Chapter 1

Introduction

Chapter 1
Introduction

1. Introduction

1.1 Neurodegenerative disorders: Alzheimer's Disease (AD)

Human brain is most complex of all the biological systems, where the mature brain consists of billions of information-processing cells called neurons. The nervous tissues involved in the command task-evoked responses include, senses, movement, emotions, communication, language, memory, and thinking (1, 2). Brain aging is described through several molecular, anatomical, and functional changes, leading to enhanced susceptibility to numerous diseases. The structural brain changes include volumetric shrinkage of the brain and changes in specific morphology of the brain observed during aging. In addition to the damage to structural integrity and neural plasticity, progressive decline in the reserves of cellular homeostasis and difference in the mechanism of calcium-dependent signaling are considered significant events associated with brain aging (3). Neurodegenerative diseases are characterized by progressive degeneration leading to changes in the structural and functional integrity of the central and peripheral nervous systems (4), causing disruptions in the connectivity and communication between neurons integral to motor, sensory, and cognitive processes. The number of cases predicted a dramatic increase with the population of aging thus posing a significant threat to global healthcare systems (5). Millions of people around the world are affected by degenerative brain diseases, and the mortality continues to rise each year, driving an increasing need for knowledge of the underlying mechanisms and development of treatments (6). Alzheimer's disease (AD) is a multifarious neurodegenerative disorder and is the leading cause of progressive cognitive and functional deficits with behavioral changes. Neuropathologically, it is characterized by the aggregation and deposition of amyloid (Aβ) peptide, extracellularly in the form of senile plaques, and hyperphosphorylated tau protein, deposited intracellularly in the form of intracellular neurofibrillary tangles

(NFTs) (7, 8). Several enzymes, i.e., acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), secretases, lipoxygenases, glycogen synthase kinase 3, sirtuins, and caspases are reported to actively participate in the pathogenesis of AD (9).

1.2 Stages of Alzheimer's Disease

The key characteristics of Alzheimer's disease stages are as follows (10):

Preclinical Stage

- The cognition status of this stage is the subtle episodic memory loss; however, the function is normal.
- The changes in behaviours include apathy, irritability, dysphoria, and a decrease in insight.

Prodromal

- It shows the apparent episodic memory loss.
- The function includes struggles to be independent with instrumental activities of daily living and decreased engagement.
- The changes in behaviours include apathy, irritability, dysphoria, anxiety, and a decrease in insight.

Dementia

- The cognition status is severe episodic memory loss.
- Loss of independence of instrumental activities of daily living and eventually basic activities of daily living (dressing, grooming, bathing, feeding, toileting, and continence) and decreased engagement.
- The changes in behaviours include apathy, irritability, restiveness to care, anger, delusions, wandering/restless behaviours, depression, anxiety, sleep issues, and a decrease in insight.

1.3 Pathophysiology of AD

In 1907, Aloïs Alzheimer identified the extensive distribution of neuronal tangles and amyloid plaques in the brain, which, along with astrogliosis, neuronal dystrophy, neuronal loss, and vascular alterations, were considered the defining features of the disorder (11). The extensive research efforts have shown that Aβ and tau proteins are the primary components of senile plaques and NFTs, respectively, which play a central role in the molecular development of the disease. Several hypotheses have been proposed considering the various contributing factors to study this multifactorial disorder. These include the cholinergic, Aβ, tau, and inflammation hypotheses.

