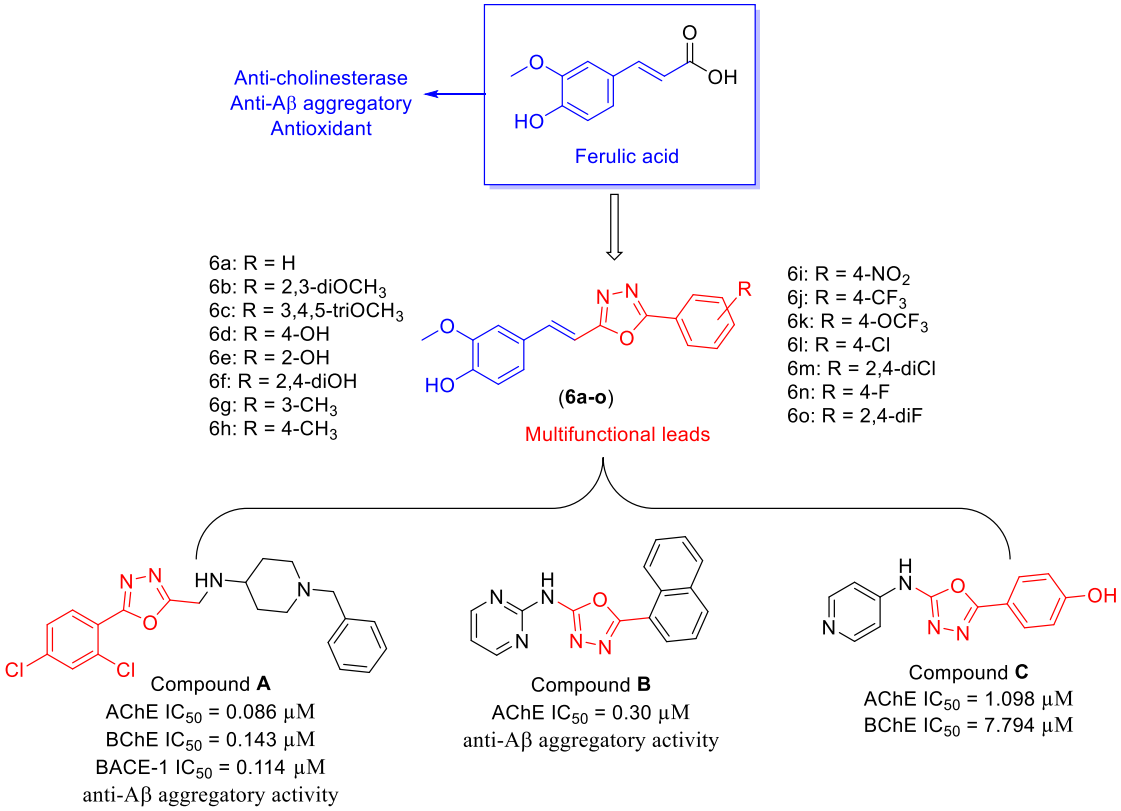

Figure 3.1. Design strategy for Part-I (Series I and II) ligands.

3.1.2 Designing of Part-II (Series III–IV) ligands

The second part (Part-II) of the thesis involved designing multifunctional molecular hybrids based on the structural frameworks of already reported molecules. Verubecestat (compound **D**), a potent BACE-1 inhibitor, and a candidate reached up to the phase III trials for the treatment of AD [Forman et al. 2013, Yan and Vassar 2014]. It contains the substituted 2-pyridyl ring and reported for its significant ability to bind with aspartate dyad residues [Malamas et al. 2010]. But unfortunately, clinical trials of verubecestat were halted recently due to a negative benefit/risk ratio [Egan et al. 2018]. Piperazines (compound **E**) and 1,3,4-oxadiazoles (compound **B**) have been considered as promising scaffolds in the development of new chemical entities due to their reported applications in the design and synthesis of derivatives against the therapeutic targets of AD. Our recent investigations proved the multifunctional activities of 1,3,4-oxadiazole [Tripathi et al. 2019b], piperazines [Tripathi et al. 2019c], and terminal phenyl ring with variable

electron-withdrawing groups (EWGs, compound **F**) [Sharma et al. 2019a] (Figure 1). The adopted design strategy is depicted in Figure 3.2.

