Dileep Kumar · Vaishali M. Patil ·
Dee Wu · Nanasaheb Thorat *Editors*

Deciphering Drug Targets for Alzheimer's Disease

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Editors

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Preface

Alzheimer's disease (AD) is a gradual, neurodegenerative, and often fatal disorder. This disease is clinically associated with a gradual loss of memory and cognitive processes, eventually leading to difficulties performing primary activities. AD is one of the leading causes of deaths in developed countries and is projected to cost almost $1 trillion in 2050 unless a therapy to slow/prevent the condition is discovered. Accountable care organizations are in a solid position to devise utilization strategies that would positively impact early detection and treatment, resulting in enhanced outcomes and reduced prices for patients, caregivers, and healthcare. The prime intent of the book entitled, *Deciphering Drug Targets for Alzheimer's Disease*, is to present a comprehensive approach based on molecular, preclinical, clinical, and translational research while addressing the novel targets and recent advances. However, covering all the broad multidisciplinary research highlights in the area of Alzheimer's would be beyond the book's purview. Instead, the book would shed light on the novel mechanistic pathways, which would undoubtedly be thoroughly studied and established in the years to come. It is gratifying to receive 15 exceptional contributions authored by a team of prominent experts focusing on various therapeutic targets related to Alzheimer's skilfully compose the book. Each chapter attempts to address thought-provoking and challenging ideas that could interest those both in and out of the AD sector.

Currently, clinically authorized AD treatments involve strategies focusing on altering various reported targets like acetylcholine esterase (AChE), ß-site amyloid precursor protein cleaving enzyme 1 (BACE-1), glycogen synthase kinase 3ß (GSK-3ß), monoamine oxidases (MAOs), metal ions in the brain, *N*-methyl-D-aspartate (NMDA) receptor, 5-hydroxytryptamine (5-HT) and histamine (H3) receptors, and phosphodiesterases. Available therapeutics is helping to improve disease condition but the complicated pathogenesis and complex network formed by the associated signalling pathways restrict the development of complete cure.

In the first and second chapters, titled "Deciphering New Drug Targets in Alzheimer's Disease" and "Novel Therapeutic Targets for Treating Alzheimer's Disease", the authors provide comprehensive emphasis on the current and emerging

pharmacological targets including cellular, nuclear, membrane receptor, and mitochondrial targets. The latter also covers AD-associated features, including mitochondrial dysfunction, and neuroinflammation.

Reports based on preclinical and clinical developments have posed the requirement of sophisticated techniques to understand the mechanisms underlying Alzheimer's pathogenesis. From a clinical point of view, another challenge would be to distinguish and diagnose the type and velocity of AD, to devise the treatment regimen. Chapter "Modified Investigation Tools and Techniques Useful in Alzheimer's Disease Research", authored by Deore et al., explores in vitro, in vivo, and ex vivo methods and in vivo brain imaging techniques developed for identifying Alzheimer's biomarkers. The authors opine that these modalities would prove to be novel and reliable in diagnosing and demarcating different types of Alzheimer's.

With the advent of technology in the healthcare sector, several signalling pathways have been attributed to the progression and development of AD. Chapter "Dual Specificity Tyrosine Phosphorylation-Regulated Kinase 1A (DYRK1A) Inhibitors: The Quest for a Disease-Modifying Treatment for Alzheimer's Disease" covers dual specificity tyrosine phosphorylation-regulated kinase1A (DYRK1A), which is imperative in the trafficking of cellular proteins by its involvement in neurofibrillary tangles (NFTs) and Aβ-governed neurodegeneration. DYRK1A has an aberrant role towards the accumulation of *p*-tau protein and formation of neurotoxic amyloid plaques, making it a fascinating disease-modifying target. The next important pathogenic enzyme i.e., BACE-1, is a key contributor towards the progression of dementia and is an attractive target for rational drug design. Chapter "β-Secretase as a Primary Drug Target of Alzheimer Disease: Function, Structure, and Inhibition", presented by Verma and Paramanick, has provided the biological structure and functions of BACE1 based on recent advances. The critical pharmacophore features essential to design selective inhibitors have been discussed.

Neurohormones and endocrine receptors have been less explored for their potential in the development of Alzheimer's therapeutics. It has been discussed in the chapter "Endocrine Receptors: The Potential Therapeutic Targets for Alzheimer's" authored by Zafar et al. Biochemical pathways and endocrine modulation with an emphasis on the mechanism of oestrogen receptors along with their modulators have been discussed. Nonestradiol compounds can potentially disrupt hormonal balance causing developmental and reproductive abnormalities. They seem to have a beneficial influence on AD symptoms and neuropathologies, acting as antioxidants, improving visual-spatial memory, lowering amyloid-ß production, and improving the survival and plasticity of brain cells. Chapter "Endocrine Receptors: The Potential Therapeutic Targets for Alzheimer's" by Zafar et al., further emphasizes the linkage among dementia and various hormones, neurohormones, and endocrine receptors. Biochemical pathways and endocrine modulations emphasizing the role of oestrogen receptors in Alzheimer's treatment have been discussed. - Chapter "Calcium Channels as a Potential Therapeutic Target for Alzheimer's Disease" by Sharma et al. explores the disruption of calcium homeostasis with the

pathology of AD brain and molecular mechanism of calcium ion channel modulators.

Astrocytes play an essential role in regulating nutritional balance, cerebral blood flow, tissue repair, and permeability in the central nervous system causing inflammatory/immune responses. Based on the microenvironment and phase of dementia, evidence has suggested neuronal effects of astrocytes. Changes in their function have been documented in people with early-onset AD. Chapter "Traversing Through the Trajectory of Pathogenic Astrocytes in Alzheimer's Disease" by Shareena et al. covers the functions of pathogenic astrocytes, their molecular pathways, and related innovative treatments. Further, the role of mitochondrial dysfunction and epigenetic mechanisms in the regulation of gene expression during early stages of disease for the prevention and treatment of AD is described in chapters "Targeting Mitochondrial Dynamics as a Restorative Approach in the Treatment of Alzheimer's Disease" and "Epigenetic Therapy for Alzheimer's Disease", respectively.

Among the numerous targets assessed in the search for possible treatments, GSK-3ß is a widely acknowledged regulator of the AD pathophysiology and several associated critical targets involving neurodegeneration. It has gained consideration as a disease-modifying diagnosis of Alzheimer's, where its anomalous expression has indeed been implicated with over-expression of Aβ-peptide, further initiating a cascade sequence leading to hyperphosphorylation of tau proteins. Authors propose approaches towards understanding the molecular basis of such over-expressed regulators in chapter "Exploring the Diverse Roles of GSK-3β Kinase in Alzheimer's Disease". The chapter concentrate on examining the inter-linkage among numerous cellular activities involving GSK-3 and elucidating its prospective inhibitors in arresting AD progression.

In chapter "Role of Target Fishing in Discovery of Novel Anti-Alzheimer's Agents: In Silico Applications", authors Murmu et al. have highlighted the limitations of traditional 'one medicine, one target' approach to handle AD complexity. In drug discovery, target fishing plays a pivotal role and it can be explored by using computational approaches. For deriving rational results, ligand- and structure-based in silico approaches are reliable. In silico tools for the optimization of target based on literature reports are explored.

Limitations associated with single target and combination therapeutics demanded exploring multi-target directed ligands (MTDLs) to channelize the complex pathologies of AD. In chapter "Multi-target-Directed Ligand Approach in Anti-Alzheimer's Drug Discovery" by Patil et al., AChE, BACE-1, PDE, metal chelation, and other target-based MTDLs from synthetic and natural origin evaluated in recent years have been explored. Hybrid analogues of potent compounds like tacrine and donepezil with dual inhibitory activity and their structural characteristics towards the rational design of MTDLs have been investigated.

Chapter "Exploring the Role of Tau Proteins in Alzheimer's Disease from Typical Functioning MAPs to Aberrant Fibrillary Deposits in the Brain" by Shareena et al. intends to capture the role of tau proteins as well as numerous successful therapies targeting tau-associated posttranslational modifications (PTMs) like hyperphosphorylation, acetylation, and methylation, and thus on AD,

which might show to be a feasible method for future advances. Since considerable research has revealed that Aβ-targeting medications are hazardous and relatively less effective in mitigating AD pathology, tau-directed therapies are of considerable interest. Although existing tau-related medications give temporary symptom relief, they do not address the condition as a whole. Thus, recent research has concentrated on dissecting the processes and intricacies of tau protein in order to develop effective disease-modifying medications.

Designing novel compounds and treatment regimens necessitates AD research at its molecular level. Research groups have been keen on exploring new targets, discovering and developing new anti-AD therapeutics. In spite of the complex aetiology and pathogenesis of AD, researchers have made efforts to explore related mechanisms and have successfully placed numerous compounds for clinical evaluation. The Food and Drug Administration (FDA) has approved some anti-AD drugs including galantamine, donepezil, rivastigmine, memantine, and aducanumab for clinical applications. Chapter "The Overview of Drugs Used in Alzheimer's Disease and Their Molecular Targets" by Kumar et al. intends to investigate AD therapies, their molecular targets, and provide an overview surrounding various treatment approaches.

