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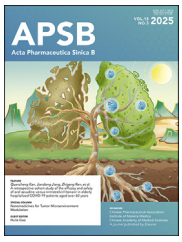

REVIEW

# Comprehensive investigation of multiple targets in the development of newer drugs for the Alzheimer's disease

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**Abstract** Alzheimer's disease, a significant contributor to dementia, is rapidly becoming a serious healthcare concern in the 21st century. The alarming number of patients with Alzheimer's disease is steadily increasing, which is contributed by the dearth of treatment options. The current treatment for Alzheimer's disease is heavily dependent on symptomatic treatment that has failed to cure the disease despite huge investments in the development of drugs. The clinical treatment of Alzheimer's disease with limited drugs is generally targeted towards the inhibition of *N*-methyl-D-aspartate receptor and acetylcholine esterase, which only elevate cognition levels for a limited period. Beyond the aforementioned molecular targets, $\beta$-amyloid was much explored with little success and thus created a feel and palpable growing emphasis on discovering new putative and novel targets for AD. This has inspired medicinal chemists to explore new targets, including microglia, triggering receptors expressed on myeloid cells 2 (Trem-2), and notum carboxylesterase, to discover new lead compounds. This review explores the functions, pathophysiological roles, and importance of all AD-related targets that address therapeutic and preventive approaches for the treatment and protection of Alzheimer's disease.



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## 1. Introduction

Neurologist Dr. Alois Alzheimer discovered the prevailing type of dementia in 1907, which was later named Alzheimer's disease (AD). The degenerative condition of the illness begins with mild memory impairment and progresses to a loss of capacity to communicate with the surroundings. AD is characterized by memory loss, linguistic problems, and impulsive or unpredictable behavior, it is defined as intracellular neurofibrillary tangles and deposition of external amyloid plaques that contribute to illness[1]. AD is classified into two subtypes based on heritability: 1) Familial (FAD) and 2) Sporadic (SAD). Furthermore, AD varieties are expressed based on the onset period as early onset (EOAD) or late onset (LOAD). Although the symptoms are mild initially, they become more severe over time[2].

AD is further described by the destruction of the cerebral cortex and neuronal death in the cortical and subcortical areas. Pathological features of AD include senile plaques, masses of the $\beta$-amyloid protein associated with neurodegenerative illnesses, and masses of tau proteins and paired helical filaments containing neurofibrillary tangles. The affected hippocampus and associative cortex area are most abundant in advanced AD. In contrast, the motor and visual cortices are relatively spared, which matches the clinical symptoms of severe memory loss and the maintenance of eyesight and movement[3].

Many risk factors such as autophagy defects, senescence, genetics apolipoprotein 4 (APOE4), Trem2, lifestyle choices, microbiota alteration, genetics, cardiovascular and traumatic brain injury, as well as environmental factors (pedagogical level, hypertension, and obesity), diametral disease, have been proposed as significant contributors to the onset of AD[4].

According to current estimates, 25 to 30 million people are supposedly suffering from AD, which is projected to triple by 2040[5]. The treatment of AD has been attempted by using numerous drugs including inhibitors of acetylcholine esterase (AChE) exemplified by donepezil, rivastigmine, and galantamine, and antagonists of *N*-methyl-D-aspartate (NMDA), memantine (Fig. 1). The afore-listed drugs alleviate symptoms, but the condition may not be cured. These attributed medicinal chemists to drift towards exploring in deep. The pathology of various mechanisms exemplified by abnormal tau protein metabolism, $\beta$-amyloid, cardiovascular disease, inflammatory response and cholinergic can offer the development of viable medicines[6] (Fig. 2).

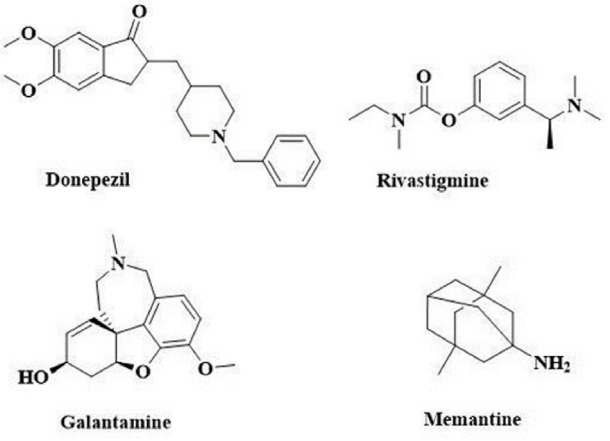



**Figure 1** The two-dimensional structures of FDA-approved drugs used in the treatment of AD[7].

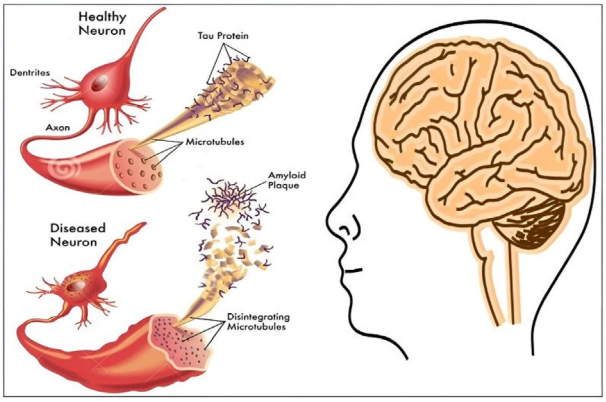



**Figure 2** Pathophysiology of Alzheimer's disease diagnosis[7].

Apart from numerous mechanisms involved in AD, depressive symptoms in AD may also be caused by genetic, neuroanatomic, vascular, and neurotransmitter imbalances. Inflammatory pathways, neurotrophic deficiency, and hypothalamic, pituitary, and adrenal axis deregulation are the possible biological mechanisms linked to depression and AD that their modulators could treat[8]. Neurotransmitters also play an important role in AD; there is a notable and disproportionate deficiency of acetylcholine (Ach) in subcortical cholinergic neurons, notably those within the basal forebrain, which give cholinergic transmission to the entire cerebral cortex. The deficiency of neurotransmitters in AD is even more intricate, involving many neurotransmitter systems, *viz* serotonin, glutamate, and neuropeptides as well as the cholinergic neurons, cortical, and hippocampal targets are affected. Cholinesterase inhibitors act by inhibiting Ach's breakdown, resulting in an increased concentration of Ach[3]. NMDA competitive antagonists decrease glutamate action by binding directly to the glutamate region of the NMDA receptor, which reduces the calcium concentration and results in minimal damage to the nerve cells[9]. There is strong evidence that oxidative brain injury has been implicated early in AD. Hence, antioxidants generally 'clear up' excess free radicals. Peroxisome proliferators activated receptor-$\gamma$ (PPAR$\gamma$) agonists improve cognitive performance and decrease A$\beta$ initiation of microglia[10,11]. $\gamma$-Secretase plays a pivotal part in the breakdown of the APP; thus, using $\gamma$-secretase inhibitors can avert the imminent process[10]. Patients suffering from AD have a persistent immune response and inflammation in their brain, and some experts believe that inflammation stands at 3rd in the major pathologic feature of AD[11]. Pro-inflammatory cytokines such as TNF$\alpha$ and IL-1$\beta$ play a crucial role in neuro-inflammation progression by activating signaling systems involving p38 MAP kinase, nuclear factor $\kappa$B, nitric oxide, COX, and Akt/mTOR[12]. The cellular prion protein (PrPC) was discovered as a key mediator in the A$\beta$ oligomers toxicity, which causes synapse loss and intellectual decline in AD. As a result, directing PrPC and its relationship with A$\beta$ oligomers or downstream mediators could be recognized as the next therapeutic link option for AD management. AD has a direct association with prion mechanisms. PrPC misfolds to form pathogenic prions, which further affects other prion proteins to misfold, eventually causing a significant elevation in abnormal protein levels, which causes brain harm[13].

## 2. Mechanism of inception and progression of AD

The two pathological attributes of AD are:

- Deposition extracellular β-amyloid senile plaques
- Intracellular neurofibrillary tangles

The deposition of β-amyloid and neurofibrillary tangles initiates the loss of synapses and neurons, leading to gross atrophy of the affected part of the brain, which is a platform for onsets in the mesial lobe. It is unknown how exactly β-amyloid peptides and neurofibrillary tangles generate such devastation. As per the amyloid hypothesis, the cumulative deposition of β-amyloid inside the brain results in neuronal necrobiosis, loss of neuronal connections, and progressive neurotransmitter deficiencies, all of the facts above lead to the behavioural symptoms of dementia[3,14]. In AD, β-amyloid in the cerebral area of the brain is deposited and tau in neurofibrillary tangles is believed to possess prion like properties for self replication. Aβ principal binding region and helix-1 epitopes could prevent Aβ binding and the Aβ-facilitated interruption of synaptic plasticity[13]. Chung and colleagues administered the monoclonal anti-PrPC antibody 6D11 intraperitoneally to APP/PS1 transgenic (Tg) mice and discovered that antibody treatment entirely rescued the Tg animals' behavioural and cognitive deficits[15]. Also, Aβ mainly accumulates in the mitochondria of Alzheimer's brain cells and hinders the activity of certain enzymes such as pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase complexes[16].

## 3. New targets in AD

The present treatment for AD is only a symptomatic approach. Tacrine gained the attention of medical practitioners in AD, but due to hepatotoxicity, it was taken off the market. Four FDA-approved drugs, namely, galantamine, donepezil, rivastigmine as AChE inhibitors, and memantine NMDA receptor antagonists, listed in Fig. 1, are available for treating AD. Even though inhibitors of cholinesterase and NMDA receptors have well established efficiency levels, clinical results of patients receiving these medications are restricted, and many authors see them as "symptomatic" therapy[3]. Several targets are being explored for drug discovery anti-Alzheimer treatment; with a few of them, therapeutic targets are previously well identified with inhibitors, whereas some are now being analysed critically for identifying or creating effective small molecules. Table 1[17-39] lists the targets with their origins and known functions.

### 3.1. The amyloid hypothesis

The amyloid cascade concept was first pointed out a couple of decades ago and is based on the Aβ peptide, a member of the intrinsically disordered proteins class (IDPs), a significant plaque element. The Aβ and CT (APP's carboxy-terminal peptide) peptides are amyloid proteins that play a pivotal part in the progress of AD[40]. The formation of the Aβ peptide from the APP molecule by the action of two proteolytic enzymes called β- and γ-secretase, which hydrolyse at the amino-terminal and carboxy-terminal of the peptide, respectively. Decreased metabolic ability to break down Aβ is evidenced in Aβ peptide aggregation in the elderly or diseased people. $A\beta_{42}$ amplifies the development of β-amyloid fibrils from senile plaques, subsequently causing tau pathology and neurotoxicity, resulting in cell apoptosis and neurodegeneration[7].

Two metabolic pathways are involved in processing APP: i) amyloidogenic path and (ii) non-amyloidogenic pathway. The amyloidogenic pathway of APP processing is represented by the APP amino-terminus (NT) by β-secretase and the cleavage of the APP CT by γ-secretase. Between M671 and D672, β-secretase cleaves APP, thus releasing the C99 fragment and an APP soluble peptide (APP). Near residue 712 of the CT, γ-secretase can cleave the CT region in V711 or I713 to form the short peptide Aβ, $A\beta_{40}$ or the long peptide $A\beta_{42}$. A CT peptide of about 50 residues from APP, sometimes called the APP intracellular domain, is also released with the Aβ peptide (AICD). In the nonamyloidogenic pathway, APP can also be processed by α-secretase (TACE), which dissects the sAPP fragment between K687 and L688, thus resulting in the formation of soluble APPα and cell membrane bound C-fragment 83 (CTF83) at residue 711 or 713. The generated CTF83 is cleaved by γ-secretase to produce AICD and a small p3 fragment. This non-amyloidogenic pathway does not make the Aβ peptide. The β-secretase produces sAPP and a 12 kDa protein segment (C99 or CTF), which is then parted by the β-secretase, resulting in Aβ. The "Aβ cascade theory" describes the association between Aβ histopathologic abnormalities, neural apoptosis, and cognitive deficit in AD[41,42].

Numerous mutations have been reported in the APP. These pathogenic APP mutations are related to a surge in the formation of $A\beta_{42}$ and an alteration in the ratio of $A\beta_{42}$ production. Remarkably, in Down's syndrome, individuals with trisomy 21 show symptoms which was assumed to be related to elevated APP and Aβ levels in the brain. Autosomal dominant EOAD with cerebral amyloid angiopathy and massive Aβ peptide accumulation is caused by APP locus duplication[43]. The PS1 or PS2 γ-secretase components are associated with familial AD mutations (Fig. 3). These APP and PS mutations are tightly connected to Aβ production, proving Aβ production and amyloid fibril development. It was previously thought that the release from proteolysis of the β-amyloid peptide from the transmembrane region of its ample precursor protein was an abnormal process that necessitated neuronal damage[44]. The rise in Aβ levels in the brain is a critical event in the amyloid hypothesis and causes synaptic impairment, thus leading to early deficiency in cognition. Synaptic failure in the olfactory bulb can cause olfactory impairments in many Alzheimer's patients. In aging APP/PS1 mice, Aβ deposition leads to functional and morphological modifications in the synapses of the olfactory processing sites[45].

#### 3.1.1. Inhibition of amyloid aggregation

Some IDP proteins tend to aggregate non-covalently to form oligomers, and Aβ can also undergo oligomerization. Amyloid aggregation in fibrils, oligomers, and plaques is thought to cause synaptic functional impairment and nerve cell death in AD. Albeit, amyloid plaques are the principal pathogenic species, and the most neurotoxic form of Aβ is soluble oligomers. Agents, which hider aggregation of Aβ monomers by binding with the monomers, are thus a rational way of containing neurotoxicity and AD[46]. Two inhibitors of such aggregation, scyllo-inositol (ELND005) and tramiprosate (Fig. 4) are explored in phase II clinical trials. In a recent trial, scylla-inositol was safe at low doses (250 mg) but did not show encouraging efficacy. In the higher doses, it was associated with severe side effects like infection and fatalities; thus, further detailed research investigations are needed to determine and measure the efficacy[47]. Phase III studies of mild to moderate patients with AD tramiprosate did not affect cognitive scores. Re-examining trial data revealed therapeutic advantages among ApoE4 homozygotic subjects of ALZ-801, a tramiprosate prodrug[48].

**Table 1** Summary of new targets in AD along with source and functions of targets.

| Sr. No. | Target | Location | Function | Ref. |
|---|---|---|---|---|
| 1 | $\beta$-secretase (BACE1) | Activity of $\beta$-secretase was found in most of the tissues, especially neural tissue and neuronal cell line. Highest activity was observed in trans-Golgi network and endosomes | Generation of A$\beta$ through BACE1 cleavage of APP | 17 |
| 2 | Butyryl cholinesterase | Highly active in heart, lung, liver, kidney and intestine | Regulates brain ACh level and cholinergic transmission in absence of AChE | 18 |
| 3 | $\gamma$-secretase: Presenilin 1 | Found in endoplasmic reticulum | Catalyses intramembranous proteolysis of the notch receptor and generates A$\beta$ by the sequential cleavage of the substrate C99 | 19 |
| 4 | Calcitonin gene-regulated peptide (CGRP) | $\alpha$CGRP is predominant in CNS and endocrine tissues. $\beta$CGRP is located in brain, thyroid gland and sensory ganglia | At the transcriptional level, CGRP receptors reduce acetylcholinesterase availability and elevate cAMP levels | 20 |
| 5 | Phosphodiesterase (PDE) | Different isoforms of PDE are found in CNS | A key function in neurons is to regulate intracellular signalling cascades initiated by neurotransmitters and control the intracellular levels of cAMP and/or cGMP | 21 |
| 6 | Muscarinic acetylcholine receptor | mAChRs located on detrusor smooth muscle and cholinergic nerve terminal of the bladder | It modulates ACh release, which can improve voiding efficiency with increased nerve traffic | 22 |
| 7 | Dopamine-2 receptor | Found in the olfactory tubercle, striatum and nucleus accumbent in large amounts and to a small amount in the amygdala, hippocampus and cortical region | Regulates various brain functions, playing vital role in regulating locomotor activity, motivation and cognition | 23 |
| 8 | $\gamma$-aminobutyric acid receptor (GABA) | Widely spread in the brain cortex, frontal, occipital, and temporal lobes | It controls cortical excitability | 24 |
| 9 | Nuclear factor-erythroid 2 related factor-2 (Nrf2) | Expressed in both the cytoplasm and nucleus of neuron hippocampi | Retaining redox balance, antioxidant and remove damaged proteins | 25 |
| 10 | Metabotropic glutamate receptor (mGluRs) | mGluRs are primarily found at the excitatory synapse, however their positions may vary depending on the type of various isoforms. It is found in the cerebellum, amygdala, dorsal striatum, olfactory bulb, nucleus accumbens and hippocampas | Long-term effects of mGluRs control neuronal plasticity, maintain CNS function, and contribute to pathological situations such as anxiety, fear extinction, and spatial working memory | 26 |
| 11 | Parkinson's disease protein (DJ-1/PARK7) | It is abundantly expressed in astrocytes in the frontal cortex and substantia nigra of idiopathic PD brains | Protection from oxidative stress | 27 |
| 12 | N-myc downstream-regulated gene | Astrocytes, epithelial tissue and glia cells | Act as an endogenous neuroprotectant in AD | 28 |
| 13 | Protein tyrosine phosphatase 1B (PTP1B) | Hippocampus, microglia | It modulates several CNS processes relevant to the physiopathology of AD and acts as a positive controller of neuroinflammation | 29 |
| 14 | Monoamine oxidase-B (MAO-B) | Expression of MAO-B is increased in the cerebral cortex and hippocampus of AD brain | Deactivates trace amines like 2-phenylethylamine (PEA), neurotransmitters like dopamine, and potentially other neuro-modulatory amines | 30 |
| 15 | NAD(P)H quinone oxidoreductase | Found in neurons of hippocampus and astrocytes of AD brain | Protects cell from oxidative injury | 31 |
| 16 | Amyloid protein precursor (APP) | Found in brain | In transfected cell lines, APP regulates cell proliferation, motility, neurite outgrowth, and survival. The soluble ectodomain, generated by APP cleavage, can replicate these actions | 32 |
| 17 | Peroxisome proliferator activated receptor-$\gamma$ (PPAR-$\gamma$) | Detected in higher quantities in the brains of Alzheimer's patients | It has helpful effects on memory and learning are specifically caused by inflammation inhibition, insulin action enhancement, or mitochondrial function within the brain | 33 |
| 18 | C−C chemokine receptor type-5 (CCR5) | Macrophages, dendritic cells, and memory T cells in the immune; in CNS | Potent inhibitor of learning and memory by inhibiting hippocampus and cortical neuronal plasticity | 34 |
| 19 | Nicotinic acetylcholine receptor (nAChR) | nAChRs are found in both neurons and glia in the central nervous system | It plays role in the development of locomotion, memory, anxiety and attention | 35 |

