Chapter 3

(Rationale, Objectives
&
Plan of Work)

3.1. Rationale and Objectives

Current treatment strategies for AD encompass the use of USFDA approved medications like AChE inhibitors (AChEIs) (donepezil, rivastigmine, and galantamine) and NMDA-receptor antagonist (memantine). However, drug therapy for this ailment is still in its infancy and fails to halt the progression of the disease. The new strategy of multi-targeted inhibitors has been adopted recently, which involves targeting multiple enzymes simultaneously with a single molecule. This strategy was built upon the fact that AD is a multifactorial disorder, linked with multiple targets. Thereby, the design and development of multitarget-directed 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 several series of ligands were designed based on the structural framework of donepezil, which is the most potent and highly prescribed drug for the treatment of advanced stage of AD. Donepezil bears *N*-benzylpiperidine as a core group, and its detailed SAR investigation suggested that modification or substitution at benzyl part of *N*-benzylpiperidine resulted in a drastic decline in AChE inhibitory activity [Kryger et al. 1999]. Also, *N*-benzylpiperidine ring of donepezil was observed to be extended into the CAS, and terminal indanone nucleus was oriented in PAS of AChE [Caliandro et al. 2018, Costanzo et al. 2016]. Moreover, *N*-benzylpiperidine ring possesses basic nitrogen moiety, which gets protonated at physiological pH and leads to its increased

affinity towards AChE and aspartate dyad of BACE-1 [Peauger et al. 2017]. All these findings have prompted us to select *N*-benzylpiperidine nucleus as a core group.

The thesis work has been carried out in two parts.

