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Amyloid beta modulators and neuroprotection in Alzheimer's disease: a critical appraisal

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Abstract

Multiple cellular changes have been identified as being involved in Alzheimer's disease (AD) pathogenesis, including mitochondrial damage, synaptic loss, amyloid beta (A β) production and/or accumulation, inflammatory responses, and phosphorylated tau formation and/or accumulation. Studies have established that A β -induced synaptic dysfunction is dependent on abnormal amyloid precursor protein (APP) processing caused by β - and γ -secretases, resulting in the generation of A β . The A β formed as a result of abnormal APP processing induces phosphorylated tau and activates glycogen synthase kinase-3 β (GSK3 β) and cyclin-dependent kinase-5 (CDK5). Here, we review the latest research on the development of A β modulators for neuroprotection in AD. We also review the use of molecular inhibitors as therapeutic targets in AD.

Keywords

amyloid beta; phosphorylated tau; synaptic damage; beta-secretase inhibitor; gamma-secretase inhibitor

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Introduction

AD is a progressive neurodegenerative disorder that generally occurs in those older than 65 years of age; however, in some instances, it is hereditary and can affect younger adults. Patients with AD can be divided into two groups: those with early-onset familial AD and those with late-onset sporadic AD. AD is caused by any one of several single gene mutations on chromosomes 21, 14, and 1. Each of these mutations causes abnormal proteins to be formed. Mutations on chromosome 21 cause the formation of A β and mutant APP, whereas mutations on chromosome 14 cause abnormal presenilin 1 protein to be made, and mutations in presenilin 2 (PS2) loci on chromosome 1 produce abnormal PS2 protein [1–3].

Late-onset sporadic AD is the most common form of AD, affecting persons 65 years of age and older. No specific gene mutation underlying this form of AD has yet been found. However, one known genetic risk factor is a variation of the apolipoprotein E (ApoE) genotype 4, on chromosome 19 [4]. Genetic variants in the genes encoding sortilin-related receptor 1, clusterin, complement component receptor 1, CD2AP, CD33, EPHA1, and MS4A4/MS4A6E also contribute to late-onset AD [5]. Modifiable risk factors for late-onset sporadic AD are life style, diet, and environmental factors (e.g., stress, risk of vascular diseases, stroke, hypertension, traumatic brain injury, and type 2 diabetes mellitus) [6].

Late-onset sporadic AD and familial AD are principally the same disease, apart from differences in their genetic makeup and the age of onset. Early-onset familial AD progresses with the same overall sequence of symptoms and impairments as does late-onset sporadic AD [7]. Major clinical symptoms of AD, whether late-onset or familial, include memory loss, poor judgment, problems with familiar tasks, confusion in time and place, personality changes, and dementia [8,9].

The *World Alzheimer's Report* estimated that, in 2015, 47.5 million people had AD-related dementia ('dementia') worldwide and projected the numbers to increase to 75.6 million by 2030 and to 131.5 million by 2050. Over 9.9 million new cases of AD-related dementia are diagnosed every year worldwide, or 1 new case every 3.2 s [10]. In high-income countries, such as the USA and Germany, seven in ten persons aged 70 years and older will pass away having had some form of dementia [10]. Dementia has a huge economic impact on society in general and on those with dementia, their families, and caretakers. The estimated total healthcare cost of dementia worldwide in 2015 was US\$818 billion [10], and dementia is foreseen to be a trillion-dollar disease by 2018.

While scientists are still trying to determine what causes AD, several cellular changes have been pinpointed that increase a person's risk for developing AD, including mitochondrial damage, synaptic loss, A β production and accumulation, inflammatory responses, phosphorylated tau formation and accumulation, cell cycle deregulation, and hormonal imbalance [11]. Aging is the main risk factor for AD and is a complex process that has been linked to DNA damage. In mammals, including humans, an accumulation of oxidative DNA damage in different tissues, including brain tissue, has been found in aging persons [12].

Here, we review the latest research on A β -induced toxicities and A β modulators in AD pathogenesis. We also review research investigating molecular inhibitors as therapeutic approaches against AD.

Cellular events in Alzheimer's disease progression

The following cellular events are associated with AD development and pathogenesis, including APP processing, A β formation, phosphorylated tau, and activation of GSK3 β and CDK5 in AD neurons.