**Table 1** (*continued*)

| Sr. No. | Target | Location | Function | Ref. |
|---|---|---|---|---|
| 20 | Angiotensin receptor I and II (AT1 & II receptor) | Blood vessels, kidney, adrenal cortex, heart, lung and basal ganglia, circumventricular organs of brain, brainstem | Rise brain A$\beta$ level *via* various mechanisms with increasing APP mRNA, $\beta$-secretase action, and presenilin expression | 6 |
| 21 | Triggering receptor expressed on myeloid cells 2 (TREM2) | Present in CSF and myeloid cells including microglia, monocytes, macrophages, neutrophils osteoclasts, and dendritic cells | Strongly increase the risk of AD development, mediates the role of AD pathogenesis | 37 |
| 22 | BDNF | Pyramidal and granule cells of the hippocampus and specific regions of the CNS | Essential for the life of adult cortical neurons in the entorhinal cortex, whose early failure causes the short-term memory loss in AD | 38 |
| 23 | GSK-3 | In the brain of AD patient | Kinase dysregulation has been shown to affect both A$\beta$ and tau metabolism and toxicity | 39 |

AT1 & II receptor, angiotensin receptor I and II; (CP)-AMPARs, (calcium permeable)-$\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; cGMP, cyclic guanosine monophosphate; GABA, $\gamma$-aminobutyric acid receptor derived neurotrop; MAO-B, monoamine oxidase-B; mAchR, muscarinic acetylcholine receptor; DJ-1/PARK7, neurotrophin brain-Parkinson's disease protein; PTP1B, protein tyrosine phosphatase 1B; PDE, phosphodiesterase; TREM2, triggering receptor expressed on myeloid cells 2.

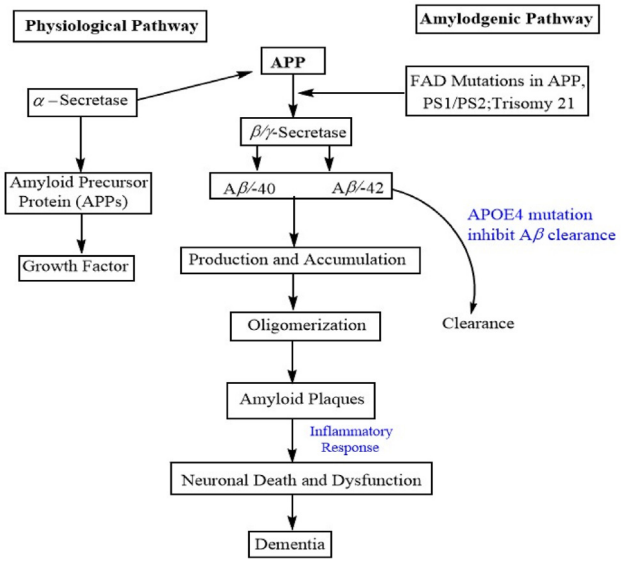



**Figure 3** Pathological pathway and amyloidogenic pathway.

### 3.1.2. *Nonpeptidic anti-aggregates*

The first class of specified agents in the suppression of aggregation are nonpeptidic anti-aggregates, of which tramiprosate is a primitive example generated from propionic acid. Subsequent phase III trials contradicted this agent's promising results in terms of safety and tolerance: the European trial avoided medical issues that could have resulted in a negative outcome in the trials of North America and proved feeble penetration of the drug in the brain and insufficient potency[49]. New nonpeptidic anti-aggregates were supposed to address these drawbacks and accumulates are thought to dissociate faster when scyllo-inositol is present. As this molecule may cross the blood–brain barrier (BBB), it can reach significant concentrations in the CNS by peripheral administration[47]. This drug also involved cell transduction modulation, cell survival and cell death regulation, and mitochondrial function. The numerous properties of this natural molecule have identified it as a potential contender, and a phase III trial with primary AD patients using epigallocatechin-3-gallate (EGCG) is now under screening consideration[50].

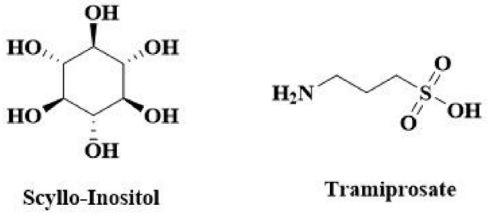



**Figure 4** The structures of amyloid aggregation inhibitors which have extended clinical trials.

### 3.1.3. *Metal chelators*

Several studies link AD pathogenesis to neocortical amyloid aggregation; this could be facilitated by abnormal A$\beta$ interaction and metal mediated oxidative stress; in the AD brain, aluminium, zinc, iron, and copper increase A$\beta$ aggregation and neurotoxicity[40]. Although the aluminium related hypothesis has some discrepancies, aluminium may play a cardinal part in developing neurofibrillary tangles and neurotic plaques in AD[51]. Using aluminium binding ligands (silicates) or deferoxamine (DFO, Fig. 5), the metal chelator, researchers have tried to slow or reverse A$\beta$ accumulation[52,53]. Two years' study by McLachlan found that DFO-treated groups had significantly lower neocortical aluminium concentrations than untreated groups, with behavioural improvement. Although DFO has an aluminium chelating effect, zinc or copper chelating effects are possible[53].

A$\beta$ can bind with metals, including copper and zinc, which are present in high amounts in neurotic plaques in AD and promote *in vitro* A$\beta$ aggregation and neurotoxicity[54]. In a masked study of APP, nine weeks of treated Tg mice escalated soluble A$\beta$ levels by 52%[55]. This hinted that metal chelators like clioquinol (Fig. 5) could inhibit A$\beta$ accumulation and thus be used to treat AD. Vital evidence indicated that soluble A$\beta$ levels correlated with cognitive dysfunction in AD Tg mice and that soluble A$\beta$ levels precede resulted in A$\beta$ plaque deposition in AD Tg mice[56,57]. It is more important to prevent both soluble and extracellular A$\beta$ plaque accumulations[58].

## 3.2. *Anti-amyloid immunotherapy*

*In vitro* investigations were the first to show that antibodies contrary to the A$\beta$ peptide might help reduce amyloid accumulation. Anti-A$\beta$ antibodies with little stoichiometries prevented A$\beta$ by

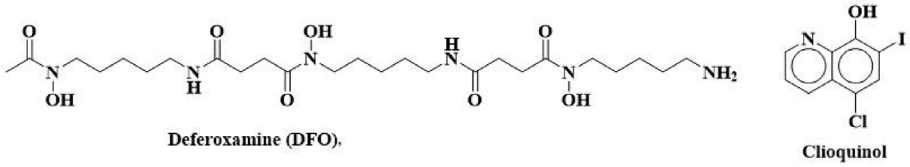



**Figure 5** Structures of metal chelators.

developing fibrils *in vitro*[59]. The study identified the EFRH epitope at 3−6 positions of A$\beta$ as a crucial target for that antibody's catalytic action[60].

As prospective Alzheimer's immunotherapies, active immunization with A$\beta$ peptide synthetic fragments hinted at a transporter protein, and passive immunization with monoclonal antibodies targeted against A$\beta$ peptide is being investigated. In AD animal models, immunization with A$\beta$ peptide provided protection and reversed pathology. Meningoencephalitis, vasogenic oedema, and microhaemorrhages have all been reported with AN1792. As of now, immunotherapy has not shown a substantial cognitive effect on Alzheimer's patients, but peptide immunotherapy is being studied against tau pathology as well[60].

### 3.2.1. *Immunotherapy mechanism*

Several hypotheses have been proposed to understand how antibodies engaged at A$\beta$ enhance peptide clearance *in vivo*[60,61].

(i) Microglial phagocytosis:

According to the elementary hypotheses, whenever anti-A$\beta$ antibodies bind to A$\beta$ peptides, their Fc component finally hits Fc receptors on the cell membrane of microglial cells, where it is phagocytized and digested. According to this theory, antibodies of anti-A$\beta$ can travel through the BBB and attach to A$\beta$ inside the central nervous system. While evidence has been found to back this theory, additional research has unequivocally shown that Fc-promoted phagocytosis is not essential for A$\beta$ clearance brought on by immunotherapy[61].

(ii) Peripheral sink:

Monomeric $\beta$-amyloid could be removed from the brain by generating antigen−antibody complexes on the outside, preventing the formation of new plaques. Another idea is that A$\beta$ could be evacuated from the brain straight into the blood by altering the A$\beta$ brain blood balance to improve clearance of soluble A$\beta$, which is supported by a rise in serum A$\beta$, the majority of which is antibody bound[60].

(i) Deaggregators:

AD immunotherapy is being studied in many forms; the first direct immunization by using synthetic A$\beta_{42}$ was tested in Tg mouse models and humans recently[61]. Passive immunization has been observed to prevent the production of new amyloid plaques and eliminate existing ones. Anti-A$\beta$ monoclonal antibody treatment wholly and quickly restored the hippocampus Ach release and uptake of high-affinity choline in mice. The anti-A$\beta$ antibody neutralizes cholinotoxic species upon binding the A$\beta$ peptide, eventually reversing the deficiency of early memory[62,63].

### 3.2.2. *Molecular understandings of active and passive immunization*

Antigen-specific antibodies are produced *via* vaccination, *i.e.*, active immunization. Antigens employed in AD include whole A$\beta$ or a portion of A$\beta$, which is conjugated to a foreign T cell epitope carrier protein. Antigen-presenting cells deliver T cell epitopes to naive T cells, resulting in a humoral immune response. Surface co-stimulatory molecule binding promotes T cell activation by providing a secondary signal to help activated T cells generate antibodies against the antigen. The soluble antigen attaches to receptors of $\beta$ cell receptors through the $\beta$ cell epitope-activated T cells to cause cellular immunity. Pro-inflammatory cytokines are released during a Type 1 T helper (Th1) reaction, while anti-inflammatory cytokines are released during a Type 2 T helper (Th2) response. Passive immunization eliminates the requirement to stimulate the immune system to manufacture antigen-specific antibodies. Anti-A$\beta$ antibodies target the peptide for elimination in both active and passive A$\beta$ vaccination (Fig. 6)[63].

Immunotherapy for AD has been met with mixed reactions. Unexpected adverse effects from the clinical trials dampened initial enthusiasm. The statistics on cognitive performance implied that some people benefited from the treatment despite its brevity. Many ways are being studied to avoid the adverse effects of active immunization trials. Managing anti-A$\beta$ titres and stopping therapy with passive immunization is appealing. High anti-A$\beta$ titres may increase cognophilic angiopathy and vascular outflow in mice, and immunotherapy trials are previously in process. One includes passive N-terminal antibody immunization, a second use of a shortened peptide vaccine to reduce T cell responses. The preceding trial's results will demand caution in future immunotherapy trials for AD. These trials may be the primary therapeutic tests of the amyloid hypothesis in AD if they are completed successfully[64].

Despite early failures, some phase II clinical trials have been conducted in the last decade with no serious adverse events. Merck (MK-8931), Novartis (CAD106), and Afiris (Affitope AD02) funded such clinical trials; however, none of them reported their findings[65]. Several passive immunization approaches are being investigated, including intravenous immunoglobulin, intravenous delivery of anti-A$\beta_{42}$ monoclonal antibodies, and so on. An intravenous immunoglobulin clinical experiment with a small number of AD patients that was followed for six months discovered a substantial effect on cognitive performance and a drop in CSF A$\beta_{42}$ levels following immunization (Fig. 6)[60].

Gantenerumab is the first anti-A$\beta_{42}$ monoclonal antibody formulation. It appears to slow the growth of amyloid deposits in AD patients, although its efficacy has yet to be proven. Limited clinical data exist on the relationship between reduced amyloid deposit formation and clinical outcomes[66]. Solanezumab is a well-tolerated anti-A$\beta_{42}$ monoclonal antibody formulation, but its efficiency is still unknown. A study of 1000 mild to moderate AD patients who were administered intravenously 400 mg Solanezumab every four weeks for 80 weeks failed to show its efficacy[67].

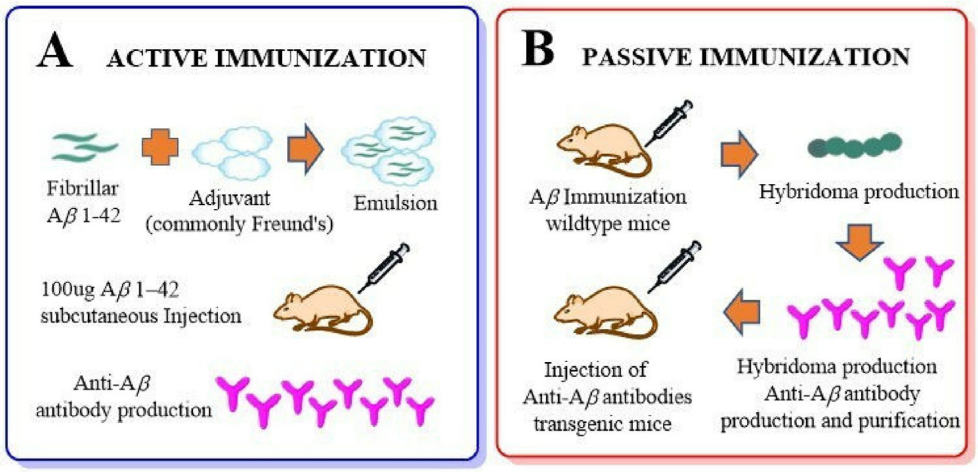



**Figure 6** Molecular basis of active and passive immunisation[61]. (Reprinted with the permission from Bentham Ref. 61. Copyright © 2011 The authors).

Six well-designed randomized critical trials by Richard et al.[68] showed that anti-amyloid $\beta$ immunotherapy does not affect AD. Second, unlike classical frequentist analysis, which merely allows us to conclude that there is no evidence, Bayes factor hypothesis testing will enable us to quantify the plausibility of six well-designed RCTs of the null hypothesis. Third, while the results of frequent meta-analysis may hint that further research is needed, the Bayesian analysis strongly suggests that anti-amyloid $\beta$ immunotherapy is no longer effective; this opens up new avenues of research that may yield additional fruit[68].

### 3.3. *Anti-amyloid strategies*

A$\beta$ amyloid targets, *i.e.* secretases are implicated in APP metabolism, selective reduction of A$\beta_{42}$ production, amyloid aggregation prevention, and anti-amyloid immunotherapy help bring down A$\beta_{42}$[69]. Although these drugs have serious side effects and should only be used in severe cases with serious care. Thus, current amyloid-based therapy research will pave the way for efficient medical care to address this deadly illness. In particular, genetic studies have assisted in strengthening the amyloid hypothesis during the previous two decades[69]. Several therapeutic strategies have been developed to block A$\beta$ peptide effects, and the advancement of effective treatment strategies for reducing A$\beta$ production or increasing A$\beta$ clearance is currently a primary focus in research. Among such approaches include the metabolism of APP by the enzyme secretases, which are discussed below.

#### 3.3.1. *$\gamma$-Secretase inhibitors (GSIs)*

The A$\beta$ peptide is formed from the APP molecule because of the action of two proteolytic enzymes $\beta$- and $\gamma$-secretase, which hydrolyze at the NT and CT of the peptide, respectively; thus, inhibitors of both secretases could benefit from the decreased levels of A$\beta$. Theoretically, inhibitors of either $\beta$- or $\gamma$-secretase could reduce A$\beta$ formation[70]. $\gamma$-Secretase is a type of aspartyl protease with multiple subunits that hydrolyze APP and another type 1 transmembrane protein. Presenilin 1 (PS 1), nicastrin (NTC), anterior pharynx defective-1 (Aph-1), and presenilin enhancer-2 (Pen-2) are the four primary elements for enzyme activity in the $\gamma$-secretase complex (Fig. 7)[71]. PS is a protein that is required for $\beta$-APP transmembrane cleavage[72] and further, Presenilin-1 (PS-1) is responsible for most A$\beta$ production, but Presenilin-2 (PS-2) can also change. PS1 and PS2 work together to form the catalytic core of $\gamma$-secretase[71]. Currently, over 200 missense mutations in the *PS-1* gene were discovered in destructive early onset FAD, with the bulk of them altering the A$\beta_{42}$/A$\beta_{40}$ ratio and meddling with APP and other $\gamma$-secretase substrate processing[73].

