Chapter 1

Chapter 1

Introduction and Literature Review

1.1. Alzheimer's Disease: A Progressive Neurodegenerative Disease

Alzheimer's disease (AD) is a progressive neurological disorder and the most common cause of dementia characterized by a gradual decline in cognitive function, memory loss, and changes in behavior and thinking abilities [1-3]. AD is a prevalent condition worldwide, with a significant impact on individuals, families, and healthcare systems. According to the World Health Organization (WHO), an estimated 50 million people worldwide were living with dementia (including AD) in 2020, and this number is expected to triple by 2050 if no effective treatments are found [4].

AD progresses through distinct stages (**Figure 1.1**). The preclinical stage is marked by subtle biological changes in the brain. As it advances to mild cognitive impairment, individuals experience mild symptoms which might not interfere with day-to-day activities but further progression shows increased memory impairment, language difficulties, and behavioral alterations. In the moderate stage, confusion deepens, daily tasks become challenging, and personality changes emerge. The severe stage brings severe cognitive decline, loss of motor skills, and the need for full-time care [5].

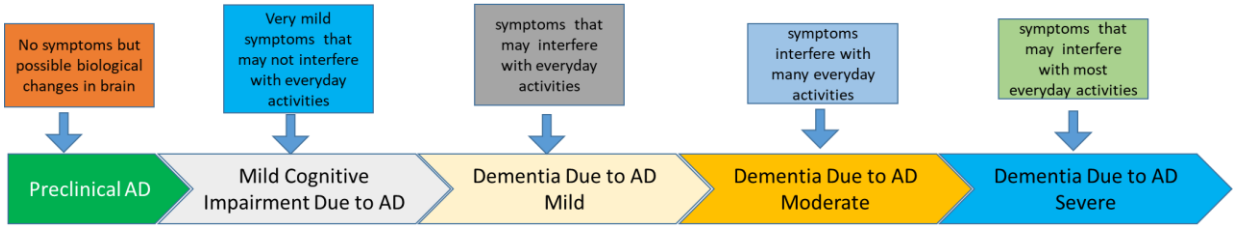


Figure 1.1. Alzheimer's Disease Continuum. The preclinical phase of AD is characterized by subtle biological alterations in the brain. Progressing to mild cognitive impairment, individuals may encounter mild symptoms that don't significantly disrupt their daily routines. However, as

it evolves, there's a noticeable increase in memory issues, language challenges, and shifts in behavior. The moderate stage is marked by deepening confusion, making everyday tasks increasingly difficult, and introducing changes in personality. Finally, in the severe stage, there's a substantial cognitive decline, a loss of motor abilities, and the necessity for round-the-clock care.

1.2. Prevalence of AD

According to global reports of the AD International, it's suggested that around three-quarters of dementia patients worldwide remain undiagnosed. This percentage may even surge to nine out of ten in certain developing countries. Statistics from 2019 indicate roughly 55 million people are living with dementia. Based on the latest data from the WHO, this number could swell to 139 million by 2050 [6].

A noteworthy aspect of this phenomenon is the evolving geographical distribution. As of now, 58% of individuals affected with dementia reside in low and middle-income countries. However, as we look ahead to 2050, this proportion is predicted to rise to 68%. The most rapid growth in the elderly population, the segment most affected by dementia, is unfolding in regions like China, India, and their counterparts in South Asia and the Western Pacific [7]. The Indian scenario is no more different, where the data collected between 2017 and 2020, indicates that approximately 7.4% of individuals aged 60 and above has dementia, which translates to about 8.8 million people across the country. A closer look reveals that dementia is more prevalent in females compared to males, with rates of 9.0% and 5.8% respectively. Furthermore, rural areas showed a higher prevalence at 8.4%, compared to urban regions with 5.3% [8].

The **Figure 1.2A** indicated that the estimated lifetime risk for AD at age 45 was approximately 1 in 5 (20%) for women and 1 in 10 (10%) for men. The risks for both sexes were slightly higher at age 65. From 2000 to 2019, recorded deaths from AD, as indicated on death certificates, surged by 145%. In contrast, deaths attributed to the primary cause, heart disease,

declined by 7.3% (**Figure 1.2B**) [7]. This uptick in death certificates mentioning Alzheimer's as the primary cause likely mirrors two patterns. Firstly, as the population gets older, AD is becoming a more frequent cause of death. Secondly, there seems to be a growing tendency among physicians, coroners, and other professionals to cite AD on death certificates over time.

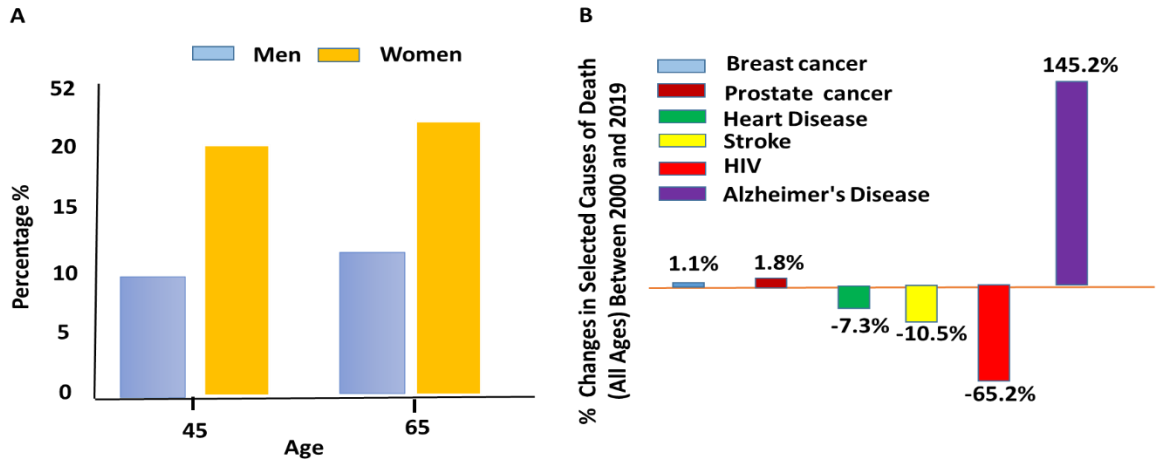


Figure 1.2. Alzheimer's Disease risk in numbers. (A) Estimated Lifetime Risk for Alzheimer's Dementia, by Sex, at Ages 45 and 65. (B) The percentage changes in selected causes of death between 2000 and 2019 [7, 9, 10].

1.3. Pathophysiology of AD

The brain is the primary control center of the body, responsible for regulating all physiological processes and cognitive functions. It processes sensory information, allowing us to interpret and interact with our environment [11]. The brain also governs emotions, memory storage and retrieval, and critical thinking [12]. Additionally, it coordinates both voluntary actions, like speaking or walking, and involuntary actions, like breathing and heartbeat. Overall, the brain's complex interplay of neurons ensures seamless communication within the body and our conscious and unconscious responses to the world [13]. However, with the onset of AD, subtle changes begin to manifest. Before any clear-cut symptoms are evident, beta-amyloid proteins

start to clump together, forming plaques. These plaques accumulate between neurons, disrupting their ability to communicate [14]. Additionally, the tau protein, which helps maintain the structure of neurons, begins to twist into neurofibrillary tangles inside the neuron, blocking the transport of essential nutrients within the cell. As the disease progresses, the density of these plaques and tangles increases, leading to widespread neuronal death. This cell death is particularly rampant in regions responsible for memory, like the hippocampus, causing the initial memory lapses that are often the first recognizable sign of AD. The brain begins to shrink as more neurons die, and the damage extends, affecting areas responsible for language, reasoning, and social behavior. Cognitive deficits become more pronounced, and individuals often exhibit personality changes, confusion, and difficulty with daily tasks. In the advanced stages of AD, the brain undergoes significant atrophy [15]. Most cortical tissues shrunk, leading to severe cognitive and functional impairments. Individuals might lose their ability to communicate, recognize loved ones, or carry out daily activities [16]. The body's systems start to fail as the brain's regulatory functions deteriorate. At this stage, comprehensive care is essential, as individuals cannot typically perform daily activities or ensure their own safety. Several hypotheses about AD have been developed including amyloid cascade hypothesis, tau hypothesis, cholinergic hypothesis, metal chelation hypothesis, oxidative stress hypothesis, and inflammation hypothesis.

1.3.1. Amyloid Cascade Hypothesis

Multiple factors contribute to the progression of AD, with the accumulation of amyloid plaques and soluble oligomers being a primary characteristic of the condition [17]. These plaques are predominantly formed by clusters of Amyloid Beta (Aβ), peptides consisting of 39 to 43 amino acids. Aβ peptides arise from the breakdown of the amyloid precursor protein (APP), an integral transmembrane glycoprotein found in the central nervous system (CNS), through what's termed

the amyloidogenic pathway [18]. Normally, the enzymatic cleavage of APP is facilitated by a trio of enzyme complexes: α-secretase, β-secretase, and γ-secretase [19].

In the amyloidogenic process, the APP protein is initially cleaved by β-secretase, releasing a significant N-terminal ectodomain known as sAPPβ into the external environment. This is then followed by γ-secretase processing, generating $A\beta_{40}$ and $A\beta_{42}$ peptides in a typical 9:1 ratio. $A\beta_{42}$ is especially prone to forming plaques due to its two additional hydrophobic amino acid residues compared to $A\beta_{40}$ (**Figure 1.3**). These Aβ peptides, produced in the amyloidogenic route, are naturally inclined to misfold and aggregate, thus playing a pivotal role in AD's pathology [20]. Interactions between these oligomeric Aβ peptides and brain cells trigger inflammatory responses, including mitochondrial dysfunction and elevated oxidative stress [21, 22]. These events disrupt cellular signaling, alter calcium metabolism, and eventually lead to neuronal apoptosis or cell death.

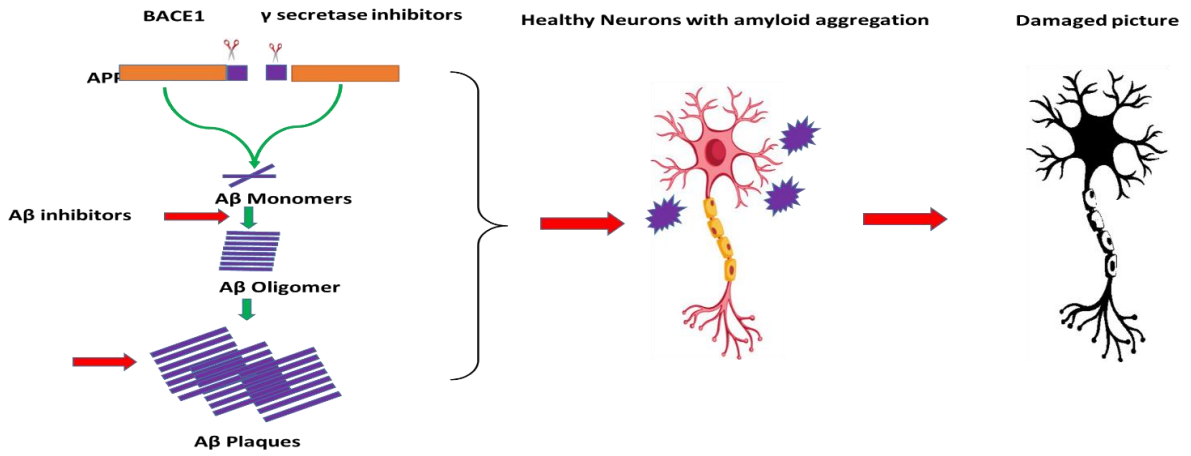


Figure 1.3. The amyloid beta hypothesis in AD. $A\beta_{42}$ is produced when enzymes called β-secretase and γ-secretase sequentially cleave the APP in the brain. The produced $A\beta_{42}$ can aggregate and form plaques, which is a hallmark of AD pathology.

Accumulation of Aβ peptides has also been suggested to negatively impact cholinergic function. Aβ can interfere with axonal transport in cholinergic neurons, impede ACh release, and may even lead to cholinergic neuron death [23, 24]. There's also evidence suggesting that

Aβ can reduce the sensitivity of nicotinic acetylcholine receptors, diminishing their responsiveness to ACh [24].