APP processing in Alzheimer's disease

APP processing occurs in two pathways, one that is nonamyloidogenic and one that is amyloidogenic. Three secretases have been identified in APP processing: α , β , and γ . In the α -secretase-based nonamyloidogenic pathway, cleavage occurs in the middle of the A β domain and generates sAPP α and C-terminal fragment C83; the latter is further cleaved by γ -secretase, producing P3 and ACID domains[DE1]. These smaller fragments are generally cleared from neurons (Figure 1). In the β -secretase-based amyloidogenic pathway, cleavage occurs at the start of the A β domain and generates sAPP β and the C-terminal fragment C99. Further cleavage of C99 produces A β (39, 40, 42 and 43)[DE2] and ACID fragments. The A β fragment(s) that accumulate in subcellular compartments, including mitochondria, lysosomes, and endoplasmic reticulum, cause(s) dysfunction of these compartments in AD neurons (Figure 1) [13].

A β -induced synaptic dysfunction and mitochondrial damage result in aberrant activation of redox-mediated events as well as elevation of cytoplasmic Ca²⁺. These events cause a further cascade of changes, including tau phosphorylation, and activation of caspases, CDK5/dynamitin-related protein 1 (Drp1), calcineurin/protein phosphatase 2B (PP2B), PP2A, GSK3 β , Fyn, cofilin, and calcium calmodulin-dependent kinase II (CaMKII). [DE3]These events result in the endocytosis of AMPA receptors (AMPA receptors) as well as NMDA receptors (NMDARs) [14]. Thus, a combination of therapies and preventive strategies will likely be needed to reduce toxicity in neurons from older individuals and patients with AD.

Research to develop drug molecules to reduce the toxicity of A β has focused on reducing A β production using inhibitors of the β - and γ -secretases, and to enhance the clearance of A β by α -secretase activators and A β sequesters. Most of these antibodies or vaccines target soluble monomeric A β and/or oligomers and/or plaques. α -secretase activators have been found to reduce APP cleavage and A β production.

Hyperphosphorylation of tau in Alzheimer's disease

The Tau protein is a neuronal microtubule-associated protein that has a crucial role in neuronal processes and in the development of neuronal polarity in the central nervous system [15,16]. The main functions of tau are the assemblage and stabilization of microtubules on neuronal axons and the inhibition of apoptosis, particularly in axons [17,18]. When tau is abnormally hyperphosphorylated, it destabilizes microtubules by decreasing the binding affinity of tau. This results in the abnormal self-aggregation of hyperphosphorylated tau in in paired helical or straight filaments, forming intracellular

neurofibrillary tangles (NFTs) [19]. NFT formation spreads to various brain areas during AD progression, ultimately causing neuronal death [20].

There are many stages in Tau hyperphosphorylation, such as initial protein–protein interactions and the resulting oxidative stress. The generation of A β is necessary for tau hyperphosphorylation. Production and aggregation of A β produces oligomeric A β , which induces the neurodegenerative triad of synaptic changes, dendritic simplification, and neuron loss via tau-dependent and -independent mechanisms. The increased production of A β and A β -induced oxidative stress results in tau phosphorylation and the formation of NFTs [21].

GSK3 β and CDK5 in Alzheimer's disease

Considerable research has focused on classifying and inhibiting the kinases responsible for pathogenic tau hyperphosphorylation. Candidate kinases that have been investigated include GSK3 β , CDK5, mitogen-activated protein kinase (MAPK) family members [extracellular signal-regulated kinase (Erk) 1 and 2, MAP kinase kinase (MEK), c-Jun NH(2)-terminal kinases (JNKs), and p38], casein kinase, CaMKII, microtubule affinity-regulating kinase (MARK), protein kinase A (PKA/cAMP-dependent protein kinase), and others. GSK3 β and CDK5 were selected because they showed promise for reducing hyperphosphorylated tau (Figure 2).

Oxidative stress has been found to induce tau hyperphosphorylation through direct interaction with GSK3 β and PP2A [22]. GSK3 β activity in mitochondria has been associated with the upregulation of oxidative stress [23]. Thus, GSK3 β appears to have a significant role in AD pathogenesis and involves A β production and A β -mediated neuronal death by elevating tau hyperphosphorylation in paired helical filaments [24]. Inhibition of GSK3 β prevents A β aggregation and tau hyperphosphorylation [25,26].

Tau phosphorylation is directly impacted by the interaction of A β and CDK5/P35, which is known to affect the calpain-activated cleavage of p35 into a p25 C-terminal fragment. These cleaved fragments have longer half-lives, are dissociated from membranes, and are able to phosphorylate additional proteins. [DE4]Therefore, CDK5 could be a key target for therapeutic gene silencing [27].