In summary, this book provides a multi-perspective of AD interpreting the potential targets outlined concisely in the form of interdisciplinary chapters. Furthermore, I want to acknowledge all authors for their valuable contributions and perseverance during the editorial process. The editors and publication teams would like to appreciate the researchers who collaborated with peer assessment, and editing. It is a great pleasure to thank Ms Humaira Hashmi, Editorial Manager of Publications, for her guidance.

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Deciphering New Drug Targets in Alzheimer's Disease

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Abstract Alzheimer's disease (AD) currently has no known treatments that can reverse the disease or cure it. Numerous pharmacological targets have already been identified for potential involvement to enhance efforts to find treatments for AD. There are roughly three basic therapeutic approaches for treating AD. The first strategy focuses on preventing the production and aggregation of Aβ oligomers as well as hyperphosphorylated tau tangles, which are the two main pathological characteristics of AD, and either causes their clearance. The second tactic controls neurotransmitter signalling. The clinically authorized therapies for the symptomatic relief of AD, including *N*-methyl-D-aspartate receptor inhibitors and cholinesterase inhibitors, make up this method. There are also mentioned other targets that try to stabilize cell signalling by altering neurotransmitters and their receptors. The third strategy includes a number of "sensitive targets" that indirectly affect the build-up of Aβ as well as tau. These targets are proteins whose activity is modified as a consequence of an accumulation in the brain cells or a direct Aβ-target interaction. This book chapter focuses on highlighting existing and novel emerging drug targets for AD.

Keywords Alzheimer's disease · Aβ oligomers · Therapeutics · Drug targets

1 Introduction

According to statistical predictions, 14 million Americans will be affected by Alzheimer's disease (AD) by 2050 (Hebert et al. 2013). The estimated cost of treating AD patients will rise to $1.1 trillion due to this severity of the condition.

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Drug development research in AD has shown to be incredibly difficult, and a treatment has not yet been found. However, the clinic has been able to modify the standards for dividing AD patients into three main stages as a result of expanding study findings (Lancet 2011). Neuropsychological evaluation or a diagnosis of exclusion was used to determine these criteria (Chapman et al. 2010), but they were unable to identify AD in its presymptomatic stage. It is thought that this later stage is when AD develops and starts the neurodegenerative process that results in inflammatory response and nerve degeneration and is seen in the final phases of dementia and MCI (Minter et al. 2016). In order to understand how the infectious proteins, A or tau, contribute to the progression of the disease, the new criteria for detecting AD rely on biochemical markers that estimate the aggregation of these proteins. Clinical studies employ the same indicators to assess the effectiveness of pharmaceutical therapies (Lancet 2017). The estimation of Aβ concentrations in cerebrospinal fluid (CSF), or the estimation of tau protein concentrations in CSF, is an example of the biochemical marker that is currently accessible (Olsson et al. 2016).

2 Amyloid and TAU Formation

The formation of insoluble oligomers of the Aβ protein, also called Aβ plaques, and intracellular generation of the microtubule-connected tau protein, commonly known as neurofibrillary tangles, are the two main pathogenic features of AD. The aspartate proteases β- and γ-secretase break down the transmembrane protein amyloid-protein precursor (APP) to yield the Aβ peptide (1-42) and a degraded C-terminus, which is where the production of Aβ begins (Frisoni et al. 2017). The amyloid then forms oligomers, dimers, and tetramers (Mantzavinos and Alexiou 2017). Synaptic dysfunction is caused by the extracellular deposition of Aβ plaques, which obstructs neurotransmitter communication between neurons (Kang et al. 1987). The neuroprotective fragment known as soluble Aβ-PP (sAβ-PP) is produced when α-secretase cleaves Aβ-PP (Zhao et al. 2012). Aβ peptide (1-40) is created when sAβPP is further broken down by γ-secretase in the plasma membrane chambers before being released into the outer space (Sengupta et al. 2016; Mucke and Selkoe 2012). This peptide has a pathogenic role in modifying synaptic function and mending blood-brain barrier (BBB) leaks (Gralle et al. 2009; Chow et al. 2010). However, it is yet under investigation what this pathogenic protein does. The tau protein that is hyperphosphorylated and generates the neurofibrillary tangles is also neurotoxic in addition to Aβ (Parihar and Brewer 2010; Hanseeuw et al. 2019). The integrity of axons and overall neuronal function is supported by the physiological stabilizing effect of tau protein on neuronal microtubules (Trippier et al. 2013). When potential kinases enzyme such as glycogen synthase kinase-3 beta (GSK3β) and cyclin-dependent kinase 5 (CDK5) are active at higher levels or malfunction, tau is hyperphosphorylated, causing it to separate from microtubules and accumulate into tangles (Trippier et al. 2013). Tau tangle accumulation in patients is shown to

coincide with cognitive loss in the initial stages of AD. Tau is hyperphosphorylated in AD, which causes it to become detached from microtubules and accumulate in tangles. Increased activity of possible kinases, such as glycogen synthase kinase-3 beta (GSK3β) and cyclin-dependent kinase 5 (CDK5), or their malfunction, is what leads to this dissociation from microtubules (Trippier et al. 2013). Cognitive deterioration happens in the primary phases of AD; simultaneously patient's tau tangles start developing.

3 Approaches Targeting Pathogenic Proteins

3.1 Suppression of Aβ Protein Aggregation

The susceptibility to form oligomeric aggregates is caused by the fact that neurons constantly produce little amounts of Aβ in a number of forms (Abushakra et al. 2017; Sharma et al. 2020). An upsurge in Aβ42 generation with an enhancement in β or γ secretase activity or by a reduction in Aβ deteriorating enzymes like as+ prolyl endopeptidase (Prep) and insulin-degrading enzyme (IDE) degradation can cause this rise in Aβ42 production. The synaptic dysfunction brought on by this oligomeric state leads to neurodegeneration as observed in AD. Several small compounds that can impede accumulation have made it into clinical research because of insufficient comprehension of the process of oligomer development (Abushakra et al. 2017). Lead compounds that obstruct the development of aggregates include 2-hydroxy-3-ethoxybenzoaldehyde, 2-amino-4-chlorophenol, 4-aminophenol, 4-aminoanisole, 3,4-dihydroxybenzoic acid, and more (McLaurin et al. 2006). Others like the tiny chemical tramiprosate—function by attaching to monomers of Aβ and keeping them in a non-fibrillar state—advanced into two sizable Phase III studies, but failed to show effectiveness in enhancing cognitive ability (Lacombe et al. 2007). In one of the latter clinical trials, additional data analytics demonstrated that Tramiprosate had a positive effect on stabilizing cognitive ability over the course of definite time period of analysis in individuals with slight cognitive disability and homozygous form of Apolipoprotein E (ApoE4) mutation, which is a health risk gene for delayed occurrence of Alzheimer's disease (Aisen et al. 2011; Abushakra et al. 2017). Despite that, the medication had undesirable adverse effects, which led to the creation of the L-Valyl Ester Prodrug ALZ-801 (Hey et al. 2018; Sharma et al. 2021). Tramiprosate pharmacokinetic characteristics were enhanced by the prodrug, and a phase I safety investigation showed that it was more acceptable. Naturally existing polyphenols such as luteolin and transilitin have been found to be powerful suppressors of the formation of Aβ fibrils and to impede Aβ accumulation and cognitive decline (Abushakra et al. 2017). Furthermore, the configuration of the soluble oligomer of Aβ is reversed and prevented from accumulating by small compounds such resveratrol, coumarin, and D737 (Churches et al. 2014; Fu et al. 2014). Several investigations have revealed that the oligomeric form of Aβ42 is the hazardous type. It is still up for dispute, however, regarding whether or not blocking

agglomerate formation is a viable therapeutic target. For example, the endogenous inositol stereoisomer Elnd005 (McKoy et al. 2012) prohibits the accumulation of the Aβ42 peptide by attaching to it. The tiny molecule demonstrated its ability to prevent Aβ42 accumulation and the production of oligomers in in vitro tests (McLaurin et al. 2000). Elnd005 is a promising option for a clinical study because it ameliorated learning impairments and retained synapse density in transgenic mice (Salloway et al. 2011). However, the drug was unsuccessful in achieving the anticipated outcomes in clinical trials, failing to outperform a placebo in terms of behavioural changes or cognitive functioning.