1.3.2. Tau Hypothesis

Tau is a protein associated with microtubules in neurons [25]. Microtubules are part of the cell's cytoskeleton and are essential for maintaining cell shape and aiding in cell division and tau helps stabilize them. However, tau is an intrinsically disordered protein, meaning it doesn't have a fixed three dimensional structure when not bound to microtubules [26, 27]. In AD, tau becomes abnormally phosphorylated at multiple sites. This hyperphosphorylated tau has reduced affinity for microtubules, leading to microtubule destabilization (**Figure 1.4**). The hyper phosphorylated tau proteins tend to aggregate and form paired helical filaments (PHFs). Over time, these PHFs accumulate and condense to form neuro fibrillary tangles (NFTs) within neurons. The presence of NFTs is believed to disrupt normal neuronal function [28]. The mechanism by which NFTs causes neuronal death isn't completely understood, but their presence correlates with the severity of cognitive impairment in AD. It's also believed that the soluble, pre-tangle forms of tau might be toxic to neurons [29-31].

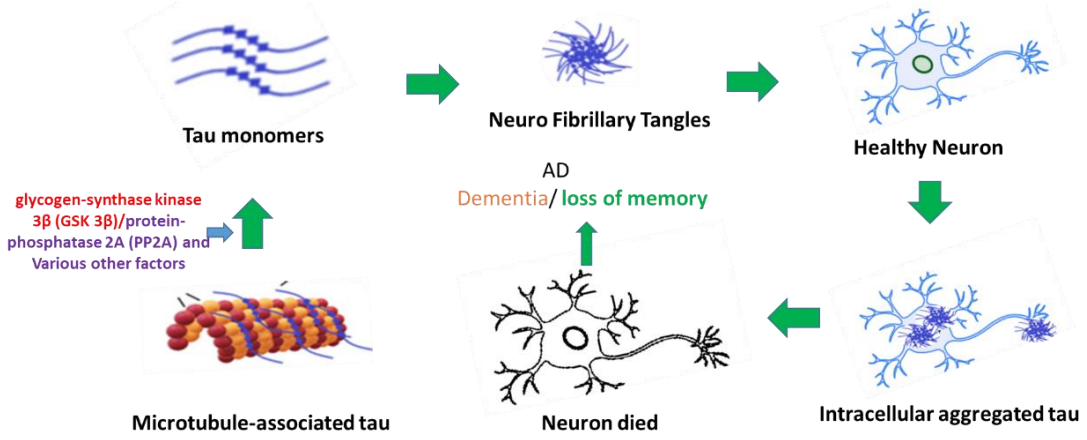


Figure 1.4. Tau hypothesis in AD. Normally, tau helps stabilize microtubules, essential for neuronal structure and function. However, in AD, tau proteins become hyperphosphorylated due to the action of kinases and phosphatases, causing them to detach from microtubules and

aggregate into NFTs. These tangles disrupt neuronal transport and impair cellular function, ultimately leading to neurodegeneration and cognitive decline.

1.3.3. Cholinergic Hypothesis

The cholinergic hypothesis of AD centers on the notion that a dysfunction in cholinergic neurotransmission, specifically in the synthesis, release, and processing of acetylcholine (ACh), is a significant contributor to the cognitive deficits observed in AD (**Figure 1.5**) [32, 33]. To delve into the mechanistic aspects, early post-mortem studies of brains from AD patients revealed a pronounced loss of cholinergic neurons in the nucleus basalis of Meynert, a critical area of the basal forebrain that supplies ACh to areas like the cerebral cortex and hippocampus. ACh is synthesized from choline and acetyl coenzyme A by the enzyme choline acetyltransferase (ChAT) [34].

After its release into the synapse and subsequent action on postsynaptic receptors, ACh is broken down by the enzyme acetylcholinesterase (AChE) into choline and acetate. In AD, there's a significant decrease in the activity of ChAT, indicating reduced ACh synthesis. Moreover, the levels of ACh in the cortex and hippocampus are also diminished. In addition to decreased ACh synthesis and release, AD brains shows altered expression of cholinergic receptors, specifically the muscarinic and nicotinic subtypes [35]. These receptors are crucial for mediating the effects of ACh in the brain. The diminished number and function of these receptors further exacerbate cholinergic dysfunction.

The mechanistic insights gained from understanding cholinergic dysfunction in AD led to the development of acetylcholinesterase (AChE) inhibitors. By inhibiting AChE, these drugs increase the availability of ACh in the synapse, thus temporarily enhancing cholinergic neurotransmission [36, 37]. This results in symptomatic improvements in cognition and function, although it doesn't address the disease's underlying progression.

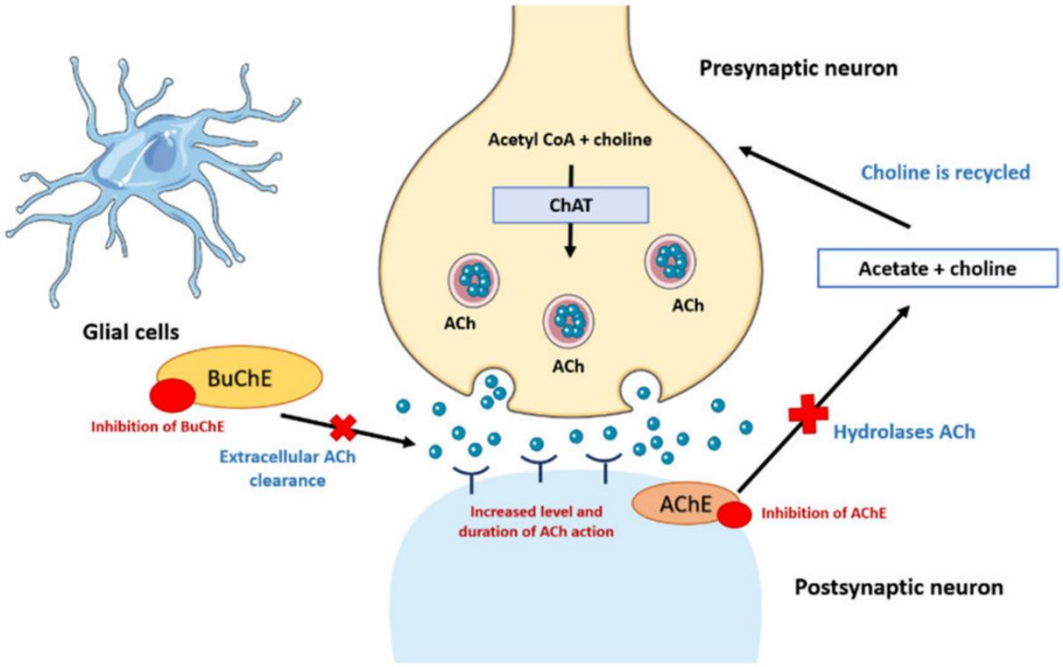


Figure 1.5. The Cholinergic hypothesis in AD. It suggests that a deficiency in the neurotransmitter acetylcholine plays a central role in the disease's cognitive symptoms. In AD, the brain's cholinergic neurons degenerate, leading to reduced acetylcholine levels, impairing memory and cognition. Cholinesterase inhibitors, such as Donepezil, are used as treatments to enhance acetylcholine levels temporarily, offering symptomatic relief, though they don't address the underlying disease mechanisms [38].

1.3.4. Metal Ion Hypothesis

The metal ion hypothesis of AD proposes that imbalances in the homeostasis of metal ions, particularly copper, zinc, and iron, play a significant role in the pathogenesis of AD (**Figure 1.6**)[39, 40]. These metal ions are known to be involved in various physiological processes in the brain, and dysregulation can have neurotoxic effects, potentially leading to the neuronal degeneration observed in AD. Researchers hypothesized that metal ions are directly and indirectly involved in causing neural damage leading to AD, that includes especially Cu^{2+} and Zn^{2+}, which can promote the aggregation of Aβ, leading to the formation of insoluble plaques that are a hallmark of AD. The interaction between Aβ and metal ions can facilitate the

generation of reactive oxygen species (ROS), which can damage neuronal cells and contribute to oxidative stress, a known factor in AD progression. Some studies also report that metal ions are involved in tau hyperphosphorylation and aggregation [41].

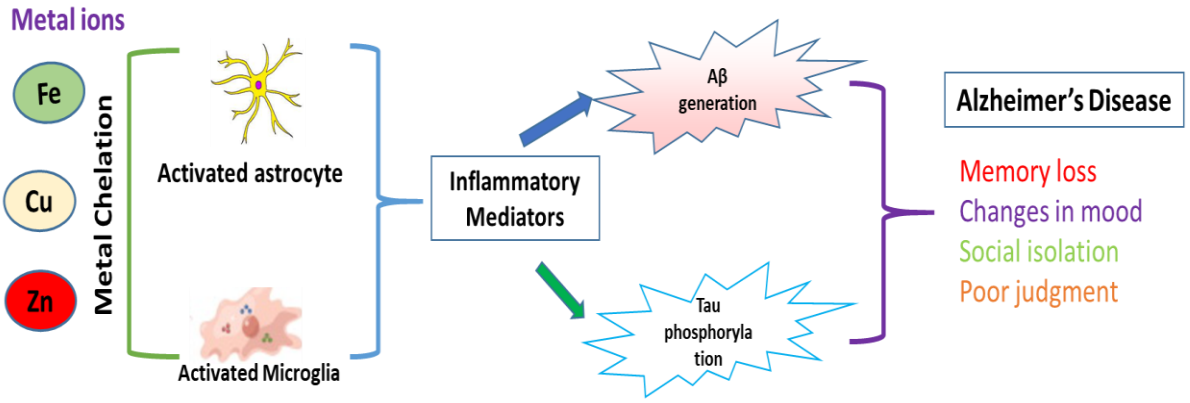


Figure 1.6. **The metal ion hypothesis in AD**. The metal ion hypothesis posits that an imbalance and abnormal accumulation of metal ions, particularly copper, zinc, and iron, in the brain contributes to the development and progression of the disease. These metals can interact with amyloid beta and tau proteins, promoting their aggregation into toxic plaques and tangles. Metal ions can also induce oxidative stress, damaging neurons.

1.3.5. Oxidative Stress and Reactive Oxygen Species Hypothesis

Oxidative stress occurs when there's an imbalance between the production of ROS and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants (**Figure 1.7**) [42]. ROS are chemically reactive molecules containing oxygen, formed as a natural byproduct of the normal metabolism of oxygen. They play a role in cell signaling and homeostasis. However, in high levels, ROS can damage cell structures, including lipids, proteins, and DNA. Increased level of markers of oxidative stress have been identified in the brains of AD patients [43]. This includes oxidized DNA, lipids, and proteins. Elevated levels of ROS can result in the damage and dysfunction of mitochondria, the energy-producing organelles in the cells. Mitochondrial dysfunction is also observed in AD and can lead to further

ROS production, creating a vicious cycle [44]. Moreover the interaction of ROS with Aβ peptide can further promote Aβ aggregation, a hallmark of AD [45].