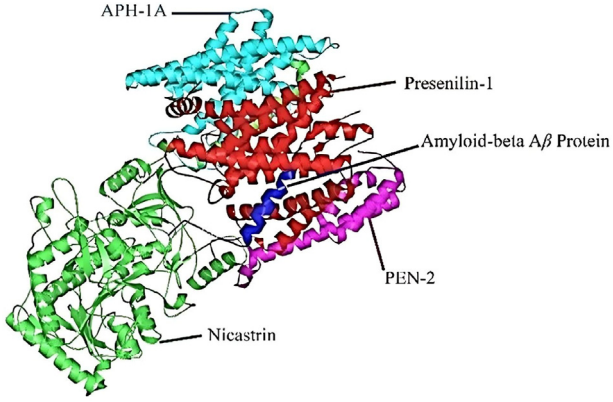



**Figure 7** Human $\gamma$-secretase complex three-dimensional structure (PDB ID: 6IYC)[74]. Reprinted with the permission from Ref. 74. Copyright © 2020 The authors.

A small molecule has been observed that can inhibit $\beta$- and $\gamma$-secretase, which are essential for controlling the A$\beta$ synthesis. In preclinical studies, the use of specific inhibitors of $\gamma$-secretase has been shown to reduce levels of soluble A$\beta$ levels and accumulation of A$\beta$[70].

##### 3.3.1.1. *Crystal structure of human $\gamma$-secretase.*

The $\gamma$-secretase complex is a member of a family of intramembrane cleaving proteases (I-CLiPs) that hydrolyze substrates in the hydrophobic environment of lipid bilayers. Membrane encased proteases are also soluble proteases, such as site two protease metalloproteases, presenilin type aspartyl proteases, and rhomboid serine proteases. I-CLiPs are found in nearly all life forms with a broad range of vital roles in biology and cut within their substrates' transmembrane domain[75,76]. Entirely several crystal structures of

γ-secretase are in the apo and inhibitors state have been identified[73,77]. Crystal structures of human γ-secretase co-crystallized with APP85, notch85, and inhibitors have been solved[73]. The catalytic subunit of the complex present in PS1 and PS2 in the aspartyl protease presenilin[78]. Complexes of presenilin (bears the active site aspartate), nicastrin, Aph-1, and Pen-2 are found in the complex with γ-secretase. The complex structure has at least eighteen transmembrane domains, thus making crystallographic experiments more challenging. It also comprises two pores (apical and basal pores) that allow water molecules to enter and a low density interior chamber. These holes may explain this remarkable intramembrane cleavage (peptide bond hydrolysis) by γ-secretase for water molecules. Two pores could allow the release of Aβ and AICD into extracellular and cytosolic regions, respectively[79].

The atomic structure of γ-secretase in a substrate free condition was disclosed in 2015 using single particle cryoelectron microscopy (cryo-EM) with a resolution of 3.4 Å. The total molecular weight of these proteins is about 170 kDa (kDa), with an extra 30−70 kDa of glycosylation present in the nicastrin extracellular domain. The catalytic component, presenilin, has nine TMs. The TM6 and TM7 regions of presenilin are autocatalytically cleaved into the NTF and the CTF upon association with PEN2. Nicastrin and APH-1 combine to form a stable subcomplex that interacts with presenilin's CTF. A sizable extracellular domain found in nicastrin is assumed to be in charge of substrate recruitment. More than 150 missense mutations found in AD patients demonstrate the critical function of presenilin in the γ-secretase complex[77].

The catalytic subunit of the complex present in PS1 and PS2 in the aspartyl protease presenilin[80]. PS1 is proteolyzed and converted into the building of two: an NTF and a CTF. The complex involves PEN2 for maturation, whereas APH1 is significant for its stability. Lately, NTC was discovered to show a vital role in APP binding (Fig. 7)[74]. The distinctive patterns YD on TM6 and GxGD protease on TM7 distinguish Presenilin from other TMs. Presenilin initiates an autocatalytic breakage during γ-secretase assembly, yielding an amino NT fragment containing TMs 1−6 and a CTF containing TMs 7−9. More than two-thirds of the 300 mutations taken from patients with FAD are localized to PS1, and roughly a third each to PS2 and APP. Nicastrin has a single TM and a huge extracellular domain that is highly glycosylated and is supposed to target the NT of substrate proteins[77].

The hydrophobic nature of the substrate binding sites on the membrane-embedded protease complex led to the detection of extremely powerful inhibitors that can pass the BBB. The inhibitor was discovered to bind with amino acids I143, M146, W165, L166, ser169, M233, F283, G384, and F388, which form a requisite motif that also contains the catalytic dyad of D257/D385 in the crystal structure of γ-secretase assembly[74].

The γ-secretase modulators (GSMs) prevent the enzyme's production of $A\beta_{42}$ without reducing total Aβ levels. This potential impact was initially detected in a subclass of NSAIDs, including naproxen, ibuprofen, and sulindac sulfide (Fig. 8). Despite their low efficacy in reducing $A\beta_{42}$ production, these compounds provided vital proof that such selective reduction could be accomplished, resulting in low nanomolar compounds with promising pharmacokinetic properties[81].

Currently, several γ-secretase inhibitors (GSIs) are being investigated, and among them, some are in clinical trials, such as semagacestat (LY450139) nonselective GSIs. Phase III trial results of semagacestat on patients involving mild to moderate AD were abruptly terminated in two of these trials[81]. It was also found that the use of GSIs was associated with adverse liver, spleen, and skin reactions. γ-Secretase activating protein, a protein that is a part of the amyloidogenic pathway, has been studied in patients who were suffering from Down's Syndrome (DS) and has been found to produce β-amyloid without displaying acute toxicity, so its inhibition might be a target for development[82]. Following the disaster of GSIs in the clinical trials, it was evident that GSMs would be a more secure solution. Because of its multiple substrates, inhibiting

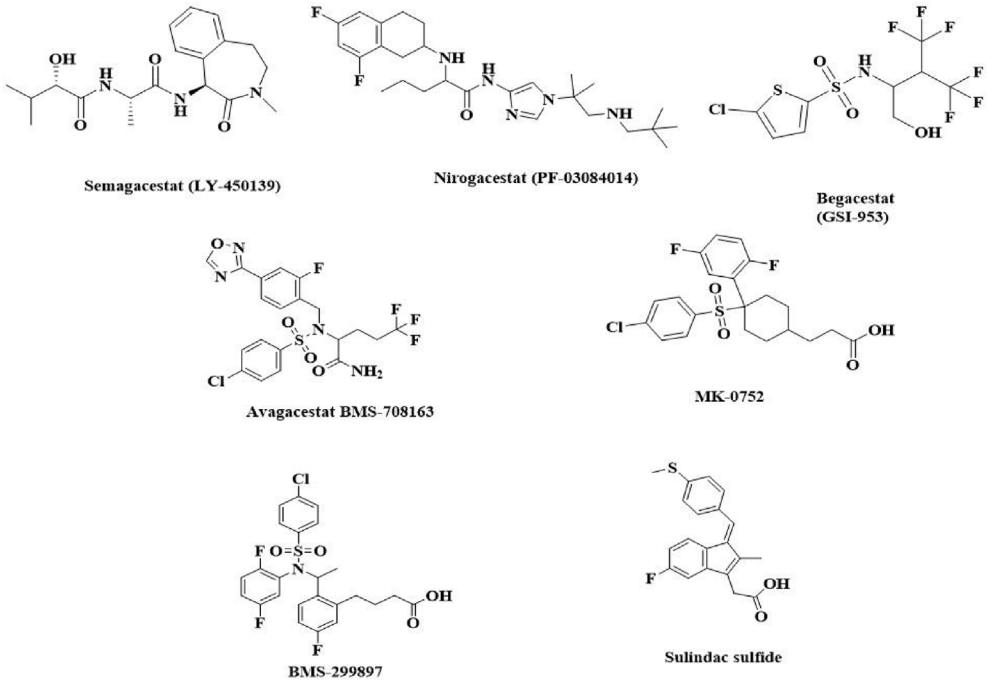



**Figure 8** The structures of γ-secretase inhibitors which have extended clinical trials.

GS may not be tolerated in a continuous dosage paradigm; however, boosting its process ability with GSMs may fix the essential molecular shortfall in FAD. Clinical trials of BMS-299897 were the first of their kind. Bristol Myers Squibb developed the inhibitor; however, no clinical data was released. Many secretase inhibitors like ELND-006 (Fig. 4), GSI-953, PF-3084014, LY-450139, MK-0752, and BMS-708163 (Fig. 8) are in clinical studies, among there the data of LY-450139 are opened for public reference. The inability of GSIs to distinguish between APP and Notch causes critical difficulties of Notch related toxicity, as depicted in Table 2[40,47,83-88].

### 3.3.2. γ-Secretase modulators (GSMs)

Inhibiting $A\beta_{1-42}$ aggregation in AD patients without increasing CTF-$\beta$ deposition, meddling with Notch signaling and APP intracellular domain (AICD) release, appears to be a logical way for containing AD causatively. GSMs with increased potency for reducing $A\beta_{1-42}$ production have been designed and developed in recent years. In $A\beta_{1-42}$ inhibitory potency has to be augmented and selectivity for other γ-secretase substrates be preserved. Some NSAIDs have been demonstrated to inhibit Notch-1's S3 cleavage site. The concentration-dependent dissociation of inhibitory effects on multiple cleavage sites appears to leave a "window of modulation". The reduction of microglia mediated cytokine release is another mechanism that may be implicated in the favourable behavioural and neuropathological effects of many NSAIDs (the best recognized being ibuprofen), reduction of microglia peroxidase and inhibition of neuronal cell death. γ-Secretase modulators like NSAIDs face challenges in brain penetration because of the high binding to plasma protein; the unbound fraction is only 3−5 percent of the total concentration, limiting the quantity of medication that may enter the CNS. Most NSAID GSMs are massive lipophilic molecules with a high prevalence of non-selective and off-target pharmacology. Finally, improved comprehension of the target(s) with which these γ-secretase modulators bind will aid future drug development for AD[41]. Tarenflurbil (Fig. 9) modulates the γ-secretase activity by reducing $A\beta_{42}$ synthesis and generating less toxic Aβ fragments while sparing other γ-secretase substrates, including Notch. Tarenflurbil is the *R*-enantiomer of Flurbiprofen that unexpectedly portrayed righteous safety and clinical results in a phase II trial[89]. The clinical advancement of γ-secretase modulators for AD treatment is shown in Table 3[41,81,90−95].

### 3.3.3. β-Secretase (BACE1) inhibitors

The β-secretase, as an enzyme responsible for the cleavage of β-site APP (BACE1; also known as Asp2, memapsin 2)[96]. BACE1 is a complex structure protein resembling other aspartyl proteases, a family of enzymes found in the human body, including pepsin BACE2, cathepsin D (CatD), renin, and cathepsin E (CatE). BACE1 hydrolyses the APP in the lumina and step that restricts speed in the production of Aβ. Inhibition of BACE1 has several advantages; it prevents Aβ formation early in the APP processing. Furthermore, knockout homozygote BACE1 mice showed a complete loss of Aβ formation with no considerable side effects. Initially, APP is broken by α- or β-secretase, followed by which

**Table 2** Clinical development of γ-secretase (Chemical structures shown in Fig. 8).

| Sr. No. | Name of compound | Pros | Cons | Clinical trial identifier | Status | Ref. |
|---|---|---|---|---|---|---|
| 1 | Semagacestat (LY-450139) | Reduces the amount of newly synthesised Aβ in healthy people's CSF. (first GSI to enter phase 3 clinical trials.) | In AD patients, it worsens cognitive function in a dose-dependent manner.<br>In AD patients, the risk of skin cancer and infection increases. | NCT01035138<br>NCT00762411<br>NCT0059456 | Discontinued | 84 |
| 2 | Avagacestat BMS-708163 | Notch sparing.<br>Lowers Aβ conc. In CSF of normal person | Increases in reversible renal tubule effects and nonmelanoma skin cancer.<br>Rises in brain atrophy rates.<br>Patients with AD have poor tolerability. | NCT00890890 | Phase II | 85 |
| 3 | Begacestat (GSI-953) | Potent Aβ reduction and *in vitro* notch processing selectivity.<br>*In vivo* plasma and CSF Aβ reduction. | Inadequate information on cerebral plaque aggregation in Tg mice.<br>Produces rebounds Aβ-dependent cognitive deficits in Tg mice | WAY 210953 | Phase II | 86 |
| 4 | Nirogacestat PF-3084014 | Notch sparing.<br>Well CNS permeation. Long term activity on Aβ conc.<br>No rebound effect on plasma Aβ in animals | Inadequate information on cerebral plaque deposition in Tg mice.<br>Inadequate knowledge regarding the behavioral impact of AD in animal models.<br>Undesirable patient's PK/PD profile | NIR-DT-301 | Discontinued | 40,87 |
| 5 | MK-0752 | Reductions $A\beta_{40}$ conc. In CSF of normal person | Prevents notch cleavage. Major gastro-intestinal toxicity in patients | NCT00645333 | Discontinued for AD | 40,88 |
| 6 | Scyllo-inositol (ELND005) | Notch sparing.<br>Well brain permeation.<br>Reduce brain Aβ load in transgenic mice | No cognitive improvement;<br>Toxicity in the greater doses (infections and death) | NCT00568776 | Phase II | 47 |

CNS, central nervous system; CSF, cerebrospinal fluid; GSI, gamma secretase inhibitor; PK, pharmacokinetic; PD, pharmacodynamic; Tg, transgenic.

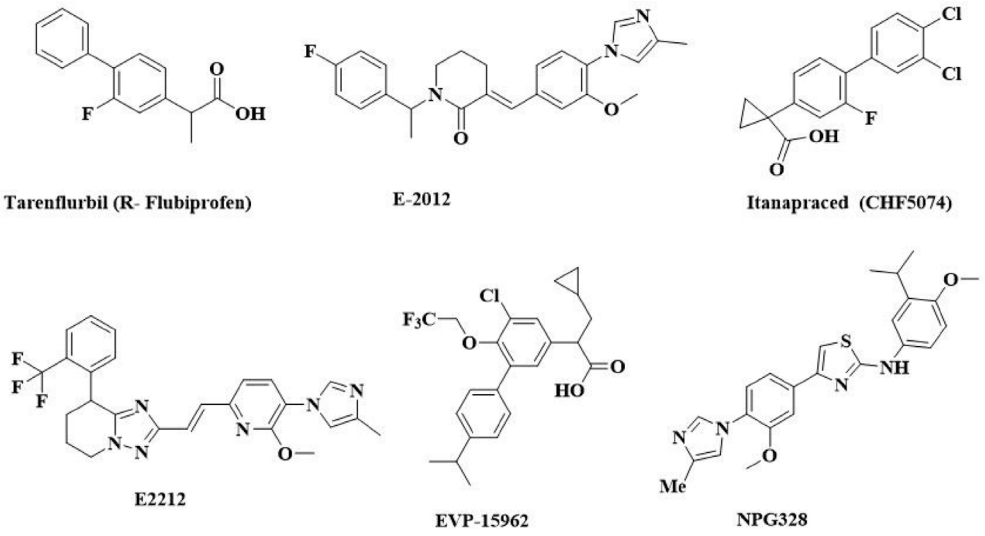



**Figure 9** The structures of γ-secretase modulators, which has extended clinical trials.

**Table 3** γ-Secretase modulators in clinical advancement for AD treatment (Chemical structures shown in Fig. 9).

| Sr. No. | Name of Compound | Pros | Cons | Clinical Trial identifier | Status of compound | Ref. |
|---|---|---|---|---|---|---|
| 1 | Tarenflurbil | High safety profile for patients with AD | Less *in vitro* effectiveness;<br>Less BBB permeation;<br>No effect in phase 3 trials | NCT00105547 | Discontinued | 90 |
| 2 | E2012 | Notch sparing without deposition of Aβ;<br>No rebound actions on human plasma;<br>Decreases plasma levels of $A\beta_{42}$ and $A\beta_{40}$ differently in healthy humans | Rats have lenticular opacity; there is a scarcity of information on brain plaque aggregation in transgenic mice;<br>Data on behavioral effects are scarce | NCT01221259 | Discontinued | 91,92 |
| 3 | CHF5074 | Notch sparing;<br>No deposition of CTFβ;<br>In Tg mice, it reduces brain pathology;<br>Increases cognitive deficit in tg mice;<br>*In vivo* neuroprotective action | Little micromolar inhibitory potency on $A\beta_{42}$;<br>Incomplete brain penetration | NCT01203384 | Phase II | 93 |
| 4 | E2212 | Well safety profile in animals;<br>High inhibitory potency on $A\beta_{1-42}$ | Inadequate information on brain plaque deposition in transgenic mice;<br>Data on behavioural effects are scarce | NCT01221259 | Phase I | Clinical trials. Gov Identifier NCT0122125G |
| 5 | EVP-0015962 | Notch sparing;<br>Well tolerated and reduces brain Aβ aggregates in Tg2576 mice;<br>Enhances cognitive deficiencies in tg mice | Cognitive effects in Tg mice found at comparatively high dose | – | Preclinical | 94,95 |
| 6 | NPG-328 | Nanomolar strength;<br>Notch-sparing;<br>In tg mice, it reduces brain pathology | Lack of information on behavioural activity | – | Preclinical | 41,81 |

γ-secretase processes the membrane-attached fragments[97]. BACE1 hydrolyses APP at the Asp+1 amino acid of the Aβ-sequence, resulting in the NT of the peptide. The hydrolysis affords two fragments: the ectodomain (APPsβ) and the second CT part, which is membrane bound. C99 is then acted by γ-secretase, thus forming the CT of the Aβ protein and the AICD.

