Chapter 7

Discussion

7.0 Discussion

Alzheimer's disease (AD) is the world's most common neurodegenerative disease that leads to a gradual loss of cognitive function. The cause of disease is presumably the impairment of cholinergic and glutamatergic excitatory neurotransmission, an important process in learning and memory, and is also associated with an increase in amyloid-β peptides and hyperphosphorylated tau proteins. A vital strategy to treat AD, therefore, may be the development and establishment of target combination, lead generation, and optimization of multi-target directed ligands (MTDLs). The current therapeutic collection of AD contains two classes of medications: the cholinesterase inhibitors (ChEIs), which include donepezil (approved for use in mild to severe AD) and galantamine and rivastigmine (both approved for use in mild to moderate AD); and the non-competitive NMDAR antagonist memantine (approved for use in moderate to severe AD). Combining ChEIs and memantine could offer greater benefits on cognition, behavior, and global outcomes. Nevertheless, these drugs provide only symptomatic benefits in AD.

Thus, the MTDLs should be designed with more rational and holistic view of target combination, ligands selection, and the balance of desired activities to enhance the efficacy, safety and therapeutic potential.

The *de novo* drug design, based on fragment growing strategy for the identification of potent chemical entities for AChE inhibition was fruitful. The development of potent scaffolds and their evaluation was the focus of study by using tiny fragment molecules. Fragments which were having good binding interaction to AChE active site were selected and allowed to grow in LigBuilder 2. The obtained optimized hit was

chemically modified to get improved binding interactions. Increase in potency from hit to optimized hit compound have proved the rationality of design strategy and inspired to search compounds with superior ChEs inhibitory profile. Using this approach, we designed, synthesized 40 compounds (**43-62**; **66-85**) and characterized all derivatives with sophisticated analytical techniques. Compound N-(3-(4-(4-chlorophenyl)-1,2-diazaspiro[4.5]dec-2-ene-3-carbonyl)phenyl)benzamide (**67**: *ee*AChE = 0.464 ± 0.166 µM; *eq*BuChE = 0.754 ± 0.121 µM; hAChE = 0.472 ± 0.042 µM; P_e = 13.92 ± 0.022 x 10^{-6} cm s^{1}) was most potent among all and showed displacement of propidium iodide from PAS-AChE in *in-vitro* assays. The X-ray crystal structure of compound N-(3-(4-(3-fluorophenyl)-1,2-diazaspiro[4.5]dec-2-ene-3-carbonyl)phenyl)benzamide (**73**) established the formation of spiropyrazoline derivatives. Hit (compound **44**) and optimized hit (compound **67**) were further assessed to support the design approach. At 50 µM, compounds N-(3-(3-(4-chlorophenyl)-1H-pyrazol-5-yl)phenyl)benzamide (**44**) and N-(3-(4-(4-chlorophenyl)-1,2-diazaspiro[4.5]dec-2-ene-3-carbonyl)phenyl)benz amide (**67**) exhibited about 90 % cell viability and diminished the metal induced $A\beta_{1-42}$ aggregation effectively at 20 µM. In i*n-vivo* behavioural study, compound **67** demonstrated better spontaneous alternation score and novel arm entries without influencing the locomotor activity. The rise in brain penetration of the compound **67** by 2.05 times as compared to compound **44**, thus strengthening the design strategy. The ChE's inhibitory potency, BBB permeability, promising data from the MC65 cell line, propitious *in-vivo* behavioural and brain penetration analysis, the compound N-(3-(4-(4-chlorophenyl)-1,2-diazaspiro[4.5]dec-2-ene-3-carbonyl)phenyl)benzamide **(67)** strongly proves an ideal hit for the future development of new chemical entities against Alzheimer's disease.