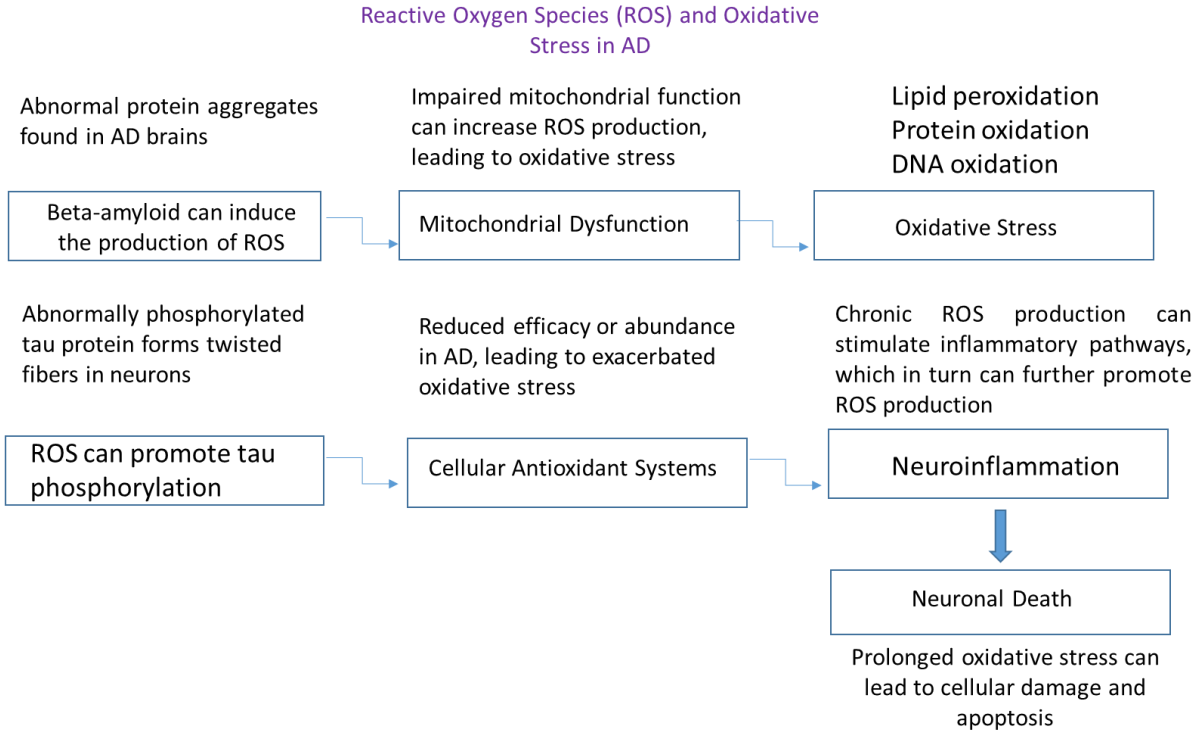


Figure 1.7. Oxidative stress and Reactive oxygen species hypothesis in AD. In AD, the hypothesis surrounding oxidative stress and ROS is gaining prominence. Within affected cells, DNA, lipids, and proteins undergo oxidation, leading to their compromised function. There's a notable increase in ROS levels in AD. Concurrently, mitochondrial dysfunction, commonly observed in AD, exacerbates this issue. Dysfunctional mitochondria produce even more ROS, setting off a self-perpetuating cycle of increasing oxidative stress.

Based on the oxidative stress hypothesis, interventions that boost the antioxidant defense mechanisms or reduce ROS production could potentially slow the progression of AD. Numerous studies have explored the benefits of antioxidant supplements in AD, with mixed results. While some show potential benefits, others have not found a significant impact on disease progression. While oxidative stress is clearly evident in AD, whether it is a primary driver of the disease or a consequence of other pathologies (like Aβ aggregation) remains a

subject of debate [46, 47]. Therapeutic strategies targeting oxidative stress need to be refined and targeted to ensure efficacy and avoid potential side effects.

1.3.6. Inflammation Hypothesis

The inflammation hypothesis suggests that chronic inflammation significantly contributes to the pathogenesis and progression of AD (**Figure 1.8**). Central to this idea is the role of Aβ plaques and tau tangles, hallmarks of AD, which stimulate inflammatory responses in the brain. Aggregated Aβ, for instance, can activate microglia and astrocytes, the brain's primary immune cells, leading to the release of pro-inflammatory cytokines. These immune cells, when overactivated, can release inflammatory mediators damaging the neurons.

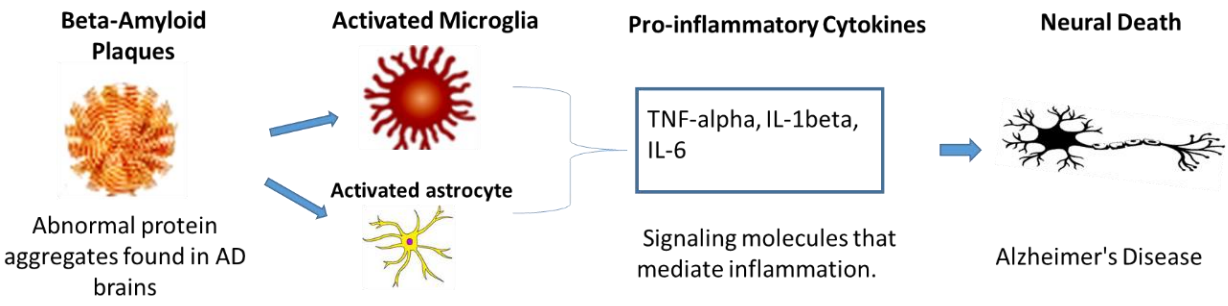


Figure 1.8. Inflammation hypothesis in AD. The inflammation hypothesis suggests that chronic inflammation in the brain plays a significant role in the AD development and progression. In response to factors like abnormal protein aggregation (amyloid plaques and tau tangles), immune cells release inflammatory molecules. These chronic inflammatory processes can damage neurons, disrupt neural communication, and contribute to cognitive decline.

Furthermore, an increase in pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6 is observed in AD, potentially exacerbating the condition. There's also growing evidence suggesting that peripheral inflammation can influence brain inflammation and the progression of AD. Genetic studies reinforce this, indicating that genes tied to the immune response, such

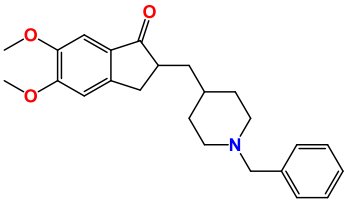
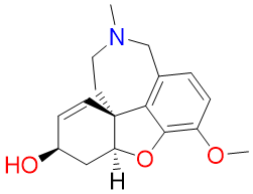
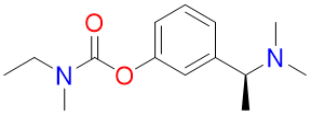
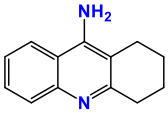
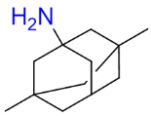
as triggering receptor expressed on myeloid cells 2 (TREM2), might increase AD risk [48]. Consequently, understanding inflammation's role has therapeutic implications.

1.4. Current Therapeutics for AD

Current therapeutics for AD focus on managing symptoms and include two main categories of drugs. Cholinesterase inhibitors (e.g., Donepezil, Rivastigmine) which enhance acetylcholine levels to temporarily improve memory and cognition. Memantine, an NMDA receptor antagonist regulates glutamate activity to mitigate cognitive decline. **Table 1.1** summarizes the current clinically available drugs for the management of AD.

It's worth noting that the landscape of AD treatment is continuously evolving, with numerous investigational treatments in clinical trials aiming at various mechanisms, including targeting tau tangles and inflammation or exploring stem cell therapies [49]. Tacrine (Cognex) stands as a noteworthy milestone, being the inaugural medication to receive FDA endorsement for AD management in 1993. Yet, concerns over its potential liver toxicity led to its eventual discontinuation from the market. Donepezil (Aricept) was granted approval in 1996, followed by Rivastigmine (Exelon) in 2000 and Galantamine (Razadyne) in 2001. These drugs were primarily approved for mild to moderate stages of AD, with Donepezil later receiving approval for all stages [50]. The next significant milestone was the approval of Memantine (Namenda) in 2003, an NMDA receptor antagonist that modulates the activity of glutamate, a neurotransmitter linked with learning and memory [51]. Memantine targeted moderate to severe stages of AD. Combining the benefits of both, Namzaric, a pill combining memantine and donepezil, emerged.

Table 1.1. Clinically available drugs for the management of AD.

Name	Structure/Molecular Formula	Target Type	Therapy Type	Approved for
Aduhelm	$C_{6472}H_{10028}N_{1740}O_{2014}S_{46}$	Amyloid-Related	Immunotherapy (passive)	AD
Leqembi Lecanemab-irmb	$C_{6544}H_{10088}N_{1744}O_{2032}S_{46}$	Amyloid-Related	Immunotherapy (passive)	AD
Donepezil		Cholinergic System	Small Molecule	AD, Dementia with Lewy Bodies (Japan)
Galantamine		Cholinergic System	Small Molecule	Mild to Moderate AD
Rivastigmine		Cholinergic System	Small Molecule	Mild to moderate AD and mild to moderate dementia related to PD AD
Tacrine		Cholinergic System	Small Molecule	
Memantine		NMDA (N-Methyl-D-Aspartate)-receptor antagonist	Small Molecule	AD

Aβ immunotherapies are the latest therapeutics approved for treating AD by targeting Aβ protein aggregates, a hallmark of the disease. These therapies mobilize the immune system to recognize and remove Aβ plaques from the brain. Active immunization involves administering a vaccine containing Aβ fragments or mimics, stimulating the patient's immune system to produce antibodies against Aβ. However, the AN1792 vaccine faced challenges in clinical trials [52, 53]. Passive immunization administers pre-made antibodies specifically designed to target Aβ. Prominent examples include Aducanumab and Lecanemab. These antibodies bind to Aβ

plaques, aiding in their clearance. Aducanumab is a human derived (IgG1) Aβ-directed monoclonal antibody mainly indicated to the patient with mild dementia and cognitive impairment in progressive neurodegenerative stage. Aducanumab is developed by Biogen and in 2021, the FDA granted accelerated approval for its use in AD, though its approval came with significant controversy due to mixed clinical trial results [54].

Aducanumab selectively target the oligomeric and fibrillar Aβ by recognizing the 3-7 amino acid residue of N terminus Aβ peptide. Crystallographic study for complex structure of Aβ peptide and antibody reveals that about 50% (506 Å²) area from the 1025 Å² area of Ala2 -Asp7 residue of Aβ peptide form the interface with Fab fragment of Aducanumab (**Figure 1.9**).

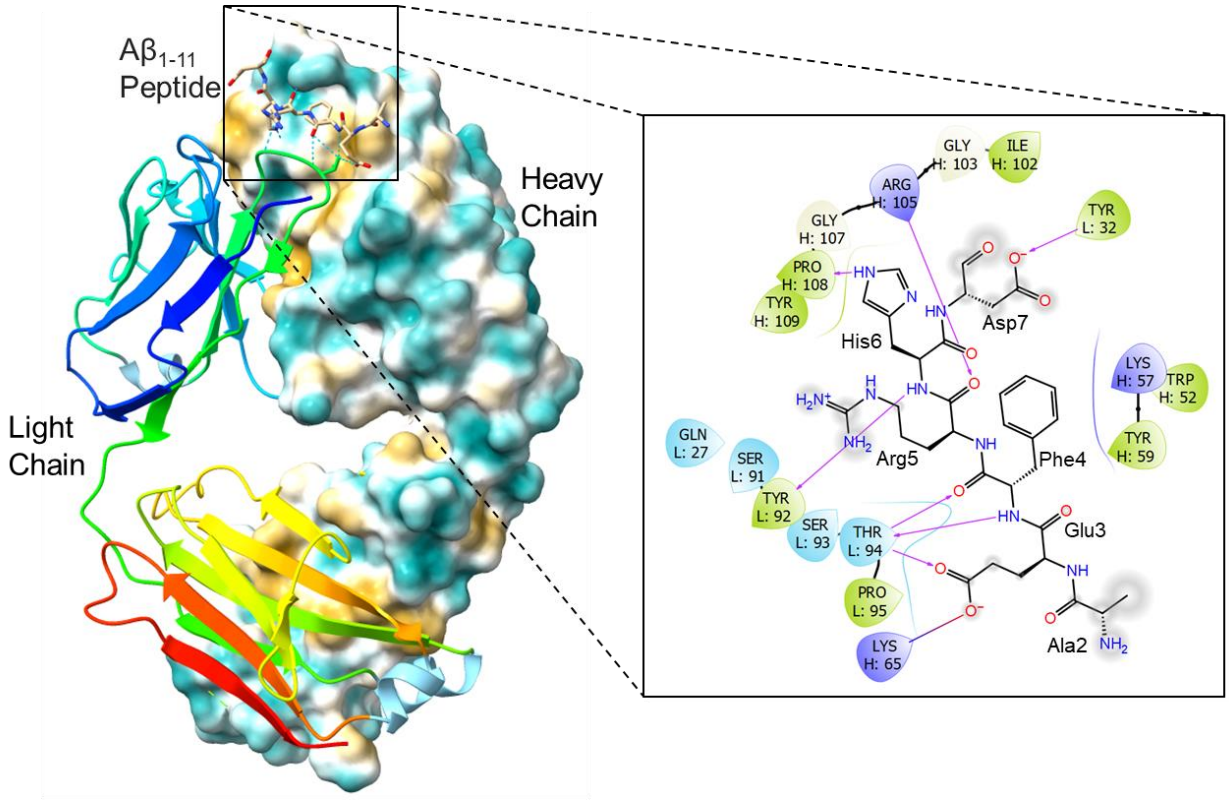


Figure: 1.9. The 3D crystal structure of the Fab fragment of Aducanumab bound to Aβ$_{1-11}$ peptide. The Aducanumab binds to the N terminus of Aβ in an extended conformation, distinct from those seen in structures with other antibodies that target this epitope. [55].