3.2 Immunization Against Aβ Using Active Immunization

Active immunization or immunotherapy that can induce the host immune reaction against eliminating Aβ plaques in the nerve cells has currently got a lot of attention. In the past generations, investigations have demonstrated that live attenuated vaccines not only hinder the development of new Aβ deposits, but also assist in the eradication of subsisting deposits (Mosher and Wyss-Coray 2014). This adaptive immune reaction is proficient in the nerve cells by microglial stimulation (Mosher and Wyss-Coray 2014). One could contend that an ageing immune system response in old persons does not operate consistently and that it can cause autoimmune adverse effects rather than the necessary antibodies (Wiessner et al. 2011). Furthermore, the role of microglia in the human nervous system is not fully implicit. This hypothesis elicits the strategy of active immunization extremely difficult. Regardless of problems, various researches in quest of active immunization have been accepted. One of these is CAD106, an innovative and novel immunotherapeutic agent developed to encourage the production of antibodies towards a brief Aβ peptide portion and prevent ensuing Aβ aggregation (Vandenberghe et al. 2017). The immunological response is then induced by coupling the peptide to a carrier that has 180 imitations of the encapsulated peptide of bacteriophage Q. CAD106 demonstrated the elimination of Aβ plaques in APP transgenic mice. Additionally, CAD106 is presently being tested in clinical studies to determine its effectiveness in Alzheimer's patients who are at threat of developing clinical signs in an effort to stop the illness before it advances (NCT02565511). The progression of further active vaccines includes MER5101, which showed promise in pre-clinical investigations (Liu et al. 2013), and Lu AF20513, that is presently undergoing pre-clinical studies (NCT02388152) (Davtyan et al. 2013) (Fig. 1).

3.3 Immunotherapy Through Passive Immunization

Passive vaccination is the strategy that has been the subject of the most researches in Alzheimer's phase III clinical trials. Bapineuzumab is a humanized anti-Aβ

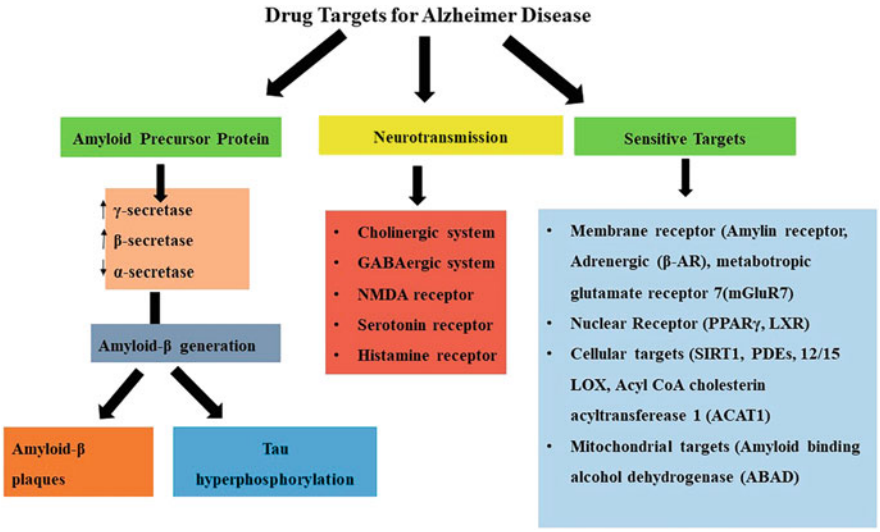

Fig. 1 Overview of therapeutic targets of Alzheimer's disease

monoclonal antibody against the A N-terminus, (Liu et al. 2016), which was first taken into consideration. However, ApoE4 messengers did not demonstrate therapy differences from the placebo group in phase II studies; non-carriers show a substantial enhancement in cognitive and functional capacities. Phase III of the antibody's development was completed without any positive results (Salloway et al. 2014). However, various passive immunization strategies are now being researched. A clinical experiment (NCT01300728) is now being conducted to determine the effects of intravenous infusion of immunoglobulin formulations (IVIg) having large concentrations of human anti-Aβ42 antibodies on cognitive ability (Relkin et al. 2017). Numerous initiatives are being made to locate monoclonal antibodies that can attach to the Aβ protein or its deposits. Sixteen of the 23 trials that were concluded did not identify a therapeutic advantage, while 7 more are still under investigation (Relkin et al. 2017). The antibodies work as an expected and hindered Aβ aggregation in the brain cells. However, no cognitive beneficial effect to individuals was established.

3.4 Aβ Deteriorating Enzymes

A new emergent therapeutic target for AD are proteases because of the fact that they can degrade Aβ. Neprilysin (NEP) is one such protease, the enhanced expression of which in transgenic mice revealed a reduction in generation of plaque but has no cognitive progression as it did not activate the oligomeric configuration (Meilandt et al. 2009). However, the overexpression of NEP can result in deterioration of additional products of NEP that may result in other adverse effects (Nalivaeva et al.

2012). A new Aβ deteriorating enzyme, IDE, particularly activates the β-integrated proteins. The expression of the protease is diminished in the primary phases of AD (Carrasquillo et al. 2010). However, enhanced expression of IDE has been revealed to diminish serum concentrations of Aβ and reduce the advancement rate to delayed beginning of AD (Kulas et al. 2019). In vivo *research* investigations demonstrated the uprise modification of AβPP to IDE; $A\beta PP^{-/-}$ mice show elevated protein, mRNA, and function of IDE in comparison with other controls (Tang 2016). Promoting IDE function with resveratrol has been revealed to increase enzyme function against deteriorating $A\beta_{42}$ parts (Krasinski et al. 2018). These research investigations show that the allosteric modification emerging of the enzyme is a viable emergent target for AD.

3.5 Immunization Against Tau

As compared to Aβ immunization, an activated antibody that can pass the blood-brain barrier or concentrate in nerve cells would then attach to tau accumulates, stimulating an antibody reaction particularly towards phosphorylated tau protein. The concept of tau immunization has gained attention in recent years, and in vivo research study shows that tau pathology can be treated by administering tau oligomer monoclonal antibodies into transgenic mice that show mutant human tau. However, neither neurofibrillary tangles nor hyperphosphorylated tau had any consequence. Anti-tau oligomer passive immunization hindered tau toxic effects and cognitive disability in tau transgenic mice (Janus et al. 2000). Active immunization against pathology of tau purports to induce the development of antibodies that activates tau neurofibrillary tangles, hinder tau accumulation, or support its elimination. In pre-clinical trial study, AADvac1-attenuated live vaccine strain revealed tolerability and efficacy in a phase 1 trial and activated the development of antibody (Brody and Holtzman 2008). Additionally, phase 2 clinical studies are recently going on in patients with less severe or slight Alzheimer's (NCT03174886).

3.6 Activating Tau Phosphorylation

Increased concentration of kinases causes tau to be hyperphosphorylated and microtubule instability. As a consequence, hindering such kinases has become a novel pharmacological method for managing AD. One of the most researched kinases that is engaged in the phosphorylation of tau is GSK3β. It is now well known that tau's Ser/Thr moieties are phosphorylated by GSK3β, resulting tau to become hyperphosphorylated and accumulate, which in turn results in tau tangle generation (Hooper et al. 2008). But no therapeutic beneficial effect was found in AD patients in a phase 3 clinical trial with the GSK3β suppressor tideglusib (Maqbool et al. 2016; Lovestone et al. 2015). Other kinase enzymes that are widely recognized to be

engaged in tau phosphorylation are P25, P35, and CDK5; neuron-specific receptors increase its function. The calcium-needed calpain protease results in the advancement of P35 and P25. This signalling results in the phosphorolysis of tau by CDK5. Tamoxifen has been recognized to maintain the activity of kinase by suppressing its stimulation by p25 pathway (Corbel et al. 2015). Another crucial possibility is that tau phosphorylation and accumulation in AD are not well understood. It has been presumed that tau phosphorylation is the main driven force for tau accumulation; moreover, it still has not been established. However, crucial kinases can change AβPP manufacturing and Aβ accumulation along with assisting the progression of neurofibrillary tangles (NFTs), which elicits significant kinase function to neuroprotective effect in AD. Moreover, comprehensive knowledge of tau phosphorylation and its significance to Alzheimer's is necessitated.

4 Approaches for Stabilizing Neuron Transmission

4.1 Cholinergic System

The increased activity of Acetylcholinesterase enzyme (AChE) and Butylcholinestearse enzyme (BuChE) results in decreased level of acetylcholine (ACh) as well as degeneration of cholinergic neurons in basal forebrain and cerebral cortex of AD patients, resulting in cholinergic deficit which is considered to be a critical pathological change that significantly contributed to cognitive decline and memory loss in AD (Mufson et al. 2008). Four out of five USFDA-approved AD drugs are acetylcholinesterase inhibitors such as tacrine (Sameem et al. 2017), donepezil (Rogers et al. 1998), rivastigmine (Rosler et al. 1999), and galantamine (Tariot et al. 2000). Therefore, cholinergic system or neuron continues to be activated for management. Thus, cholinergic system is a promising target for the development of novel therapeutic drug in the AD treatment.