1.3.1 Cholinergic hypothesis

The cholinergic hypothesis emerged and acquired momentum in the seventies and early eighties, based on the examination of brain samples from AD patients. Certainly, the autoptic material from AD patients consistently found with cholinergic deficit from a group of neurons in the basal forebrain, specifically the nucleus basalis magnocellularis of Meynert, to the cortex and hippocampus. The remarkable decrease is observed in the reliable marker of cholinergic neurons and synapses activity, including the enzyme catalyses, synthesis of acetylcholine (ACh) and choline acetyltransferase (CAT), in pathological samples from the brain region of cortex and hippocampus of AD patients (12). The cholinergic hypothesis of age-related memory impairment, proposed by Bartus et al. in 1982, suggested that the functional disturbances occurred in cholinergic activity in healthy older adults' brains and those with dementia contributed to memory decline and associated cognitive issues. Therefore, restoring cholinergic function could help to reduce the severity of cognitive decline (13). This hypothesis is backed by the observation that cholinesterase inhibitors have demonstrated positive effects on cognition in patients with AD (14).

1.3.2 Amyloid beta (Aβ) hypothesis

In AD, the pathology is mainly driven by the prime suspects, Aβ deposits, which are the focus of therapeutic and diagnostic developments. Aβ deposits, an extensively studied pathological biomarker, are closely related to AD pathogenesis and indirectly contribute to other toxic entities (15). According to the amyloid hypothesis, Aβ species originate from the sequential proteolytic cleavage of the amyloid precursor protein (APP) by β-secretase (BACE-1), followed by γ-secretase in the amyloidogenic pathway (16) (**Figure 1.1**). The monomeric Aβ peptides of varying lengths, along with amyloidogenic preferences, are formed on the cleavage of the C-terminal fragments by the γ-secretase enzyme. The presence of a hydrophilic N-terminus region and a hydrophobic C-terminus in the structure of the Aβ monomer contributes towards its aggregation to minimize the unfavourable interactions with the surrounding aqueous environment (17). Monomeric Aβ tends to form Aβ species, oligomers, and fibrillary polymers, including $A\beta_{40}$ and $A\beta_{42}$, where $A\beta_{42}$ shows a higher tendency to aggregate, which results in amyloid plaques and deposits (18). $A\beta_{1–42}$ is the principal constituent of the senile plaques, containing additionally two hydrophobic residues at the c-terminus (17, 19).