The Fab fragment of Aducanumab form seven hydrogen bond, one salt bridge and hydrophobic interactions with the Aβ1-11 peptide. Specifically, Glu3 of Aβ peptide interacted with Thr94 of the light chain of Aducanumab through a hydrogen bond and with Lys65 of the heavy chain of Aducanumab through a salt bridge. Further, Phe4 of Aβ peptide interacted with Thr94 of the light chain of Aducanumab through two hydrogen bonds. Arg5 of Aβ peptide formed a hydrogen bond with Arg105 of the heavy chain of the Aducanumab. Moreover, His6 and Asp7 residues of the Aβ peptide interacted with Pro108 of the heavy chain and Tyr32 of the light chain of Aducanumab through hydrogen bonding. Though Aβ peptide interact to the interface between the heavy and light chain of antibodies but the confirmation and deepness of binding of Aβ peptide varies with antibodies.

Lecanemab is a humanized IgG1 monoclonal antibody which protects against aqueous fibril synaptotoxicity and targets selectively for Aβ protofibril over insoluble fibrils. The in-vivo studies on both animals and humans shows that Lecanemab is decreasing the Aβ accumulation. In 2023, lecanemab received approval for use in the treatment of early AD. Gantenerumab and Donanemab are other passive immunotherapeutics under clinical trials [56]. While Aβ immunotherapies have shown promise, they face challenges like side effects, variable patient responses, and the timing of treatment initiation. Ongoing studies are assessing their safety and efficacy [57]. Despite hurdles, Aβ immunotherapies remain a critical avenue in the search for effective AD treatments, given the central role of Aβ in the disease's progression [58]. In addition to these medications, managing AD often requires treatments like antidepressants, antipsychotics, anxiolytics, and sleep aids to address mood, behavioral, and other symptoms [59]. Beyond pharmaceuticals, lifestyle interventions like regular physical activity, balanced diets like the Mediterranean or Mediterranean-DASH intervention for neurodegenerative delay (MIND) diet, cognitive stimulation, and social engagement play a pivotal role in managing AD symptoms [60].

1.5. Need of New and Effective Treatment for AD

In light of the ongoing global demographic shift towards an aging population, the pressing need for new and efficacious AD treatments becomes increasingly apparent. Several compelling factors underscore the imperative demand for potential AD drugs. The growing prevalence of AD, which the WHO estimated to afflict approximately 50 million people worldwide in 2020, with AD contributing to the majority of cases, is set to surge with the expanding elderly demographic [61]. Current medications, such as Donepezil, Rivastigmine, and Memantine, predominantly offer symptomatic relief without disease-modifying capabilities [62]. The limited availability of disease-modifying agents, notably Aducanumab and Lecanemab used primarily for mild cognitive impairment, further compounds the urgency to discover transformative treatments [63]. The profound economic impact of AD, encompassing substantial healthcare costs and caregiver productivity loss, underscores the potential financial relief that new drugs capable of slowing disease progression could provide [64]. Existing treatments' numerous adverse effects underscore the necessity for novel, disease-modifying agents [65]. Effective drugs, poised to modify the disease's course, have the potential to enhance cognitive functions and bolster patient independence. Moreover, the pursuit of AD treatments extends beyond symptom management; it contributes to a more profound comprehension of AD, informing enhanced diagnostic tools and preventive strategies.

1.6. Protein Aggregation in AD

Protein aggregation in AD involves the abnormal accumulation of misfolded proteins, primarily Aβ and tau, in the brain as summarized in sections **1.3.1** and **1.3.2** respectively. Aβ proteins clump together to form amyloid plaques outside neurons, while tau proteins aggregate into neurofibrillary tangles (NFTs) inside neurons. These aggregates disrupt cellular function, trigger inflammation, and induce oxidative stress, ultimately leading to neuronal damage and

cognitive decline. Protein aggregation is a hallmark of AD pathology, and understanding its mechanisms is crucial for developing potential disease-modifying therapies [66].

1.7. Mechanism of Protein Aggregation

Protein aggregation refers to the process by which abnormal and misfolded proteins accumulate or clump together. Such aggregates can range from small oligomers to larger insoluble fibrils [67]. There are various factors responsible for protein aggregation such as environmental factors like pH shifts, changes in temperatures, and oxidative stress. Certain genetic mutations can result in the production of proteins that are more prone to misfolding and aggregation as well as various post-translational modifications. The overall mechanism of protein aggregation consists of three steps namely, primary nucleation, elongation, and **s**econdary nucleation (**Figure 1.10**). Primary nucleation is the initial and important step in protein aggregation leading to the formation of a stable aggregate or nucleus without the influence of pre-existing aggregates. In primary nucleation small protein fragments come close together to form large aggregates or nuclei that act as a template for further aggregation. Primary nucleation is typically a slow step in the aggregation pathway because of the thermodynamic barrier in the formation of the stable nucleus. Once the critical nucleus is formed, the addition of more monomers to the aggregate is energetically favorable and can proceed more rapidly. Understanding primary nucleation is crucial not only for grasping the fundamental principles of protein aggregation but also for designing strategies to intervene in diseases where aggregation is a central feature [68].

Elongation is the second stage in protein aggregation which refers to the rapid growth phase that follows the formation of a stable aggregate nucleus. The nucleus, which is formed during the primary nucleation step, acts as a template or seed for the addition of monomeric protein molecules. The elongation process contributes to the formation of larger aggregated structures, such as fibrils. [68]. Secondary nucleation in protein aggregation refers to the process by which

new aggregates or nuclei form in the presence of already-existing aggregates. Unlike primary nucleation, where nuclei form solely from monomeric proteins in the absence of aggregates, secondary nucleation arises due to interactions between monomers and pre-existing aggregates, particularly fibrils. Each of these smaller pieces can then act as a seed or nucleus for further aggregation. This fragmentation process can dramatically increase the number of aggregates in the system and is a form of secondary nucleation.

Secondary nucleation plays a pivotal role in the spread and amplification of protein aggregates, especially in the context of neurodegenerative diseases like AD and Parkinson's Disease. Understanding this process in detail is key to deciphering the underlying mechanisms of these diseases and developing potential treatments.

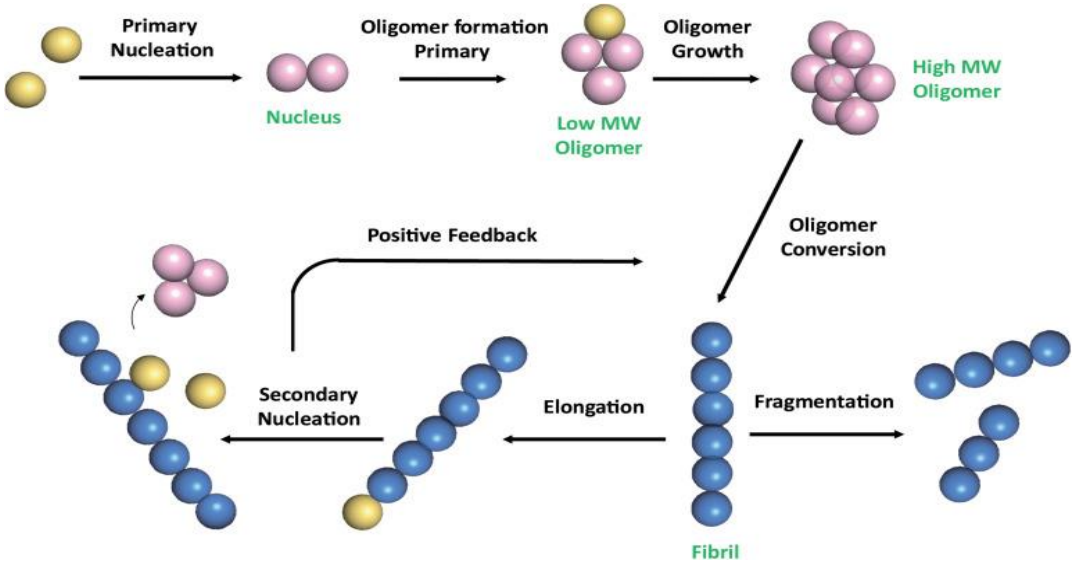
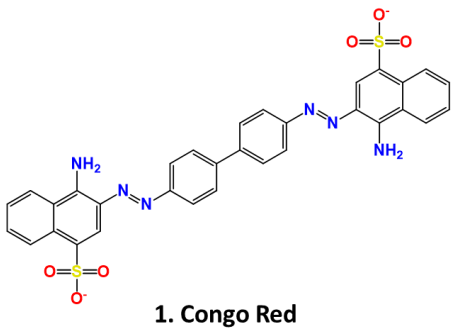


Figure 1.10. **Mechanism of Protein Aggregation in AD**. Protein aggregation in AD comprises primary nucleation, where misfolded proteins form initial nuclei, followed by elongation, where additional proteins join to create larger aggregates. Secondary nucleation occurs when existing aggregates induce the formation of new nuclei, perpetuating aggregation. These processes lead to the buildup of toxic Aβ and tau aggregates, contributing to neurodegeneration and cognitive decline in AD patients. Understanding and targeting these stages is vital for developing potential therapies to halt or slow the disease progression [69].

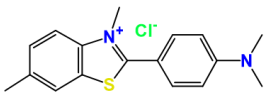
1.8. Aβ Aggregation Inhibitors

Amyloid β aggregation inhibition has been a prominent drug target in AD research, reflecting the significance of Aβ in the disease's pathology. While several drugs targeting Aβ aggregation have been developed and tested, results have been mixed, with many clinical trials failing to show significant benefits. The design, synthesis and pre-clinical evaluation of small molecule Aβ aggregation inhibitors have been nicely reviewed [70-72] and some of the widely studied Aβ aggregation inhibitors are discussed below.

Congo red (**1**) and thioflavin T (**2**) (often abbreviated as ThT) are dyes that have traditionally been used to stain and detect the presence of amyloid fibrils. However, their interaction with amyloid fibrils, especially Aβ in the context of AD, has also sparked interest in understanding their potential inhibitory effects on fibril formation. However, while Congo Red can bind to and interact with Aβ fibrils, it is not typically used as a therapeutic agent to inhibit Aβ aggregation in AD patients due to concerns about its toxicity and its inability to cross the blood-brain barrier [73, 74].

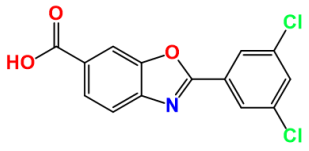


1. Congo Red

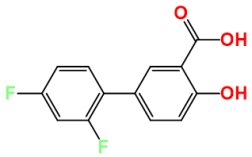


2. Thioflavin T

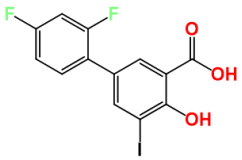
Transthyretin (TTR) is a protein that has been found to bind to Aβ peptides and prevent their aggregation. It also modulates the deposition, processing, and toxicity of Aβ peptides. Several small molecules, such as tafamidis (**3**), diflunisal (**4**), and iododiflunisal (**5**), which stabilize TTR, have shown inhibitory effects on Aβ aggregation. In 2020, Ellen and his coworkers reported the effect of TTR is increased in presents of iododiflunisal in mice model [75].