4.2 GABAergic System

Management of the γ-aminobutyric acid (GABA) neurotransmitter system as a therapeutic target has received attention as a result of recent discoveries about the neurotransmitter system and its remodeling in Alzheimer's disease (Govindpani et al. 2017). In learning and memory, GABA plays a crucial role in controlling the excitatory/inhibitory balance between neurons. When activated by the neurotransmitter GABA, the corresponding receptor causes hyperpolarization and a shift in membrane potential, which in turn causes the regression of excitatory response (Mann et al. 2009). Learning and memory events are coupled with the equilibrium between both excitatory and inhibitory states in neurons, which is further mediated by GABA transmission. Decreased cognitive ability in mice has been linked to

Aβ-induced disruption of the system (Froemke 2015). Triggering the release of neurotransmitter at presynaptic cleft results in disturbed neuronal excitement because Aβ promotes synaptic failure by depleting synaptic vesicles and increasing intracellular ionic concentration of calcium ions. Patients with mild cognitive impairment are enrolled in phase II clinical trial of levetiracetam (NCT03461861, NCT03489044), a drug that works by balancing the excitatory and inhibitory states of neurons. Although patients with MCI have substantially different hippocampus pathology than those with AD, the findings of the trial may be relevant to the research study and advancement of remedies for AD. By controlling the GABAergic system, perhaps neuron signaling can be stabilized. However, it has been established that there are shifts in GABA receptor subunit expression in AD (Marcade et al. 2008). Based on the current level of knowledge on the regulation of GABA receptors in the AD brain, attention for research should be directed against learning more about the GABA processes that govern the transformation of nerve cells and contribute to cognitive disability as a whole. After a promising phase II clinical trial, etazolate, a modulator of GABA receptors, was discontinued. Since GABA receptor modulators might cause off-target symptoms including seizures and anxiety, more exploration is required to establish the efficacy of drugs in AD and whether or not they can be safely used for long-term treatment (Govindpani et al. 2017).

4.3 N-*Methyl* D-*Aspartate Receptor*

Glutamate excitotoxicity via *N*-methyl-D-aspartate receptor (NMDAR) is a fundamental concept in AD pathology. Persuasive manifestations implicate that it is essential for synapsis plasticity and viability of nerve cells is supposed to underlie learning and memory in AD (Gu et al. 2014). However, if NMDA receptor stimulation leads to overproduction of Aβ plaques and oligomers attachment, still more research is required to understand the association between NMDA receptor activation and AD pathology (Wang and Reddy 2017). Glutamic acid (glutamate) is a non-essential amino acid and one of the most readily available endogenous neurotransmitters of excitatory nature in the mammalian central nervous system required to maintain normal neurophysiology including a spectrum of cognitive capabilities, long-term potentiation, and involvement in synaptic plasticity, believed to be a cellular pathway of cognition and memory. However, over-activation of *N*-methyl-D-aspartate (NMDA) type of glutamate receptors, or decreased reuptake of glutamate by microglial cells results in an elevated level of glutamate due to its excessive release in the synapse causing glutamate-mediated excitotoxicity, which contributes to impaired neuronal signaling followed by cell damage and killing of cells underlying the powerful process of nerve degeneration in AD (Wang and Reddy 2017). Memantine is a non-competitive NMDA antagonist that reduces intracellular calcium levels through calcium channels blockage and entrapping it in its open configuration (Parsons et al. 2007).

4.4 Serotonin Receptors

Aiming serotonin (5-HT) receptors has received increasing consideration recently as growing number of pre-clinical studies supported that antagonism of 5-HT receptor showed not only increased cholinergic neurotransmission, promotion of neurogenesis, and neuronal plasticity, but also helped in reducing the burden of Aβ plaques in AD animal brains (Cochet et al. 2013). These results were further confirmed by significant reduction in Aβ level in CSF of transgenic mice upon treatment with selective serotonin reuptake inhibitor (SSRI) citalopram (5 mg/kg) and Fluoxetine (10 mg/kg) (Upton et al. 2008). Furthermore, perturbed cholinergic-serotonergic function seems to be connected to the cognitive dysfunction, memory, and learning impairment in AD patients, even though deposition of Aβ plaques has been proven to alter neuron development and monoaminergic neurogenesis (Shahidi et al. 2018). Paroxetine, an SSRI, was not found to have a positive effect on Aβ pathology or tau hyperphosphorylation when used chronically in Alzheimer's disease. Depending on their structure and function, serotonin receptors are divided into seven distinct categories. Only two of them, 5-HT4R and 5-HT6R, have been identified as a potential therapeutic target for AD. Agonism at the 5-HT4R receptor triggers the AβPP non-amyloidogenic pathway (Shahidi et al. 2018). In contrast, it has been found that blocking the 5-HT6R receptor improves cognitive abilities in AD. After a phase III clinical trial of the selective 5-HT6R antagonist idalopirdine failed to show a positive impact in individuals with Alzheimer's disease, the drug was taken off the market (Shahidi et al. 2018). Recent research indicates that activating the 5-HT7R can restore Aβ-impeded neuronal and synaptic plasticity. The reduction in Aβ plaque accumulation and neurodegeneration that resulted from its agonistic activity was also associated with improved cognitive and memory functions. The role of serotonin receptors in Alzheimer's disease is currently being studied, and further study is needed to identify whether or not they are directly related to the clinical characteristics seen in AD.

4.5 Histamine Receptors

Modulation of histaminic receptors, and in particular the H3 receptor, is developing as a viable emergent therapeutic target for the management of multiple central nervous system illnesses, including Alzheimer's disease (Govindpani et al. 2017). Blockage of the H_3 receptor by using selective antagonist can increase the release of various neurotransmitters that are involved in cognitive process such as acetylcholine, dopamine, GABA, noradrenaline, and 5-HT from non-histaminergic neurons (Mann et al. 2009). Antagonism of the H3 receptor has been exhibiting increased alertness and improved cognitive performance, suggesting its potential as a therapeutic intervention strategy for the treatment of Alzheimer's disease (Nathan et al. 2013). GSK239512, an extremely selective H3 receptor antagonist, has recently

moved into human testing. In phase II, however, patients having mild to moderate AD demonstrated improvement in episodic memory, but no change in executive skills or working memory. Although early research indicated a good impact on focus and memory, the medicine was ultimately pulled from the market by GlaxoSmithKline due to sedative side effects. The cognitive benefits of other H3 receptor antagonists, such as ABT-288, have not been demonstrated in clinical trials (Nathan et al. 2013). However, it is still unclear whether or not a specific neurotransmitter with its receptor could be a viable emerging target for AD (Fig. 1).

5 Approaches for Identifying Sensitive Targets in AD

5.1 Membrane Receptor Targets

When Aβ oligomers interact with membrane receptors, they can cause either direct disruption of receptor function or set off a cascade of toxic events (Costantini et al. 2005). This overview focuses on three recently discovered membrane receptors. The amylin receptor is the first target for research because the mechanism by which it is influenced is not yet known. It is still unclear whether Aβ-mediated toxicity is driven by Aβ binding to its receptor or amylin-caused activation of the receptor is the primary mechanism. Second, people with Alzheimer's disease have been found to have higher levels of p75 neurotrophin receptor (p75NTR) (Perini et al. 2002; Tsukamoto et al. 2003). The precise process by which Aβ binding to p75NT would result in necrobiosis is yet being studied. Nevertheless, the c-Jun N-terminal kinase (JNK) pathway has been proposed as a possible mechanism, as it translocates NF-kappa B and activates p53. Caspases-8,9 and 3/7 are activated as a result of this translocation, ultimately leading to apoptosis (Gu et al. 2014). Activation of p21-activated kinase also inhibits metabotropic glutamate receptor 7 (mGluR7) modulation of NMDARs, which disturbs Ca^{2+} hemostasis (Nguyen et al. 2014). The p75NT ligand LM11A-31 was demonstrated to reduce phosphorylation of tau and misfolding in a transgenic mouse model of Alzheimer's disease, thereby protecting against cognitive impairments and neurite degradation. Recent evidence suggests that Aβ binding to p75NT mediates activated microglia secretion of proinflammatory factors, TNF-α and IL-1β (Zhou et al. 2013). These results provide substantial evidence that A-p75NT interaction causes apoptosis and tau pathology. The third receptor of importance is the β2-adrenergic receptor (β2-AR), which belongs to the category of G-protein-coupled receptors (GPCR) involved in the pathological control of cognition, memory, and synaptic plasticity (Ji et al. 2003). Therefore, more research is required to determine whether or not β2-AR is involved in Aβ-induced neurotoxicity.

5.2 Nuclear Targets

Further evidence for the fascination of various nuclear targets is the control of various genetic expressions that trigger a neuroprotective as well as anti-inflammatory reaction. This chapter discusses peroxisome proliferator-activated receptor gamma (PPARγ) and liver X targets (LXR), two intriguing nuclear targets. The transcription of various genes, comprising apolipoprotein E4, is controlled by these nuclear genes (Jiang et al. 2012). There is some evidence that PPARγ contributes to improved cognition and memory performance via a biochemical mechanism. However, PPARγ agonists have a number of drawbacks, including the fact that they cannot cross the BBB (Kirchgessner et al. 2016) and that they often cause unpleasant side effects in humans. Therefore, more research is needed to determine the molecular mechanism by which PPARγ receptors are modulated, and perhaps to identify a particular target that is controlled by PPARγ. Agonists that target LXRs and can preferentially attach with the brain nuclear receptor have been studied, but their role in in vivo models of AD is not yet evident. BMS-852927 is a new LXR-selective drug that was reported to increase plasma LDL-cholesterol and diminish neutrophils in blood circulation in a study for atherosclerosis conducted during a phase I clinical trial (Fessler 2018). These unwanted effects were not anticipated by models based on nonhuman primates, and it is still unclear whether they were off-target impacts or were mediated by LXR.