A β modulators

α -secretase inducers and Alzheimer's disease

Nonamyloidogenic α -secretase activity accomplished by the disintegrins ADAM10 and ADAM17 arises in the middle of the A β domain of APP, from which it liberates the large sAPP α neuroprotective fragment. Given that the activation of α -secretase was recently highlighted as a promising therapeutic approach to treat AD, the identification of natural compounds able to elevate this cleavage is required. Expression of ADAM10 can be enhanced by targeting nuclear retinoic acid receptor β , resulting in further nonamyloidogenic APP processing. ADAM10 and ADAM17 are not APP specific and cleave various substrates. The use of ADAM10 to increase the levels of α -secretase has been associated with tumorigenesis and tumor progression in AD brains. As a result, ADAM10 has been avoided by researchers in their investigation in AD. Thus, further research is

needed to understand the toxicity resulting from the effects of ADAM10 in AD neurons (Figure 3) (K. Jacobsen, Licentiate thesis, Stockholm University, 2010).

Curcumin-based modified compounds have previously been described as α -secretase activators. These compounds include the amino-acid conjugates curcumin-isoleucine, curcumin-phenylalanine, and curcumin-valine, all of which have been found to promote α -secretase activity and to increase ADAM10 immunoreactivity [28,29]. This led to the discovery of natural α -secretase-inducing compounds, such as ginsenoside and those contained within leaves of *Ginkgo biloba*, which were found to regulate nonamyloidogenic APP-processing pathways [30].

Triptolide is another naturally occurring compound that is extracted from *Tripterygium wilfordii*, a Chinese medicinal herb that is commonly used to treat inflammatory diseases. Triptolide also inhibits the processing of amyloidogenic APP as well as the expression of β APP-cleaving enzyme-1 (BACE1) both *in vivo* and *in vitro* [31,32].

These naturally occurring compounds have all been found to reduce A β 42 levels, with the exception of triptolide, which was found to reduce A β 40 levels. In addition to reducing A β 42, curcumin and ginkgo biloba have also been found to reduce A β 40 and A β 25-35 levels (Table 1) [33,34].

Thus, based on current research, α -secretase activators generally exhibit positive effects. Recent research also showed that α -secretase activators, particularly ADAM10, show [DE5] tumor progression in AD brains. Further research is required to understand the basic functions of these agents and their beneficial and adverse effects in patients with AD.

β -Secretase inhibitors and Alzheimer's disease

Inhibition of BACE1, the key enzyme in the generation of A β peptides, appears to be the most attractive target to prevent the formation of A β oligomers in AD. BACE1 expression and activity are enhanced by oxidative stress, leading to metabolic dysfunction and apoptosis of neurons in AD [35,36].

A β peptides are generated by cleavage of APP at an N terminus to the start of the transmembrane domain by BACE-1 and cleavage within the transmembrane domain by γ -secretase. One possible approach is to reduce A β using BACE1 inhibitors. Thus, the partial inhibition of BACE1 has become a [DE6]prime therapeutic target for anti-amyloid therapy (Figure 4).

Curcumin, asiaticoside, and tryptoline were the first naturally occurring compounds to be identified as possible BACE1 inhibitors. *In vivo* and *in vitro* studies verified the ability of curcumin to prevent A β aggregation and fibril formation. Curcumin was also found to reduce A β 40 and A β 42 in AD mice by inhibiting BACE1 and inducing α -secretase [37]. Tryptoline was found to bind the catalytic sites [DE7]Asp32 and Asp228 to BACE1, which inhibited β -cleavage of APP [38]. Asiaticoside, a highly polyphenolic compound, prevented amyloidogenesis by reducing A β 42 levels in AD neurons [39]. Hilpert *et al.* developed fluoro-oxazines derivatives (e.g., CF₃) as BACE1 inhibitors. They substituted oxazine for

BACE1 inhibitors in rats at oral doses as low as 1 mg/kg, which reduced A β 40 and A β 42 levels significantly [40].

More recently, potent third-generation small-molecule BACE1 inhibitors have been developed. These inhibitors exhibited acceptable blood–brain barrier (BBB) permeability and robust A β reduction *in vitro*. Researchers are also investigating the ability of β -secretase inhibitors to reduce A β in patients with AD (Table 2). Human clinical trials are currently investigating the β -secretase inhibitors AZD3293 (Phases II/III), MK-8931 (Phases II/III), E2609 (Phases II/III), and HPP854 (Phase I). A Phase II toxicology investigation of the β -secretase inhibitor LY2886721 was discontinued because of adverse toxicity that damaged the pigment epithelium of the eyes of rats. However, other studies of LY2886721 were found to reduce A β levels in amyloidogenesis, particularly of A β 5–40, A β 1–34, and sAPP β . Studies of the β -secretase inhibitors AZD3293, CTS-21166, and MK-8931 are currently investigating the ability of these inhibitors to reduce A β 40 and A β 42 levels. Furthermore, MK-8931 has been found to bind to the conserved extracellular domain of APP and to inhibit the cleavage of APP (Table 2) [41].

