DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL PROTEIN AGGREGATION MODULATORS AS POTENTIAL THERAPEUTICS AGAINST ALZHEIMER'S DISEASE

Thesis submitted in partial fulfillment
for the Award of Degree

Doctor of Philosophy

By

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Chapter 7

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Summary & Conclusion

Alzheimer's disease (AD) is a progressively debilitating neurodegenerative disorder marked by cognitive decline, memory impairment, and behavioural changes. Its aetiology involves the aggregation of amyloid-beta (Aβ) plaques and tau tangles in the brain, leading to neuronal degeneration. The precise molecular mechanisms underpinning these protein accumulations remain subjects of investigation, but they disrupt neural signalling, provoke inflammation, and induce oxidative stress. This cascade ultimately results in widespread brain atrophy, particularly impacting regions crucial for memory and cognition, such as the hippocampus.

In the context of AD, the aggregation of specific proteins, namely Aβ and tau, assumes a central role in the pathogenesis. Aβ proteins aggregate extracellularly into amyloid plaques, while tau proteins form intracellular neurofibrillary tangles. These proteinaceous aggregates compromise neuronal function, disrupt inter-neuronal communication, and trigger inflammatory responses, culminating in neuronal demise. Consequently, the accumulation of these protein aggregates represents a pivotal event in the progression of AD.

α-Ketoamides and their derivatives have garnered significant attention within the realm of medicinal chemistry due to their notable inhibitory activity against a range of enzymes, with a particular emphasis on proteases. This property positions them as prospective therapeutic agents across a spectrum of diseases. Several derivatives of α-ketoamides have display broad biological activities, arising from their dual electrophilic and nucleophilic characteristics, establishing them as pivotal in chemical biology.

My PhD research work is spread over four studies which are summarised in **Figure 7.1** and discussed below.

Study I (**Chapter 3**) details the synthesis of N-benzyl-4-(4-chlorophenyl)-2-oxobutanamide from a specific α-hydroxythioester. The compound's 3D structure was determined via X-ray diffraction and optimized using DFT calculations. Spectroscopic validations were made using FTIR and NMR, with further theoretical analyses including the TD-DFT approach. Intermolecular interactions in the crystal structure were studied in-depth. Energy framework calculations indicated significant electrostatic and repulsion forces. Moreover, DFT-based analyses revealed detailed interactions within the compound. Compound-1 modulated Aβ42 aggregation without cellular toxicity, making α-ketoamides potential scaffold for amyloid beta aggregation modulation.

In Study II (**Chapter 4**), we synthesized and characterized twenty-eight piperazine and piperidine-based ketoamide derivatives in highy purity. The compounds were evaluated for their modulation of Aβ aggregation through an in vitro Aβ ThT assay, with most showing inhibition or delaying of the Aβ fibril formation. Compound BD23 was further selected as lead compound based on its good solubility, blood-brain barrier permeability determined using PAMPA-BBB assay and exhibited strong inhibition of tau aggregation in vitro. In vivo studies in an Aβ-induced cognitive impairment mouse model suggested BD23's effectiveness in improving cognitive function. Moreover, in silico studies including molecular docking and molecular dynamics simulation supported the favourable interaction of the compound BD23 with Aβ monomer, fibril and tau structures.

In Study III (**Chapter 5**), we have further explored the ketoamide derivatives by synthesizing indole and piperidine-based derivatives. A total of twenty-four molecules were synthesized by suitable methods and characterized by NMR spectra and HRMS. These compounds were evaluated for their modulation of Aβ aggregation through an in vitro Aβ ThT assay, with most showing inhibition or delaying of the Aβ fibril formation. Compound MD08, selected based on its good solubility, blood-brain barrier permeability in a PAMPA-BBB assay and exhibited

strong inhibition of tau aggregation in vitro. In vivo studies in an Aβ-induced cognitive impairment mouse model suggested MD08's effectiveness in improving cognitive function. Moreover, in silico studies including molecular docking and molecular dynamics simulation supported the favourable interaction of the compound MD08 with Aβ monomer, fibril and tau structures.