The synthesis of A$\beta$ in the brain is attributed to the successive proteolytic breaking of APP by $\beta$- and $\gamma$-secretase[96,98].

*3.3.3.1. Crystal structure of BACE1.* According to electron microscopy, regular and dystrophic presynaptic terminals are where BACE1 primarily produces subcellular[99]. BACE1 crystal structures with and without inhibitor bound in the active site have been resolved repeatedly. An NT protease domain, a linking strand, a transmembrane domain, and a cytosolic domain are all present in BACE1[100]. It has an aspartic protease structure; however, its active site is much more open than pepsins. The substrate binding site (the "cleft") is situated in the middle of the NT and CT lobes, with the catalyzed dyad of asp32/asp228 at the center of the cleft[101]. A flap or hairpin loop (aa, residues 67−75) is situated at the NT lobe and controls substrate access through conformational variations. The flap of BACE1 was found to implement an open conformation in apo structures (Fig. 10)[102]. However, due to the considerable pharmaceutical attention paid to developing BACE1 inhibitors, some structures of complexes of BACE1 crystallized with nonpeptide inhibitors have been identified in recent years, and a diversity of flap conformations has been reported. Numerous side conformations of the conserved flap amino acid residue Y71 and BACE1 crystal structures, particularly self-inhibitory ones, have also been identified[101,103]. Numerous pharmaceutical industries have developed inhibitors of BACE1 to combat this terrible disease, many of which have proceeded to clinical studies, and inhibition of BACE1 is thus considered a key AD treatment strategy[98].

Numerous hurdles must be overcome to build BACE1 inhibitors with low unintended consequences. Selectivity is critical in BACE1 inhibition without harming other proteases to avoid off-target side effects[103]. The size of BACE1's active site, which includes catalytic aspartic acid residues, a flap, and a 10S loop, has also been identified as a challenge[97]. Another concern is the potential of these chemicals to cross the BBB. Additionally, several of the discovered BACE1 inhibitors were susceptible to P-glycoprotein (Pgp) efflux, a limiting factor that hampers drug entrance into the brain even when BBB penetration is achieved[103].

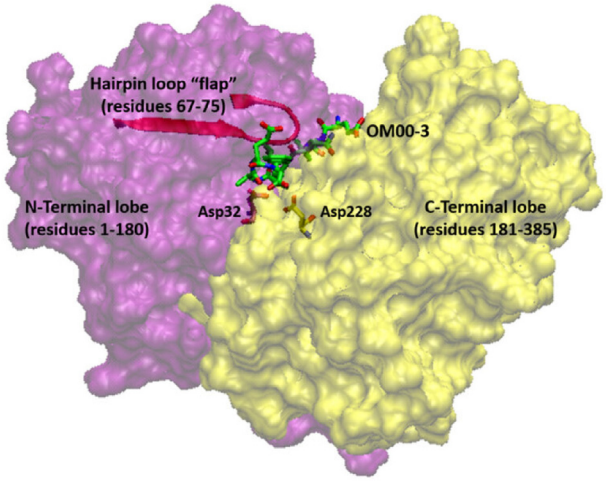



**Figure 10** Human BACE1's three-dimensional structure (PDB ID: 1FKN). The NT lobe is magenta in colour, whereas the CT lobe is green. The active site is shared by the co-crystallized inhibitor OM00-3 and is located in the centre of the two lobes[74]. Reprinted with the permission from Ref. 74. Copyright © 2020 The authors.

Despite the challenges above, numerous laboratories have succeeded in generating potent, selective, and orally bioavailable BACE1 inhibitors. In clinical trials, a number of them have displayed hopeful results, but one has reached the FDA approval stage. Clinical trials are currently conducted with several $\beta$-secretase inhibitors, including PF-05297909, AZD3293, LY2886721, and MK-8931. In its phase I clinical trial, CTS-21166 proved a dose dependent decline in plasma A$\beta$ levels (Fig. 11)[104]. AZD3293 is presently being studied in phase II/III clinical trials to test its disease modifying properties[105]. A few of these new classes of BACE1 inhibitors have advanced to clinical development in recent years, including AZD3839 and LY2811376, which have also advanced to phase II a/b clinical development. CoMentis conducted clinical development of CTS21166, which was shown to reduce human plasma A$\beta$. Further structure based design efforts aim to meet varied challenges posed by therapeutic inhibition of the BACE1 target[104]. The clinical advancement of $\gamma$-secretase modulators for AD treatment is shown in Table 4[105-110].

*3.3.4. $\alpha$-Secretase activators*

The metalloprotease ADAM10, also known as $\alpha$-secretase, cleaves APP primarily in the transmembrane domain, inhibiting the formation of A$\beta$[111]. Several proteases, including the ADAM family (A$\beta$ disintegrin and metalloprotease) ADAM9, ADAM10, ADAM17, and tumour necrosis factor-$\alpha$ convertase (TACE), meet few of the $\alpha$-secretase criteria[112]. Despite amyloidogenic processing, APP is converted by $\alpha$- and $\gamma$-secretase in the non-amyloidogenic pathway. $\alpha$-Secretase proteolyses APP at the domain, particularly between K16 and L17. Interestingly, along with the formation of A$\beta$, it elevates the release of NT fragment, sAPP$\alpha$, a neuroprotective and neurotrophic that enhances long-term potential released by $\alpha$-secretase cleavage. In AD patients, decreased concentrations of sAPP were reported in CSF[113,114]. Following the breakage of APP by $\alpha$- and $\gamma$-secretase that cleaves the 83 residues membrane-anchored C-terminal fragment C83, retaining p3 (isoforms $A\beta_{17-40}$ and $A\beta_{17-42}$) and AICD is released[115]. *In vitro*, p3 isoform $A\beta_{17-42}$ induces neuronal apoptosis, but less potently than $A\beta_{1-42}$[116]. AICD is a second $\alpha$-secretase cleaved product that may be neuroprotective. Increasing AICD levels improved memory and synaptic plasticity in transgenic mice. AICD is produced by both APP processing pathways (Fig. 12) but mostly by the non-amyloidogenic APP processing pathway. $\alpha$-Secretase proteolysis of APP stops pathogenic A$\beta$ peptide formation and releases neuroprotective APP cleavage products. This suggests that this enzyme activity may protect healthy people against AD and prevent the generation of A$\beta$ aggregates and plaques[117,118]. $\alpha$-Secretase appears to be specific for various peptidic bonds, not only the scissile bond. The enzyme's proximity to the membrane appears to be important, as 12 and 13 residues of the N-terminal to the membrane are cleaved by $\alpha$-secretase[104].

$\alpha$-Secretases can cleave APP depending on APP's position on the plasma membrane, the site of processing (endosomes or membrane), and the pH of the environment, resulting in non-amyloidogenic compounds[119]. Protein kinase C (PKC) can be stimulated to increase $\alpha$-secretase, which has been shown to prevent the formation of $A\beta_{42}$ in test animals. An agonist of the M1 receptor has been shown to elevate APPs' non-amyloidogenic proteolysis and to reduce $A\beta_{42}$ levels. Activation of the $\alpha$-secretase, specifically ADAM-10, may be more advantageous than other targets for treating AD because it inhibits A$\beta$ peptide formation[112].

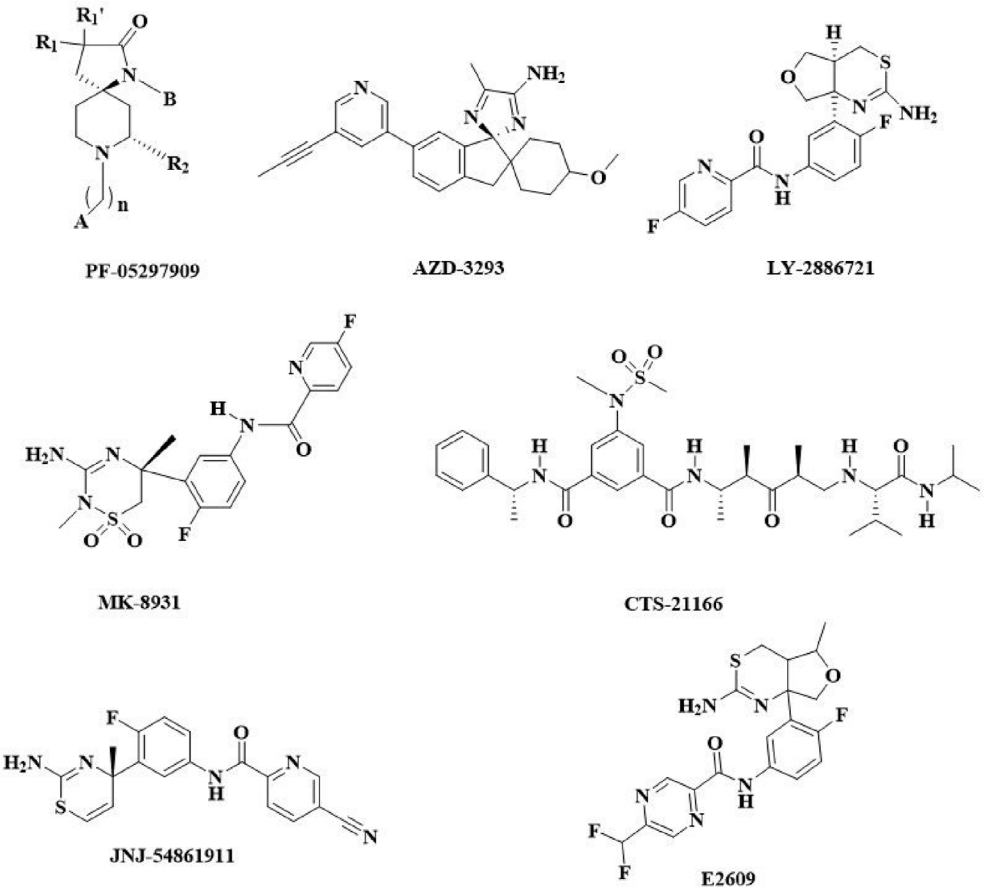



**Figure 11** The structures of $\beta$-secretase inhibitors.

### 3.4. *Tau targeting therapy*

Neuropathologically, AD is described as the occurrence of neurofibrillary lesions in the intraneuronal space constituted of tau proteins[121]. Tau, a microtubule associated protein, polymerizes tubulin into microtubules and assists in maintaining complex neuronal cell microarchitectures, such as microtubule formation and stabilization, mainly in the axon. The MAPT gene transcripts express six main tau splicing isoforms from exons 2, 3, and 10[122]. In the NT projection domain of tau, exons 2 and 3 express twenty-nine residue acidic inserts, while exon 10 encodes a 31 residue microtubule attaching repeat in the CT domain. Microtubule binding repeats of 352−441 amino acids are found in whole tau isoforms. Tau is a phosphoprotein whose activity is controlled by phosphorylation. Its isoforms are susceptible to disorder in aqueous solutions because of their high polar, glycine, proline, and

**Table 4** $\beta$-Secretase inhibitors in clinical advancement for AD treatment (chemical structures shown in Fig. 11).

| Sr. No. | Name of compound | Pros | Cons | Status of compound | Ref. |
|---|---|---|---|---|---|
| 1 | PF-05297909 | Well tolerated. Reduction in plasma A$\beta$ (A$\beta$40 and A$\beta$42) | No functional or cognitive progress; Well tolerated and more side effects | Discontinued in 2018 | 106,107 |
| 2 | AZD 3293 | Reduction of CSF A$\beta$ | No functional or cognitive progress | Discontinued status for AD | 105 |
| 3 | LY2886721 | Potent small molecule, active site inhibitor of BACE1 | Rat toxicology; Pigment epithelium of the eye | Terminated in 2013 | 108 |
| 4 | MK-8931 | Not any significant outcome | No functional or cognitive progress; More side effects | Discontinued | 109 |
| 5 | CTS21166 | Reduction in plasma A$\beta$ and plaque load | Reduces central penetration/exposure | Terminated in 2014 | 107 |
| 6 | JNJ-54861911 | Reduction of CSF A$\beta$ concentrations by 80% | Worse cognition; More adverse effects (hepatotoxicity) | NCT02406027 (II) NCT02569398(II/III) | 110 |
| 7 | E2609 | Dose dependent reduction in A$\beta$40 and A$\beta$42 Guinea pigs in levels in brain and CSF of SD rats | Abnormal liver biochemical tests. | Terminated phase II clinical trial | 107 |

BACE1, $\beta$-site APP cleavage; CSF, cerebrospinal fluid; SD, Sprague−Dawley.

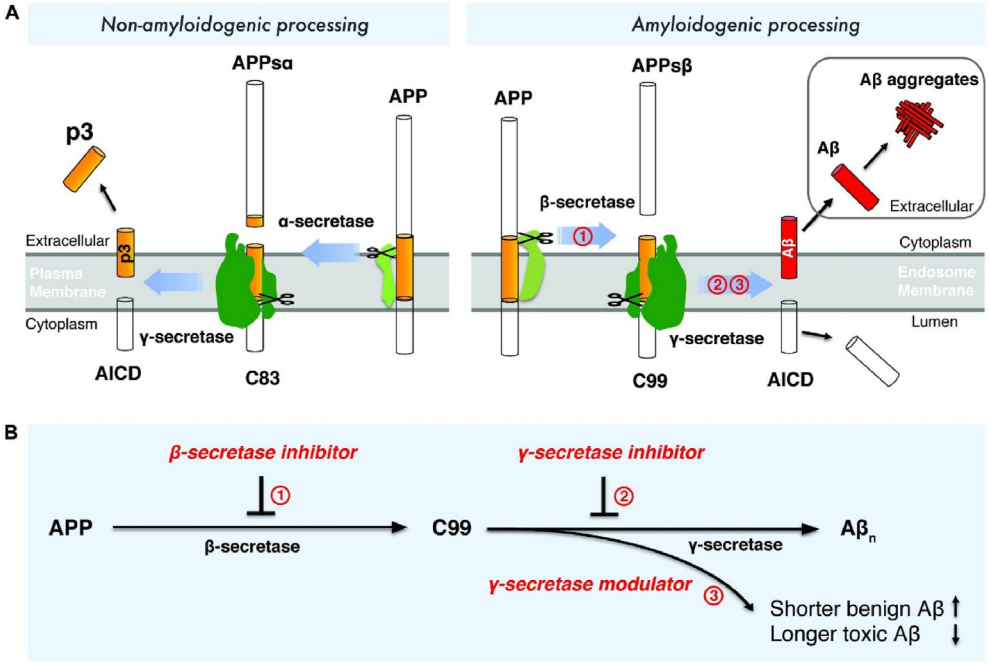



**Figure 12** APP is cleaved by either $\alpha$- or $\beta$-secretase in the non-amyloidogenic and amyloidogenic pathways. The non-amyloidogenic pathway generates sAPP-$\alpha$ and C83 (left). The amyloidogenic pathway produces sAPP-$\beta$ and C99 (right). Gamma-secretase cleaves C99 to produce AICD and A$\beta$ (right)[120]. Reproduced integrally with permission from Ref. 120. Copyright © 2011 The authors.

low hydrophobic content. Some of them have cysteine residues, which can react with oxygen and produce adsorbed molecules. Solvent exposed hydrophobic orders create the vital areas of filamentous clumps in tauopathies[123]. When the control of kinases and phosphatases are imbalanced, that disconnects the aggregated tau from microtubules[124]. It can also impact the plasma membrane and microtubules in neurons. Moreover, tau increases neuronal development and synaptic function in synapses and dendrites[125]. Alterations in synaptic supply and disturbance of synaptic protein interactions can impair neuronal activity and cause AD. If the abnormal inclusions occur in neuronal cell bodies, they are known as NFTs and threads if they are produced in dendrites or axons. This research suggested that AD could be caused by tau mis-sorting[43].

The aberrant tau hyperphosphorylation is distinct from typical and temporary tau hyperphosphorylation in development, anaesthesia, and hypothermia. ADP-tau is sedimentable/oligomeric and likely promotes neurodegeneration by sequestering normal microtubule associated proteins and disturbing the microtubule network. In frontotemporal dementia, tau abnormalities may induce neurodegeneration by increasing aberrant tau hyperphosphorylation. Paired helical or straight filament (PHF/SF) creates neurofibrillary tangles from AD P-tau. Tau truncation in the AD brain enhances PHF/SF self-assembly and does not sequester MAPs or damage microtubules like AD P-tau. Thus, preventing aberrant tau hyperphosphorylation may be a promising treatment for AD and tauopathies (Fig. 13)[126,127].

*3.4.1. Development of tau directed therapies*

Several target pathways could be imagined based on tau biology and its role in neurodegeneration. Post translational tau changes could be addressed by blocking kinases like glycogen synthase kinase-3$\beta$ (GSK3$\beta$) and protein phosphatase (PP2A) or altering phosphorylation and tau aggregation is another objective. Another target might be total tau or pathologically mutated tau species. Few drugs that target tau or its principal target, microtubules, have made it to clinical trials. TRx0237 (modified methylene blue, Rember) is a tau aggregation inhibitor, BMS-241027 (epothilone D) is a microtubule stabilizer, and medications to lower tau protein or phospho-tau are available (davunetide, sGC-1061, immunization approaches).

