Summary and Conclusion

Chapter 6

6. Summary and Conclusions

In summary, we designed and synthesized novel diarylurea-hydroxyamidine analogs as multifunctional agents for treating AD. These compounds were evaluated as potential ChEs (*h*AChE and *eq*BChE) inhibitors. Among the synthesized compounds, compounds **3q** and **6e** exhibited the most potent AChE inhibition with (**3q** IC_{50}: 1.72 ± 0.15 μM, **6e** IC_{50}: 0.91 ± 0.016 μM), and BChE inhibition (**3q** IC_{50}: 6.69 ± 0.28 μM, **6e** IC_{50}: 1.19 ± 0.026 μM respectively). The synthesized compounds, **3q** and **6e** were further evaluated for enzyme kinetics study. The data from the enzyme kinetic study proved that **3q** and **6e** showed mixed inhibition of AChE and competitive inhibition of BChE. Significantly, compounds **3q,** and **6e** exhibited remarkable antioxidant activity (IC_{50} 16.15 ± 1.05 & 15.17 ± 0.07 μM, for **3q** & **6e**, respectively) in the DPPH assay among all tested molecules. PAMPA experiments indicated that compounds **3q** and **6e** exhibited permeability (Pe) values exceeding 4, suggesting their potential to cross the blood-brain barrier and effectively target brain sites. Interestingly, **3q** and **6e** could effectively inhibit self-induced full-length tau and $A\beta_{1-42}$ aggregation. **3q** and **6e** were found to curtail microglial cell proliferation, mitigating the damage arising from LPS and ATP-induced ROS and MMP. In addition, acute toxicity tests in mice showed that **3q** and **6e** had no toxicity at the dose of 2000 mg/kg. The **3q** and **6e** inhibited NLRP3 inflammasome and reduced microglial cell proliferation in response to LPS and ATP-induced ROS and MMP. It also demonstrated the ability to reverse scopolamine-induced amnesia by enhancing spatial and cognitive memory in the AD mice model. Additionally, compared to the scopolamine treatment group, these compounds upregulated neuroprotective biomarkers, including BDNF and TRKB. The above results indicate that compounds **3q** and **6e** could be new multi-target-directed ligands for treating AD.

We further extend the SAR and lead to developing a new series of compounds derived from the RIV fragment template **15d** and **15e**, explicitly focusing on enhancing anti-cholinesterase, antioxidant, and metal chelation properties. In summary, we designed and synthesized novel tryptamine-RIV hybrid analogs as multifunctional agents for treating AD. These compounds were evaluated as potential ChEs (*h*AChE and *eq*BChE) inhibitors. Among the synthesized compounds, compounds **15d** and **15e** as the lead molecules with a potent inhibitor against AChE (**15d**, IC_{50}**:** 0.99 ± 0.009 nM and **15e**, IC_{50}: 7.97 ± 0.016 nM and BChE (**15d**, IC_{50}: 27.79 ± 0.21 nM and **15e**, IC_{50}: 0.79 ± 0.005 nM respectively), compared to the marketed drug Riv (AChE, IC_{50}: 6630 ± 0.76 nM, BChE IC_{50} = 91 ± 0.40 nM, respectively). Compound **6e** exhibited remarkable radical scavenging activity in the DPPH assay (IC_{50}: 22.91 ± 1.73 µM) compared to RIV (% radical scavenging activity: 3.71 ± 0.09 at 200 µM) among all tested molecules. PAMPA experiments indicated that compounds **15d** and **15e** exhibited permeability (Pe) values exceeding 4, suggesting their potential to cross the blood-brain barrier and effectively target brain sites. Lead molecules **15d,** and **15e** demonstrated the capability to counteract oxidative stress and amyloid-induced neuronal death in SH-SY5Y cells. Interestingly, **15d** and **15e** could inhibit self-induced full-length $A\beta_{1\text{-}42}$ aggregation. In addition, acute toxicity tests in mice showed that **15d** and **15e** had no toxicity at the dose of 175 mg/kg. It also demonstrated the ability to reverse scopolamine-induced amnesia by enhancing spatial and cognitive memory in the AD mice model at doses as low as 0.3 and 0.5 mg/kg. Additionally, compared to the scopolamine treatment group, these compounds upregulated neuroprotective biomarkers, including BDNF and TRKB. The above results indicate that compounds **15d** and **15e** could be new multi-target-directed ligands for treating AD.