5.3 Cellular Targets

Toxic effects of Aβ oligomers can also be initiated by their interaction with intracellular targets, and this has been argued by some researchers as the more likely scenario, given the widespread belief that Aβ oligomers are generated within cells (Sebastian et al. 2012). Sirtuin histone deacetylase 1 (SIRT1) is a good example; it has been associated with nerve plasticity and cognitive activity, overexpressed in neurons, and acts as a neuroprotective that is linked with cognitive impairment (Michan et al. 2010). Neurons vulnerable to age-related illnesses have altered SIRT1 pathway activity, and this has implications for synaptic plasticity (Wang et al. 2010). SIRT1 has been connected to the Rho-dependent kinase 1 (ROCK1) mechanism, which enhances APP manufacturing via α-secretase and thereby regulates Aβ synthesis (Qin et al. 2006). Cilostazol-induced in vitro activation of SIRT1 suppressed Aβ-induced tauopathy (Lee et al. 2014). SIRT1 activation, along with several other apparent processes outlined in the cited chapter, has brought it to the forefront as a probable new treatment target for AD (Wong and Tang 2016). The second utmost target to produce 12- and 15-hydroxyeicosatetraenoic acid (12-HETE and 15-HETE), as well as 13-hydroxyoctadecadienoic acid (13-HODE), is 12/15Lipoxygenase (12/15-LO) which oxidizes arachidonate substrates. By performing this function, the enzyme is able to modulate anti-inflammatory effects

during the adaptive and innate immune responses (Uderhardt and Kronke 2012). Aβ levels are reduced, tau neuropathology is attenuated, synaptic integrity is enhanced, and autophagy is triggered by PD146176, an extremely specific and precise pharmacological inhibitor of 12/15-LO (Di Meco et al. 2017). Moreover, many other studies indicated direct function for 12/15-LO in AD pathogenesis, notably in maintaining neuronal synaptic integrity, making it a promising therapeutic target. The second messengers mainly cyclic guanosine monophosphate (cGMP) as well as cyclic adenosine monophosphate (cAMP), are degraded by enzymes like phosphodiesterases (PDEs), which are the ultimate target. Therefore, they regulate the absolute and temporal levels of cyclic nucleotides, thereby governing the signaling of second messengers within cells (Bender and Beavo 2006). PDEs are involved in a number of neurodegenerative illnesses (Maurice et al. 2014), and their cellular location makes them important controllers of cell activity. This includes results on target transport and nerve transmission. According to a review conducted on clinical trials with PDE inhibitors, the results of the initial trials including PDE3, PDE4, and PDE9 inhibitors in AD patients were unsatisfactory. Moreover, inhibitory small compounds produced to date have been poorly selective. In addition, it is not yet clear which variant kinds of PDE will prove to be effective therapeutic candidates in Alzheimer's disease (Prickaerts et al. 2017). One of the four cellular targets, i.e. acyl-CoA cholesterin acyltransferase 1 (ACAT1), transfers the fatty acyl group of fatty acyl-CoA to the 3-hydroxy moiety of cholesterol, creating esters of cholesteryl (Maurice et al. 2014). Inhibiting ACAT has been shown to be beneficial in numerous animal studies. However, testing on ApoE mutant mice, in which cholesterol transport of cholesterol is diminished, will be necessary to substantiate the target.

5.4 Mitochondrial Targets

Targeting mitochondria is warranted, as recent studies have demonstrated that mitochondrial dysfunction starts early in Alzheimer's disease (AD) (Du et al. 2012). Yan et al. and its co-workers reported in a yeast two-hybrid model for Aβ binding substances (Du et al. 2012), and amyloid-binding alcohol dehydrogenase (ABAD) has been the most extensively investigated Aβ binding protein. An oxidoreductase enzyme found in mitochondria, ABAD converts a variety of substrates containing alcohol or carbonyl functionalities into a form that neurons can use for energy (Yan et al. 2000). Importantly, ABAD was found to be localized in the same brain regions as those initially afflicted by AD. A beta-amyloid decapeptidase (ABAD) was observed to co-localize with Aβ aggregates in immunofluorescence pictures. Increased enzyme levels have been observed in neurons from AD patients and experimental animals of the disease (Morsy and Trippier 2019). Compared to estradiol, a weaker oestrogen hormone, estrone, is an inferior indicator of brain function (Morsy and Trippier 2019). ABAD controls the conversion of estradiol to estrone. According to reports, when Aβ binds to ABAD, the enzyme undergoes a conformational shift that increases its activity in degrading estradiol. Estradiol

inhibits tau hyperphosphorylation by preventing GSK3 from being phosphorylated (pY194). Estradiol also reduces Aβ synthesis by inhibiting BACE processing of AβPP (Morsy and Trippier 2019). With the new inhibitor AG18051, estradiol levels were restored in SH-SY5Y cells after Aβ damage when the enzyme activity of ABAD was inhibited in vitro (Morsy and Trippier 2019). Cell death and ROS production were both increased in cultured neurons from mAβPP/ABAD double transgenic mice compared to control neurons, and this difference was accompanied by a greater degree of DNA breakage (Morsy and Trippier 2019). The overexpression of ABAD in AD has also been connected to two other proteins. After Aβ binds to ABAD, an antioxidant called peroxiredoxin-2 (Prdx-2) is upregulated. The latter is related to the higher Prdx-2 expression observed in AD patients and AD transgenic mice. However, when CDK-5 concentrations are high in the cytoplasm, Prdx-2 is phosphorylated and gets inactivated. Endophilin-1 (Ep-1) is the second protein, and it plays a crucial role in synaptic function by controlling endocytosis of synaptic vesicles, mitochondrial activity, and receptor trafficking. Ep-1 expression increases in response to Aβ-ABAD binding (Ramjaun et al. 2001). Ep-1 overexpression in presynaptic neurons has been found to increase synaptic glutamate release, disrupting normal neurotransmitter signaling and neuronal connectivity (Yarza et al. 2015). Further, Ep-1 causes neurotoxicity in AD by activating the stress kinase JNK 9. Taken together, these results identify ABAD as a hitherto unrecognized target of Aβ-induced toxicity (Reddy et al. 2005). The mitochondrial enzyme ABAD controls synapsis by changing Ep-1 expression, and numerous investigations have established a connection between mitochondrial oxidative stress and synaptic dysfunction. As a result, our research provides evidence that ABAD is involved in the onset of mitochondrial and synaptic dysfunction in AD (Du et al. 2012) (Fig. 1).

6 Conclusion

Drug development for Alzheimer's disease has reached a pivotal point, necessitating both in-depth analyses of existing research and novel approaches to the problem. Considering that advancing age is a significant factor in the progression of Alzheimer's disease, a multi-target approach towards the research and development of effective prophylactic treatments for the disease, which involve a variety of powerful mechanisms and pathways, is necessary. The outcomes may not be achieved by focusing on just tau instead of amyloid β, or any other single target. Medication repurposing and combination drug therapy research for AD in recent years may fare better if they use genetic and omic approaches with big data analysis from electronic health records.

Adopting a multi-target approach for the development of AD medicines is difficult to accomplish. When compared to single-target clinical studies, trials for multi-target drug candidates and combination therapies are more complex, expensive, and design-dependent, and they also produce data that are more difficult to

explain. Finally, the FDA may reject drug approval applications that do not disclose a specific single target or unambiguous mechanism. A multi-target strategy for the development of AD medications may be the best solution given the urgent need to treat such a devastating disease.

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Novel Therapeutic Targets for Treating Alzheimer's Disease

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Abstract Alzheimer's disease is a progressive neurodegenerative disorder which is characterized by Amyloid β (Aβ) plaques and neurofibrillary tangles (NFTs). These characterized features cause mitochondrial dysfunction, oxidative stress, synaptic dysfunction, cognitive deficits, neuroinflammation, and ultimately lead to neurodegeneration. Although the current AD treatments are successful, they are limited due to their only symptomatic treatment. In the past few decades, much research has been focussing on targeting Aβ and NFTs which are hypothesized to prevent neurodegeneration. These strategies failed clinically, thus shifting the focus onto newer targets. In the present book chapter, we will emphasis on the current therapeutic targets, focussing on mitochondrial dysfunction, synaptic dysfunction, and neuroinflammation.