3. Tafamidis

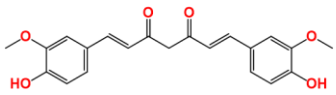


4. Diflunisal

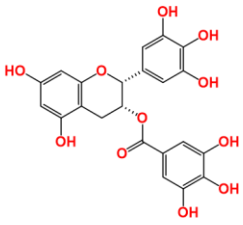


5. iododiflunisal

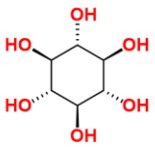
Curcumin (6) is a natural compound found in turmeric, and it has been studied for its potential to inhibit several drug targets. In 2004, Yang et al. reported that curcumin inhibited Aβ aggregation (IC_{50}= 0.8 µM) as well as disaggregated fibrillary $Aβ_{40}$ (IC_{50} = 1 µM), indicating favorable stoichiometry for inhibition under aggregating condition in vitro [76]. Epigallocatechin-3-gallate (7, EGCG) is a polyphenol found in green tea, known for its antioxidant and anti-inflammatory properties. It has been investigated as an Aβ aggregation inhibitor and has shown some ability to disrupt and reduce Aβ aggregation. Scyllo-inositol (**8**), also known as ELND005, is a synthetic compound that has been shown to inhibit Aβ aggregation and reduce the toxicity of Aβ aggregates. It has undergone clinical trials for the treatment of AD but didn't reach the clinics [77].



6. Curcumin



7. Epigallocatechin gallate (EGCG)



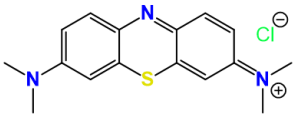
8. Scyllo-inositol

1.9. Tau Aggregation Inhibitors

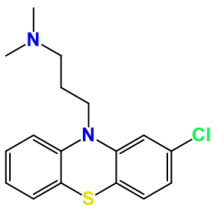
Targeting tauopathy in AD is a promising avenue for potential therapies, as abnormal tau protein aggregation is a key pathological feature of the disease. Several strategies have been explored including tau-targeted immunotherapies, which aim to clear abnormal tau aggregates from the brain, tau kinase inhibitors which target enzymes responsible for tau hyperphosphorylation, microtubule-stabilizing agents which seek to enhance tau's normal

function in stabilizing neuronal structures and small molecules to interfere with tau aggregation or promote its clearance. Several research groups have explored small molecule inhibitors of tau aggregation and the efforts towards the development of potential tau aggregation therapeutics have been critically reviewed [78]. Some of the widely studied tau aggregation inhibitors are summarized below.

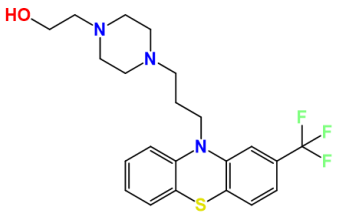
Methylene blue (**9**) is a synthetic dye that has been investigated for its potential as a tau aggregation inhibitor [79]. It has shown some ability to interfere with tau aggregation and reduce the formation of tau tangles. Certain aniline derivatives, such as Congo Red analogs, have been studied for their ability to inhibit tau aggregation. These compounds can bind to tau fibrils and disrupt their formation [80]. In 2017, Jonas Schartner and his team unraveled the therapeutic intervention of methyl blue and congo red on tau and Aβ by developing attenuated total reflection Fourier transform infrared spectroscopy (ATR–FTIR) sensor [81]. Other Phenothiazines that includes drugs like chlorpromazine (**10**) and fluphenazine (**11**), have been investigated as tau aggregation inhibitors.



9. Methylene Blue



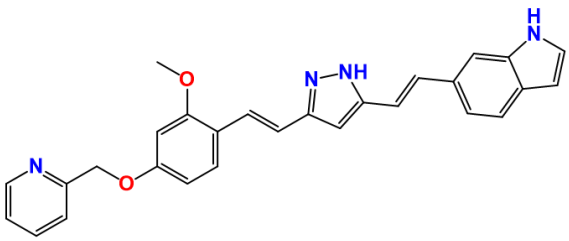
10. Chlorpromazine



11. Fluphenazine

These compounds have shown some ability to reduce tau aggregation in experimental studies. EGCG has also been studied for its potential to inhibit tau aggregation. It has shown some ability to interfere with tau fibril formation and reduce tau-induced toxicity. Okuda and his coworker synthesized different derivatives of curcumin and evaluated their activity against Aβ and tau aggregation, among all the synthesized derivatives they found compound **12** as a potential protein aggregation inhibitor having an IC_{50} value of 1.2 ± 0.2 µM and 0.66 ± 0.13

μM, respectively for Aβ and tau aggregation inhibition [82]. They reported that the compound has a good pharmacokinetic profile. Dolai and his coworkers reported clicked sugar-derivatives of curcumin (**13**) and evaluated their biological activity against Aβ inhibited Aβ aggregation at 8 nM and tau aggregation at 0.1 nM concentrations, which is far more effective than curcumin [83].



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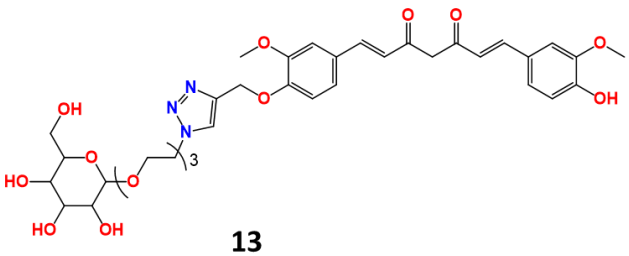
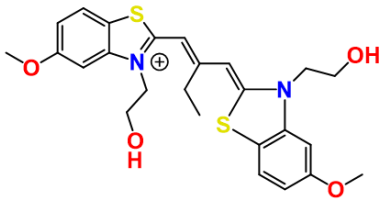


13

Chirita and colleagues employed a fluorescence-based assay to discover the molecule N744 (**14**), which inhibited tau fibrillization. Their research involved screening an array of small molecules in the presence of arachidonic acid-induced htau40. N744 was found to not only impede the aggregation of tau filaments, likely by disrupting their nucleation at particular concentrations compared to total tau (with an IC_{50} value of 294 ± 23 nM), but also to encourage their disaggregation. When evaluating its specificity for tau against Aβ and α-synuclein, N744 showed no effect on Aβ and α-synuclein aggregation. Consequently, in vitro experiments suggest N744 as a promising therapeutic agent for addressing tau fibrillation in neurodegenerative conditions [84]. Bulic and his team pinpointed a rhodanine analog as a tau aggregation inhibitor through high-throughput screening. Subsequent adjustments to the rhodanine derivative eventually resulted in the identification of the highly potent compound, RH-1 (**15**). This compound exhibited nanomolar efficacy in both inhibiting and disaggregating tau aggregation [85].



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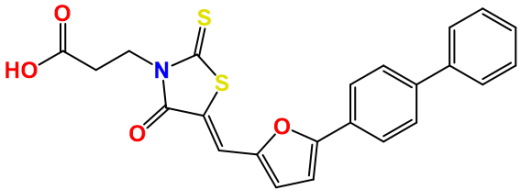
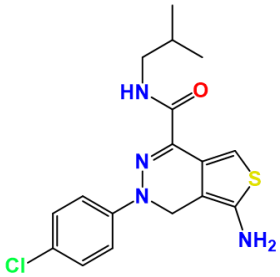


15

Ballatore and his associates crafted and assessed a range of new analogues that not only inhibit tau fibrillization but also demonstrate notable brain-to-plasma exposure ratios when administered to mice. Notably, compounds **16** and **17** stood out in their findings as most promising candidate as tau inhibitors [86].



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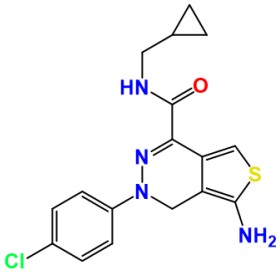

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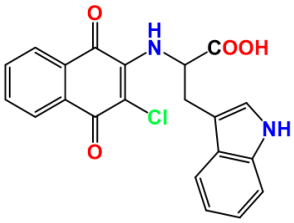


18

Frenkel-Pinter and colleagues identified a compound, referred to as compound **18** which displayed potential in inhibiting tau aggregation both in vitro and in vivo. When larvae of Drosophila were treated with Cl-NQTrp (**18**), there was a marked reduction of about 70% in total tau levels. Furthermore, this treatment effectively mitigated the symptoms associated with tauopathy by preventing tau accumulation in the eye tissue of the treated flies [87].

1.10. Computational Modeling in Drug Design

Computational modeling, commonly referred to as computer-aided drug design (CADD), has revolutionized the field of drug discovery by playing a pivotal role in accelerating the identification of potential drug candidates while significantly reducing the associated costs and time [88, 89]. At the heart of this powerful approach lies a diverse array of computational tools and techniques that serve as indispensable allies in the multifaceted drug development process.

One of the fundamental roles of CADD is in target identification and validation, where computational tools excel in predicting the functions of biological molecules, primarily proteins [90]. By virtually characterizing these proteins and their roles in disease pathways, CADD contributes to the discovery of new drug targets, providing researchers with a starting point for designing novel therapeutics. Within the realm of structure-based drug design (SBDD), CADD offers two essential techniques: docking and molecular dynamics (MD) simulations [91]. Docking enables the prediction of how small molecules, potential drug candidates, bind to specific target proteins. By virtually "docking" these compounds to their protein targets, researchers can identify molecules with the highest binding affinity, which is a critical factor in drug development. Complementing this, MD simulations provide dynamic insights into how molecules move over time, particularly in response to the binding of potential drugs. This dynamic perspective helps researchers assess the stability and feasibility of drug-target interactions.

Ligand-based drug design (LBDD) harnesses computational techniques such as quantitative structure-activity relationship (QSAR) and pharmacophore modeling. QSAR correlates the chemical structures of compounds with their biological activities, allowing researchers to predict the activity of new molecules based on known compounds. Pharmacophore modeling, on the other hand, identifies the essential chemical features necessary for a molecule to interact with a specific target [92]. These features serve as a guide for designing new molecules with the desired properties, optimizing their potential as drug candidates. Virtual screening is a hallmark application of CADD. Instead of the time-consuming and costly process of physically testing thousands of compounds in a laboratory setting, computational methods can efficiently sift through vast libraries of molecules to identify those with the desired properties [93]. By prioritizing the most promising candidates, virtual screening significantly expedites the drug discovery pipeline.

1.10.1. Molecular Docking

Molecular docking is one of the computational techniques that plays an important role in drug discovery. Molecular docking is a pivotal computational technique in drug discovery, simulating how a small molecule, such as a potential drug, interacts with its target protein. Think of it as matching a lock (the protein) and a key (the drug molecule) [94]. The process starts with either identifying or predicting the binding site on the protein where the drug will fit. Once this is established, the orientation and conformation of the molecule within this site, called "pose prediction," is determined. To gauge the effectiveness of this binding, a scoring system is employed, assessing the predicted binding affinity. This docking technique plays several roles in drug discovery. For instance, it aids in identifying potential drug candidates from a vast compound database and fine-tuning these candidates to improve their binding efficiency [93]. Furthermore, it can offer insights into the mechanism by which a drug operates and even predict unintended drug interactions by evaluating its binding with various proteins. However, there are challenges. The accuracy of prediction, especially the scoring functions, remains a concern. Additionally, accounting for the dynamic nature of proteins and molecules, along with the effects of the aqueous environment in the body, can be complex. To navigate these challenges, researchers utilize various software tools like AutoDock, GOLD, and Glide [95-97]. Ultimately, while molecular docking provides invaluable insights, it is most effective when combined with experimental data and other computational methodologies in the drug discovery journey.