3.1.1 Designing of Part-I (Series I) ligands

The first part (Part-I) of the thesis involved designing a series of ligands using computational screening frameworks, such as pharmacophore modeling, HTVS, docking-post processing (DPP), MM-GBSA, etc. to screen the Maybridge Hitfinder database of 18930 ligands. SEW06622 was identified as the most potential hit bearing *N*-benzylpiperidine nucleus connected with terminal 2,3-dimethoxyphenyl group via methanimine linker. The AChE was demonstrated to be involved in the promotion of Aβ aggregation by binding specifically to the PAS residues. AChEIs bound to the active site and PAS could not only result in AChE inhibition but also prevent the Aβ production and deposition [Inestrosa et al. 1996]. Therefore, 2,3-dimethoxy functionality present on the phenyl group of SEW06622 was substituted with several electron-donating (EDGs) and withdrawing (EWGs) groups to improve the binding characteristics with PAS and increase the inhibition of Aβ aggregation. Further, binding with aspartate dyad residues of BACE-1 was cemented with the reduction of central methanimine functionality to methanamine, while retaining the PAS-AChE binding characteristics (Figure 3.1).
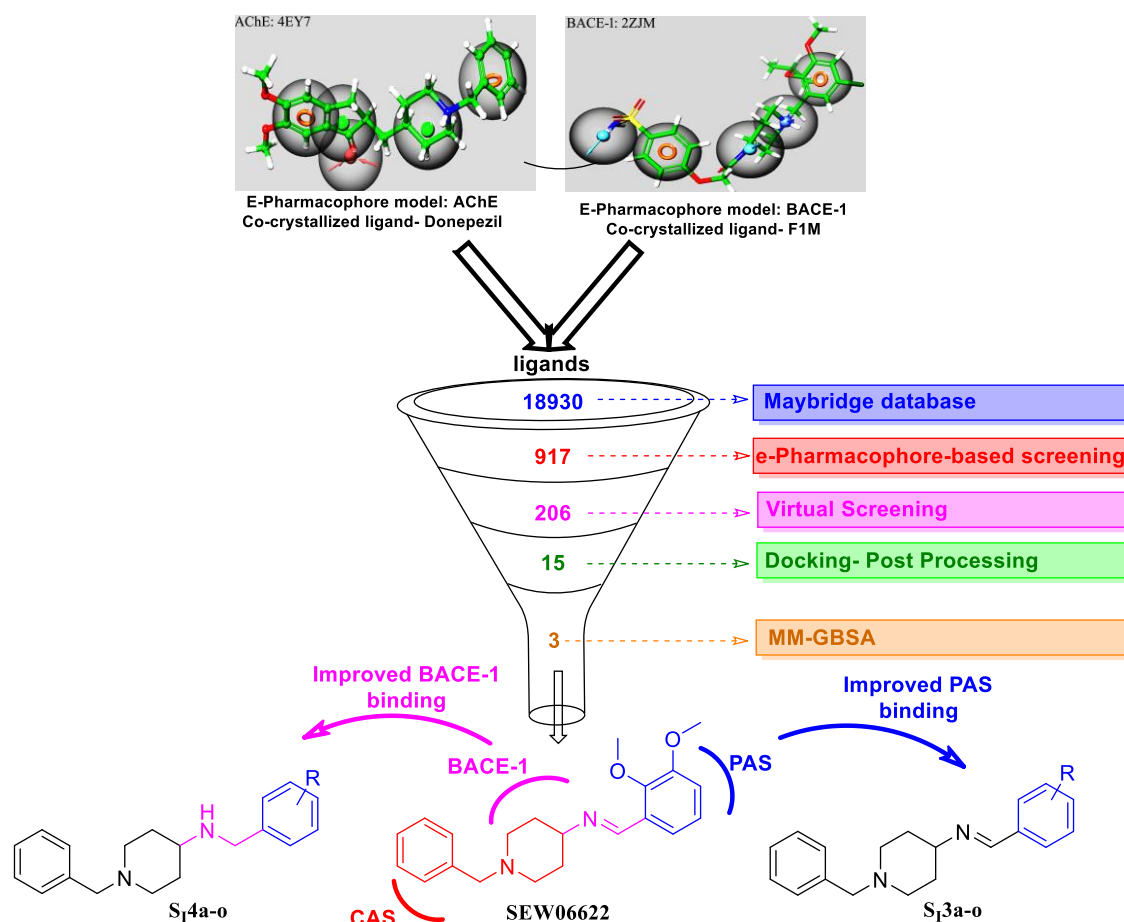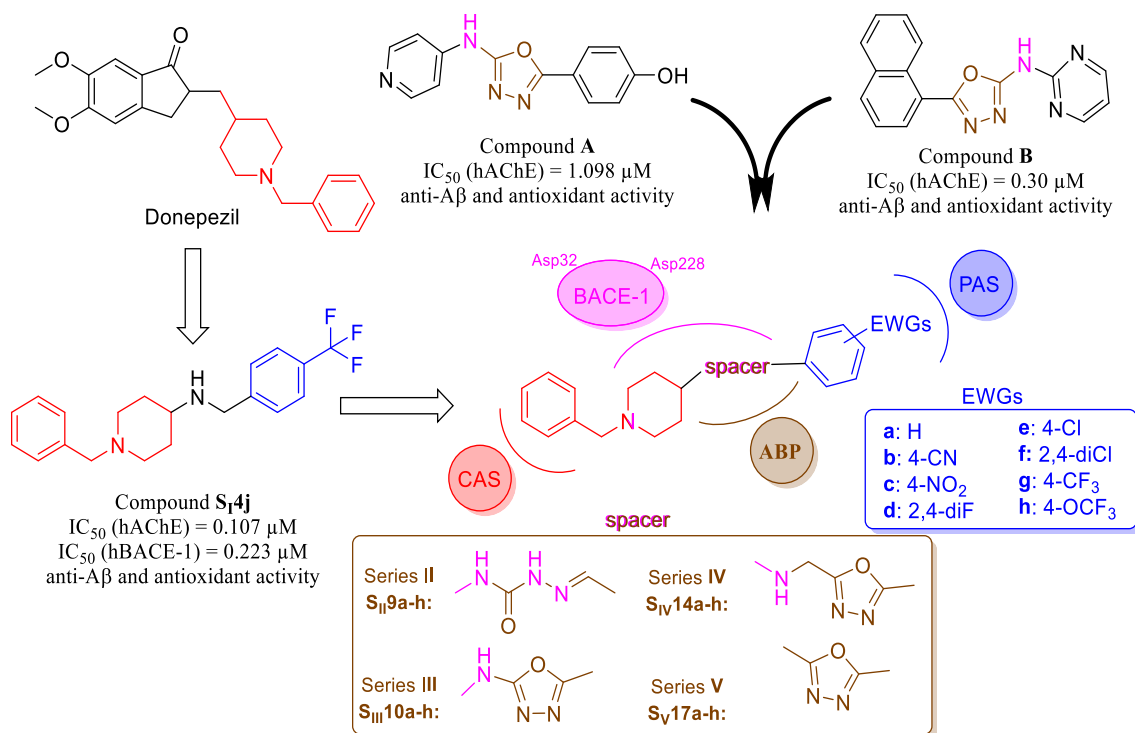
Figure 3.1. Design strategy for Part-I (Series I) ligands.