Molecules that reduce the load of p-Tau protein are also in development, which is supposed to be the direct cause of symptoms in AD. In animal studies, many tau vaccinations have demonstrated efficacy and safety. The drug that was tested on mice showed an excellent safety profile and even motivated a positive immune reaction in a human patient[127]. Microtubule associated tau has undergone many post translational modifications in several neurodegenerative complications, including AD. These changes are strongly linked to tau aggregation in AD. Tau hyperphosphorylation, truncation, glycosylation, glycation, nitration, and ubiquitination are among the post-translational modifications identified in tau[128].

Several therapies and drugs targeted other diseases that affect tau, such as propylthiouracil (PTU) (Fig. 14), an antithyroid molecule that causes tau phosphorylation in AD patients by boosting pro-inflammatory cytokines. Radiotherapy was used to treat numerous types of brain cancers, can expose the brain to ionizing radiation. In AD patients, radiation exposure to a 0.5 or 2 Gy beam causes amplification of tau phosphorylation. Simultaneously, oxidative stress, a factor from ionizing radiation, entices the same progression[122]. Clinical trials for AADvac1 (liposomal based vaccination) have commenced, while trials for ACI-35 (liposomal-based vaccine) have also begun. This treatment aims to prevent clusters of paired, helically twisted strands of hyperphosphorylated tau from forming in neurofibrillary tangles. There has been research into tau protein phosphorylation inhibitors, such as tideglusib (Fig. 14), an irreversible GSK

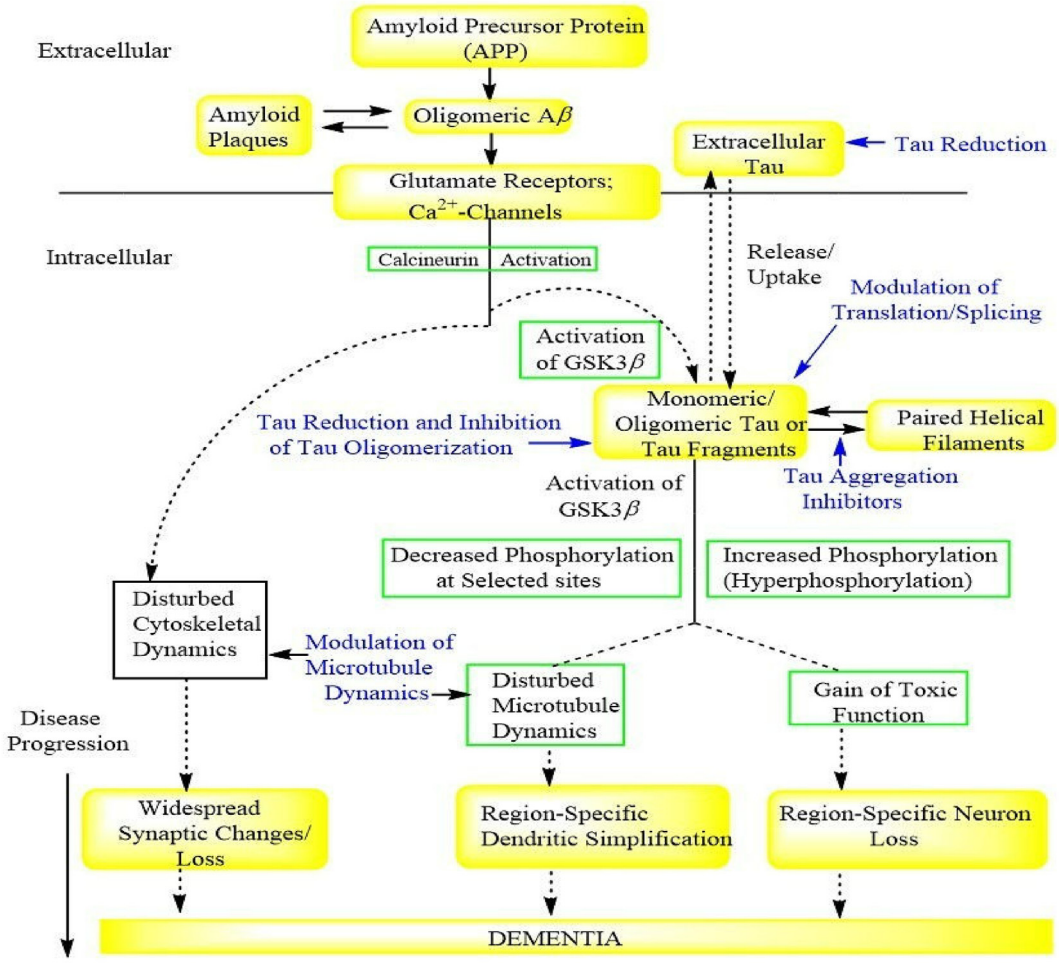
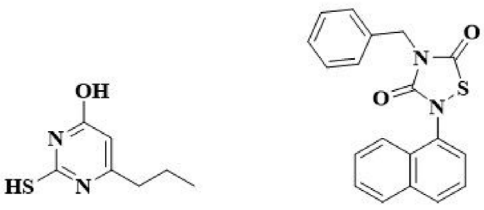



**Figure 13** The neurodegenerative triad and the altered amyloid cascade hypothesis in AD. Oligomeric A$\beta$ causes the neurodegenerative triad of synaptic alterations, dendritic simplicity, and neuron death through tau-dependent and tau-independent pathways. The locations of potential tau disease-targeted therapies are highlighted in blue. For more information, see the text. GSK3$\beta$ stands for glycogen synthase kinase 3$\beta$[127]. Reprinted with permission from Ref. 127. Copyright © 2016 The authors.




**Propylthiouracil** **Tideglusib**

**Figure 14** Structures of anti-thyroid drug and an irreversible GSK inhibitor respectively.

inhibitor, but no statistically meaningful benefits have been discovered yet. Other promising inhibitors include *N*-phenylamines, anthraquinones, phenyl thiazolyl-hydrazides, rhodamine, phenothiazines, and benzothiazoles[129].

*3.4.1.1. Kinase inhibitors.* The hyperphosphorylation of tau by protein kinase is required for its toxicity; however, kinases play a vital part in regulating cell activity and maintaining a healthy physiological state. A tau targeted treatment is difficult to create since kinase interactions are redundant, and it is unclear which enzyme catalyzes the phosphorylation[130].

The first family of tau inhibitors lowers associated kinase activity since an imbalance between GSK3$\beta$ and PP2A promotes Tau hyperphosphorylation and NFT formation. This is supported by GSK3$\beta'$s influence on cellular signalling and gene transcription[131]. GSK3 is a ubiquitously expressed, constitutively active serine/threonine kinase involved in various physiological activities, including gene transcription and glycogen metabolism. GSK-3 interacts with several parts of the amyloid system that form plaques and take part in the phosphorylation of Tau. This microtubule binding protein helps generate neurofibrillary tangles and affects presenilin and other AD associated proteins. Mammalians have two closely related GSK3 isoforms, GSK-3$\alpha$ and $\beta$, with similar biochemical properties and 98% homology in their catalytic domains. The catalytic domains of the isoforms are similar, but their N-terminal sections differ dramatically[132].

According to studies, GSK3$\alpha$, but not GSK3$\beta$, has been shown to restrict APP cleavage, leading to an increase in the production of A$\beta$. Neuronal exposure to A$\beta$ increases GSK3$\beta$ activity by inhibiting PI3 kinase signalling. Blocking either GSK3$\beta$ expression or activity

prevents A$\beta$ induced neurodegeneration. Increased GSK3 activity would serve to enhance A$\beta$ production and in turn, tau hyperphosphorylation and neuronal degeneration in both FAD and sporadic instances, following the amyloid cascade hypothesis of AD, even though it is not the major cause of illness in this scenario[133].

A polymorphism in the GSK3 promoter has recently been linked to a risk factor for late onset AD, which may explain changes in GSK3 expression in illness. However, it is acknowledged that there is currently little direct evidence for this and that some research shows no alteration in GSK3 activity or reduced GSK3 activity. These findings collectively suggest that GSK3 activity might be increased in AD through changes in its phosphorylation state in addition to expression levels[133]. GSK3$\beta$ is newly discovered and is responsible for 31 and 16 pathogenic tau phosphorylation sites and colocalized with NFTs in the postmortem brain[134]. Toxic A$\beta$ enhances GSK3$\beta$ activity, implying that GSK3$\beta$ is a possible therapeutic target. The search for GSK-3 inhibitors is a particularly active area in both academic institutions and pharmaceutical firms[135].

In preclinical studies, a non-ATP competitive GSK3$\beta$ inhibitor NP-031112 (NP-12) reduces tau phosphorylation and the amyloid burden, thus preventing cell death and improving spatial memory[136]. Several GSK3 inhibitors, including cations like lithium or small compounds have been studied as a promising strategy for treating AD. Lithium lowered tau phosphorylation and restored tauopathy in animal models but not in Alzheimer's patients[137].

Organic GSK-3 inhibitors of both natural and synthetic origin are highly varied in structure and span a variety of chemical regions. Most of the effects seen are from *in vitro* and cellular research. SB216763 corrects responses such as GSK3 activity and is connected with elevations in amounts of p-Tau, caspase-3, neuronal DNA fragmentation, the tau kinase phospho-c-jun N-terminal kinase (pJNK), and gliosis. In contrast, a single administration was linked with the induction of neurodegenerative markers and behavioural deficits. A negative result ended the phase 2b trial, and some GSK3 inhibitors from the paullone, indirubin, and maleimide families are in the process but are hampered by concerns about cytotoxicity[138]. The maleimide compounds SB415286 and SB216763 (Fig. 15) are neuroprotective but might not specifically target cyclic dependent kinases (CDKs); they decreased GSK3 activity by battling for the ATP binding site. The thiadiazolidinones are ATP non-competitive, albeit these findings need to be confirmed. Most recently, AstraZeneca revealed a powerful and selective inhibitor of GSK3 (AR-A014418) that is effective against cdk2, cdk5, and additional kinases tested[139].

CDK5 is another kinase linked to tau disease. Pathological tau phosphorylation is caused by the CDK5 regulatory protein present in the AD brain. Preclinical CDK5 selective inhibitors have been shown to permeate BBB and thus lower the increased A$\beta$ levels by controlling CDK5. Insufficient efficacy or significant side effects led to the discontinuation of trials for other medicines targeting different protein kinases[140].

*3.4.1.2. Tau aggregation inhibitors.* Prevention of tau aggregation or promoting tau assembly disassociation are two alternative options. Preclinical evidence showed that Rember (methylene blue) can reverse learning deficits, and a phase II trial showed reduced AD progression with high bioavailability. Also known as leucomethylthioninium (TRx0237), it has increased absorption, bioavailability, and tolerance. TRx0237 has been extensively studied since 2008 with evidence that it increases neuroprotection, A$\beta$ clearance in transgenic mice, and spatial learning in rats. Few literatures indicated the anti-aggregation characteristics, and phase III investigations are still ongoing[6]. Phase II/III RCT enrolling persons with dementia are in the evaluation on six month regimen of 4 mg TRx0237 (Fig. 16) twice daily to a placebo[130].

Most tau stabilizers (*e.g.*, paclitaxel and epothilone D) (Fig. 16) had hazardous adverse effects. TPI 287 has shown promising results in mild to moderate AD patients, chronic supranuclear palsy, and corticobasal syndrome, with positive effects on cognitive function and/or nerve cell activity. In a mouse model, nicotinamide lowers phosphorylated tau and protects microtubule stability[141]. Phase II clinical trial is in progress in people with mild-to-moderate AD.

### 3.5. *Neurotransmitter related putative therapies*

Depletion of acetylcholine and synaptic impairment are two hallmarks of AD. Consequently, two hypotheses were proved: cholinergic and glutamatergic. FDA approved therapies, AChE inhibitors, and NMDA receptor antagonists were created to alleviate AD symptoms. Even though medications that regulate transmitter

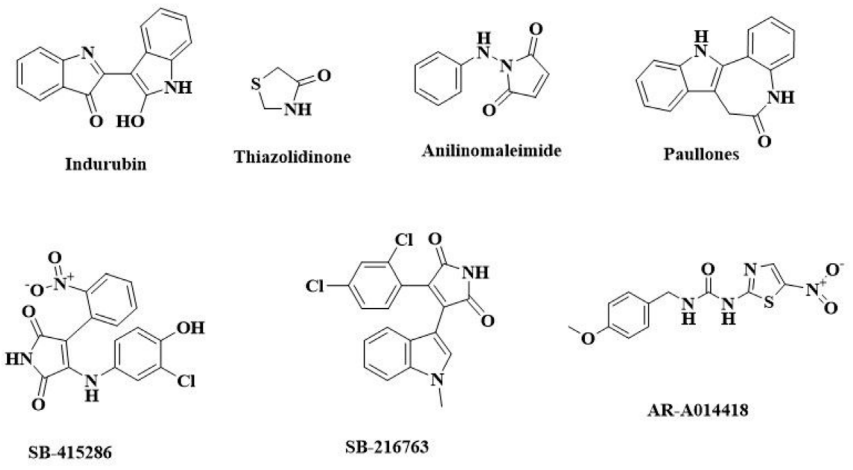



**Figure 15** The structures of GSK3 inhibitors which has extended clinical trials.

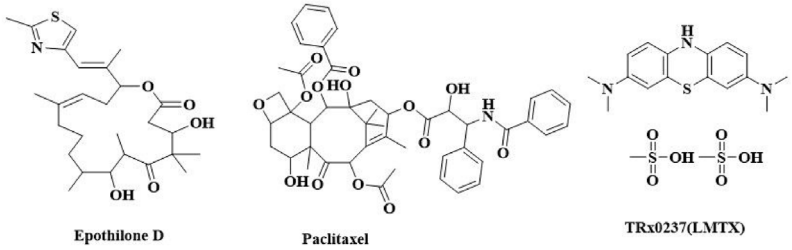



**Figure 16** Structures of tau aggregation inhibitors (TRx0237) and tau stabilizers (Paclitaxel and epothilone D).

generation, release, and recycling cannot halt AD progression, the quest for new receptor agonists and antagonists continues[10].

The cholinergic impairment changes cognitive and neuropsychiatric disorders in AD patients. Acetylcholine, a key neurotransmitter, is degraded by acetylcholinesterase and butyrylcholinesterase (BuChE). The main therapeutic focus of cholinesterase inhibitors (ChEI) therapy for AD has been AChE inhibition. AChE positive neurons modulate cortical processing and reactions to novel inputs. These neurons may be important in attention, executive function, emotional memory, and behaviour. Inhibiting BuChE may thus provide further benefits. The substrate selectivity, expression, enzyme kinetics, activity in multiple brain regions, and gene regulation complex of the two enzymes are significantly different. According to a new study, AChE and BuChE play roles other than co-regulatory esterase actions at the end of Ach-mediated neural transmission[142].

### *3.5.1. Cholinesterase inhibitors*

For more than a quarter century, AD pathogenesis has been connected to a lack of the brain cholinergic neurotransmitter Ach, based on research that linked cholinergic system problems to cognitive decline[143]. The formation and degradation of Ach is schematically portrayed in Fig. 17. As a result, one potential therapeutic approach is to reduce acetylcholinesterase's biological function to increase cholinergic levels in the brain. AChEIs are used to limit ACh breakdown, which can improve brain cell function by boosting AChE concentration[144].

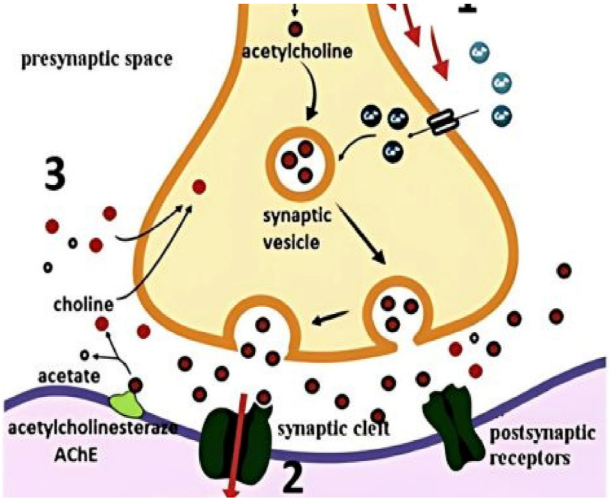



**Figure 17** The acetylcholine release cycle and cholinergic hypothesis of AD are depicted schematically. AChE catalyses the breakdown of acetylcholine, and choline molecules are taken up by the presynaptic neuron. (1) Action potential causes $Ca^{2+}$ influx and subsequent membrane docking of synaptic vesicles. (2) Acetylcholine binds to receptors and initiates a graded depolarization in the postsynaptic cell. (3) Acetyl-CoA is acetyl coenzyme A; ACh is acetylcholine; and AChE is acetylcholinesterase[146]. Reproduced integrally with permission from Ref.146. Copyright © 2019 The authors.

AChE hydrolyses ACh, releasing choline and acetate ions. It possesses a big hydrophobic cavity along with an esteratic subsite and an anionic substrate binding site. Also, other cationic substrates and inhibitors can be bound by the active site. Ser200, Glu327, and His440 comprise the ES catalytic triad. Approximately 20 from the enzyme surface, at the end of a gorge that deepens towards the bottom. Ser200 is involved in proton transfer hydrolysis of choline esters. A cation interaction occurs between quaternary ammonium of Ach and aromatic amino acid. The AS of Torpedo California AChE (TcAChE), a prototype ACh binding protein, contains many aromatic residues (14 amino acids). When Trp84 is substituted for alanine, the reactivity of the AChE is reduced by 3000-fold. AChE also has an acyl pocket that specifies substrates and an oxyanion hole that interacts with negative oxygen ions throughout catalysis, increasing the efficiency of AChE[145,146].