Overall, β -secretase inhibitors are promising agents that have been shown to inhibit A β levels in AD mice and patients with AD. However, these agents did not improve cognitive functions in patients with AD and older individuals. Furthermore, these β -secretase inhibitors had adverse effects in patients with AD. Thus, further research is needed to understand the normal function of these agents and the causal factors of their adverse effects in patients with AD.

γ -Secretase inhibitors and Alzheimer's disease

γ -Secretases are a group of widely expressed, intramembrane-cleaving proteases involved in many physiological processes associated with AD. As a result of alterations on chromosome 1, 14, and 21, genes on chromosome 14 encode PRESENILIN 1 (PSEN1) and on chromosome 1 encode PRESENILIN 2 (PSEN2). PSEN1 and -2 lead to γ -secretase activity, which has been associated with the formation of A β in AD [41]. Based on research investigating the γ -secretase complex in AD progression, PSENs were found to be important in maintaining the γ -secretase complex[DE8] . Two other subunits [nicastrin and anterior-pharynx defective-1 (APH1)] were identified as co-factors essential to PSEN [42]. All four γ -secretase enzymes together generate an active and stable complex (Figure 4) that cleaves APP at the end of A β domain in APP. Thus, inhibition of these enzymes redirects the amyloidogenic pathway towards the nonamyloidogenic pathway by reducing A β production.

γ -Secretase inhibition has been frequently studied over the past decade. Diverse classes of γ -secretase inhibitor (GSI) and modulators (GSM) have been found to reduce levels of A β peptides or to modulate the composition of A β [43,44]. GSIs can be classified into three subgroups, depending on where they bind to the γ -secretase complex: active site-binding GSIs, substrate docking site-binding GSIs, and alternative binding-site GSIs. This latter class of GSIs can be further divided into carboxamide- and arylsulfonamide-containing GSIs [45]. The ability of some GSIs and GSMs to reduce A β has been hampered by poor BBB penetration or severe adverse effects [46–48].

Initially developed as a GSI, L-685,458 has been used to examine whether PSEN provides a catalytic core for γ -secretase [49]. Results revealed that L-685,458 does not distinguish among different complexes or substrates; thus, it has not been studied in any clinical trials for AD chemotherapies [49,50]. More studies have been carried out on N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine tbutyl ester (DAPT) and have established it as a potential drug target that inhibits γ -secretase activity in AD. DAPT showed a moderate IC₅₀ of 20 nM for A β in cellular assays and was orally active [51]. Furthermore, the carboxamide- and arylsulfonamide-containing GSI, semagacestat was introduced to reduce A β levels rapidly, but it was discontinued because of its adverse effects on the cognition of patients with AD.

SCH697466 is an arylsulfonamide series that was tested as a γ -secretase inhibitor. Although SCH697466 resulted in sufficient notch signaling[DE9], it did not downregulate A β levels [52]. Researchers then sought to develop notch-sparing inhibitors of the arylsulfonamide series, such as avagacestat [53] and begacestat [51]. Avagacestat exposed notch-related problems in a similar manner to that seen with semagacestat[DE10] [53]. A recent clinical study investigated the notch-sparing compound ELND006 to determine its γ -secretase activity. However, the study was stopped because of non-mechanism-based liver toxicity [54,55]. By contrast, CHF-5074 and NIC5-15 are γ -secretase inhibitors that have been found to reduce A β levels in patients with AD (<https://clinicaltrials.gov/ct2/show/NCT01203384>; <https://clinicaltrials.gov/ct2/show/NCT01303744> [56]). The Phase II trial investigating NIC5-15 is ongoing, whereas the Phase II trial studying CHF-5074 was terminated because of adverse effects (Table 3) (<https://clinicaltrials.gov/ct2/show/NCT00470418> [57]).

To improve the therapeutic benefits of GSI and GSM, new chemical moieties need to be developed. These require safer and faster pharmacokinetics profiles and need to be derived from natural elements [DE11]. At the same time, the pathological relevance of γ -secretase involvement in preventing cancer and other disorders suggests that the development of specific GSM is healthier than that of GSI[DE12].

Overall, γ -secretase inhibitors are promising agents to reduce A β levels in AD neurons. However, γ -secretase inhibitors have failed in clinical trials with patients with AD mainly because of adverse effects in organs other than the brain. γ -Secretase inhibitors cause *in vivo* toxicities by blocking proteolysis of the essential notch receptor. Thus, further research is required to understand the normal function of γ -secretase inhibitors and their physiological relevance.

GSK3 β inhibitors and Alzheimer's disease

In 1980, GSK3 was found to be a regulatory serine/threonine protein kinase. It is encoded by two genes (*GSK3a*, located on chromosome 19, and *GSK3b* located on chromosome 2) and mediates the addition of phosphate molecules onto serine and threonine amino acid residues. GSK3 α has a molecular mass of 51 kDa, whereas that of GSK3 β is 47 kDa. These two isoforms are highly homologous at the kinase domain, but differentiate at the N- and C-terminal regions [58]. Activation of GSK3 β has been implicated in a large number of human diseases, including cancer, cardiovascular, and neurodegenerative diseases [59].