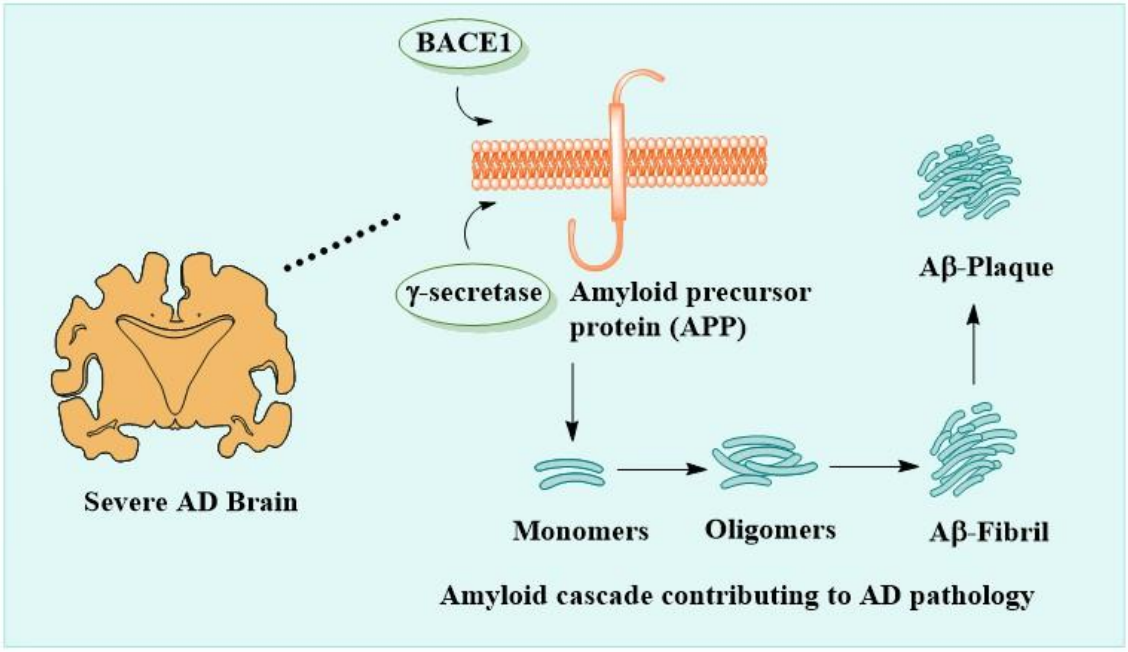

Figure 1.1. Schematic depiction of amyloid-β aggregation (amyloid cascade).

1.3.3 Tau hypothesis

NFTs comprise hyperphosphorylated aggregates of the microtubule-associated protein tau, which are considered the pathological hallmark of AD. Tau, a microtubule-associated protein found in axons, helps to stabilize microtubules by interacting with tubulin (**Figure 1.2**). Recent studies reveal additional role of tau in the prevention of neuronal apoptosis by stabilizing ß-catenin and regulating axonal transport by modulating both kinesin-driven anterograde and dynein-driven retrograde transport (20). In AD, an imbalance between kinase and phosphatase activities leads to the accumulation of hyperphosphorylated tau (p-tau). While, the cause of this imbalance remains unclear, factors such as Aβ, age, genetics, and the environment have been suggested to have some role. Since p-tau pathology is also found in other tauopathies, the tau hypothesis suggests that biochemical factors beyond Aβ may play a role in initiating the disease (21).

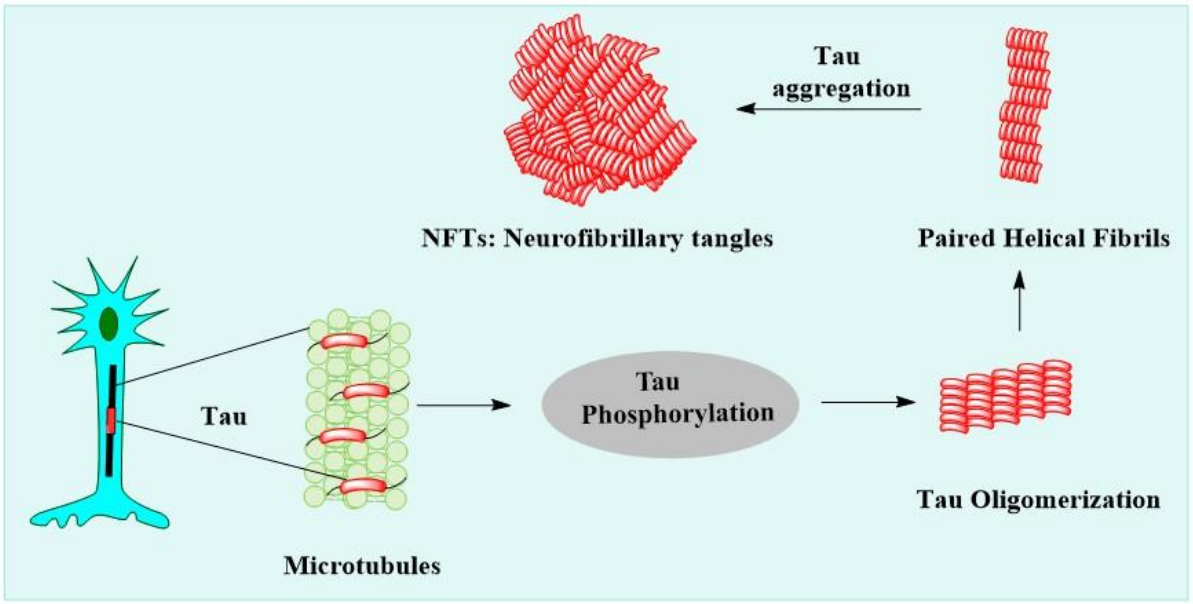

Figure 1.2. Schematic depiction of tau aggregation and formation of NFTs.