AChE hydrolyses 90% of ACh in the normal human brain and abandons its function further; BuChE plays a minor role. AChE levels drop in AD patients, whereas BuChE levels rise in some brain areas[142]. Recent research suggests that selective or nonselective inhibition of BuChE may have neuroprotective and disease modifying effects. These include indolinone, coumarin-3-carboxamides with *N*-benzyl piperidine moiety, coumarin-3-carboxamides with tryptamine moiety, 7-hydroxycoumarin derivatives, tacrine analogues and indole based hydrazide-hydrazone derivatives[147].

The three drugs include inhibitors of AChE exemplified by donepezil, rivastigmine, and galantamine are in clinical use (Fig. 1). However, these medications have limited efficacy and have exhibited varied dose related adverse effects, especially at greater doses[148]. Galantamine and donepezil suppress AChE, while rivastigmine inhibits AChE and BuChE. The damage of cholinergic neurons in the CNS and the loss of nerve transmission are the primary reasons for cognitive function impairment in AD patients[149]. Another important element in AD pathogenesis is the increased synthesis and accumulation of amyloid[150]. As a result, dual inhibitors based on tacrine hybrids[151] and donepezil is being designed to decrease both AChE activity and A$\beta$ accumulation. Numerous AChE and A$\beta$ cleaving enzyme 1 dual inhibitors have been created using computational techniques[152].

Early dementia is accompanied by cholinergic neuron loss, so both preclinical and clinical investigations revealed that

cholinesterase inhibitors improved memory and learning[153]. Later, in animal models, a strong link between weak cholinergic neuronal activity and memory loss was established[154]. Thus, improving the cholinergic system, including binding acetylcholine nicotinic receptors, excites the postsynaptic neuron, which is crucial for long term potentiation (LTP) and memory development. After completing the phase I/II trials that demonstrated safety and well tolerance, EVP-6124 was recently studied in the phase III trials to assess cognitive aids. Also, some trials with nicotinic agonists are in various stages of clinical trials (ladostigil hemitartrate, phase II), including RO5313534 or Pozanicline (ABT-089) (Fig. 18)[35]. Serotonin (5-HT) is a neurotransmitter affecting cell death and memory loss. Memory and learning deficits could be improved by increasing cholinergic transmission by inhibiting the 5-HT6 receptor. 5-HT6 antagonists have been shown to reverse anticholinergic drug-induced amnesia[10,155]. Two HT antagonists, PRX-03140 (5-HT4 antagonist) and Intepirdine (SB-742457; 5-HT6 antagonist) have been evaluated in clinical phase II trials. Idalopirdine (Lu AE58054; 5-HT6) has newly entered phase III evaluation consisting of 930 mild to moderate Alzheimer's patients[10].

### 3.6. *N-methyl-D-aspartate receptor (NMDAR) antagonist*

NMDAR antagonists pose a greater therapeutic potential in a variety of CNS illnesses. When administered at levels within their presumed therapeutic range, several NMDA receptor antagonists generated highly potential effects. As a result, it has been concluded that NMDAR antagonism is a viable treatment strategy[156]. The excitatory glutamatergic nerve transmission is regulated by the NMDAR which is required for synapse formation and cell survival. However, elevated NMDAR activity results in excitotoxicity and cell death thus implicating a probable nerve degenerative process in AD. The huge number of excitatory neurotransmissions in the human CNS are mediated by glutamate and its receptors, especially ligand gated ionotropic glutamate receptors (iGluRs). iGluRs perform critical roles in synaptic plasticity, the chemical mechanism governing learning and memory. Disruption of normal signaling *via* iGluR has been connected to a variety of neuropathological illnesses and diseases. These conditions include epilepsy and brain injury, AD, PD, Huntington's disease, and multiple sclerosis[157].

#### 3.6.1. *NMDAR subtypes linkage to neurological disorders*

It is now more evident that NMDAR hyperactivity or hypofunction can have negative repercussions. Changes in NMDAR

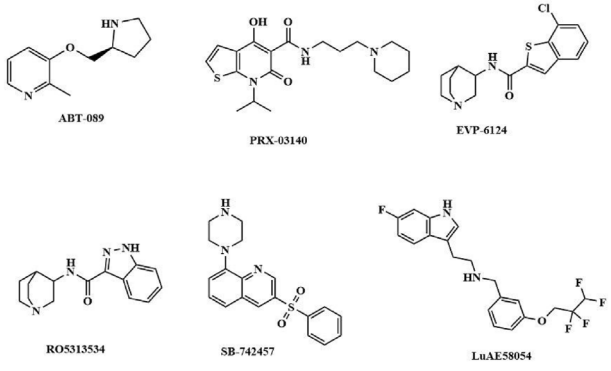



**Figure 18** The structures of cholinesterase inhibitors which has extended clinical trials.

presence/function can add value to CNS disease in several ways: their over activation can induce neuronal death, as in stroke and possibly in Huntington's disease, or their inhibition can alter the balance of inhibition and excitation in neural circuitry, influencing CNS functions, as is likely in schizophrenia. For example, improved NMDAR action on excitatory neurons may result in improved synaptic plasticity of excitatory neurons, whereas improving NMDAR function on inhibitory neurons is likely to increase inhibition (reducing excitation as a result) and decrease in excitatory neurons' synaptic plasticity as a result. Accordingly, alterations in NMDAR expression or activity, depending on their locus, can influence the ratio of excitation to inhibition, impairing circuit and brain function[158].

The membrane topology of all ionotropic glutamate receptor subunits, such as the seven GluNs, is defined by three transmembrane parts (M1, M3, and M4) and a re-entrant pore loop (M2). The long N-terminus is extracellular, while the C-terminus is intracellular and engages with a variety of cytosolic proteins. Glutamate binds to GluN2 subunits in an NMDAR binding pocket formed by two regions in the proximal N-terminal domain and the lengthy extracellular loop among both M3 and M4 (S1 and S2, respectively). The re-entrant M2 loop is a component of the channel pore and contains a crucial asparagine residue that defines the channel's calcium permeability and facilitates the magnesium blockade[159]. There are seven NMDAR subunits namely, NR1, four NR2 (A, B, C, and D), and NR3A and NR3B. The NR1 subunit has eight splice variants, as do the NR2 and NR3 subunits (excluding NR2A). Generally, NMDARs are hetero tetramers with two NR1 and two NR2 subunits each and each receptor of NR1−NR2 dimer is considered the basic useful structure. NR3A can form a receptor complex with NR1/NR2, also some NMDARs are heterotrimers containing two NR1 and two NR2 subunits[160]. NR3A protein expression drops from neonatal week 7 to week 21, while NR3B protein expression increases and NR3A was found to be low while NR3B was reported high in adults. In humans, NR1 expression begins to rise during pregnancy and then plateaus until puberty. NR3A levels are low during the embryonic day, quickly surge post-birth and then slowly decline (Fig. 19)[161,162].

The majority of NMDARs have receptors for GluN1, GluN2B, GluN2A, or a combination of the two. NMDAR subunit expression varies throughout the brain and changes dramatically during its development. In the temporal area of the cerebral cortex and the hippocampus of the embryonic brain, these genes are expressed mildly, but they are widely expressed in the neonatal brain. Following that, it gradually declines to adult levels, reaching adult levels by the third postnatal week. Glutamate overstimulation of NMDA receptors has the potential to cause neuronal depolarization and $Ca^{+2}$ influx. Several $Ca^{+2}$ dependent enzymes are induced by intracellular $Ca^{+2}$ over accumulation and these enzymes have the potential to harm and kill neurons (excitotoxicity).

As a successful method to slow the detrimental effects of excitotoxicity in the evolution of several disorders, including AD, ischemic stroke, neuropathic pain, PD, and depression, selective antagonism of GluN2B subunit-containing NMDA receptors has developed[163]. In the fatal brain, inside the hippocampus and temporal portion of the cerebral cortex, the NR2B subunit is barely expressed[159]. In adult granule cells that integrate into circuits with strong and linked synaptic activity, GluN2B-containing NMDARs encourage synapse activation. Thus, depending on the environment, GluN2B-containing NMDARs can have a bidirectional effect on synapse formation[164]. Ifenprodil (Fig. 20) is a brand-new NMDA receptor antagonist that specifically inhibits NR2B

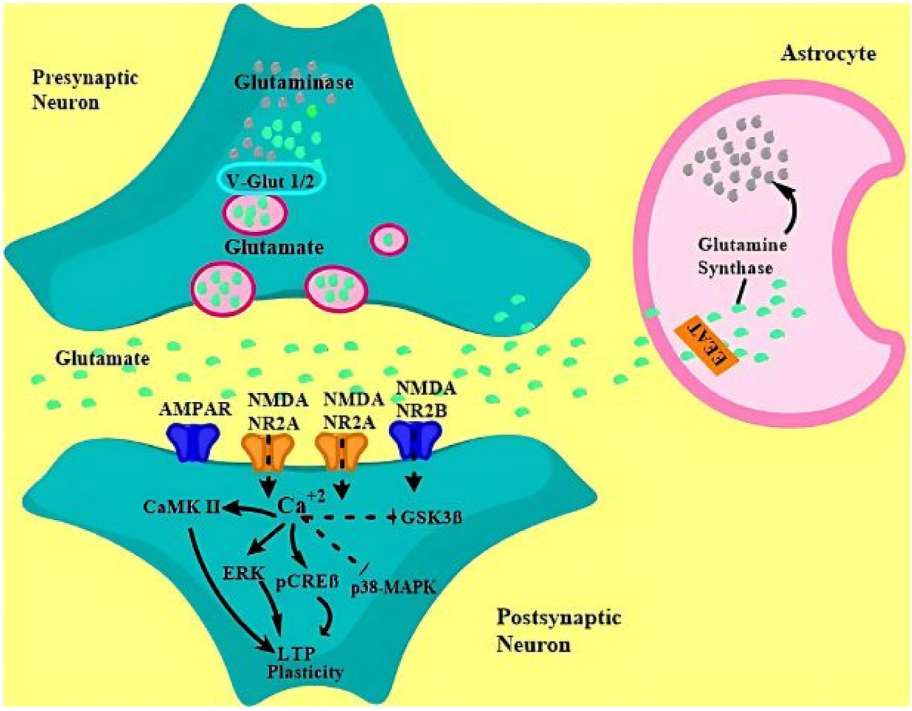



**Figure 19** Transmission of glutamates in a healthy brain. By activating the postsynaptic terminal glutamate receptors, glutamate released from presynaptic terminals has an effect. Multiple metabolic pathways, specifically CaMK, ERK, and CREB, which are in charge of anabolic activation and long-term potentiation (LTP) process activation, are promoted by the interaction among glutamate and the NMDA receptor. Excess glutamate is carried into astrocytes by the excitatory amino acid transporter (EAAT) and converted to glutamine there by the glutamine synthase. Glutaminase then transforms glutamine into glutamate, which is then packaged into vesicles using certain transporters (VGlut). Vesicular glutamate transporter (VGlut); excitatory amino acid transporter (EAAT); *N*-methyl-D-aspartate NR2A and NR2B subunits (NMDANR2A and NMDANR2B subunits); extracellular signal-related kinase (ERK); calcium calmodulin-dependent kinase II (CaMKII); phosphorylated cyclic AMP response element binding protein (pCREB); p38-mitogen activated protein kinase (p38-MAPK)[162]. Reprinted with the permission from Ref. 162. Copyright © 2014 copyright holder.

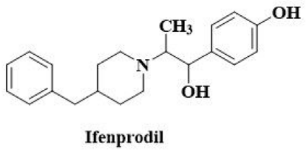



**Figure 20** Structure of a brand-new NMDA receptor antagonist.

subunit containing receptors. As a result, it has found widespread usage as a tool for molecular investigations of the characteristics and control of NMDA receptors, as well as a method for studying NMDA receptor subtypes both *in vitro* and *in vivo*. Ifenprodil's mode of action may entail a rise in proton inhibition of NMDA receptors and exhibit an uncommon type of activity dependence. Analogues or derivatives of ifenprodil, some of which may be lead substances for therapeutically effective NMDA antagonists, possess several characteristics. Such antagonists can be used as analgesics, neuroprotectants, anticonvulsants, and for treating Parkinson's disease and other nervous system illnesses.

Some of the mutations have been reported in NMDARs; it is tacit that the mutation of the postsynaptic NR2B subunit is linked to alterations in synaptic architecture. Numerous coding variations on the risk haplotype encoding *rs1806201* may have a part in the susceptibility to AD. An increased frequency of the Ht2-AG haplotype in AD patients revealed that the GRIN3A mutation may be a risk factor for AD[165,166]. People with insCGTT, an NMDAR NR3B subunit mutation that adds four bases to the coding area, are predisposed to schizotypal personality features[167]. The p. N615K mutation in GRIN2A deletion mutation in a little girl with epileptic encephalopathy reduced $Ca^{2+}$ permeability in NR1−NR2A receptor currents. The NR2 subunit of NMDARs' electrical equilibrium is disrupted, resulting in various neuropathologies[165].

A study found that regulating NMDA receptors reduces glutamate-induced excitotoxicity and improves AD symptoms. This novel neurochemical technique differs from the cholinomimetic process employed in all currently approved AD treatments[167]. NMDAR antagonists approved for the treatment of moderate to severe AD in the United States and Europe may have the potential to alleviate other neurological conditions such as vascular dementia and Parkinson's disease. Memantine has been shown in animal models to be a neuroprotective agent that improves both vascular and neurodegenerative mechanisms. While high glutamate levels cause neurotoxicity due to the overactivation of NMDARs, memantine, as a partial NMDAR antagonist, inhibits the NMDA glutamate receptors to normalize the glutamatergic system and enhance cognitive and memory deficits[168]. A therapeutic approach with high affinity antagonists of NMDA receptor like MK-801 and phencyclidine is impractical because of unwanted effects; however, memantine, a low, moderate affinity,

non-competitive, and strongly voltage dependent NMDA receptor antagonist, is well tolerated and was finally approved by the US Food and therapies for the cure of moderation[169].

### 3.7. *Triggering receptor expressed on myeloid cells 2*

TREM2 is an immunoglobulin superfamily transmembrane receptor that binds lipids, lipoproteins (apoE, apoJ, apoAI, apoAII), and ligands linked to damage or pathogen-related molecular patterns (*e.g.*, lipopolysaccharides, bacteria)[170]. It has an extracellular domain, transmembrane part, and cytoplasmic tail and is encoded by a gene on chromosome 6p21.1. *TREM2* gene mutations and polymorphisms have been related to an elevated risk of AD[171,172]. Unusual *TREM2* gene mutations have been shown to raise an individual's risk of AD by up to thrice[173]. Polycystic lipo membranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL; also known as Nasu-Hakola disease NHD) and frontotemporal dementia are caused by an unusual biallelic gene mutation[174]. TREM2's basic roles in the brain must be better understood before new therapeutic targets may be discovered.

#### 3.7.1. *TREM2 variants and risk for AD*

TREM2 has 46 genetic variants that have been linked to AD, out of these genetic variants, p. Arg47His (rs75932628), has been demonstrated to double or triple the risk of AD in numerous European and North American populations[175]. A proxy of rs75932628 was discovered to be associated with AD in black patients[173] and pArg47His was not observed in late onset AD patients or healthy controls in Chinese cohorts[176,177]. No such link was discovered in the Iranian[178] or Japanese population[179], indicating that TREM2 is demographic specific. Recent studies have related AD to polymorphisms in microglia expressed genes such as TREM2[37], CD33[180] and CR[181]. The R47H missense mutation is encoded by the rs75932628T polymorphism in TREM2, which carries the highest risk. TREM2 deficits in neurological illness models like cuprizone-induced demyelination[182].

#### 3.7.2. *TREM locus and TREM2 expression*

TREM2 is situated within a gene cluster on chromosome 6p21.1, in proximity to *TREM1*, triggering receptors expressed on myeloid cells like *TREML1*, *TREML2*, *TREML3P*, *TREML4*, and natural cytotoxicity triggering receptor 2 (*NCR2*) genes. The genes in this cluster share a lot of similarities and are primarily immunological[173]. TREM2 is an immunoglobulin superfamily transmembrane receptor expressed in myeloid cells such as microglia and osteoclasts that modulates the immune system[183]. TREM2 transcripts have the greatest signal in the basal ganglia, spinal cord, medulla oblongata, and corpus callosum. TREM2 interacts with anionic ligands such as bacterial lipopolysaccharides, DNA, and phospholipids[184].

Ligand binding to TREM2 initiates a signalling cascade that phosphorylates the TREM2-associated intracellular adaptor TYROPB. Phosphorylated TYROBP binds to a tyrosine kinase found in the spleen (SYK), which further activates phosphatidylinositol 3 kinases (PI3K), AKT serine/threonine-kinase (AKT), mitogen activated protein kinase (MAPK), $Ca^{2+}$ mobilization and other downstream substrates[183]. The proliferation and differentiation of cells are regulated by these mechanisms (Fig. 21)[174,183].