In recent years, several studies have concentrated on revealing the role of GSK3 β in AD pathogenesis, mainly because of its known role in causing the phosphorylation of tau and in increasing levels of A β and A β deposits in AD brains, AD mouse models, and AD fly models [60,61]. In primary rat cortical neuronal cultures stimulated by the combination of the copper chelator, cuprizone, and oxidative stress (Fe²⁺/H₂O₂), tau phosphorylation was significantly increased because of the elevated activity of GSK3 β [62].

It has been proposed that GSK3 β activation is mainly caused by two conditions: (i) the interaction of the A β peptide with GSK3 β and (ii) oxidative stress caused by A β and its associated overproduction of reactive oxygen species (ROS). This overactive GSK3 β enzymatic state leads to tau phosphorylation [63]. By contrast, recent studies implied that, in AD, increased GSK3 β activity also induces A β formation through its action on γ -secretase via PES1 mutations [64,65]. The role of GSK3 β in causing the phosphorylation of tau, and in producing increased levels of A β and A β deposits in AD brains, AD mouse models, and AD fly models prompted researchers to investigate the role of GSK3 β in AD pathogenesis [60,66–69]. They found that inhibition of GSK3 β reduces AD pathology (Figure 5) [70–72]. Additional research using AD models found ameliorated cognitive decline in AD mice.

Given the A β -induced toxicity of GSK3 β , researchers sought to investigate therapeutic agents capable of inhibiting GSK3 β to reduce the symptoms of AD. Multiple GSK3 β inhibitors were identified as being capable of reducing A β levels as well as tau hyperphosphorylation in neuronal and non-neuronal cells. Additionally, GSK3 inhibitors were found to enhance adult hippocampal neurogenesis *in vivo* as well as *in vitro*. The metal ion hypothesis states that the irregular metal homeostasis with an A β imbalance is one of the causes of AD, because metal chelators can maintain the balance of metal ions [73,74]. Recent studies using lithium revealed that GSK3 β inhibitors can be neuroprotective in AD and several other diseases [75].

Manzamine A derivatives function as non-ATP competitive inhibitors, inhibiting GSK3 β and CDK5. Such derivatives have been obtained from the marine sponge *Acanthostrongylophora* [76–78]. Hymenialdisine, a potent ATP competitive GSK3 β inhibitor, is also obtained from a marine sponge and inhibits CDKs and casein kinase-1 [79].

Kenpaullone and alsterpaullone inhibited GSK3 β in concentrations with IC₅₀ values of 0.4 and 0.23 mM, such that they were selected as targets for mammalian GSK3. Kenpaullone has been established as a first-generation GSK3 β inhibitor. The common problem with these analog inhibitors is their lack of selectivity to GSK3 [80]. The non-ATP-competitive GSK3 β inhibitor tideglusib is currently in Phase IIa and Phase IIb clinical trials.

Methylene blue is a tau aggregation inhibitor. 6-bromoindirubin, another ATP-competitive inhibitor obtained from molluscan Tyrian purple, showed more selectivity towards GSK3 than towards any CDK [81]. Heteroannular compounds, such as heteroaryl-pyrazolo[3,4-b]pyridine, 3-(7-azaindoly)-4-arylmaleimides and 3-anilino-4-arylmaleimides, were established as second-generation GSK3 inhibitors [82–84]. Among known GSK3 β inhibitors, CHIR-98023 has been found to be the more selective [85]. Oxazolo[4,5-

b]pyridine-2-one-based 1,2,3-triazoles showed maximum inhibition of GSK3 β with a IC₅₀ value of 0.19 μ M (Table 4) [86].

Overall, GSK3 β inhibitors appear to have an important role in reducing A β -induced synaptic toxicity in AD neurons. However, recent clinical trials using GSK3 β inhibitors did not have positive results. Further research is needed to understand the physiological relevance of GSK3 β inhibitors and their protective and adverse effects in AD neurons.

CDK5 inhibitors and Alzheimer's disease

CDK5, a unique member of the CDKs, has been reported to be intimately associated with AD pathogenesis. CDK5 is important in the development of the central nervous system and neuronal movements, such as neuronal migration and differentiation, synaptic functions, and memory consolidation. However, when neurons are exposed to pathological stimuli, CDK5 becomes hyperactive and causes the aberrant hyperphosphorylation of various substrates of CDK5-like APP and tau NFTs, resulting in neurodegenerative diseases, such as AD (Figure 6).