Keywords Alzheimer's disease · Neurofibrillary tangles · Amyloid β (Aβ) plaques · Mitochondrial dysfunction · Neurodegeneration

Abbreviations

Ach	Acetylcholine
AD	Alzheimer's disease
APP	Amyloid precursor protein

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Aβ	Amyloid β
BuChE	Butyrylcholinesterase
CREB	Cyclic AMP response element-binding protein
D2	Dopamine (D2) receptor
GABA	Gamma-aminobutyric acid
HD	Huntington's disease
IMM	Inner mitochondrial membrane
LTP	Long-term potentiation
MAO-B	Monoamine oxidase B
MS	Multiple sclerosis
mtDNA	Mitochondria contain their own DNA
NAD	Nicotinamide adenine dinucleotide
NFT	Neurofibrillary tangles
NMDA	*N*-methyl-D-aspartate
PD	Parkinson's disease
PDE	Phosphodiesterase
PGC-1α	Peroxisome proliferator-activated receptor γ coactivator-1α
PPARα	Peroxisome proliferator-activated receptor alpha
PS1	Presenilin-1
RCC	Respiratory chain complexes
ROS	Reactive oxygen species
SP	Senile plaques
VPA	Valproic acid

1 Introduction

One of the most prevalent neurodegenerative diseases, Alzheimer's disease (AD), is primarily characterized by amyloid (Aβ) plaques and neurofibrillary tangles (NFTs), which lead to dementia. Globally, about 47 million people live with dementia. By 2050, it is anticipated that this figure would surpass 131 million (Chaudhary et al. 2018). The "amyloid cascade hypothesis" states that the amyloid precursor protein (APP) is processed by aβ and γ-secretase to produce Aβ40 and Aβ42 peptides, which go on to form oligomers and aggregates and deposit Aβ plaques. Additionally, tau protein hyperphosphorylation results in NFT production. The hallmarks of AD, including synaptic failure, vascular damage, increased oxidative stress, neuronal and axonal injury, microglia-regulated neuroinflammation, and mitochondrial dysfunction, are facilitated by intraneuronal NFTs and extra-neuronal senile plaques (SP) comprised of Aβ peptides.

To date, cholinesterase inhibitors (donepezil, rivastigmine, and galantamine) and *N*-methyl-D-aspartate (NMDA) receptor inhibitors (memantine) have been used to treat AD. None of these medications are curative or disease-modifying; instead, they merely temporarily or symptomatically relieve some AD patients. There is a

continuous quest for innovative therapeutic targets because these medications are only marginally effective, unable to stop cognitive deterioration, and also have numerous undesirable side effects.

The key pathological hallmarks of AD, extracellular Aβ deposition, and the emergence of intracellular NFTs have been the subject of growing investigation in recent years. Aβ-peptides were once thought to be one of the most promising AD treatment candidates. Unfortunately, despite promising clinical results so far, many clinical investigations based on the Aβ cascade theory were unsuccessful (Doody et al. 2014, p. 3; Salloway et al. 2014). Clinical trials targeting NFTs, which are known to impede axonal transport and cause synaptic dysfunction, have not been able to enhance cognition (Mohandas et al. n.d.; Pedersen and Sigurdsson 2015). The clinical failure of numerous Aβ- and NFT-based treatments gave rise to the idea that AD is a multifactorial illness. While other treatment targets still need to be researched, these regions nonetheless promise the development of AD therapeutics (Calvo-Flores Guzmán et al. 2018).

Currently, many targets including beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), Gamma Secretase, Butyrylcholinesterase (BuChE), Phosphodiesterase (PDE), Gamma-aminobutyric acid (GABA), Dopamine (D2) receptor, Nrf2, Acetylcholine (Ach) receptor, Amyloid precursor protein (APP), and Monoamine oxidase B (MAO-B) are being considered for anti-Alzheimer's drug discovery (Chaudhary et al. 2018). These targets are found in different regions of the brain like Hippocampus, astrocyte, glial cells, temporal, frontal lobe, cortex, Striatum, thalamus, cerebellum, and Basal forebrain Nucleus Basalis (NB). These parts of the brain correspond to various functions like synaptic plasticity, long-term potentiation (LTP), memory formation, oxidative stress, neuronal apoptosis, anti-inflammatory, cell survival, etc. Some of these targets are already having known inhibitors, while others are still being investigated for designing suitable ligands against them.

Apart from these established targets, some novel therapeutic targets are emerging due to increasing need for the effective treatment of AD. Such targets include Purinergic receptor (P2X7R), PPAR-α, proteins associated with synaptic dysfunction, and mitochondrial dysfunction (Table 1). In this chapter, we focus on the above targets and their therapeutic efficacy in AD.

Table 1 Alzheimer's disease therapeutic targets

Established targets	Novel targets
• Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1)	• Purinergic receptor (P2X7R)
• Butyrylcholinesterase (BuChE)	• Proteins associated with synaptic dysfunction
• Phosphodiesterase (PDE)	• Proteins and enzymes associated with mitochondrial dysfunction
• Gamma-aminobutyric acid (GABA)	• Peroxisome proliferator-activated receptor alpha (PPAR-α)
• Dopamine (D2) receptor	
• Acetylcholine (Ach) receptor	
• Amyloid precursor protein (APP)	
• Monoamine oxidase B (MAO-B)	

2 Novel Therapeutic Targets for Alzheimer's Disease

2.1 Purinergic Receptor (P2X7R)

Purinergic receptors are well known for their therapeutic role in different diseases, including Multiple Sclerosis (MS), AD, Huntington's disease (HD), cancer, rheumatoid arthritis, ischemia and inflammatory pain, and Parkinson's disease (PD). P2X7 receptor (P2X7R) belongs to the class of Purinergic receptors (P2), which is highly expressed in immune cells, particularly in those engaged in the innate immune response such as macrophages, monocytes, and specifically microglia (Di Virgilio et al. 2018; Wei et al. 2018).

P2X7R structure includes large extracellular domain (282 amino acids), short intracellular N-terminal domain (26 amino acids), an intracellular C-terminal domain (239 amino acids), and 2 short transmembrane domains (24 amino acids each) constituting a total of 595 amino acids (Jiang et al. 2013). Intracellular domain is important in regulation of Ca^{2+} influx and activation of ERK ½ pathway contributing to the permeation of channel. When compared to other subtypes of P2X receptor, P2X7R contains a large C-terminal domain which consists of many motifs and sub-domains related to multiple functions. These sub-domains include LPS-binding motif, Src homology 3 binding domain, death domain, and binding sites for various cytoskeletal proteins (Chen et al. 2021).

Highest density of P2X7Rs is located at CNS microglia. ATP is the physiological agonist for P2X7R. Extracellular aggregation of Aβ peptides triggers glial cell activation and the release of ATP, therefore stimulating purinergic receptors, especially P2X7R (Illes et al. 2019). In support of this, upregulation of P2X7R has been found near Aβ plaques and microglia (Parvathenani et al. 2003; McLarnon et al. 2006). Also, it is reported that activation of P2X7R enhances the migration of senile plaques through microglia (Martínez-Frailes et al. 2019). Activation of P2X7R converts the resting microglia to activated microglia, in which the latter generates pro-inflammatory cytokines including IL-1β, IL-6, IL-18, TNF-α, several types of reactive oxygen species (ROS), and chemokines such as CCL2 and CCL3 (Shieh et al. 2014; He et al. 2017).

The surface of microglia is expressed with collection of pattern recognition receptors (toll like receptors-TLRs) that stereotypically detect pathogen-associated molecules (such as lipopolysaccharide; LPS) or danger-associated molecular patterns (DAMPS) (such as ATP). There are two signals involved in the production of IL-1β. One is through TLRs which recognize DAMPs, Aβ, LPS, etc. and activate NFkB pathway, thus translation of pro-IL-1β to IL-1β. P2X7R activation is the other signal. However, activation of this receptor induces assembly and activation of NLRP3 inflammasome (which is composed of NLRP3-nucleotide binding, leucine-rich repeat, pyrin domain containing 3, ASC-apoptosis-associated speck-like protein-containing caspase recruiting domain, and pro-caspase-1). Then, NLRP3 inflammasome converts pro-caspase-1 to caspase-1, which thereby cleaves the biologically inactive pro-interleukin-1β to interleukin-1β (IL-1β) (Fig. 1)

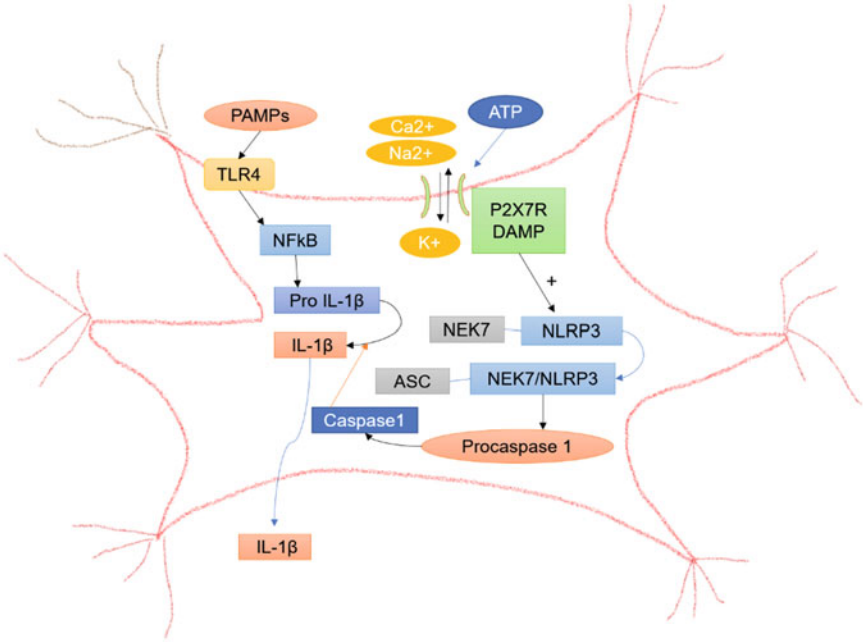

Fig. 1 P2X7R in microglia [generation of interleukin-1β (IL-1β)]: pathogen-associated molecular protein (PAMPs), danger-associated molecular protein (DAMPs), Toll-like receptor 4 (TLR4), NIMA-related kinases (NEK7), nucleotide-binding leucine-rich repeat pyrin domain containing 3 (NLRP3), and apoptosis-associated speck-like protein (ASC)

(Muñoz-Planillo et al. 2013). Upon LPS priming, P2X7Rs also enhance inflammatory cytokine response sequentially by IL-1β, IL-6, and tumour necrosis factor-α (TNF-α) (Young and Górecki 2018; Bhattacharya and Jones 2018). The critical role of P2X7Rs in the secretion of IL-1β makes it an attractive therapeutic target.