Limitations and Future Directions

Although promising results were obtained for compounds **3q, 6e, 15d**, and **15e**, the study has certain limitations. Firstly, pharmacokinetic data such as bioavailability, half-life, and metabolic stability were not included. These studies are essential for understanding the correlation between in vitro potency and in vivo efficacy and should be addressed in future work. Secondly, although our compounds demonstrated aggregation inhibition, the exact molecular mechanism remains unclear, and further studies, such as NMR or co-crystallization with Aβ/tau aggregates, would be valuable to elucidate the mechanism.

In in vivo experiments, only a limited range of doses was evaluated, primarily due to compound potency and feasibility constraints. Additionally, variability in animal handling procedures between collaborative laboratories (e.g., BITS Hyderabad and our lab) resulted in differences in scopolamine dosing, which may affect direct comparisons. Despite these limitations, our study establishes a solid foundation for designing multifunctional agents and warrants further optimization and preclinical evaluations, including ADME profiling, PK studies, chronic toxicity, and behavioral assessments to support potential translation into clinical candidates.

Based on the above findings, we have compared the data of both series in **Table 18** to provide a comprehensive overview of the pharmacological profiles of the lead compounds.

Table 18. Comparison of the pharmacological profiles of the lead compounds from both series.

Property	3q and 6e	15d and 15e
AChE Inhibition	3q: IC_{50} = 1.72 ± 0.15 μM 6e: IC_{50} = 0.91 ± 0.016 μM	15d: IC_{50} = 0.99 ± 0.009 nM 15e: IC_{50} = 7.97 ± 0.016 nM
BChE Inhibition	3q: IC_{50} = 6.69 ± 0.28 μM 6e: IC_{50} = 1.19 ± 0.026 μM	15d: IC_{50} = 27.79 ± 0.21 nM 15e: IC_{50} = 0.79 ± 0.005 nM

ChE Inhibition Mode	Mixed-type for AChE Competitive for BChE	Pseudoirrversible inhibition
Antioxidant (DPPH assay)	3q: IC_{50} = 16.15 ± 1.05 µM 6e: IC_{50} = 15.17 ± 0.07 µM	15d: Not tested 15e: IC_{50} = 22.91 ± 1.73 µM RIV: only 3.71% at 200 µM
$A\beta_{1-42}$ Aggregation Inhibition	✓ (Effective inhibition for both 3q and 6e)	✓ (Effective inhibition for both 15d and 15e)
Tau Aggregation Inhibition	✓ (Tested and effective)	✗ (Not tested)
Neuroprotection (SH-SY5Y)	✓ (Against H_2O_2-induced toxicity)	✓ (Against Aβ-induced oxidative stress)
Anti-inflammatory Activity	✓ (NLRP3 inhibition, reduced ROS and MMP, inhibited microglial activation)	✗ (Not evaluated)
BBB Permeability (PAMPA)	Pe > 4 for both compounds (suggesting good BBB penetration)	Pe > 4 for both compounds (suggesting good BBB penetration)
In vivo Acute Toxicity	Safe at 2000 mg/kg (single oral dose in Swiss albino mice)	Safe at 175 mg/kg (single oral dose in mice)
In vivo Efficacy (Y-Maze test)	Dose: 5.25 & 10.5 mg/kg Reversed scopolamine-induced amnesia	Dose: 0.3 & 0.5 mg/kg Reversed scopolamine-induced amnesia
Effect on Neurotrophic Markers	Upregulated BDNF and TRKB	Upregulated BDNF and TRKB