Chapter 8

Summery and conclusions

8. Summary and conclusions

Neurodegenerative disorders are the leading cause of age-related loss of motor and cognitive function and subsequent death, affecting over 55 million people worldwide. The timely diagnosis of neurodegenerative disorders, including AD, is not only crucial for the management of the disorders but also in determining treatment approaches. The timely diagnosis, management, and treatment of AD is the major challenge being faced. Accordingly, the work delineated the development of theranostic agents with both diagnostic and therapeutic potential and multi-target-directed ligands (MTDLs) for the treatment of AD. Series of theranostics probes and MTDLs were designed using various design strategies, including scaffold hopping, fragment-based design, hybrid drug design, and lead optimization. The theranostic agents, featuring a rational structural framework that integrated lead therapeutic scaffolds and fluoroprobes with an electron donor–acceptor architecture, demonstrated promising diagnostic and therapeutic potential.

The first series of 1, 3-dimethylbarbituric acid-based theranostic agents endowed with potential anti-ChEs activity. The optimal compound/ probe **39** (1,3-Dimethyl-5-(4-(4-(pyridin-2-yl)piperazin-1-yl)benzylidene) pyrimidine-2,4,6(1H,3H,5H)-trione) (*ee*AChE IC_{50} = 0.886 ± 0.068 μM) and **43** (*ee*AChE **IC_{50} = 0.806 ± 0.0431 μM**; *eq*BuChE **IC_{50} = 9.908 ± 0.017 μM**) exhibited potent inhibitory activities. The obtained results of BBB assay anticipated excellent BBB permeability of the lead compounds. Significant fluorescence emission profiles of the synthesized probes were observed. The DFT calculation studies used to understand the relationship between these probe's fluorescence characteristics and structure. The $A\beta_{1-42}$ aggregates detection ability of the probe **39** indicated through the significant enhancement in the fluorescence properties and high apparent binding constant of K_d = **76.98 μM**. The *in-situ* visualization of Aβ aggregation and colocalization with ThT signified the binding affinity of probe **39** with

Aβ aggregates. The computational binding mode analysis with AChE & Aβ species advocated potential of probe **39.** Acute oral toxicity studies demonstrated no sign of any toxicity upon administration of compound **39**. The Lead compound **39**, at oral dose of 20 mg/kg, demonstrated a substantial improvement of the cognitive and special memory impairment in the scopolamine-induced cognitive deficit mice model. Further, brain AChE inhibitory potential of lead **39** was affirmed through *ex vivo* biochemical analysis with reduced AChE and increased ACh levels along with antioxidant properties.

To improvise the Aβ detection ability and ChEs inhibitory potential, benzothiazolium and indolium theranostic agents were developed. Where, compound/probe **18** ((E)-3-Ethyl-2-(4-(4-(pyridin-2-yl)piperazin-1-yl)styryl)- benzo[d]thiazol-3-ium Iodide) exhibited significant inhibition ChEs (AChE; **IC_{50} = 0.172 ± 0.011 µM**; BuChE; **IC_{50} = 1.376 ± 0.141 µM**), indicating its strong therapeutic potential. Remarkable photophysical properties and enhancement in the fluorescence response upon binding of probe **18** with $A\beta_{1-42}$ aggregates was observed in the binding study assay. The high affinity of probe **18** toward $A\beta_{1-42}$ aggregates was established in the *in vitro* saturation binding assay (**K_d = 0.731 µM**). Probe **18** indicated its ability to detect the Aβ deposit in elavGAL4 >UAS Aβ, the *Drosophila* model for AD, which was comparable to the standard Thioflavin T dyes. It presented no sign of any toxicity. Moreover, *in vivo* behavioural studies of compound **18** demonstrated a significant improvement in cognitive and spatial memory deficits in the scopolamine-induced cognitive impairment mouse model. The *ex vivo* analysis showed a reduction in AChE and an increase in the levels of ACh, indicating the significant inhibition of brain AChE. The assessment of MDA and CAT showed the antioxidant potential of test compound **18.** Compound/probe **18** demonstrated excellent properties as a contrast fluorescent agent along with therapeutic activity, evidencing its potential as a theranostic agent, a safe and effective lead compound for AD.

An additional significant component of the study focused on the development MTDLs, guided by a rational and holistic design approach, that also demonstrated promising therapeutic potential.

A series of chalcone derivatives bearing *N*-aryl piperazine moiety, designed through a scaffold-hopping-based MTDLs approach, were evaluated for their multi-targeting ability. Compound **41** ((E)-3-(4-(4-benzylpiperazin-1-yl)phenyl)-1-(4-bromophenyl) prop-2-en-1-one) bearing an unsubstituted benzylpiperazine fragment and para-bromo substitution at the chalcone scaffold exhibited potent AChE inhibitory activity, **IC_{50} = 14.84 ± 1.562 µM**, and significant BuChE inhibition. The notable inhibition of $A\beta_{1-42}$ aggregation with maximal inhibitory potential at 20 µM inhibitor concentration was observed in both self and AChE-induced assay, which indicated the multi-targeting potential of compound **41**. The *in silico* ADMET and molecular properties analyses and BBB permeability were also determined. Furthermore, compound **41**, evaluated on the scopolamine-induced amnesia model, produced a significant reversal of learning and memory functions. The *ex vivo* biochemical analysis indicated a comparable reduction in AChE and an increase in ACh levels. The treatment of compound **41** attenuated the levels of MDA and remarkably increased the levels of CAT. Based on the findings, it was inferred that the presence of a carbon linker joining the piperazine and aryl ring-bearing electronegative substituent at the para position improved activity.

The last part of the study, focused on the lead optimization-based strategy for the development of multi-targeted ligand for the AD. The chalcone scaffold was optimized to derive the pyrazoline analogs with different substituents at the *N*-aryl piperazine moiety. Among all the tested derivatives, **48** (1-(5-(4-(4-Benzylpiperazin-1-yl)phenyl)-3-phenyl-4,5-dihydro1H-pyrazol-1-yl)ethan-1-one)(*ee*AChE **IC_{50} = 2.89 ± 0.706 µM**; *eq*BuChE **IC_{50} = 0.151 ± 0.089 µM**) possessed outstanding

inhibitory profile. SAR studies signified the importance of various structural features responsible for the inhibitory potency of the derivatives. Our findings outlined that the presence of the *N*-acetylation on the pyrazoline scaffold bearing the *N*-benzylpiperazine fragment was vital for the potent inhibitory activity. Additionally, compound **48** also showed a remarkable inhibitory potential against *h*BACE-1. At 20 μM, compound **48** exhibited maximal anti-$A\beta_{1-42}$ aggregation potential in self-induced and AChE-induced assays, which indicated the multi-targeting potential of lead compound **48.** The optimal binding orientation was observed for compound **48** with AChE and BACE-1 in molecular docking studies. The protein–ligand complex stability against AChE was evaluated with MD simulation studies. Compound **48** demonstrated a dose-dependent increase in % spontaneous alternations in the scopolamine-induced amnesia model. At a dose of 20 mg/kg, it produced a significant reversal of learning and memory functions. The promising data of *ex vivo* analysis showed a significant reduction in the AChE and an increase in levels of ACh. The assessment of oxidative biochemical markers, including MDA and CAT, showed the antioxidant potential of lead compound **48**. Overall, the findings favored compound **48** as the most promising multi-target directed ligand from the series and a lead for the treatment of AD.

Theranostic agents and MTDLs hold significant potential and could serve as promising leads for the development of integrated diagnostic and targeted therapeutic strategies and eventually may enhance the management and treatment outcomes for AD.