It is reported that IL-1β is involved in the formation of Aβ plaques, hyperphosphorylation of tau, and synaptic plasticity impairment (Smith et al. 2012). Besides this activation of NLRP3, inflammasome promotes deposition of tau protein in a mouse model of Frontotemporal dementia (FTD) (Lemprière 2020). Some studies confirmed that P2X7R upregulation in activated microglia was parallel with AD progression by using two different mouse models of AD (APP/PS1 mice and J20 mice) (Lee et al. 2011; Martínez-Frailes et al. 2019). A recent study reported that PS2-deficient mice are most sensitive to Aβ-induced neuroinflammation due to the upregulation of P2X7R in both glial and neuronal cells in a transcription factor Sp1 (SP1)-dependent manner (Qin et al. 2017). Different studies using both in vitro and in vivo approaches postulated that P2X7R might be one of the factors controlling APP processing (Delarasse et al. 2011; León-Otegui et al. 2011; Diaz-Hernandez et al. 2012; Darmellah et al. 2012). Furthermore, role for P2X7R in the phagocytosis of Aβ peptides was also reported to contribute to Aβ clearance. Another study reported that P2X7R might also down-regulate pathological microglial activation in AD (Martin et al. 2019).

Further, pharmacological blockade or knocking out the P2X7R in different AD mouse models has shown neuroprotective effects by reducing neuroinflammation (Ryu and McLarnon 2008; Chen et al. 2018; Martin et al. 2019). Initial studies demonstrated that in vivo pharmacological inhibition of P2X7R by Brilliant Blue G (BBG) attenuated inflammatory response and diminished leakiness of BBB in $A\beta_{1-42}$-induced AD model (Ryu and McLarnon 2008). In accordance, later study revealed that in vivo inhibition of P2X7R by BBG prevented the spatial memory impairment and cognitive deficits in AD mouse model (Chen et al. 2014). The reversal of the $A\beta_{1-42}$-induced morphological and cognitive effects by BBG proved the involvement of P2X7Rs. In another study, administration of oxidized ATP (o-ATP), a P2X7R antagonist, attenuated microglial activation and neuronal damage in LPS-induced AD model (Choi et al. 2007). Moreover, APP/PS1/P2X7R-deficient mice exhibited smaller cognitive deficit and better synaptic plasticity than APP/PS1 mice (Martin et al. 2019). Another study demonstrated that P2X7R plays a critical role in Aβ peptide-mediated release of chemokines, particularly CCL3, which is associated with pathogenic $CD8^+$ T cell recruitment. This study highlights a novel detrimental function of P2X7R in chemokine release and supports the notion that P2X7R may be a promising therapeutic target for AD (Martin et al. 2019).

Another pathological feature of AD is impaired phagocytosis ability. A genome-wide association study revealed various genes associated with phagocytosis function of microglia such as TREM2 and CD33 (Efthymiou and Goate 2017). Further, it is reported that reduced phagocytic capacity results in increased amyloid deposition in AD mouse model (Parhizkar et al. 2019). It is believed that P2X7R shows scavenger activity. A study reported that high level of P2X7R mediates phagocytosis of apoptotic lymphocytes in HEK-293 cells were tranfected with P2X7R and macrophages to acquire the ability to phagocytose apoptoticlymphocytes (Gu et al. 2011). This study explains that involvement of P2X7R in its un-activated state acts as scavenger receptor. Further, experiments on microglia have shown that P2X7R activation attenuated their phagocytic capacity (Janks et al. 2018; Martínez-Frailes et al. 2019).

ROS is another effector of microglia by P2X7R activation. Several pieces of evidence point to the fact that P2X7R may be the primary receptor involved in the generation of ROS (Ex: H_2O_2) by activating microglial cells (Nuttle and Dubyak 1994). In vitro studies revealed that fibrillar $A\beta_{1-42}$ causes ROS production generated via P2X7R activation induced by ATP released from rat microglial cells in an autocrine manner (Kim et al. 2007; Liu et al. 2020). Hence, P2X7R upregulation in microglial cells may result in excessive ROS production induced by Aβ which contributes to the synaptic toxicity associated with the early stages of AD (Lee et al. 2011). In vivo administration of selective P2X7R antagonist A438073 avoided ROS production and oxidative DNA damage induced by P2X7R activation in spinal cord dorsal horn neurons (Munoz et al. 2017). Furthermore, P2X7Rs drive proliferation and activation of microglia, upregulating their surface expression of immunomodulatory proteins and becoming efficient in producing a variety of cytokines, chemokines, and ROS (Monif et al. 2009, 2010). All these studies suggest that BBB permeable compounds and selective P2X7R antagonists might be considered as

good therapeutic drugs to treat chronic neuroinflammation associated with AD. Therefore, P2X7R antagonists may become general anti-neuroinflammation and anti-neurodegeneration remedies, also improving late-onset AD.

2.2 Proteins Associated with Synaptic Dysfunction

Synaptic plasticity events are crucial for synaptic functions including learning and memory processes, where short-term alterations in synaptic strength are converted to long-lasting memories. Apart from the presynaptic terminal and the postsynaptic compartment, synapse also includes astrocytes and the extracellular matrix creating a tetrapartite synapse. Synaptic transmission strength is based on changes in neuronal activity where long-term potentiation (LTP) and long-term depression (LTD) represent the functions of learning and memory. Synaptic transmission majorly relies on multiple cellular mechanisms which include biosynthesis of neurotransmitters (NTs) from amino acids and delivery of synthesized NTs to synaptic sites. This requires proper formation of synaptic vesicles, intact microtubule tracts, and timely removal of NTs from synaptic cleft after neurotransmission (Pelucchi et al. 2022).

N-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-364 isoxa-zolepropionic acid (AMPA) receptors together regulate excitatory synaptic transmission and plasticity in brain, thus playing critical role in learning and memory. Altered internalization of AMPA receptors affects synaptic plasticity inducing synaptic dysfunction and loss of dendritic spines. Aβ-induced excitotoxicity in postsynaptic neurons associated with more Ca^{2+} influx leads to increased ROS production, tau hyperphosphorylation, and lipid peroxidation, altogether contributing to synaptic dysfunction. Moreover, Aβ-induced tau hyperphosphorylation destabilizes microtubules which alter axonal trafficking of mitochondria and translocation of tau to dendritic spines. This further contributes to NMDA receptor destabilization and excitotoxicity and has a detrimental effect on synaptic function (Tönnies and Trushina 2017).

Synaptic dysfunction is one of the common pathogenic traits in many neurological disorders. In AD, the degeneration of synapses can be detected at the early pathological progressions before achieving complete neuronal degeneration, supporting the hypothesis that synaptic failure is a major determinant of AD. Most of the Aβ plaques generate and form oligomers at the synaptic region. All the elements constituting the tetrapartite synapse are altered in AD and can synergistically contribute to synaptic dysfunction (Marsh and Alifragis 2018). Moreover, the two main hallmarks of AD, i.e. Aβ and NFT's, collectively cause synaptic deficits. Deciphering the mechanisms underlying synaptic dysfunction is relevant for the development of the next-generation therapeutic strategies, aimed at modifying the progression of AD.

The targets of Aβ at synapse have been identified as dendritic or axonal compartments (overexpression of APP) and plasticity in nearby neurons, ultimately leading to reduction in spine density (Marcello et al. 2012). It has been hypothesized that Aβ

peptides enhance neurotransmitter (NT) release. Several reports suggested that key proteins which regulate the interaction of synaptic vesicles (SVs) with the presynaptic membrane or the availability of SVs to participate in NT release are affected by Aβ peptides (Yang et al. 2015; Russell et al. 2012). Proteins involved in SV docking and fusion that regulate NT release are Syntaxin 1a (Stx1a), Synaptophysin (Syp1), dynamin, and Synapsin1 (Snp1) (Kelly et al. 2005; Liu et al. 2019) (Fig. 2).