1.3.4 Inflammation

Reactive gliosis and neuroinflammation are key features of AD. As supported by recent genetic and transcriptomic studies, microglia-related pathways are central to AD risk and progression (22). A growing of evidence suggests that microglia play a pivotal role in the pathogenesis of AD. During the early stage, microglia along with TREM2 and the complement cascade contribute to synaptic pruning. Activity-dependent and long-term synaptic plasticity represent essential cellular mechanisms that form the basis of learning and memory, often evidenced by their role in modulating long-term potentiation. Subsequently, activated microglia and astrocytes accumulate around amyloid plaques, releasing various pro-inflammatory cytokines. This neuroinflammatory response is considered a key early event in the progression of AD (23) (24).

1.4 Diagnostic approaches for AD

Diagnosis of AD is primarily based upon the clinical examination of the patients, neuropsychological tests. There is a dire need for viable tools for the early diagnosis of AD patients. The diagnosis of AD encompasses different approaches such as the

combination of clinical assessments, cognitive screening, and available advanced neuroimaging techniques (25). Different techniques have been used for brain imaging, including computed tomography (CT) scan, magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), positron emission tomography (PET), and optical imaging. The most commonly used MRI has the limitation of lower sensitivity, and PET use is limited by its higher cost. SPECT also has an issue with poor blood−brain barrier (BBB) penetration (26). The optical imaging probes, such as the development of near-infrared fluorescence (NIRF) imaging, provide widespread application compared to other imaging techniques (27).

1.4.1 Diagnostic markers

1.4.1.1 Aβ species

Aβ deposits are one of the significant AD biomarkers suggested by the Alzheimer's Association and the National Institute on Aging. The imaging of Aβ can provide more insights into the monitoring and diagnosis of AD (28). $A\beta_{1–42}$ is the major constituent of the senile plaques, containing additionally two hydrophobic residues at the c-terminus (17, 19). The Aβ species are the main and potential targeting biomarker for fluorescent probes to visualize AD progression. The structural features of AD probes and properties need to be optimized to improve their biological and optical properties for precise and selective targeting, as well as for efficient BBB crossing. The $A\beta_{(1-42)}$ has the amino acid sequence of DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA, which consists of the hydrophobic region (G, A, F, V, L, I, M), negatively charged (D, E), and the polar (Y, H, Q, N, S) amino acids (29, 30) (**Figure 1.3**). The potential binding sites are A, B, and C **(Figure 1.4**). A and B sites come across at the hydrophilic N-terminal portion and the hydrophobic mid-region, whereas, the C site consists of hydrophobic C-terminal and hydrophilic N-terminal from two different Aβ peptides (31).

The small designed molecules targeting Aβ plaques were found to have a binding affinity towards the B site (31). Distinct phenomena such as an emission wavelength alteration, a large stroke shift, and a significant increase in fluorescence emission were observed on the binding of small molecule fluorescent probes to the Aβ species.

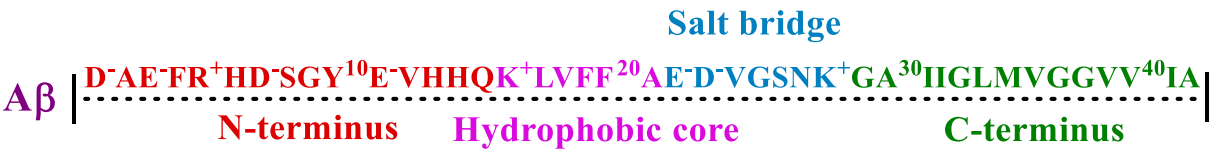

Figure 1.3. $A\beta_{1-42}$ Sequence with sub-regions formed through the amyloidogenic pathway.