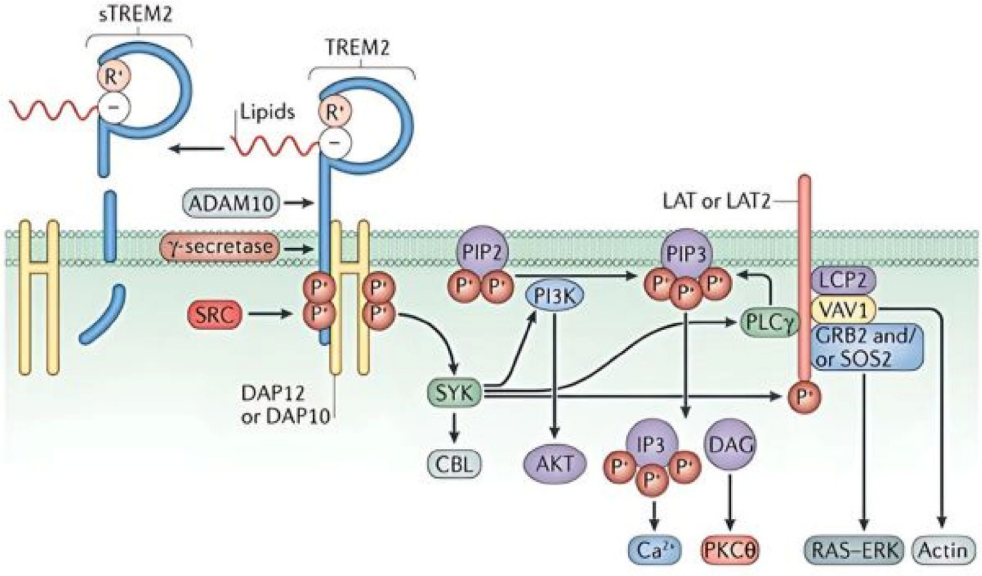



**Figure 21** TREM2 signalling pathways. TREM2 binds positively charged arginine residues in its extracellular domain (R+) to negatively charged lipid ligands. The protein kinase SRC tyrosine phosphorylates the TREM2-associated adaptor DAP12 upon ligand binding, recruiting the tyrosine protein kinase SYK. Signalling proteins include phospholipase C (PLC), lymphocyte cytosolic protein 2 (LCP2; also called as SLP76), protooncogene vav (VAV1), growth factor receptor-bound protein 2 (GRB2), and others. All of these pathways result in the stimulation of protein kinase C (PKC), activation of the RAS-ERK pathway, and actin remodelling. SYK also stimulates the PI3K−AKT signalling pathway and CBL, an E3 ubiquitin-protein ligase that inhibits the TREM2 signalling pathway. TREM2 is also linked to DAP10, a PI3K attractant and activator. TREM2 is released from the cell surface by secretase, disintegrin, and metalloproteinase domain-containing protein 10 (ADAM10) (sTREM2)[183]. Reprinted with the permission from Ref. 183. Copyright © 2016 Macmillan Publishers Limited.

TREM2 signalling has been found to reduce toll like receptor responsiveness in dendritic cells [185].

### 3.7.3. TREM2 cleavage and sTREM2

TREM2 can be sequentially proteolyzed, after the p. H157Y residue. α-Secretases and metalloproteinase (ADAM10 and ADAM17) cleave the protein. Thus releasing the ectodomain and soluble TREM2 followed by α-secretase mediated cleavage of the C-terminus[186,187].

*3.7.3.1. TREM2 and AD.* TREM2 inactivating mutations were originally discovered in Nasu−Hakola disease (NHD) patients, this condition causes PLOSL in the brain and bones[188,189]. Given that microglia and osteoclasts express TREM2 signifying that these cells need TREM2 to function effectively in the CNS and bones. A low frequency TREM2 variation was recently revealed as a genetic problem for nonfamilial AD in genome wide association studies (GWAS). In two huge patient cohorts, the mutation has generated an extracellular Ig domain arginine-47-histidine (p.R47H) substitution which increased the prevalence of AD[171].

The discovery of a TREM2 variation as an AD risk element confirmed the long-held idea that changed microglial activity contributes to the disorder's etiology[182]. GWAS have found uncommon variations of immune receptor genes produced by microglia as risk elements for AD. Some examples are the inhibitory receptor myeloid cell surface antigen (CD33)[189] and another system method identified immune-related gene networks as AD regulators. The function of TREM2, its adaptor DAP12, and the downstream signalling pathway was emphasized [190].

Discovery of the R47H variant linked to AD has inspired comprehensive studies of TREM2 polymorphisms in the human population that disclosed the less common variants including the D87N substitution (rs142232675) in human population[191]. Additional TREM family receptor variants, including TREM and TREM like transcript protein 2 (TREML2) have been linked to AD susceptibility or safety the independent of a TREM2 genetic linkage. Some mutations were detected in the extracellular TREM receptor exons, while others were discovered in intronic areas that affect gene expression and/or splicing. An example of a TREM1 risk allele lowered TREM1:TREM2 expression[183].

### 3.7.4. TREM2 mediated neuroprotection in microglia

TREM2 is expressed by myeloid cells such as microglia, granulocytes, dendritic cells, bone marrow, and monocyte derived macrophages as well as tissue macrophages (*e.g.*, Osteoclasts)[174]. TREM2 delivers intracellular signals *via* DAP12 after ligand binding. TREM2−DAP12 complex then recruits Src followed by phosphorylation of various downstream cascades (*e.g.*, PLC, PI3K, and ERK). TREM2 signalling dysfunction causes aberrant phagocytosis, cytokine secretion, defective cell proliferation, and survival. In other words, a reduction in TREM2 brain expression may contribute to a neurodegenerative microglia phenotype[171,192]. TREM2's role in AD is complicated by its participation in Aβ reactive microgliosis. This procedure causes microglia to congregate around Aβ plaques thus allowing Aβ elimination. Recent evidence suggests that TREM2 deficiency reduces the amount of microglia cells around plaques. TREM2 deletion lowers phagocytosis, although haplo-deficiency lessens plaque compaction and axonal dystrophy without affecting amyloid phagocytosis. Aβ lipidation phase in TREM2 mediates microglial polarisation, processing, and plaque encapsulation. TREM2 is also activated by lipidic components of a lipoprotein complex, allowing it to perceive the milieu and phagocytize dead neurons, myelin, and amyloid plaques[193,194]. These findings support the concept that TREM2 is responsible for brain structure modeling and the pathophysiology of AD.

### 3.7.5. TREM2 directed therapeutics

TREM2 has been linked to a variety of NDDs, implying that it could be a target for a variety of disorders. TREM2 directed treatments may be a novel target for neurodegenerative disorders, in addition to its biomarker potential. Several factors influence the design of TREM2 therapies, TREM2 variants increase the risk of AD as much as one ApoE4 allele, and the minor allele occurrence of TREM2 variants is much lesser than ApoE4173[195]. Some have proposed that rectifying TREM2 mutations may be a useful therapeutic method, but this is unlikely to be a widely applicable way. Rather, investigating TREM2 polymorphisms associated with neurodegenerative disorders will elucidate immune response components critical to disease control and will pave the way for creating immune-directed treatments. Even in the case of AD, just activating or suppressing TREM2 does not appear to be beneficial. sTREM2 levels in CSF have been reported to differ between male and female subjects in certain investigations, but not all[196,197]. Similarly, in women, a TREM2 variation was associated with indicators of systemic inflammation. Raising or lowering TREM2 is unlikely to be a universal treatment for neurodegenerative disorders due to the absence of strong biomarkers and the variation in clinical development across patients[198].

### 3.7.6. Future perspectives of TREM2

Numerous mutations have been identified and one such mutation is p. R47H, it is unclear if the other TREM2 variations reported in AD patients are also dysfunctional. Protein expression and folding may be affected by the glutamine-33-stop (Q33X) and T66M mutants allied to NHD and frontotemporal dementia[184,191]. It will also offer structural support for the participation of specific amino acid residues in lipid binding. TREM2 has the ability to bind non-lipidic ligands like heat shock protein 60 (HSP 60) or act as a co-receptor for the transmembrane semaphorin Sema6D[190,199]. Also, soluble fusion proteins with the TREM2 extracellular domain can bind to numerous cells, especially near amyloid plaques[200].

## 3.8. Bridging integrator (BIN1)

BIN1 was first discovered as a cancer suppressor containing a BAR (Bin1/Amphiphysin/RVS167) domain, a CT SH3 domain, and a MYC interacting domain. It is now the second utmost prevalent genetic susceptibility locus in LOAD next to APOE and it is expected to have an impact on AD risk primarily through the Tau pathway. BIN1 is engaged in membrane trafficking and clathrin-mediated endocytosis. As this gene family is involved in membrane transport and actin dynamics and BIN1 can influence Aβ processing, production, and clearance[201]. However, a study found that the strongly related SNP at BIN1 had the highest odds ratio and population attributable fraction among non-APOE risk loci. BIN1 expression is altered in transgenic aging mouse models of AD[201], AD brains and elevated levels of BIN1 expression have been linked to a later age of onset in AD patients[202].

### 3.8.1. Biochemical features of BIN1

Biochemical features of BIN1 were initially identified with 19 exons, but a missed exon 6a was discovered between exons 6 and

7[203]. BIN1 transcripts undergo substantial differential splicing and a wide group of BIN1 splice variants with variable tissue spread is formed[204]. The primary versions differ principally in the presence of 4 exons namely 6a, 10, 12 (which included a sequence of alternate brain specific exons, 12A−D), and 13[205,206]. Exon 12 encodes a central insert domain that interacted with clathrin and AP2/a-adaptin, while some brain splice variants have a potential coiled coil part in the BAR domain, in which exon 6a encodes a 31 residue attachment (NT insert domain) incorporated. A 15 residue region expressed by exon 10 in the muscle-specific isoform comprises a putative nuclear localization sequence and lipid binding sequence. Exon 13, which encodes a portion of the MYC binding domain and is tissue independently spliced. According to a study, two of the BIN1 cDNAs seem to encode splice variants that lack a Carboxy terminal SH3 domain[201].

Numerous BIN1 splice variants have aberrant electrophoretic motilities, as seen on polyacrylamide gel electrophoresis, implying that post translational alteration of this protein might show a role. Even though the longest BIN1 transcript in humans, rats, and mice have a projected mass of just 65 kDa, the brain BIN1 isoforms is 85 kDa in humans and 92 kDa in rats. Likewise, the muscle isoform transfers at a position corresponding to 60−70 kDa in polyacrylamide gels despite its expected size of 50 kDa[201].

PICALM, a gene connected to AD, is also linked to endocytosis. The average expression of BIN1 and PICALM has newly been revealed to be more significant in the white matter of the CNS[189]. The N-terminal N-BAR domain, which adheres to lipid membranes and starts membrane curvature in T-tubules in muscular cells, endocytic pits in neuronal and non-neuronal cells, and probably cytoplasmic endosomes, also present in all known alternatively spliced BIN1 forms[207,208]. BIN1 seems to connect the microtubule cytoskeleton to the cellular membrane through tubular membrane structures[208], which may have an impact on the production of neurofibrillary tangles. Lastly, BIN1 is required to activate cell senescence and apoptotic[209,210] Similarly, BIN1 has been found to play a vital role in oncogene-induced senescence in primary cells, preventing cancer in its early stages[211].

#### 3.8.2. *A possible role for BIN1 in AD*

The concept of the amyloid cascade may be important further than the monogenic forms of AD, as LOAD is linked to a minor but pervasive dysfunction in the capacity to remove peripheral A$\beta$ peptides[212]. Additional discoveries, however, suggested that drug discovery goals must concentrate more on emerging disease pathways such as endocytosis, synaptic damage, immune system, and lipid metabolism[61,213]. Unlike APOE, clusterin (CLU), and CR1, which have vast information from the perspective of AD, the in-depth role of BIN1 on neurodegeneration is still a mirage. Thus, more investigation is required into the function of BIN1 in the pathogenesis of AD and putative pathways that affect AD risk. Even though the mechanisms behind BIN1's harmful character in AD are unknown, various plausible pathways have been discovered. Furthermore, BIN1's activity in multiple circumstances may aid us in grasping a potential function in AD[214].

#### 3.8.3. *BIN1 as a potential AD therapeutic target*

Overall, the potential functions of BIN1 in the development of neurodegeneration create various new avenues for research into AD. The relations with Tau pathology must be prioritized, among them since it has the potential to alter neurofibrillary tangles. Further prominently, it has been shown that knocking down BIN1 reduces Tau mediated neurotoxicity[215], proposing that targeting BIN1 could be a novel approach to nerve protection and AD treatment. As previously indicated, blocking IDO1, a BIN1 immunoregulatory target, has proven therapeutic advantage for AD[216]. Provisionally, BIN1 may influence Ca(v)1.2 trafficking and calcium channel blocker selectivity, which could reduce Tau load and increase autophagy role[217], a trait that could be crucial for effective treatment. Indeed, autophagy has been a critical reason for numerous neurodegenerative disorders, including frontotemporal dementia, AD, Parkinson's disease, and Huntington's disease. In AD, the pathogenesis has also been attributed to the endosomal lysosomal system precisely A$\beta$ amyloidogenesis. Functional abnormalities in lysosomal pathways and overexpression of hydrolases lead to the formation of elevated levels of A$\beta$[218].

### 3.9. *Targeting infectious agents*

Several medications in the current AD therapeutic development pipeline target infections and inflammation. COR388 inhibited the generation of gingipain by *P. gingivalis* and blocked A$\beta_{1-42}$ production by reducing neuroinflammation and thus rescuing neurons in the hippocampus of mice[219]. One study found that the Herpesviridae family, Epstein Barr virus (EBV), Herpes simplex virus-1 (HSV-1), Human herpesvirus 6 (HHV-6), and Chronic progressive nephropathy (Cpn) infection were related to a greater risk of AD in a meta-analysis of case control studies[220].

HSV-1 antibodies were found in people with schizophrenia in the 1960s and 1970s. Some studies analyzed nucleic acid sequences of HSV-1 in the brains of manic and psychiatric patients and detected the HSV-1 genome in brain samples from elderly dementia patients. Various researchers looked for a link between viral infection and late onset sporadic AD however, some of such studies were futile. Hepatitis B virus (HBV) and influenza A and B viruses were among the viruses studied. Serum antibody titres to CMV, adenovirus, HSV, influenza A/B/measles virus, *Coxiella burnettii*, chlamydia group B, influenza A/B/measles virus and Mycoplasma pneumonia were not associated with AD[221,222]. In certain cases, negative results can be attributed to the approaches that were not sensitive enough to detect viral genomes. Utilizing the polymerase chain reaction, other researchers found HSV genomes in the serum or brains of AD patients. Previously, spirochetes were suspected of causing AD. Recently, two additional bacteria, Chlamydia pneumonia, and Helicobacter pylori were linked to AD and developed a vascular hypothesis[222].

### 3.10. *Carboxylesterase Notum*

Notum, a secreted palmitoyl protein carboxylesterase, has newly been discovered to be a Wnt signalling negative modulator. Notum works by taking away a crucial palmitoyl moiety from Wnt proteins thus converting them to inactive form. It may be a more tractable therapeutic target for regulating Wnt signalling since it has a known high resolution crystal structure[223]. Notum appears to play a crucial role in human disease and study results suggest that targeting Notum may change a new therapeutic approach for the treatment of osteoporosis, cancer, and neurodegenerative disorders[224].

Planarians through humans are all metazoans that have notum orthologues and they all have the recognizable S−H−D sequence catalytic triad of $\alpha/\beta$-hydrolases. Early theories suggested that

Notum may hydrolyze the glycosaminoglycan chains of glypicans thereby changing their capacity to interact with Wnts and somehow modify signaling activity. This idea was sparked by Notum's sequence resemblance to plant pectin acetyl esterases. Later, it was shown that Notum causes cultured cells to release glypican, possibly *via* cleaving the GPI anchor. It is currently believed that Notum is a phospholipase that is peculiar to glypicans. However, glypican based interactions also affect Hedgehog, fibroblast growth factor, Dpp (*Drosophila* TGF), and Wingless signalling. Therefore, it stands to reason that these bio-pathways would be susceptible to Notum-induced glypican release. Wnt signalling activates notum expression in *Drosophila*, planarian worms, zebrafish, and hepatocarcinoma; on the other hand, Notum appears to preferentially inhibit Wnt signalling[225].