1.10.2. Molecular Dynamics

Molecular dynamics (MD) is a computer simulation technique where the time evolution of a set of interacting atoms or molecules is followed by numerically solving Newton's equations of motion [98]. This allows researchers to study the physical properties of molecular systems over time, giving insights into their dynamical behavior. In order to get thermodynamic better,

comprehend the physical motion of protein and ligands, MD simulation provide a major contribution to the drug discovery. It also provides a dynamic view of biomolecular structures, such as proteins or nucleic acids. This is in contrast to static images obtained from techniques like X-ray crystallography [99]. Understanding these dynamics can reveal how a drug molecule affects the target's motion and function. By observing the trajectory of a drug molecule as it approaches and binds to its target, researchers can understand the detailed binding process, identify key interactions, and gain insights into the factors determining binding specificity and affinity. Lately, researchers are exploring the use of MD simulations to correctly estimate the binding affinity of a drug molecule to its target, giving insights into how well a potential drug might work.

1.10.3. Artificial Intelligence in Drug Discovery

Artificial intelligence (AI) is revolutionizing the field of drug discovery, offering transformative capabilities that expedite the identification and development of novel therapeutics while significantly reducing costs [100]. AI-driven approaches are becoming indispensable across various stages of the drug discovery process as summarized in **Figure 1.11.**

At the beginning of the process of drug discovery, by analyzing vast biological datasets, AI can predict the functions of genes and proteins, aiding researchers in identifying potential drug targets quickly and accurately. This accelerates the selection of promising targets, a crucial first step in drug discovery. Later on, AI can predict molecular structures and properties with remarkable precision. Generative models, such as deep learning-based neural networks, have the ability to generate novel molecular structures tailored to specific criteria, optimizing the lead discovery process. This not only expedites drug design but also enhances the chances of discovering innovative and effective compounds. Moreover, AI-driven virtual screening is invaluable in the drug discovery pipeline. By leveraging machine learning algorithms, AI can

swiftly assess extensive compound libraries and prioritize molecules with the highest likelihood of binding to target proteins [101]. This predictive screening significantly reduces the need for extensive and costly laboratory testing, expediting the identification of potential drug candidates. Furthermore, AI has a role in optimizing clinical trial design. By analyzing patient data, AI can assist in identifying suitable patient populations, thereby increasing the chances of successful trials and shortening development timelines. AI-enhanced predictive modeling also helps in evaluating potential safety concerns and side effects, ensuring the development of safer drugs [102].

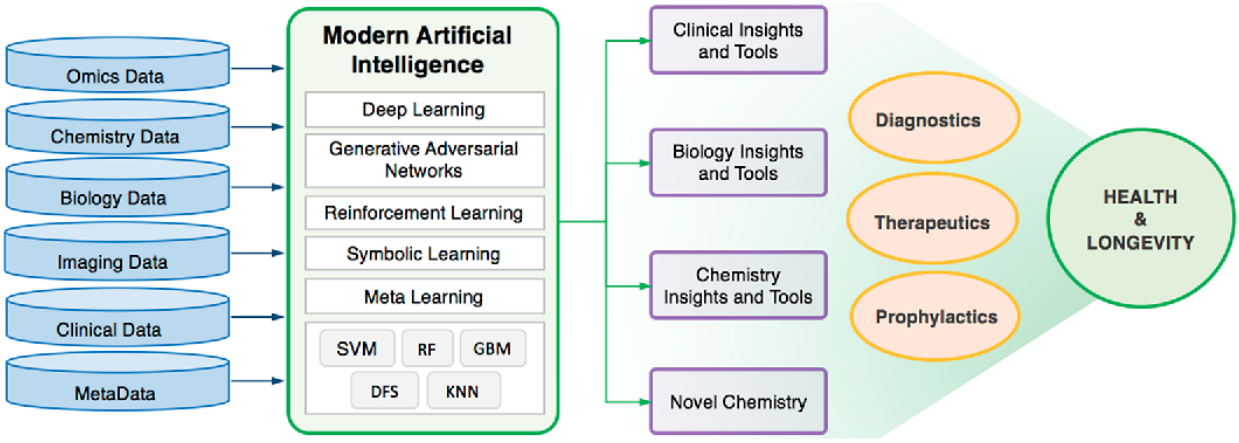


Figure 1.11. Role of Artificial Intelligence (AI) in drug discovery. Reprinted (adapted) with permission from Reference Zhavoronkov et al). Copyright (2018) American Chemical Society [103].

1.10.4. Limitations and Challenges of Using Computational Modeling for Drug Discovery

Computational modeling is a pivotal tool in drug discovery, but it comes with inherent limitations and challenges. Models' accuracy relies on data quality and assumptions, which may not fully capture biological complexity [104]. Scoring function accuracy in predicting binding affinities can be problematic due to simplifications. Limited or variable data quality, computational resource demands, and predicting novel targets pose additional hurdles. Flexibility in proteins and molecules, solvent effects, overfitting, and the need for experimental

validation add to the complexity [105]. Ethical concerns arise from generating numerous untested compounds, while regulatory approval still relies on extensive experimental data. Despite these challenges, computational modeling is indispensable, aiding compound prioritization, understanding drug-target interactions, and optimizing candidates. Its effectiveness is maximized when integrated with experimental approaches, guiding and complementing laboratory work.

1.11. α-Ketoamides

α- Ketoamides represent a class of organic molecules that have gained significant attention in medicinal chemistry due to their potential therapeutic applications. Characterized by the presence of a keto (carbonyl) group adjacent to an amide functionality, these compounds have been found to be versatile intermediates for the synthesis of a wide range of bioactive molecules. Due to their unique structural features, α-ketoamides have demonstrated the potential to modulate various biological targets, making them attractive scaffolds for drug discovery and development.

Over the years, research on α-ketoamides has expanded, leading to the identification of potential lead compounds in various therapeutic areas such as antiviral, anticancer, and anti-inflammatory research. α-Ketoamides, owing to their unique dicarbonyl and amide motif, do possess an interesting structural and electronic feature that provides a distinct mode of interaction with biological targets [106, 107]. This increasing interest underscores the importance and promise of α-ketoamides in the realm of medicinal chemistry [108].

1.11.1. Chemistry of α-Ketoamides

The α-ketoamide scaffold provides a rich array of potential chemical reactions due to the presence of the carbonyl and amide functional groups [106, 107]. These groups can undergo a variety of chemical reactions, including reductions, oxidations, condensations, and nucleophilic

substitutions, allowing the creation of diverse compound libraries for drug discovery [106, 109]. The carbon atom of both the carbonyl group acts as electrophilic centers for various nucleophilic attacks. α-Ketoamides compounds are characterized by a carbonyl group (keto) situated next to an amide functional group, exhibit unique electrophilic and nucleophilic centers that underpin their diverse chemical reactivity. **Figure 1.12** contains reactive ambident electrophile and nucleophile moiety, displaying two possible nucleophilic reaction sites together with two electrophilic centers, whose reactivity can be augmented through the selection of specific activation modes [110].

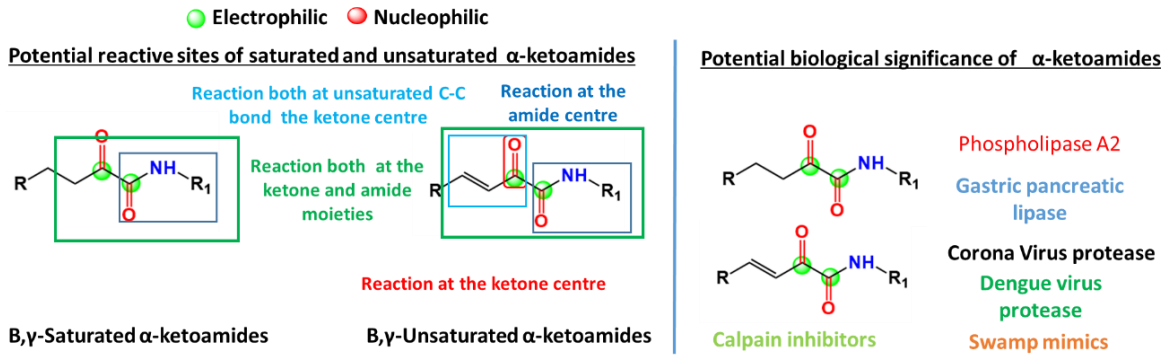


Figure 1.12. The biological importance and potential reactive sites of α-ketoamides.

Many natural products contain *α*-ketoamide in their main scaffold owing the potential therapeutic efficacy motivating researchers to design *α*-ketoamide derivatives to develop compounds tailored to interact with different biological targets for providing therapeutics for the clinical management of a number of pathological conditions [111, 112]. In another word, 1,2-dicarbonyl compounds are important life-related structures that are abundantly found in natural products. In comparison to other carbonyl derivatives, such as *α*-ketoacids and *α*-ketoesters, *α*-ketoamides have been shown to possess better pharmacokinetic properties [113, 114]. The dicarbonyl motif in *α*-ketoamide can interact with the proteins in the body and enhances its bioavailability together with the amide functionality which is known for solubility enhancement effect making it a highly useful moiety for lead optimisation studies in the early

phase drug discovery [110, 115]. The electrophilic sites mainly reside at the carbonyl carbon of the keto group, owing to its polarized C=O bond, and to a lesser extent at the carbonyl carbon of the amide group, although the latter's reactivity is dampened due to resonance.

On the nucleophilic front, the carbon positioned between the keto and amide groups can, when deprotonated, form an enolate that acts as a powerful nucleophile. This enolate can engage in reactions such as Michael additions or aldol condensations. Furthermore, the amide's nitrogen, due to its lone pair, might also serve as a weak nucleophile, although its potential is often restrained by the resonance of the amide group. In essence, the dual presence of keto and amide functionalities grants α-ketoamides their intriguing reactivity profile, where the specific outcome depends on both the molecule's substituents and the external conditions applied. The β,γ-unsaturated carbonyl group present in β,γ-unsaturated α-ketoamide acts as Michael acceptor and shows various reactivity towards other functional groups [116]. Michael acceptors are important in biological systems because they can react with nucleophilic amino acid residues in proteins (e.g., cysteine) through a process known as covalent protein modification. This can have significant implications for drug design and chemical biology [117].

The α-ketoamide scaffold often imparts drug-like properties to compounds, such as good stability, bioavailability, and the ability to interact with specific biological targets. These properties make ketoamides a preferred choice in drug design and discovery. Ketoamides can be designed and synthesized to specifically inhibit certain enzymes or targets, which is particularly beneficial in the treatment of diseases such as viral infections (e.g., SARS-CoV-2), cancer, and neurodegenerative disorders.

α-Ketoamides, due to their well-defined structure and reactivity, can be easily modified to study structure-activity relationships, an essential aspect of drug discovery. Different functional groups or substituents can be added or removed to see how these changes affect the compound's biological activity. α-Ketoamides can be synthesized from readily available starting materials

using a variety of well-established chemical reactions, making them relatively easy and cost-effective to produce in a laboratory setting. Given these attributes, the ketoamide scaffold will likely continue to play a key role in the future of medicinal chemistry and drug discovery. It's worth noting, however, that while α-ketoamides have shown promise in preclinical and early clinical studies, more advanced trials are necessary to fully understand their safety and efficacy in humans. **Figure 1.12.** describes different reactive centers as well as biological significance of α-ketoamides.

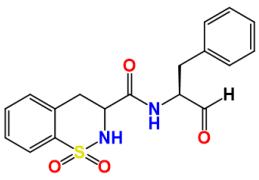
1.11.2. Role of α-ketoamides in Neurodegenerative disease

α-Ketoamides derivatives have been largely studies for a variety of disease drug targets. However, the reports involving their evaluation on AD drug targets are limited. Some of the studies are summarized below. Some ketoamides have been investigated for their ability to inhibit the aggregation of Aβ peptides, which is a critical step in the formation of amyloid plaques in the brain. By preventing or slowing down this aggregation, ketoamides could potentially help reduce the buildup of Aβ plaques and their neurotoxic effects [118].