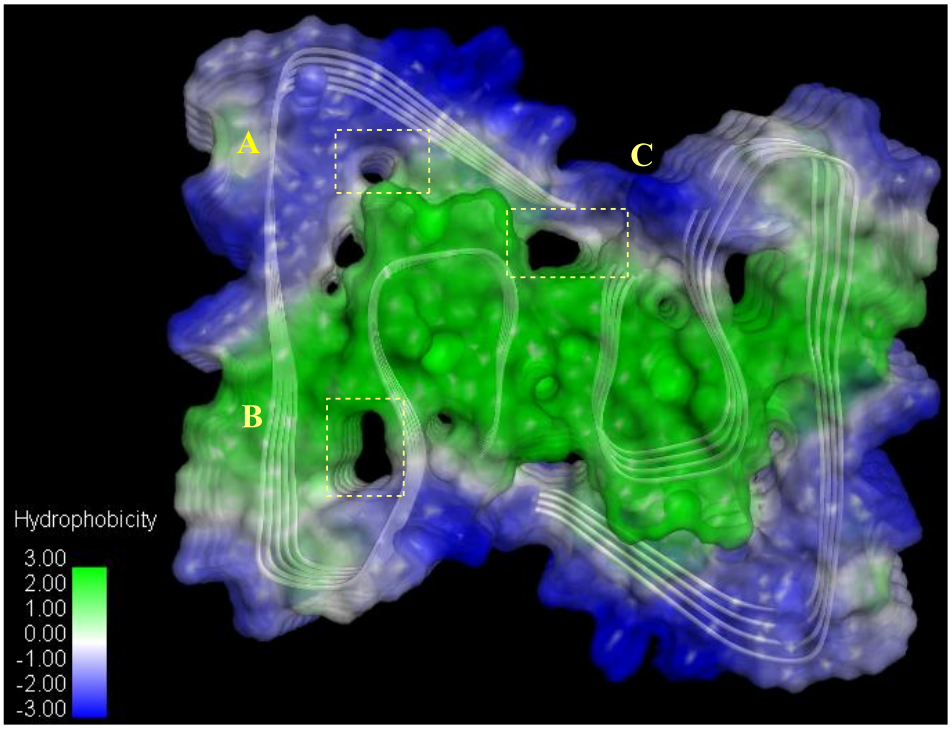

Figure 1.4. Representation of the possible binding sites of $A\beta_{1-42}$ protein (PDB ID: 5OQV**)** (32), (31).

1.4.1.2 Hyperphosphorylated tau peptide

In addition to the Aβ, the tau biomarker for AD is also widely recognized as an important diagnostic parameter for the detection of AD pathology antemortem and is recommended by the recent clinical and research guidelines (33). The abnormal hyperphosphorylation

of tau protein leads to increased self-aggregation. This self-aggregated form of tau is the core component of autopsy findings such as pre-tangles, paired helical filaments, neurofibrillary tangles, and ghost tangles (34). Since the formation of hyperphosphorylated tau in the neocortex is viewed as a subsequent event following Aβ deposition in AD, NFTs and paired helical filaments are rarely observed in the neocortex during the asymptomatic stages of AD (35). Detecting the pre-tangles, paired helical filaments, neurofibrillary tangles, and ghost tangles in AD is considered a major approach.

1.5 Therapeutic drug targets in AD

1.5.1 Cholinesterase Inhibitors

Cholinergic neurotransmission is crucial in the cognitive dysfunction seen in AD and other adult-onset dementia disorders. At present, improving cholinergic neurotransmission remains a primary strategy for the symptomatic treatment of cognitive and behavioral symptoms in the mild and moderate stages of AD (36). A low level of ACh is the key pathologic hallmark of AD, as the dysfunction of cholinergic activity results in a decline in memory and recognition (37). Thus, the activation of cholinergic function via the inhibition of cholinesterase enzymes may prove clinically useful for the symptomatic treatment of AD (38). The ACh is hydrolytically degraded in the brain by two Cholinesterase's (ChEs), AChE and BuChE (38). The rational approach to treat AD is to improve ACh level by inhibiting AChE with highly selective inhibitors (39). The BuChE can also hydrolyse ACh, but does not have the same affinity as AChE (37). In the advanced Braak stages of AD, the AChE level decreases, while BuChE is highly increased (40). The ratio of BuChE/AChE changes from 0.5 in the healthy brain to 11 in the AD brain**,** suggesting that ACh hydrolysis may occur at a higher rate via BuChE catalysis (41). The inhibition of BuChE plays an essential role in increasing ACh levels