In vitro, the full-length human tau aggregates slowly, but quick tau aggregation is carried out by the mutation of an N-terminally truncated tau (tau187) to form NFTs identical to those formed by full-length tau. CDK5 was recognized as a potential disease tau kinase because CDK5-phosphorylated tau was found at hyperphosphorylated sites in AD brains. CDK5 participation in tau phosphorylation was revealed by the increase in tau enzymatic activity in MT-2 cells in the presence of roscovitine or CDK5 small interfering (si)RNA [DE13][87]. Hence, CDK5 could be a key target candidate for the therapeutic silencing of the *APP* gene [27].

Another important kinase involved in the regulation of tau phosphorylation is p38. This kinase might have a role in tau hyperphosphorylation under pathological conditions [88]. CDK5 is activated by p35, which is associated with membranes via the myristoylation of the p35 N-terminal region. p35, a protein with a short-life span, is proteasomally degraded.

Whenever AD neurons are exposed to oxidative stress, A β plaques, or NFTs, calpain is activated and cleaves p35 into a p25 C-terminal fragment [89]. Given the longer half-life of p25, p35 dissociates from membranes and phosphorylates tau, among other proteins [90,91]. CDK5/p25 is responsible for the formation of A β peptide aggregations (i.e., the formation of extracellular plaques) and tau hyperphosphorylation (i.e., the formation of intracellular NFTs) [92].

Most of the drug molecules that target GSK3 β inhibitors, such as such as hyemialdisine [93], 6-bromoindirubin [94], and analogs of manzamine A, also reduce CDK5 levels [95]. M Paullones, a new class of benzazepines, has been reported to be an efficient ATP competitive inhibitor of CDKs, and purvalanol A, also a new class of benzazepines, has been found to selectively inhibit CDK2 and CDK5 kinases at IC₅₀ values of 4–70 nM and 75 nM, respectively [96]. Para-carboxylate of purvalanol B prevents an H-bond (known to exist in the active form of CDK2 and CDK5) between the side-chain amine of K89CDK2/5 in the C-

terminal kinase lobe and the backbone carbonyl oxygen of I10 CDK2/CDK5 in the N-terminal lobe, rendering kinases inactive (Table 5) [97].

Dysfunction in these pathways could result from A β oligomers that directly or indirectly affect mitochondrial fission proteins, such as Drp1, and mitochondrial matrix proteins, including ABAD and CypD, via interactions such as A β -Drp1, A β -ABAD, A β -CypD, and A β -VDAC. In these interactions, the A β complexes can lead to mitochondrial dysfunction, bioenergetic compromise, and consequent synaptic dysfunction and loss. No selective therapeutic targets have been identified to prevent these interactions. Further research is needed to understand abnormal interactions between A β and other mitochondrial and/or synaptic proteins, and phosphorylated tau and mitochondrial and/or synaptic proteins. Further research is also needed to understand the physiological relevance of these interactions in AD disease progression and pathogenesis.

Concluding remarks and future directions

A β -induced synaptic dysfunction is a complicated process involving multiple pathways, components, and biological events, such as oxidative stress, kinase activation, and protein interactions. These complexities lead to the formation and accumulation of A β and phosphorylated tau, mitochondrial dysfunction, and, ultimately, neuronal death in AD. Despite the many clinical trials conducted to identify drug targets that could reduce mutant protein toxicity in AD, such targets remain unidentified. Currently, multiple therapeutic strategies are being investigated using preclinical (animal models) and human AD clinical trials, including anti-inflammatory, antioxidant (mitochondria-targeted molecules), cholinergic, cell cycle, hormonal, and small-molecule inhibitors. These alternative approaches could provide new information on which to base the development of future therapeutic drugs to treat patients with AD. The development of drugs capable of targeting the underlying mechanisms of disease pathogenesis is needed to slow the progression of and, someday, eliminate, AD.

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Highlights

1. Multiple cellular changes have been identified as being involved in AD pathogenesis.
2. Studies concluded that abnormal APP processing causes A β -induced synaptic dysfunction.
3. A β induces tau phosphorylation and activates GSK-3 β and CDK-5, ultimately causes neuronal dysfunction.
4. A β modulators are potential therapeutic drugs to treat patients with Alzheimer's disease.

Figure 1.

[DE19]Proteolytic processing pathways of the amyloid precursor protein (APP): nonamyloidogenic and amyloidogenic. The nonamyloidogenic APP is cleaved by α -secretase, which removes an ectodomain fragment designated APPs α and a membrane-bound fragment, C83. γ -Secretase cleaves C83 to produce the nonamyloidogenic P3 and AICD peptides. The amyloidogenic pathway. The cleavage of APP by the β -secretase β APP-cleaving enzyme-1 (BACE1) removes the sAPP β ectodomain fragment, yielding the C99 transmembrane fragment. Further cleavage of the C99 C-terminal fragment via γ -Secretase produces amyloidogenic A β_{39-42} peptides and AICD as part of the amyloidogenic pathway. A β aggregates into small multimers known as oligomers

Figure 2.