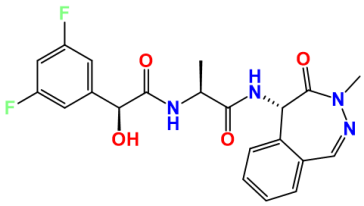
Certain ketoamides have shown promise in stabilizing the tau protein, preventing its abnormal aggregation into neurofibrillary tangles. α-Ketoamides have been explored for their potential neuroprotective effects, which could help prevent or slow the loss of neurons in the brain. This neuroprotection may help preserve cognitive function and delay the progression of AD. Some ketoamides have demonstrated anti-inflammatory properties, and chronic inflammation is believed to play a role in the development and progression of AD. By reducing inflammation in the brain, ketoamides could potentially mitigate some of the damaging effects of the disease. Oxidative stress is another factor contributing to AD pathology. Some ketoamides possess antioxidant properties that could counteract the harmful effects of reactive oxygen species and protect neurons from oxidative damage. Researchers focus on designing α-ketoamides-based

drugs due to their wide range of potential biological activities, chemical stability, and promising pharmacological profiles.

In a 2004 study, Bihovsky et al., synthesized and characterized novel derivatives of 1,2-benzothiazine 1,1-dioxide α-ketoamide. The inhibitory activity of these derivatives was subsequently assessed against Calpain_I. Notably, among the evaluated compounds, compound **19** emerged as the most active entity [119]. Prasad et al., reported design of benzodiazepinones as peptidomimetics. They investigated structure– activity relationships of diazepinones and synthesized orally active γ-secretase inhibitors. Among all compounds compound **20** (BMS-433796) was identified as potent molecule with an acceptable pharmacodynamics and pharmacokinetic profile [120].

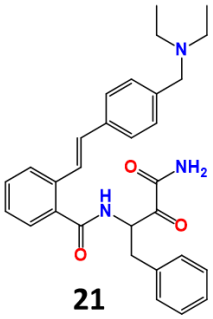


19
Calpain I: IC_{50} = 7 nM



(**20**) BMS233796
γ-Secretase: IC_{50} = 0.3 nM

In 2008 C. Sinjoanu et al., reported compound **A-705253 (21)** which potently inhibited oligomeric Aβ-induced dynamin 1 and tau cleavage in hippocampal neurons. Quantitative Western blot analysis indicated that the incubation of these with **21** prior to the addition of oligomeric Aβ reduced both dynamin 1 and tau cleavage in a dose-dependent manner. In addition, their results showed that this calpain inhibitor significantly ameliorated the cleavage of these proteins when added simultaneously with oligomeric Aβ [121]. In 2011 Yong Jae lo et al., reported several derivatives of cinnamoyl ketoamides and among the synthesized derivatives, compound **22** emerged as most potent inhibitor of calpain I (IC_{50} = 0.13 μM) and also exhibited strong antioxidant activities in DPPH and superoxide anion radical scavenging and lipid peroxidation inhibition assay systems [122].



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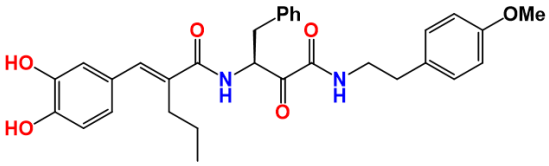
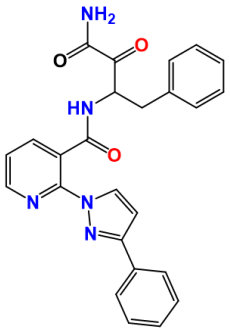


22

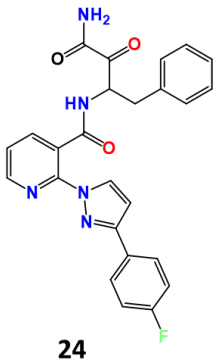
Calpain I: IC_{50} = 0.13 µM

In 2017, Kling and his coworkers synthesized different derivatives of 2-(3-phenyl-1H-pyrazol-1-yl) nicotinamides, among them they found compound **23** and **24** were identified as potent and reversible inhibitors of calpain with high selectivity versus related cysteine protease cathepsins, other proteases, and receptors at low nanomolar concentration [123].



23

Ki Calpin I = 18 nM



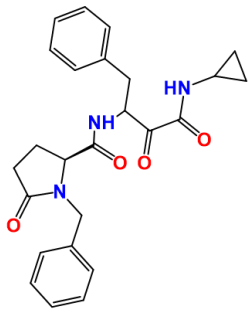
24

Ki Calpin I = 34 nM

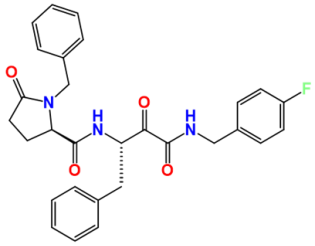
In a 2019 study, Jantos et al., identified molecule ABT-957: 1-Benzyl-5-oxopyrrolidine-2-carboxamide (**25**) as a selective inhibitor of calpain. The researchers further elucidated that in preclinical models pertinent to AD, the compound **25**, exhibited efficacy in preventing both NMDA-induced neurodegeneration and Aβ-induced synaptic dysfunction. Consequently, it advanced to Phase I clinical trials under the designation Alicapistat (ABT-957) [124].

Later it also went phase II clinical trial and it is an orally active selective inhibitor of human calpains I and II for the potential application of AD. Alicapistat mitigates the metabolic liability of carbonyl reduction and inhibits calpain I with an IC_{50} value of 395 nM.

In 2020, Ammar and his coworkers reported the compound **26** while exploring the compound ABT-957. This allowed the exploration of stereoselective inhibition of calpain-1 (IC_{50}=78 nM) [125].



(**25**) ABT957 (Alicapistat)
Calpain I: IC_{50} = 395 nM

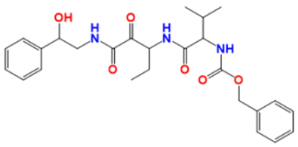


26
Calpain I: IC_{50} = 78 nM

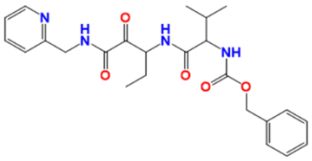
1.11.3. Biological importance of α-Ketoamide based molecules in other disease

Besides the neuroprotective potential of α-ketoamides, it also possesses tremendous biological significance in other diseases. This scaffold, in various studies, has showcased potential anti-inflammatory properties and roles in cellular regulation. Such versatile implications make α-ketoamides an important molecule of interest in biomedical research. Furthermore, there's an increasing interest in understanding its mechanism of action and potential therapeutic applications. The biological significance of α-ketoamide has been well discussed in the previous literature [106] .

In 2015, Zhaozhao Li et. al. synthesized novel dipeptidyl α-ketoamide derivatives and evaluated their inhibitory activity against cysteine protease calpain I, calpain II and cathepsin B. From the total 89 compounds, compound 27, **28** and **29** were found as best inhibitors for calpain II at K_i 15 nM, 17 nM and 22 nM respectively whereas compound **28** at Ki 19 nM and **29** at K_i 31 nM exhibited the significant inhibitory activity on Calpain I and cathepsin-B respectively [126].



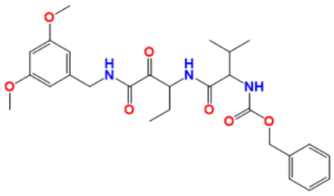
27. Ki = 15nM



28

Calpain I: Ki = 19 nM

Calpain II: Ki = 17 nM



29

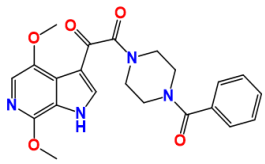
Calpain II: Ki = 22 nM

Cathepsin B: Ki = 31 nM

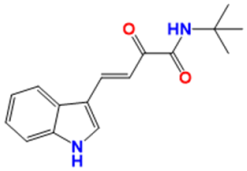
In 2009, Tao Wang et. al., synthesized azaindole derivatives from the lead compound 1-(4-benzoylpiperazin-1-yl)-2-(1*H*-indol-3-yl) ethane-1,2-dione by substituting each unfused carbon atom in phenyl ring of the indole with nitrogen and they evaluated their potency to inhibit the HIV-1 attachment. Further optimization of these azaindole compounds, led to the development of BMS-488043 (**30**) (EC_{50}=0.88±0.46 µM) which progressed into clinical studies, and was shown to effectively reduce viremia in HIV-1 infected individuals when administered as monotherapy for 8 days [127]. In 2011, based on the styryl pharmacophore combined with ketoamide to create an electrophilic trap for catalytic serine residues of viral protease, Christian Steuer et. Al., developed the fragment-like lead compounds and tested against west Nile virus protease and thrombin which also showed the ability to selectively inhibit the flaviviral protease. Moreover, some selected compounds they screened to evaluate the antiviral activity in reporter-gen cell-culture assay of Dengue replication, compound **31** was shown to inhibit the Dengue Virus replication in dose dose-dependent manner by achieving a more than 1000-fold reduction in virus titers at nontoxic concentration. The percent inhibition of Dengue virus at its 50 µM concentration was about 39.1% [128].

In 2014, Nicole Trager et. al., demonstrated the effect of SNJ-1945 (**32**, calpain inhibitor) on neurodegeneration and immunomodulation in murine models of multiple sclerosis. In-vivo study showed that a 50 mg/kg SNJ1945 treated model for 9 days was found to reduce the inflammatory response. As calpain is the substrate for axon, cytoskeletal, and myelin protein

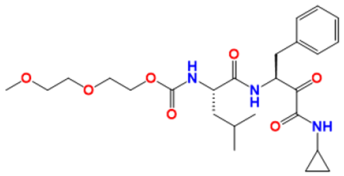
degradation which is responsible for neurodegeneration SNJ-1945 treatment helps in neuroprotection [129].



30. BMS-488043
EC_{50}=0.88 ± 0.46 µM



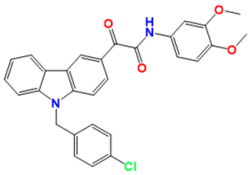
31. Conc. 50 µM = 39.1% Inhibition



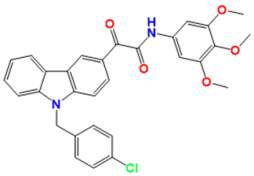
32. SNJ-1945

In 2016, S.N.C Sridhar et. al., synthesized the twenty-four 2(carbazole-3-yl) iodoacetamide derivatives and evaluated their inhibitory activity against pancreatic lipase (PL). In PL inhibition assay compounds **33**, **34,** and **35** inhibited pancreatic lipase at IC_{50} 6.31 µM, 8.72 µM and 9.58 µM respectively [130].

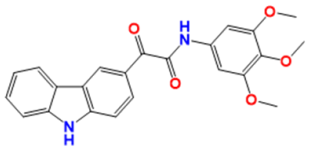
In 2016, Shashidhar Nizalapur et. al., synthesized N-acrylisatin based glyoxamide derivatives through the process of ring opening in N-acrylisatin using acyclic and cyclic amines and amino acid esters to evaluates its inhibitory activity against quorum sensing. Compound **36** exhibited the best quorum sensing inhibitory activity against *P. aeruginosa* (MH602) and *E.Coli* (MT102) about 48.7% and 73.6 % respectively at 250 µM concentration [131].



33. IC_{50} =6.31 µM
X_{i50} =21.85

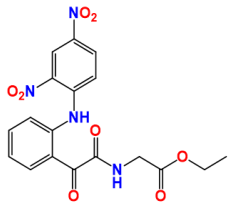


34. IC_{50} = 8.72 µM
Xi50 = 21.94

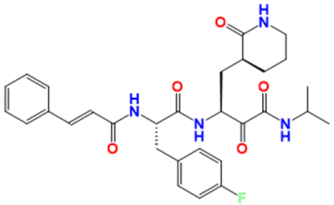


35. IC_{50} = 9.58 µM
Xi_{50}=26.2

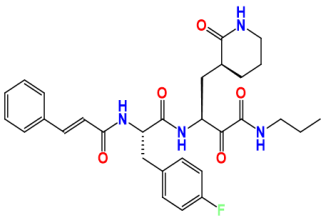
In 2016, Debin Zeng et. al. reported α-ketoamide derivatives having enterovirus protease inhibitory activity. SAR study of these derivatives indicated that substitution of benzyl group with para-fluoro benzyl enhances the inhibition potency from which compounds **37, 38** and **39** showed satisfactory inhibitory activity at IC_{50} value 1.32±0.26, 1.882 ±0.35 and 1.522 ±0.31 µM respectively [132].