3.1.2 Designing of Part-II (Series II–V) ligands

The second part (Part-II) of the thesis involved designing ligands based on the outcomes of the Series I, which revealed the remarkable inhibitory activity of AChE, BACE-1, and Aβ aggregation, by compounds in which terminal phenyl group was substituted with EWGs (particularly 4-CF₃, compound S_I4j) rather than EDGs. Thereby, only EWGs were selected for substitution at the terminal phenyl group in Series II-V. The outcomes from Series I ligands, also leave a room for further optimization by increasing length of the linker chain, thereby, assisting the extension of *N*-benzylpiperidine moiety further deep into the CAS. Linker with —NH functionality was given prominence based on its ability to increase the affinity of molecules toward BACE-1. Also, the presence of a lone pair of electrons at linker site would give an edge to the designed molecules by imparting antioxidant potential [Markesbery 1997]. Therefore, based on these

conjectures, we have initially tethered the pharmacophores by an open-chain spacer group, methylenehydrazine-1-carboxamide (Series II, **S$_{II}$9a–h**). However, compounds **S$_{II}$9a–h** were prone to the acidic hydrolysis and degradation owing to the presence of an imine functionality [Cordes and Jencks 1962, Cordes and Jencks 1963]. Several other hybrids reported by our lab particularly, compounds **A** and **B** signified remarkable propidium iodide (PI) displacement from PAS-AChE [Mishra et al. 2019, Tripathi et al. 2019]. The planer conformation and H-bond acceptor ability of 1,3,4-oxadiazole make it a suitable candidate for achieving the requisite orientation within the active pocket of target enzymes. These findings prompted us to replace the open-chain linker with 1,3,4-oxadiazole hybrids. Thereby, we have designed series of compounds bearing *N*-benzylpiperidine and substituted 5-phenyl-1,3,4-oxadiazoles tethered with an —NH linker (Series III, **S$_{III}$10a–h**), an —NHCH$_2$ linker (Series IV, **S$_{IV}$14a–h**), and without linker (Series V, **S$_V$17a–h**). The adopted design strategy is depicted in Figure 3.2.

Figure 3.2. Design strategy for Part-II (Series II–V) ligands.

3.2 Plan of work

3.2.1 Computational studies

- Pharmacophore modeling
- HTVS and DPP
- MM-GBSA
- Molecular docking study
- Molecular dynamics simulations study

3.2.2 Synthesis of N-benzylpiperidine analogs

- **Series I**: Substituted phenyl methanimines/methanamines
- **Series II**: Substituted benzylidenehydrazine-1-carboxamides
- **Series III**: Substituted 5-phenyl-1,3,4-oxadiazoles tethered with an —NH linker
- **Series IV**: Substituted 5-phenyl-1,3,4-oxadiazoles tethered with an —NHCH$_2$ linker
- **Series V**: Substituted 5-phenyl-1,3,4-oxadiazoles tethered without a linker

3.2.3 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.

3.2.4 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

- Neurotoxic liabilities against SH-SY5Y cell lines by MTT assay

B) In vivo and ex vivo studies

- Acute oral toxicity study

- Scopolamine-induced amnesia model: Y-maze test

- *Ex vivo* studies: AChE estimation and antioxidant activity

- Aβ-induced AD phenotypic model: Morris water maze test

- Western-blot analysis

- Immunohistochemical analysis

C) 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)

3.3 Significance of the study

Currently, available therapeutics for AD provides only modest improvement in memory and cognitive function, but they do not prevent progressive neurodegeneration. The etiology of AD remains elusive, but multiple factors, such as ACh, Aβ, BACE-1, and oxidative stress biomarkers, play a significant role. Therefore, from both fundamental and practical perspectives, ligands affecting multiple pathways simultaneously for the treatment of this fatal neurodegenerative disorder are desirable. Thus, Our hypothesis states that by targeting multiple pathways (AChE, BChE, BACE-1, and oxidative stress) simultaneously, the lead candidates could successfully block the actual progression of AD.