Chapter 6
(Conclusion)

6.1. Conclusion

Owing to the multifaceted pathophysiology involved in AD development and its progression, a single molecule-multiple target approach has been adopted. The current therapy of AD provides only symptomatic relief hence MTDL strategy is believed to be advantageous in halting development and progression of AD. Using e-pharmacophore models of hAChE and hBACE-1 enzymes with their co-crystallized ligands like donepezil and F1M, a potential hit bearing quinazoline moiety was identified. The pharmacophoric features of the identified hit (**ZINC000015441499**) containing quinazoline moiety was analysed as it was quite different from the identified hit (**SEW06622**) from the previous studies. Also, the MD simulation analysis of both these identified hits revealed that **ZINC000015441499** hit showed better interaction fraction and binding profile as compared to the previously identified hit (**SEW06622**). Based on this identified hit (**ZINC000015441499**), a series containing quinazoline nucleus along with desired pharmacophoric features was designed and evaluated for hAChE and hBACE-1 inhibitory potential. The molecular docking and MD simulation studies of compound **AV-2** showed good binding profile and stability against both the hAChE and hBACE-1 enzymes.

The *in-vitro* results suggested that three compounds **AV-1**, **AV-2** and **AV-3** from the series showed promising activities against hAChE and hBACE-1 in sub-micromolar range. Amongst which compound **AV-2** demonstrated good PI displacement from PAS-AChE and also demonstrated good anti-Aβ aggregation potential in both self and AChE-induced experiments. At a maximum tested concentration (80 μM), compound **AV-2** showed no neurotoxicity against differentiated SH-SY5Y cell lines.

In-vivo, studies including the scopolamine-induced amnesia model (Y-maze test) and Aβ induced Morris water maze test demonstrated that compound **AV-2** significantly ameliorated learning and cognitive impairments in a dose-dependent manner. *Ex-vivo* and biochemical analysis of investigated compound suggested significant inhibition of AChE and an elevated ACh levels in the hippocampal brain homogenate. The oxidative stress biomarkers like MDA was declined and similarly SOD, GSH, and catalase levels were improved in a dose dependent manner after treatment with compound **AV-2**. Moreover, the western blot analysis suggested a decrease in the molecular expression levels of APP, Tau, and BACE-1 which indicated the multi-targeting capability of compound **AV-2**. The IHC studies also inferred reduced molecular expression of Aβ and BACE-1 levels in hippocampal brain sections. The pharmacokinetic studies suggested that the compound **AV-2** showed good oral absorption along with *in-vivo* brain permeability. Overall findings indicated that the compound **AV-2** was considered to be a promising lead and multi-targeting agent for AD therapy and can be explored further.

In second part of the thesis, as complex pathophysiology is responsible for AD development and its progression, the MTDL ligands containing quinazoline scaffold were rationally designed based on the lead identified from our previous work and optimized through bioisosteric replacement and a molecular hybridization approach. The designed derivatives were synthesized, characterized, and biologically evaluated against hChE and hBACE-1 enzymes. Amongst them, compound **AK-2** has shown the most encouraging results (hAChE, IC_{50}: 0.283 μM; hBChE, IC_{50} > 10 μM; hBACE-1, IC_{50}: 0.231 μM) as compared to the standard donepezil and rivastigmine. The compound **AK-2** also showed Aβ aggregation inhibition potential with non-neurotoxic liabilities and BBB permeability.

The *in-vivo* Aβ-induced ICV rat model of AD revealed a significant improvement in learning and memory after being treated with compound **AK-2**. The *in-vivo* AD

drosophila eye phenotypic model also suggested a significant reversal of drosophila eye phenotype post **AK-2** treatment. The *ex-vivo* IHC analysis also demonstrated decreased molecular expression of both Aβ and BACE-1 protein levels indicating the multi-targeting nature of the compound **AK-2**. The brain histopathology demonstrated an intact neuronal population. The *in-silico* findings also corroborated the *in-vitro* and *in-vivo* results. The pharmacokinetic studies demonstrated good oral bioavailability of compound **AK-2**. The experimental findings of both the compounds (**AV-2** and **AK-2**) suggested that these compounds demonstrated good MTDL activities in AD. However, we notice that lead optimization from compound **AV-2** to **AK-2** improved *in-vivo* BBB permeability (*In-vivo* BBB permeability of **AK-2** at 10 and 20 mg/kg doses = 0.663 and 0.977 μg/mL; **AV-2** at 10 and 20 mg/kg doses = 0.015 and 0.054 μg/mL). Overall, the compound **AK-2** showed multi-targeting effects, and therefore the quinazoline scaffold can be explored further to design MTDL for AD therapy.

6.2. Scope and future directions

AD development and progression are influenced by its complex pathophysiology. The current treatment regimens for AD only address the symptoms; they do not stop the disease's progression. By interacting with several targets simultaneously, the multitarget-directed ligand strategy has already demonstrated proof of concept in addressing the complex nature of AD and slowing the disease's progression.

Here, using computational e-pharmacophoric and molecular hybridization techniques, we have found putative lead candidates for the treatment of AD. The results of research conducted both *in-vivo* and *in-vitro* to validate our MTDL concept. The current study clearly identifies the pharmacophoric properties of quinazoline and benzylpiperazine moieties, which may be further exploited to create a multi-targeted lead drug with enhanced potency for the treatment of AD. Overall, the data showed that the compounds

AV-2 and **AK-2** are multi-targeted potential lead candidates with encouraging inhibitory actions against oxidative stress, BACE-1, Aβ, and cholinesterases (AChE and BChE).

Because of the complexity of the factors involved in the onset and progression of brain illnesses like AD, scientists and researchers confront significant obstacles in their efforts to successfully treat the condition. With our found leads, a thorough preclinical study on transgenic animal models might be started. These preclinical investigations will aid in the development of a promising candidate that will actually stop the disease's progression in addition to relieving symptoms. To prepare our drugs for clinical trials, additional toxicological and biological investigations may be necessary.