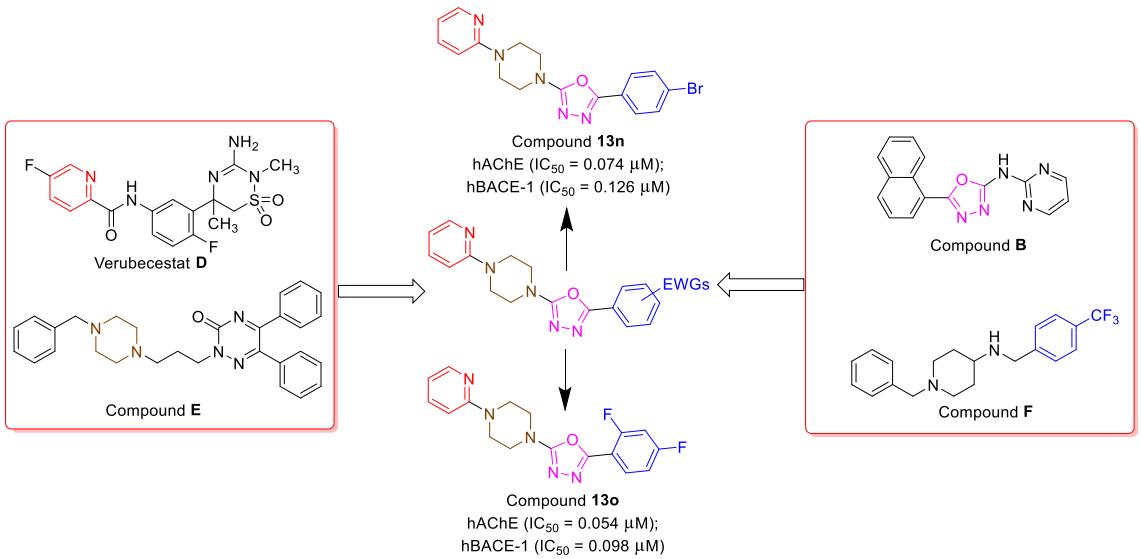

Figure 3.2. Design strategy for Part-II (Series III and IV) ligands.

3.2 Plan of work

3.2.1 Synthesis

- **Series I**: Ferulic acid analogs with substituted 3-(4-Hydroxy-3-methoxyphenyl)acrylohydrazide
- **Series II**: Substituted 2-phenyl-1,3,4-oxadiazole tethered with Ferulic acid
- **Series III**: Pyridyl piperazine analogs with substituted N'-Benzylideneacetohydrazide
- **Series IV**: Substituted 2-phenyl-1,3,4-oxadiazole tethered with Pyridyl piperazine

3.2.2 Characterization of the synthesized compounds

- Physicochemical characterization including melting point and TLC (R_f value)
- Structural confirmation by FT-IR, ^{1}H NMR, ^{13}C NMR, and Mass spectra.
- Determination of percentage purity by HPLC.

3.2.3 Biological Evaluation

A) In-vitro studies

- Cholinesterase (AChE and BChE) inhibition by Ellman assay
- Enzyme kinetics study
- BACE-1 inhibition assay
- Propidium iodide displacement assay
- Parallel artificial membrane permeation assay (PAMPA-BBB)
- Aβ aggregation (self- and AChE-induced) inhibition by thioflavin T assay
- AFM study
- Neuroprotective MTT assay against SH-SY5Y neuroblastoma cell lines

B) In-vivo behavioral studies

- Acute oral toxicity study
- Scopolamine-induced amnesia model: Y-maze test
- Aβ-induced AD phenotypic model: Morris water maze test.

C) Ex-vivo studies

D) Pharmacokinetic studies

- Maximal plasma concentration (C_{max})
- Time to reach maximum plasma concentration (T_{max})
- Area under curve (AUC)
- Elimination half-life ($t_{1/2}$)
- Mean residence time (MRT)

3.2.4 Computational studies

- *In-silico* molecular docking study
- Molecular dynamics simulations study