and improving cognition (42). Currently approved drugs for AD include ChEs inhibitors such as donepezil, galantamine and rivastigmine (**Figure 1.5**).

1.5.2 Aβ Aggregation Inhibitors

Aβ is one of the primary targets for AD that forms insoluble senile plaque (18). It exists in many forms, such as monomers, dimers, higher oligomers, and fibrillary polymers of $A\beta_{40}$ and $A\beta_{42}$ (7). The fibrillary aggregates of 40- and 42-residue Aβ peptide can produce extracellular deposition of a large number of senile plaques. The deposited senile plaque causes a cascade of events such as inflammatory responses, microglia activation, cytokine release, and astrocytosis, which are responsible for progressive neurodegenerative injury, neuronal deficits, and cognitive dysfunction (7). The development of Aβ aggregation inhibitors has received continuous attention (43).

1.5.3 BACE-1 Inhibitors

BACE is a membrane-bound aspartic protease type B-site APP cleaving enzyme 1 (BACE-1). The enzyme is responsible for the initiation of Aβ production by cleaving the extracellular domain of APP and plays a significant role in the pathogenesis of AD (8). The inhibition or modulation of the BACE-1 enzyme is one of the prime and attractive therapeutic goals for reducing cerebral Aβ concentration in patients with AD (44). The designing approaches for BACE-1 inhibitors must focus on permeation through the BBB to attain a clinically significant amount of drug inside the CNS, and good drug-like properties, i.e. ADME-absorption, distribution, metabolism, and excretion (45). At present, most of the small molecule BACE-1 inhibitors failed to live up to expectations due to toxicity and efficacy issues in the late phase of clinical trials (46).

1.5.4 Tau Aggregation Inhibitors

In AD, the aggregates of intracellular NFTs are observed in the brain due to the accumulation of microtubule-associated tau protein, which is toxic to neurons (47). Tau

protein, as such, is highly soluble and contains hydrophilic residues. It plays a major role in stabilizing microtubules in the region of neuronal axons, as it tracks for axonal transport and as cytoskeletal elements for the growth of axons (48). Neurodegeneration involves the abnormal hyperphosphorylation of tau protein, and this hallmark of AD is called neurofibrillary tangles, neuropil threads, and dystrophic neurites. Abnormal hyperphosphorylation of tau sequesters normal tau, microtubule-associated protein 1 (MAP1), and MAP2 (49). The key factor responsible for tau aggregation is the binding to a non-specific substrate, which possesses a higher affinity to the tau-tau binding domain in the repeat region and undergoes propagation once it has been initiated (50). Two major approaches that consider and address tau aggregation include i) inhibition of kinases that participate in the abnormal phosphorylation of tau and ii) direct inhibition of the tau process (51).

1.5.5 Metal-induced protein aggregation inhibitors

The increase in the concentration of metal ions such as Cu, Fe, and Zn has been associated with the aggregation of β-amyloid (Aβ) plaques in the AD brain, along with causing oxidative stress (52). The metal-ion chelation therapy can be used to minimize oxidative stress as well as disrupt Aβ pathology.

