Chapter 2

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Research Envisaged

In AD research, rational drug design adopts a methodical approach, formulating therapies rooted in our comprehensive knowledge of the biological and biochemical mechanisms intrinsic to the disease. A myriad of key mechanisms has been implicated in AD, each presenting potential targets for systematically conceived pharmaceutical interventions. Among these mechanisms, protein aggregation has recently emerged as a therapeutic focal point. The quintessential build-up of Aβ plaques in AD brains underscores the disease's pathology. There is burgeoning interest in targeting the nascent phase of Aβ aggregation, not only to impede the aggregation trajectory but also to modulate the process by transmuting toxic Aβ oligomers into benign, mature fibrils. Concurrently, the manifestation of neurofibrillary tangles, composed predominantly of tau proteins, remains a diagnostic hallmark of AD. Rational drug design in this context might emphasize averting tau hyperphosphorylation, thwarting the tau aggregation mechanism, catalyzing the removal of tau conglomerates, or shielding neuronal structures from the deleterious impacts of such aggregates.

Simultaneously, α-ketoamides have exhibited significant biological importance in the context of several diseases as discussed in **Chapter 1**, **Section 4.** Given the significance of protein aggregation, especially the aggregation of tau and Aβ, in the context of potential therapeutic targets for AD, our research objective is to design innovative protein aggregation modulators with potential therapeutic implications for AD. Keeping in mind the therapeutic potential of the α-ketoamide scaffold, we have explored a number of its derivatives as potential protein aggregation modulators for their development as future therapeutics for AD.

Initially, we embarked on the synthesis and characterization of N-benzyl-4-(4-chlorophenyl)-2-oxobutanamide, and α-ketoamide derivative utilizing appropriate synthesis methodology (**Chapter 3**). Further, to elucidate its structural and crystallographic attributes, we determined

its 3D structure using single-crystal X-ray diffraction technique. In silico quantum chemical studies were performed using density functional theory (DFT) to gain insights into its thermal properties, electronic behaviors of constituent atoms, and potential interactions with biological proteins. Furthermore, the in vitro assessments, encompassing the Aβ aggregation modulation assay and cytotoxicity assay, established compound 1 as a non-toxic Aβ aggregation modulator (**Figure 2.1**).

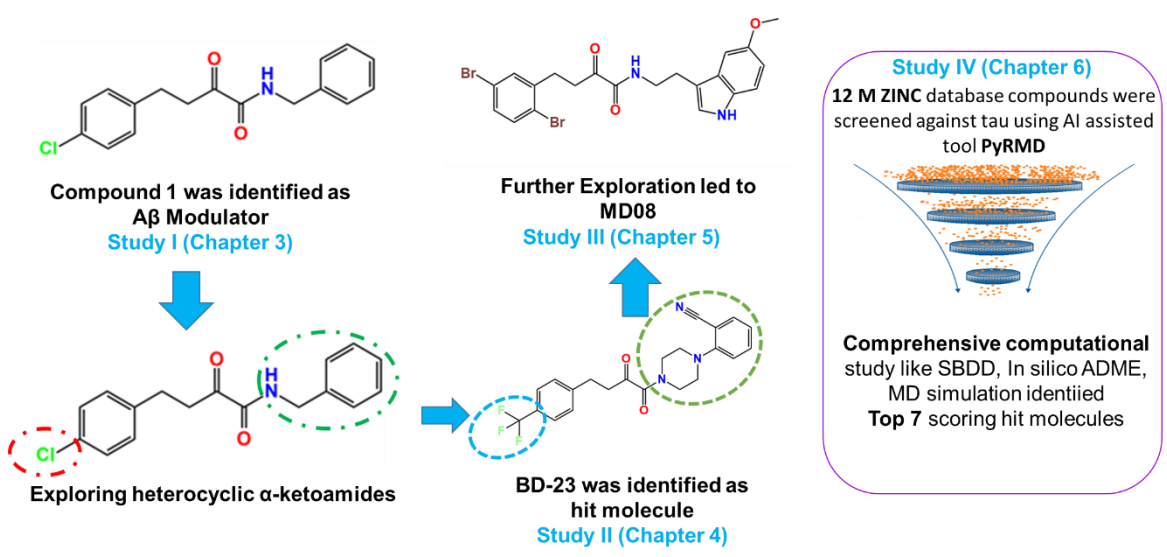

Figure 2.1. The schematic representation of research work done.

These promising findings motivated us to further explore this scaffold by synthesizing a broader range of heterocycle substituted α-ketoamide derivatives with potential activity against protein aggregation in AD. Consequently, in **Chapters 4** and **5**, we undertook the synthesis of diverse heterocyclic derivatives and assessed their bioactivity through both in vitro and in vivo methodologies.

In **Chapter 4**, we have synthesized a total of twenty-eight piperazine and piperidine-based α-ketoamide derivatives were synthesized and characterized with the aim of investigating their potential as treatments for AD. These compounds underwent assessment for their capacity to modulate Aβ aggregation through an in vitro Aβ ThT assay, revealing significant promise in impeding Aβ fibril formation for the majority of these compounds. Among the compounds,

BD23 was singled out due to its favorable solubility profile, and it was subjected to a PAMPA assay to assess its potential for crossing the blood-brain barrier. Additionally, in vitro experimentation demonstrated that BD23 exhibited robust inhibitory effects on tau aggregation. Subsequent in vivo investigations conducted in a mouse model with cognitive impairment induced by Aβ suggested that BD23 effectively improved cognitive function at a 5 mg/kg dose. Furthermore, molecular docking and dynamic simulation studies provided corroborative evidence of BD23's stable interaction with key proteins relevant to AD.

Following the promising results observed with BD23, there emerged a compelling rationale to investigate heterocyclic α-keto amides in relation to their effects on Aβ and tau. Consequently, in **Chapter 5**, our focus shifted to the potential of indole derivatives in the context of AD. We synthesized an array of twenty compounds, subsequently subjecting them to a ThT Aβ aggregation assay to evaluate their therapeutic potential against AD. A solubility assessment of the most promising molecules led to the identification of compound MD08 as a particularly potent candidate. In vitro analyses confirmed that MD08 exerted pronounced inhibitory effects on tau aggregation, specifically at concentrations of 100 and 200 μM. Further in vivo studies, undertaken using a mouse model manifesting Aβ-induced cognitive impairment, indicated that MD08, when administered at dosages of 5 and 10 mg/kg, brought about a significant enhancement in cognitive performance. Complementing these findings, molecular docking and dynamic simulation studies offered consistent evidence supporting the stable interaction of MD08 with pivotal proteins implicated in AD.

Upon examining various α-ketoamide derivatives for their potential against protein aggregation, our subsequent strategy leveraged the capabilities of AI to pinpoint potential inhibitors of tau. Recognizing the limitations of traditional synthesis methodologies, which can be both time-intensive and costly, in **Chapter 6** we streamlined our efforts by screening a massive dataset of 12 million compounds using the AI-enhanced PyRMD tool. Given the

intrinsically disordered nature of tau, we sought to obtain its 3D structure. To this end, a REMD simulation was conducted, yielding 10 distinct conformations of tau. Subsequently, through rigorous computational methodologies, encompassing molecular docking, in silico ADME assessment, and molecular dynamics studies, we discerned seven potential tau inhibitors.