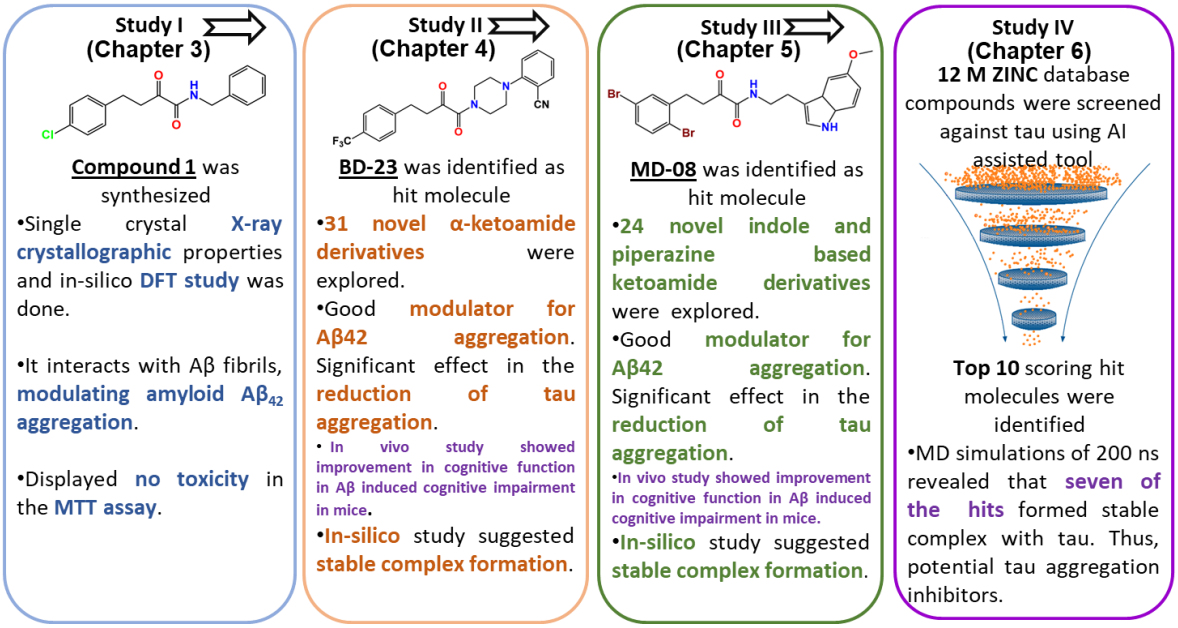

Figure 7. 1. Overall summary of the research work carried out in the thesis.

In Study IV (**Chapter 6**), we used a fully automated AI-assisted ligand-based virtual screening tool, PyRMD to screen a library of twelve million compounds from the ZINC database to identify potential tau aggregation inhibitors. The preliminary hits from virtual screening were filtered for similar compounds and Pan-Assay Interference compounds (the compounds containing reactive functional groups that can interfere with the assays) using RDKit. Further, the selected compounds were prioritized based on their molecular docking score with the binding pocket of tau where the binding pockets were identified using replica exchange molecular dynamics simulation. Thirty-three compounds showing good docking scores for all the tau clusters were selected and were further subjected to in silico pharmacokinetic prediction. Finally, top 10 compounds were selected for molecular dynamics simulation and MMPBSA

binding free energy calculations resulting in the identification of UNK_175, UNK_1027, UNK_1172, UNK_1173, UNK_1237, UNK_1518, and UNK_2181 as potential tau aggregation inhibitors.

In conclusion, we have focused on discovering novel α-ketoamides derivatives as dual Aβ and tau aggregation inhibitors which are the major pathologies involves in AD. The compounds were synthesized in good yield and high purity and possesses potent activity against both Aβ and tau aggregation. Additionally, the vivo studies using an Aβ-induced cognitive impairment mouse model, corroborating the potential of the lead molecules BD23 and MD08 to ameliorate the cognitive deficit. Furthermore, an AI-assisted virtual screening endeavour led to identification of several potential tau aggregation inhibitors. Overall, this research work opens up new avenues for development of α-ketoamides as novel potential therapeutics for AD and related neurodegenerative diseases.