Factors causing tau phosphorylation. Amyloid beta ($A\beta$) oligomers direct oxidative stress and increase levels of glycogen synthase kinase 3 β (GSK3 β). Subsequently, the interaction of increased GSK3 β and $A\beta$ causes tau hyperphosphorylation. $A\beta$ simultaneously interacts with P25/cyclin-dependent kinase-5 (CDK5), which also induces tau hyperphosphorylation. These events converge to result in tau protein aggregation and autophagic dysfunction, leading to neurodegeneration and cell death in Alzheimer's disease (AD).

Figure 3.

ADAM10 and α -secretase activity. ADAM10 increases α -secretase processing of amyloid precursor protein (APP), which in turn reduces amyloid beta ($A\beta$) while increasing the generation of $APP\alpha$. $APP\alpha$ is thought to have neuroprotective properties, including the inhibition of $A\beta$ production. γ -secretase cleaves C83 to produce the P3 and AICD peptides.

Figure 4.

β - and γ -secretase inhibitors. β APP-cleaving enzyme 1 (BACE1) is responsible for β -secretase activity and a complex of presenilin, nicastrin, Aph-1, Pen-2, and γ -secretase activity. A major research effort is aimed at reducing amyloid beta ($A\beta$) production with BACE1 inhibitors and γ -secretase inhibitors and modulators. Inhibition of BACE1 terminates the β -cleavage of amyloid precursor protein (APP), subsequently causing the α - and γ -cleavage of APP. By reducing presenilin, nicastrin, and Aph-1, Pen-2 activity prevents cleavage at the γ position of APP. Both mechanisms force the system towards the nonamyloidogenic pathway.

Figure 5.
[DE20]Glycogen synthase kinase 3 β (GSK3 β) and Alzheimer's disease (AD) pathology. Amyloid beta (A β) induces elevated GSK3 β activity, a key event in abnormal amyloid precursor protein (APP) processing, increased A β production, tau phosphorylation, and synaptic pathology in AD.

Figure 6.

[DE21]. Cyclin-dependent kinase-5 (CDK5), an inactive catalytic subunit. CDK5 is activated by the p35 CDK5 activator, which is concomitantly recruited to membranes because p35 associates with membranes via myristoylation of its N-terminal region. p35 is a protein with a short life span and is proteasomally degraded. When neurons are exposed to stress or encounter death signals, calpain is activated and cleaves p35 into a p25 C-terminal fragment. p25 has a longer half-life and is dissociated from membranes, where it phosphorylates additional proteins.

Table 1 **α -Secretase activators**

Drug	Study design	Gene/Protein	Result	Refs
Curcumin	<i>In vitro</i> and <i>in vivo</i> (mouse)	↑ ADAM10, BACE1/ α -secretase	↓ A β 40 and ↓ A β 42	[98,99]
Curcumin –Ile; Curcumin –Val; Curcumin –Phe	<i>In vitro</i>	↑ ADAM10	↓ A β 42	[28]
Ginkgo biloba	<i>In vivo</i> (mouse)	ADAM10	A β 25-35 and ↓ A β 42	[33,34]
Ginsenoside	<i>In vivo</i> (rat)	ADAM10, BACE1	↓ A β 42	[30]
Triptolide	<i>In vivo</i> (mouse)	ADAM10	↓ A β 40	[31,32]