We have also delineated a scaffold hopping guided MTDLs strategy for the development of multifunctional agents against AD. A series of thirty-three novel structural analogs (**29A-39A** and **86A-107A**) of benzo[d]oxazol-5-amine were synthesized. The synthesized compounds were initially investigated for their ability to inhibit ChEs (*ee*AChE and *eq*BuChE) and blood-brain barrier permeability. Amongst all the tested derivatives, (4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-bromophenyl) methanone (**92A**, *ee*AChE IC_{50} = 0.052 ± 0.010 μM; *eq*BuChE IC_{50} = 1.085 ± 0.035 μM) and (4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-fluorophenyl)methanone (**95A**, *ee*AChE IC_{50} = 0.073 ± 0.010 μM; *eq*BuChE IC_{50} = 0.817 ± 0.021 μM) possessed the most significant inhibitory profile. Moreover, SAR studies indicated various structural features which are responsible for the inhibitory potency of the derivatives. In particular, our findings revealed the requirement of carbonyl linker between the piperazine and aryl ring bearing electronegative substituent at *para* position. The molecular docking analysis demonstrated that lead analog (4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-bromophenyl)methanone (**92A**), well-resided in the PAS and CAS sites with optimal binding orientation within the active site of AChE, which was further supported by propidium iodide displacement assay (**92A**, 40.74 % inhibition at 50 μM). Compound **92A** at 20 μM concentration, exhibited higher anti $A\beta_{1-42}$ aggregation property in self-induced and AChE-induced assays. The *in-vitro* neuroprotection assay on SH-SY5Y cell lines of the lead compound demonstrated better cell recoveries at 40 and 80 μM concentrations. Furthermore, the potent AChE inhibitor ((4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-bromophenyl)methanone **92A**) with neuroprotection and anti $A\beta_{1-42}$ aggregation properties presented dose-dependent increase in % spontaneous alternations in scopolamine-induced amnesic rodents. In the

Y-maze test, the compound at a dose of 10 mg/kg showed no significant difference with DNZ (5 mg/kg) treated group and *ex-vivo* AChE activity indicated significant inhibition of brain AChE. The Morris water maze test also indicated that compound **92A** improved spatial memory and cognitive abilities in $A\beta_{1-42}$ treated rats. The promising data from these *in-vivo* and *in-vitro* experiments led us to report compound (4-(5-aminobenzo[d]oxazol-2-yl)piperazin-1-yl)(4-bromophenyl) methanone (**92A)** as a potent analog for the further development of disease-modifying agents against AD.

To establish the multi modal approach with ChEs, NMDAR antagonism, $A\beta_{1-42}$ peptide aggregation and neurotoxicity, we investigated novel triazole bridged cycloaryl hybrids (**22B-28B** and **31B-37B**). All the fourteen derivatives were successfully synthesized with good yields and assessed with various experimental approaches on AD relevant targets i.e. inhibitory potencies on enzymes (AChE and BuChE), NMDAR (GluN2B). Furthermore, anti Aβ aggregation and neuroprotective profiles were also evaluated. It is evidenced that the adamantine containing analogs (**31B-37B**) demonstrated better inhibitory profiles in comparison to cycloheptane analogs (**22B-28B**). The *para*-bromo substituted derivative of adamantine analog (N-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)adamantan-1-amine (**32B**): AChE IC_{50} = 0.086 μM; BuChE IC_{50} = 2.54 μM; GluN1-1b/GluN2B IC_{50} = 3.41 μM) and *para*-chloro substituted derivative (N-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)adamantan-1-amine (**33B**): AChE IC_{50} = 0.135 μM; BuChE IC_{50} = 3.79 μM; GluN1-1b/GluN2B IC_{50} = 3.29 μM) turned out to be potent multi modal ligands. Furthermore, these derivatives also displayed notable anti-Aβ aggregatory activity in self- and AChE-induced experiments and Neuroprotection assays at concentrations of 20 μM and 10 μM, respectively. The presence of triazole ring in these hybrids (**32B-33B**) may be considered as responsible for the better

activities against Aβ aggregation and neuroprotection. Compounds N-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)adamantan-1-amine (**32B**) and N-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)adamantan-1-amine (**33B**) of the triazole bridged cycloaryl hybrids can be designated as lead compounds with balanced potency and drugability.