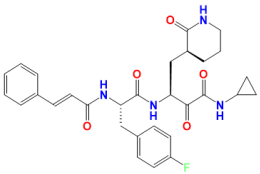
36. (Conc. 250 µm) *P. aeruginosa* =48.7% E.Coli = 73.6 %



37. IC_{50}=1.32±0.26 µm

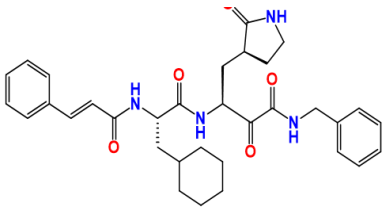


38. IC_{50}=1.882±0.35 µm

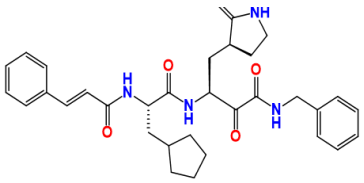


39. IC_{50}=1.522±0.31 µm

To get the broad-spectrum antiviral compounds against alpha, beta coronavirus and enterovirus, In 2020, Linlin Zhang et. al., designed the peptidomimetic α-ketoamides on the basis of protease structure of coronavirus and enterovirus. In the cell based assay compounds **40** and **41** was found to inhibit the protease activity at IC_{50} 4.7±0.94 µm,1.93±0.43 µm,1.27±0.34 µm and 1.69±0.47 µm,0.95±0.15 µm,0.71±0.36 µm concentration on EV-A71 $3C^{pro}$, CVB3 $3C^{pro}$, SARS-CoV M^{pro} cells respectively whereas compound **40** exhibited significant antiviral activity against Middle East respiratory Syndrome coronavirus at picomolar range i.e. at EC_{50} = 0.0004±0.0003 µm [133].



40. IC_{50}
EV-A71 3Cpro =1.69±0.47 µm
CVB3 3Cpro =0.95±0.15 µm
SARS-CoV Mpro =0.71±0.36 µM
Huh-7MERS-CoV(EC_{50})=0.0004±0.0003 µm



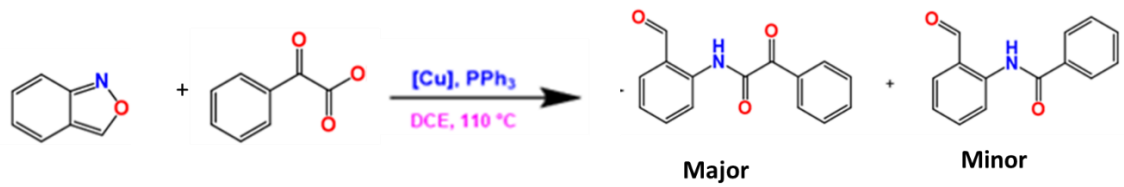
41. IC_{50}
EV-A71 3Cpro= 4.7±0.94 µm
CVB3 3Cpro=1.93±0.43 µm, SARS-CoV Mpro =1.27±0.34 µm

1.11.4. Methods of synthesis of α-ketoamides

Various routes have been reported for the synthesis of α-ketoamides including oxidation, amidation approaches, ionic disconnection, coupling reaction, double carbonylative amination

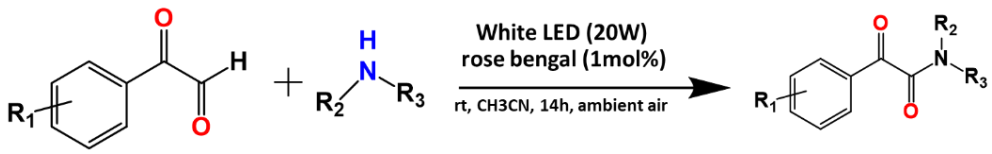
etc. The different methods for the synthesis of α-ketoamides along with mechanism have been reported in the previous literatures [107]. Here we have summarized some recent approaches for the synthesis of α-ketoamides.

In 2019, Li et. Al., reported direct synthesis of α-ketoamides by copper catalyzed coupling of anthranils and α-keto acids (**Scheme 1.1**). This process involves N–O/C–O bond cleavages and C–N bond formation. Furthermore, the decarboxylation of α-keto acids can be successfully suppressed under redox-neutral conditions [134].



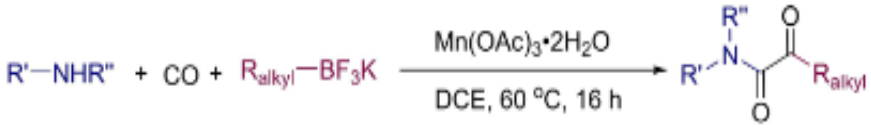
Scheme 1.1. Synthesis of α-ketoamides by copper-catalyzed coupling of anthranils and α-keto acids.

In 2019, Monga et. al., reported the synthesis of visible light-mediated synthesis of photocatalytic amidation of α-keto aldehydes using rose bengal as photocatalyst at room temperature under ambient air and under irradiation of a 20 W white LED bulb (**Scheme 1.2**). The method is mild, efficient and environmentally benign [135].



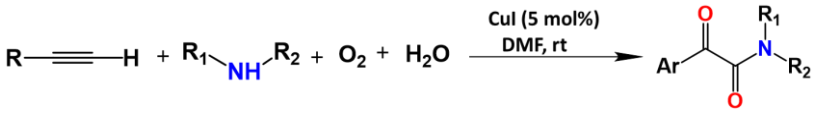
Scheme 1.2. Synthesis of α-ketoamides via visible light mediated photocatalytic amidation of α-keto aldehydes.

In 2021, Chen et. al., reported the synthesis of different α-ketoamides by using easily available potassium alkyl trifluoro borates or Hantzsch esters as the starting material, and cheap and non-toxic Mn(OAc)3·2H2O as the promotor (**Scheme 1.3**) [136].



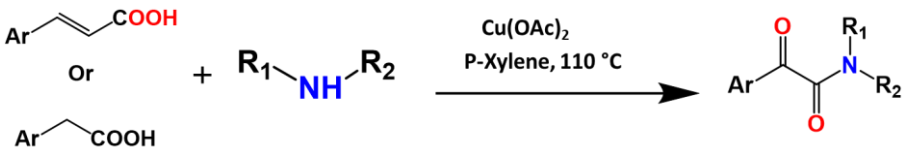
Scheme 1.3. Synthesis of α-ketoamides via manganese (III)-Promoted double carbonylation of anilines.

In 2020, Xiao et. al., reported synthesis of α-ketoamides using water and dioxygen as the oxygen source. This copper-catalyzed reaction is carried out in a tandem manner constituted by the hydroamination of alkyne, hydration of vinyl–Cu complex, and subsequent oxidation (**Scheme 1.4**) [137].



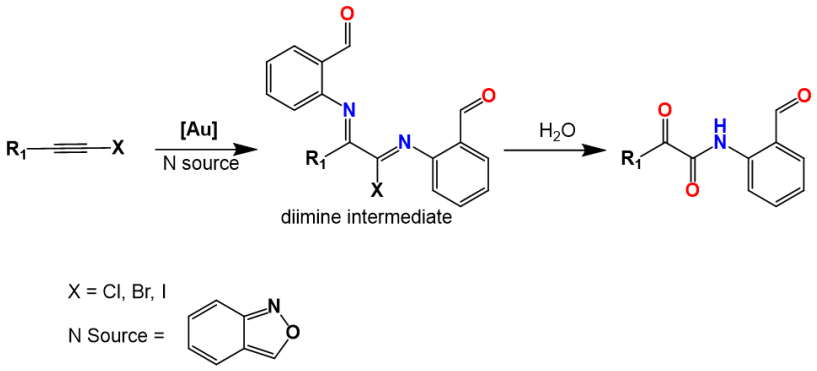
Scheme 1.4. Cupper catalyzed synthesis of α-ketoamides by hydroamination of alkyne using water and dioxygen as the oxygen source.

In 2019, Anup et. al., reported synthesis of α-ketoamides by reaction of cinnamic acids/arylacetic acids with 2° amines in the presence of Cu-catalyst. The reaction between cinnamic acid and amine involves the formation of enamine followed by its aerobic oxidation, whereas the reaction of arylacetic acid with amine involves amide formation followed by benzylic methylene oxidation (**Scheme 1.5**) [138].



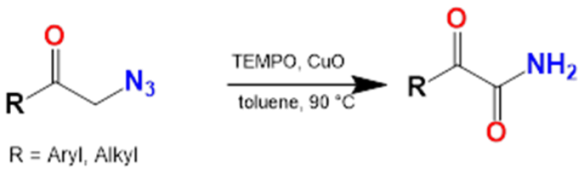
Scheme 1.5. Synthesis of α-ketoamides by reacting cinnamic acids/arylacetic acids with secondary amines in the presence of Cu-catalyst.

In 2022, Zuo et. al., reported the synthesis of various substituted ketoamides via a gold-catalyzed 2-fold reaction of a bromoalkyne with anthranils that led do intermediate diimine. When the intermediate undergoes subsequent hydrolysis α-ketoamide formed (**Scheme 1.6).** [139]



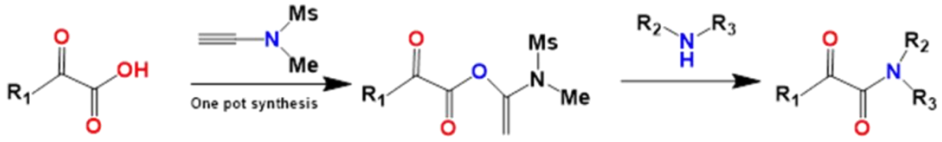
Scheme 1.6. Synthesis of α-ketoamides by gold-catalyzed 2-fold reaction of a bromoalkyne with anthranils.

In 2022, Wang et. al., documented the creation of primary α-ketoamide derivatives using a copper(II)-driven denitrogenation/oxidation method. This process utilized α-azido ketones as the starting material and TEMPO for oxidation. During the procedure, α-azido ketones were converted in-situ into an iminoketone intermediate. This intermediate then underwent a series of radical-based transformations to produce α-ketoamides (**Scheme 1.7**) [140].



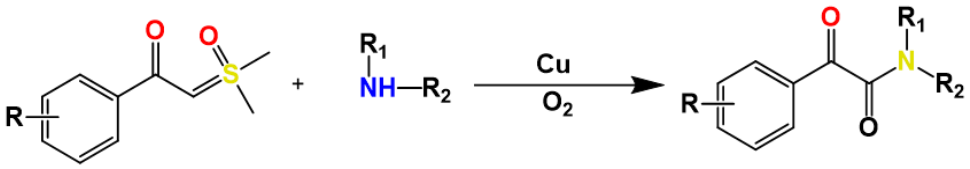
Scheme 1.7. Synthesis of α-ketoamides by using a copper(II)-driven denitrogenation/oxidation method.

In 2022, Jang et. al., crafted 36 varied structural variants of α-ketoamides with a yield reaching 98%. They employed a single-step technique that combined α-keto acids and amines, using ynamides as coupling agents (**Scheme 1.8**) [141].



Scheme 1.8. One pot synthesis of α-ketoamides by using ynamides as coupling agents.

In 2023, Wang et. al., synthesized thirty-eight derivatives of α-ketoamides from sulfoxoniumylides and secondary amines by using copper catalyst [142]. This approach accommodated aryl, heteroaryl, and tert-butylsulfoxoniumylides, leading to a diverse range of α-ketoamides with favorable yields (**Scheme 1.9**).



Scheme 1.9. Synthesis of α-ketoamides from sulfoxoniumylides and secondary amines by using copper catalyst.

1.12. References

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