1.6 Clinical drugs for the treatment of AD

The drugs traditionally used for the treatment of AD can be categorized into two classes: AChEIs, tacrine, donepezil, rivastigmine, galantamine, and NMDA receptor antagonists, memantine (**Figure 1.5**) (53, 54). Tacrine was also approved for the treatment of AD in 1993 but was withdrawn from the market in 2013 due to its liver toxicity. Second-generation AChEIs, including donepezil, rivastigmine, and galantamine, are found to be more selective (55). The Memantine, an FDA-approved NMDA receptor antagonist, is used to treat moderate to severe stages of AD. It works by modulating glutamate

transmission and dopamine receptors, showing some effectiveness in enhancing cognitive function, daily living skills, and behavior in patients. (56). The recent treatments of AD include Aducanumab, lecanemab, and donanemab, which are monoclonal antibodies targeting Aβ, each of which has met with differing outcomes. In 2021, Aducanumab received FDA accelerated approval (57). Lecanemab gained traditional approval in 2023, whereas, Donanemab has completed phase III trials and is in the process of market authorization. All the three drugs have demonstrated effectiveness in reducing Aβ plaque and slowing cognitive decline; however, the risks of amyloid-related imaging abnormalities (ARIA) and the associated treatment costs are significant considerations (53).

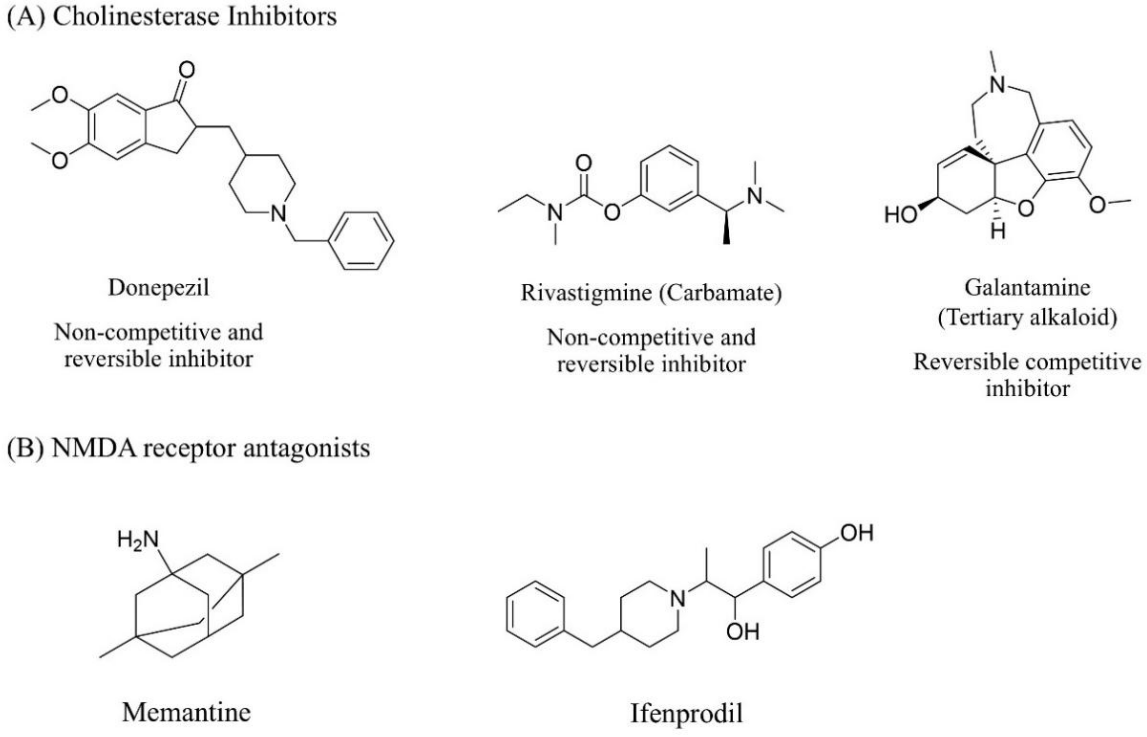

Figure 1.5. Clinical drugs for the treatment of AD.