Wnt proteins are a type of lipoprotein that binds to a wide range of cell surface receptors and co-receptors to trigger multiple intracellular signalling pathways. Three crucial Wnt signalling pathways that impact cytoskeleton remodelling and/or changes in gene expression are planar cell polarity (PCP), canonical Wnt/$\beta$-catenin, and Wnt/$Ca^{2+}$. The well-studied Wnt pathway regulates the expression of Wnt target genes by stabilizing cytoplasmic $\beta$-catenin and promoting cellular cytoskeleton remodelling. GSK3 and CK1 phosphorylate $\beta$-catenin in the absence of Wnt ligands, forcing it to disintegrate[226]. Wnts are morphogens that are produced and are required for embryonic homeostasis. Wnt signalling dysregulation is linked to a variety of developmental defects and illnesses, including cancer, fibrosis, and osteoporosis. When Wnts interact with cell surface receptors, both $\beta$-catenin dependent and independent pathways are activated. Frizzled, LRP 5/6 (low density lipoprotein receptor associated proteins), and G protein coupled receptors. Because the palmitoleate group fits into the frizzled cysteine rich domain's hydrophobic groove for better engagement with receptors frizzled, a conjugation between the mono-unsaturated palmitoleic acid and a serine residue is vital. Wnt secretion requires palmitoylation, which is mediated by the ER enzyme Porcupine. Wnt secretion is inhibited by either

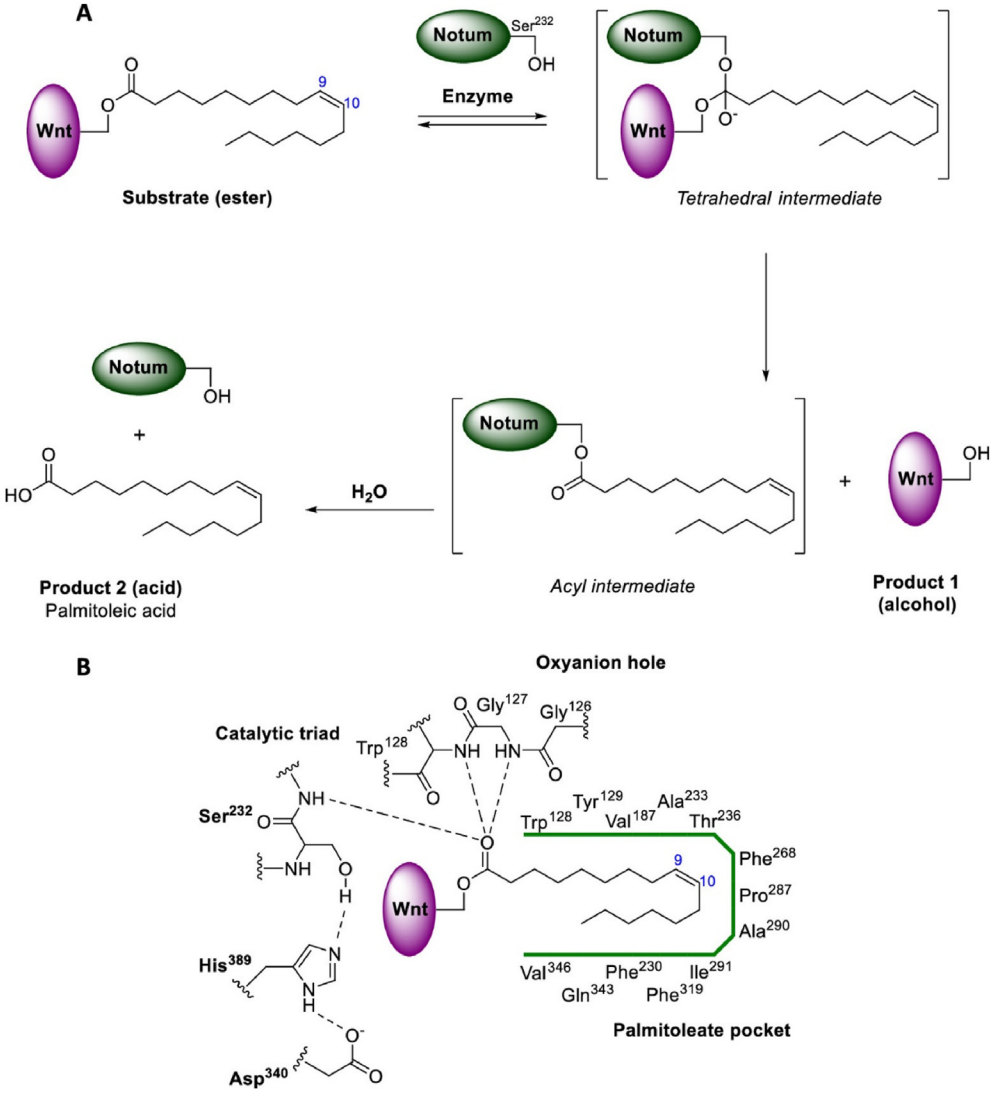



**Figure 22** (A) Chemical reaction involving Notum's carboxylesterase activity operating on Wnt to release the palmitoleate group. (B) A 2D representation of the binding interactions. In addition to the traditional Ser232−Ala233 and Gly126−Gly127 amides, the Gly127−Trp128 amide plays role in the generation of the oxyanion hole. The catalytic triad (Ser232, His389, Asp340) is shown in bold[230] 

changing the conserved serine residue in Wnt or reducing PORCN enzymatic activity, which stops Wnts from binding with their carrier protein Wntless (WLS)[227].

### 3.10.1. *Wnt pathway and Notum function determination*

Two groups studying the regulation of *Wingless* signalling in *Drosophila* independently discovered Notum. An enhancer trap screen for genes activated by *Wingless* signalling led to the discovery of a mutation that caused the expansion of presumptive wing tissue in Gerlitz and Basler's group[228]. At the same time, Giraldez et al.[229] discovered that overexpression of the same gene resulted in the opposite phenotype, wing tissue loss, and an enlargement of the notum, an anatomical structure at the back of the fly. The latter phenotype prompted the authors to name the gene notum, which somehow surpassed *wingful*. Both studies found Notum to be a target of Wingless signalling and its protein product to be a powerful inhibitor of *Wingless* signaling[230].

The fact that a signal peptide was present early on suggested that Notum functions in the extracellular environment. Given that Notum and plant pectin acetyl esterase have proteins with similar amino acid sequences, it is possible that Notum could change the glycosaminoglycans of glypicans, which are known to bind to Wnts and other growth factors *via* glycosylphosphatidylinositol anchored proteoglycans[231]. Further biochemical studies, however, challenged this theory and proposed that Notum might be a phospholipase that breaks down proteins the GPI anchor of glypicans, releasing the glypicans from the cell surface along with any bound Wnt[232]. Since many extracellular proteins other than Wnts bind to glypicans, one would anticipate additional pleiotropic effects if the molecular target of Notum was a glypican (*e.g.*, Hedgehog, FGF, BMP). The phospholipase model was ruled out by structural analysis and enzymatic assays, which revealed that Notum is a carboxylesterase (Fig. 22)[230].

### 3.10.2. *Crystal structure of Notum*

The Notum structure is based on the "canonical"/$\beta$-hydrolase superfamily protein shape, with an eight-stranded $\beta$-sheet core hidden by $\alpha$-helices ($\alpha$B, $\alpha$C and $\alpha$F) and loops. The helices $\alpha$A, $\alpha$D, and $\alpha$E and loops make up a movable lid domain, which can be 'opened' or 'closed' by shifting the helices far from or closer to the catalytic pocket, which is a unique property of lipases (Fig. 23A). An open state enables substrate entry, whereas a closed state is believed to allow catalytic substrate processing. The palmitoleated substrate bound Notum (S232A) structure demonstrates this by acquiring a closed conformation. However, few small molecule inhibitors are attached to Notum in an open conformation. The crystal structures of Notum exhibit a distinct, large (about 380 Å) active site with a hydrophobic pocket next to the catalytic triad created by S232, H389, D340 that houses the Wnt palmitoleate group (PDB id: 4UZQ) (Fig. 23B). Extended fatty acid chains need a turn in their structure to fit into the hydrophobic binding pocket, which can take lengthy carbon chains up to C8−10. The conventional S232−A233 and G126−127 peptides and the G127 to W128 amide produce the oxyanion hole, which offers additional stability to the tetrahedral transition state throughout ester hydrolysis. Entry into the pocket is relatively narrow, yet it has excellent flexibility. Because of these structural characteristics, this Notum pocket has been classified as highly druggable[223].

Notum's crystal structure also exhibited a hydrophobic pocket that might hold *cis*-palmitoleate, concluding that Notum might hydrolyze the O-linkage of palmitoleate to Wnts. MALDI analysis of palmitoylated peptides treated with recombinant Notum confirmed this *in vitro*. Surprisingly, the structural study revealed multiple heparin binding sites on Notum's surface. These sites

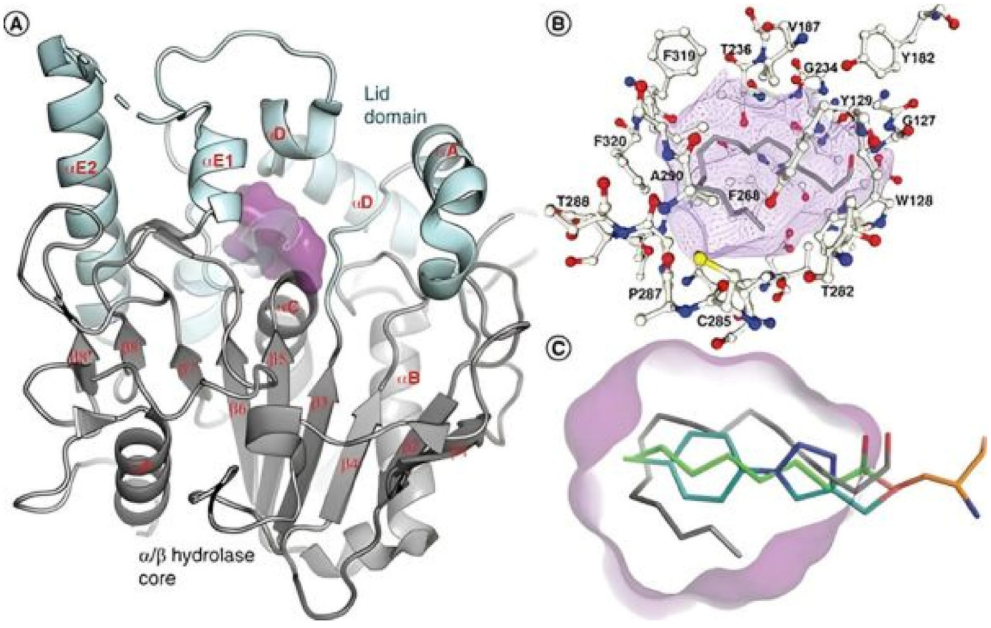



**Figure 23** Notum structure as illustrated in a cartoon. (A) A grey cartoon of the enzyme core is shown, with the lid domain in pale cyan. A purple surface delineates the lipophilic pocket. (B) Notum pocket-forming residues (white ball and sticks) and palmitoleic acid substrate (grey sticks) within the pocket (purple mesh). (C) A close-up view of a pocket (purple) showing the alignment of Wnt palmitoleate (grey) and ghrelin octanoyl lipid (green/orange) substrates, as well as a representative inhibitor (teal)[223]. Reprinted with the permission from Ref. 223. Copyright © 2021 The authors.

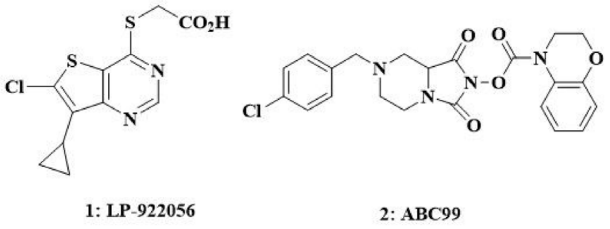



**Figure 24** The structures of cholinesterase inhibitors.

explain Notum's potential to bind glypicans and give a molecular foundation for the genetic interactions among Notum and glypicans that have been recorded[229]. This pocket is where Notum's substrates and most inhibitors bind (Fig. 23C)[223].

### *3.10.3. Notum inhibitors*

Inhibitors of notum can bring back Wnt signalling, which could help treat diseases like osteoporosis and AD. Notum inhibitors have been shown to revitalize aged colon stem cells, enhance cortical bone strength and width, and raise elderly neuronal progenitors. They are being studied as a possible therapy for neurodegenerative diseases like AD, in which Wnt signalling is frequently downregulated. The discovery of 4-(indolin-1-yl)-4-oxo butanoate esters, a new class of covalent Notum inhibitors, has been reported. According to high resolution crystal structures, the nucleophile S232 and hydrolyzed butyric esters create a common covalent adduct in the Notum inhibitor complexes[232].

Only a few NOTUM inhibitors, which are heteroaryl fused thiophenes, have been described so far. These compounds are considered reversible type inhibitors, but their specificity inside and outside the serine hydrolase family is unclear. Ureas and activated carbamates have previously been shown to be dynamic serine hydrolases that are irreversible inhibitors, particularly once combined with activity based protein profiling to optimize effectivity and selectivity. Suciu et al.[233] isolated *N*-hydroxy hydantoin (NHH) carbamates from a structurally varied set of activated carbamates and urease to serve as specific and compelling inhibitors of NOTUM. In animals treated with ABC99, inhibition of Notum generated by Paneth cells led to the regeneration of aging intestinal epithelium[234]. In the brain, the Notum controls Wnt signaling, which regulates neurogenesis in the subventricular zone (SVZ), and inhibition of the Notum with ABC99 (Fig. 24) activates Wnt signaling and increases more in the SVZ[235].

Notum melatonin complex crystal structure could assist in developing more highly effective brain accessible drugs that could help cure neurodegenerative disease. Melatonin has previously shown neuroprotective action in aging and AD animal models by reducing the deposition of A$\beta$ and hyperphosphorylated Tau. Stimulation of Wnt signalling can prevent GSK3, a component of the "destruction complex", thus minimizing tau hyperphosphorylation. Zhao et al.[236], investigated the XChem platform output to find Notum inhibitors, and the fragment hit *N*-[2-(5-fluoro-1*H*-indol-3-yl) ethyl]-acetamide ($IC_{50}$ 37.2 µmol/L; PDB 6TR7) was emphasized because of its structural resemblance to melatonin. By immersing (PDB 6TR5) and *N*-acetyl serotonin (PDB 6TR6) in Notum crystals (Fig. 25), various structural studies were done, and high-resolution structures of related groups were produced. Among these structures, two compounds interact with Notum: one at the catalytic region of the enzyme and the other at the pocket's edge against the substrate entrance[236].

Caffeine and, to a lesser extent, theophylline can inhibit notum activity (Fig. 26). Both biochemical and biophysical methods were used to characterize the caffeine Notum interaction thoroughly. High resolution structures of (PDB 6TV4) and (PDB 6TUZ) show that both compounds bind in the palmitoleate pocket but in distinct manners. This structural information could aid in the discovery of more potent Notum inhibitors[229].

In the development of 1-phenyl-1,2,3-triazoles and 5-phenyl-1,3,4-oxadiazol-2(3*H* )-ones, (1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl) methanol ($IC_{50}$ 11.5 µmol/L) was a notable hit from this fragment set and was chosen as the beginning point for a hit-to-lead programme (Fig. 27). According to the X-ray structure

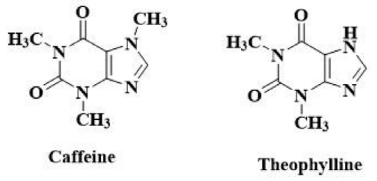



**Figure 26** Caffeine and theophylline are Notum inhibitors.

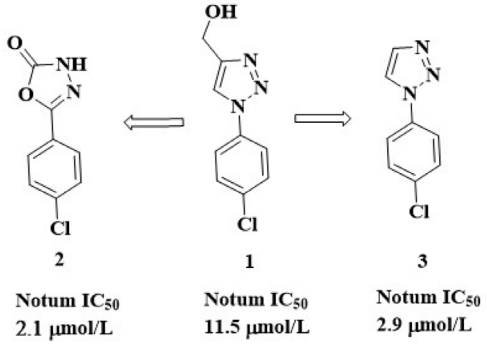



**Figure 27** Oxadiazole **2** and triazole **3** were two complimentary leads produced by a fragment.

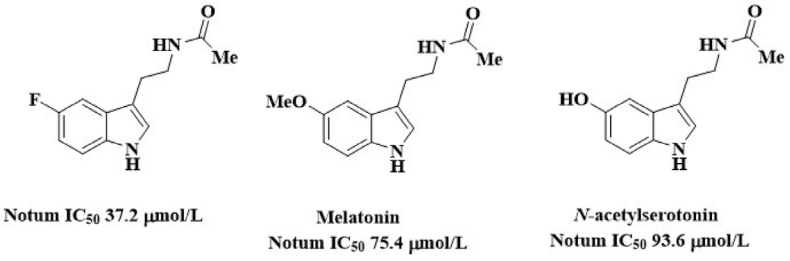



**Figure 25** Notum inhibitors associated with the hormone melatonin.

of 1 (PDB 6ZUV), the 4-chlorophenyl ring occupied the palmitoleate pocket and formed a stacking contact with the residue Phe268. Trp128 and the triazole head group may interact *via*—-stacking, and the triazole's N2 and Trp128's peptidic backbone may form a hydrogen bond. Moreover, residue Trp128 forms a hydrogen bond with the methyl alcohol group's oxygen. Modification of 1 by alteration of the heterocyclic head group found two complimentary leads: oxadiazole 2 and triazole 3[229].

## 4. Conclusions

Alzheimer's disease is one of the dreadful diseases that has blown a full whistle for the cure of patients. Numerous pathophysiological changes have been recognized involving multiple signaling systems for the gradual increase in the deteriorating condition of AD. According to the existing data, one must instead consider a causal polymicrobial community that impacts immune/inflammatory reactions in the brain and the periphery and interacts with many factors such as genetics, environment, and age. Thus, AD should be viewed as a complicated illness involving the dysfunction of the brain's immune system. Despite technical advancement and artificial intelligence, early AD detection is needed for an hour; once detected, the treatment depends on the use of the drug for symptomatic treatment. Future treatments (and prevention) of AD will not be a single simple molecule but a multimodal complicated approach. Putative molecular mechanisms in the progression of AD have indicated the involvement of amyloid proteins and complicated neuroinflammation, TREM2, notum, tau accumulation, and degeneration, etc., with numerous supporting scientific proofs. A few significant numbers of new minor molecule hits have been reported against the modulation of the aforementioned molecular targets, and some have advanced to higher stages of clinical trials. The successful molecule as a clinical drug for treating AD is eagerly anticipated in the present and future.

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## Author contributions

All of the authors approved the final version of the manuscript.

## Conflicts of interest

All the authors express no conflict of interest with any agencies.

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