Table 2**β-Secretase inhibitors**

Drug	Natural/Synthesized	Study design	Gene/Protein	Result	Status	Refs
In vitro screened drug molecules						
Amidine						[38]
Curcumin	Natural	<i>In vitro</i> and <i>in vivo</i>	BACE1/α-secretase	↓ Aβ40 and ↓ Aβ42		[98,99]
Tryptoline		<i>In vitro</i>	BACE1	↓ Aβ		[100]
Asiaticoside	Natural	<i>In vivo</i>	----	↓ Aβ42		[101]
Fluoro-oxazines	Synthesized	<i>In vivo</i>	BACE1	↓ Aβ40 and ↓ Aβ42		[40]
Clinical trials						
AZD3293	AstraZeneca	Phase II/III	BACE1	↓ Aβ40, ↓ Aβ42, and ↓ sAPPβ	On going	[102]
CTS-21166	CoMentis	Phase I	BACE1	↓ Aβ40 and ↓ Aβ42		
E2609	Eisai/Biogen Idec	Phase II/III	BACE1	↓ Aβ, ↓ sAPPβ	Ongoing	[103–105] https://clinicaltrials.gov/ct2/show/NCT01600 https://clinicaltrials.gov/ct2/show/NCT01511783
HPP854	High Point	Phase I	BACE1		Ongoing	https://clinicaltrials.gov/ct2/show/NCT01482013
LY2886721	Lilly	Phase II	BACE1	↓ Aβ, ↑ Aβ5-40, ↓ Aβ1-34, ↓ sAPPβ	Discontinue	d [106,107]
MK-8931	Merck	Phase II/III	BACE1	↓ Aβ1-40, ↓ Aβ1-42, ↓ sAPPβ	Ongoing	[108] https://clinicaltrials.gov/ct2/show/NCT01496170 ; https://www.clinicaltrials.gov/ct2/show/NCT01739348 ; https://clinicaltrials.gov/ct2/show/NCT01953601
PF-05297909	Pfizer	Phase I	BACE1	↓ Aβ, ↓ sAPPα		[109]
RG7129	Roche	Phase I	BACE1	↓ Aβ		[110]
TAK-070	Takeda	Phase I	BACE1	↓ Aβ		[111]
VTP-37948	Vitae/Boehringer Ingelheim	Phase I	BACE1	↓ Aβ		[112]

Table 1 **α -Secretase activators**

Drug	Clinical stage	Adverse effects	Aβ level	Refs
α -Ergocryptine		NA	↓ A β	[113]
2-Bromo- α -ergocryptine		NA	↓ A β	[113]
Avagacestat (BMS-708163)	Phase II	Diarrhea, nausea, vomiting, rash	↓ A β 1-38, ↓ A β 1-40, ↓ A β 1-42, ↑ A β 1-14/15/16, discontinued	[51,114] https://clinicaltrials.gov/ct2/show/NCT00890890
Begacestat	Phase I	NA	Terminated	[49]
CHF-5074	Phase II	NA	= A β 1-40, = A β 1-42, ↓ CD40; terminated	[56] https://clinicaltrials.gov/ct2/show/NCT01203384 ; https://clinicaltrials.gov/ct2/show/NCT01303744
DAPT			↓ A β 42	[115]
Dihydroergocristine		NA		[116]
ELND006	Phase I	Liver	↓ A β halted	[52,55]
EVP-0015962	NA	NA	↓ A β 42, ↓ A β 38	[117]
L-685,458	NA	NA	↓ A β	[46,48]
NIC5-15	Phase II	NA	Ongoing	https://clinicaltrials.gov/ct2/show/NCT00470418
PF-3084014	Phase I	NA	↓ A β	[50]
SCH 697466	NA	NA	↓ A β	[118]
Semagacestat (LY450139)	Phase III	Placebo in cognition and patients	↓ A β 1-38, ↓ A β 1-40, ↓ A β 1-42, ↑ A β 1-15/16; discontinued	[119–123]

Table 4**GSK3 β inhibitors**

Drug	Study design	Gene/Protein	Result	Refs
2-Chloroacetyl-4,5-dichlorothiophene	<i>In vitro</i>	GSK3	Inhibited	[124]
6-Bromoindirubin		GSK3	↓ GSK3	[94]
Alsterpaullone				[125]
CHIR-98023		GSK3	↓ GSK3	[85]
Hymenialdisine	<i>In vivo</i>	GSK3	↓ Tau-GSK3	[93]
Intranasal glulisine			Terminated	
Intranasal Humulin R			On going	
Kenpaullone				[125]
Lithium	<i>In vivo</i>	GSK3	GSK3-Li	[126,127]
LMTX (methylene blue)	Phase IIb	Tau aggregation inhibitor	Terminated	https://www.clinicaltrials.gov/ct2/show/NCT01689
Manzamine A	<i>In vivo</i>	GSK3	↓ GSK3	[128]
Oxazolo[4,5-b]pyridine-2-one-based 1,2,3-triazoles	Rat-paw edema model	GSK3	↓ GSK3-Tau	[86]
Thieno[2,3-b]pyridines	<i>In silico</i>	GSK3	On going	[129]
Tideglusib (NP031112)	Phase IIb	GSK3	↓ GSK3, terminated	https://clinicaltrials.gov/ct2/show/NCT01350362

Table 5**CDK5 Inhibitors**

Drug	Gene/Protein	Result
Hymenialdisine	GSK3	↓ Tau- GSK3 [93]
N ⁶ -Isopentenyladenine	CDK5	↓ CDK5 [130]
Olomoucine	CDK5	↓ CDK5 [131]
(R)-Roscovitine	CDK5	↓ CDK5
Purvalanol A	CDK5	↓ CDK5-Aβ [96]
Purvalanol B	CDK5	↓ CDK5-Aβ