The prolonged phosphorylation of Snp1 would enhance neurotransmission by increasing the availability of SVs that would dock to the active zone. Furthermore, disruption of the Syp1/VAMP2 complex (VAMP2 known as Synaptobrevin 2) on these vesicles would increase the accessibility of VAMP2 to the other SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins, promoting the SNARE complexes formation and enhancing the probability of NT release (Marsh and Alifragis 2018) (Fig. 2). Moreover, an Aβ-mediated increase of Ca^{2+} levels inside the pre-synapse would also enhance SV fusion and the release of glutamate. This aberrant release of glutamate would initially activate NMDARs, but eventually induce excitotoxicity. In the long term, the extensive use of SVs combined with endocytic defects and recovery due to the inactivation of dynamin and sustained phosphorylation of Snp1 would gradually deplete these vesicles from the synapse, thereby reducing synaptic activity. However, depletion of SV reserve pools after prolonged exposure to Aβ in neuronal cultures has been reported (Parodi et al. 2010; Kelly et al. 2005). Further, Park et al. showed that exposure of neurons to Aβ reduces the activity-dependent lateral dispersion of SVs, providing significant evidence that Aβ reduces SV mobility (Park et al. 2017).

The sustained phosphorylation of Snp1 might be the underlying cause for the inhibition of inter-synaptic vesicular movements, thereby disrupting the Syp1/VAMP2 complex by Aβ which could be one of the contributing factors for this inhibition. Collectively, these effects would have a substantial impact on the gradual progression of synaptic dysfunction and ultimately cause synaptic deficit, which is a key hallmark of AD pathology.

Another major hallmark in AD is tau which is involved in synaptic dysfunction. Pathological modifications of tau protein alter its binding affinity and lead to aberrant aggregation and migration to different brain regions, which eventually lead to tauopathy in AD (Chen et al. 2019). Hyperphosphorylation of tau leads to its detachment with microtubules and further impairs axonal transport. Some studies revealed that uptake of extracellular localized tau by neurons triggered tau accumulation in axons and dysregulated the axonal transport of membrane organelles (Swanson et al. 2017; Wu et al. 2013). Some studies reported that abnormal tau binds to synaptic vesicles by synaptogyrin-3, thus disrupting presynaptic functions (McInnes et al. 2018; Zhou et al. 2017). Further, it is observed that accumulation of tau in presynaptic vesicles induces significant increase in NT release by intracellular calcium release, leading to synaptic depression (Moreno et al. 2016). Further, tau infiltration in dendrites results in reduced clustering of AMPA and NMDA receptors, which leads to compromised synaptic transmission and memory deficits (Hoover et al. 2010).

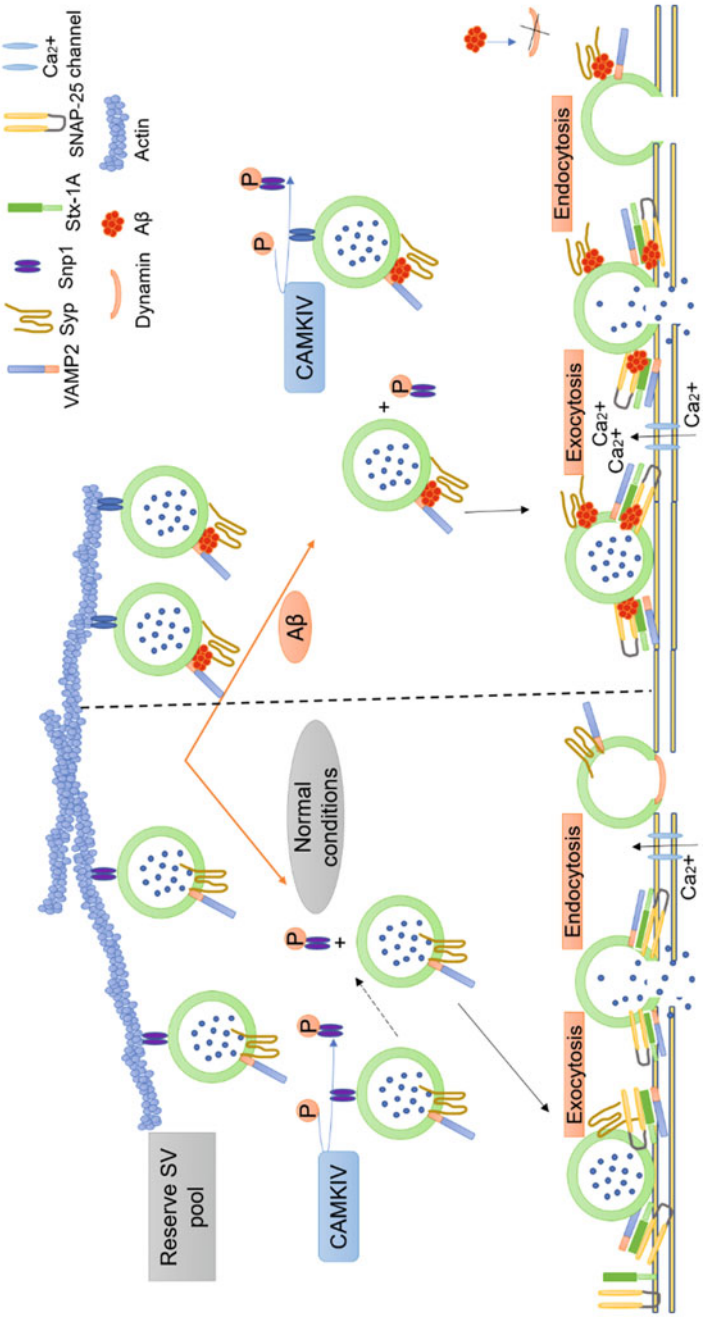

Fig. 2 Synaptic dysfunction associated with Amyloid β in Alzheimer's disease: (1) Under normal physiological conditions, release of neurotransmitter (NT) from synaptic vesicles (SVs) is tightly regulated by Snp1, Syp1/VAMP2 complex, Stx-1A, and dynamin1. (2) In the presence of Aβ, release of NT from SVs will be disrupted by Aβ by binding to Syp1/VAMP2 complex, thus leading to uncontrollable involvement of SVs in aberrant NT release. Further, this also leads to dysregulation of endocytosis by reducing the levels of dynamin1. This will eventually lead to disruption of SV pools

It is well established that primary kinase involved in the tau phosphorylation includes glycogen synthase kinase (GSK-3) and cyclin-dependent protein kinase 5 (Cdk-5). Multiple studies demonstrated the benefits of inhibiting GSK-3 which majorly includes reversing synaptic dysfunction. A study demonstrated that selective GSK-3 inhibitor AR-A014418 prevented LTP impairment and tau hyperphosphorylation induced by Aβ in wild-type mice (Shipton et al. 2011). Another study reported that GSK-3 inhibitors (lithium and kenpaullone) rescued LTP by upregulating mTOR pathway in AD mice model (Ma et al. 2010). Later, a study revealed that specific GSK-3 inhibitor CT-99021 has prevented Aβ-induced LTP in hippocampal cultures (Jo et al. 2011). Besides GSK-3, inhibition of Cdk-5 with roscovitine or butyrolactone prevented the Aβ-mediated block of LTP induction (Wang et al. 2004). All the above studies suggest that abnormal tau phosphorylation is an important factor in synaptic dysfunction.

Valproic acid (VPA) has been recognized which could be used to abrogate some of the early presynaptic defects (Marsh et al. 2017). VPA is a short-branched chain fatty acid, most commonly used to treat epilepsy and bipolar disorder. Studies on pre-clinical models suggest that VPA plays key roles such as affecting long-term potentiation (LTP) which could be therapeutic potential to combat AD (Zhang et al. 2003; Leng et al. 2008; Qing et al. 2008). Moreover, it has also been shown that it prevents Aβ-induced reduction in SV recycling and that it can induce clustering of Snp1 in developing neurons (Williams and Bate 2016; Hall et al. 2002). Although many evidences highlight the significance of Aβ peptides and tau hyperphosphorylation in the deregulation of NT release and dysfunction of SV dynamics, this area as new therapeutic target has been largely overlooked. Targeting these defects of synaptic function could serve as a target for crucial early intervention and diagnosis of AD.

2.3 Targeting Mitochondrial Dysfunction

Mitochondria are defined as the powerhouse of the cell because every cell in the human body relies on the energy provided by these organelles to sustain its vital functions. Mitochondrial energy production (process of oxidative phosphorylation) takes place at the inner mitochondrial membrane (IMM) through the activity of respiratory chain complexes (RCC), generating an inner membrane potential (mtΔΨ) that is used by the ATP-synthase enzyme complex to synthesize ATP (Cenini and Voos 2019). This process depends on the supply of reducing equivalents by the end-oxidation of nutrients via the Krebs cycle or β-oxidation in the mitochondrial matrix compartment (Stock et al. 2000). Mitochondria contain their own DNA (mtDNA) located in the matrix that encodes mainly 13 protein subunits of the RCC. Hence, the maintenance of an entire and functional mitochondrial proteome requires a fine-tuned and well-coordinated sequence of many reactions and a close integration of organellar and cellular biogenesis processes (Pfanner et al. 2019). A master regulator of mitochondrial biogenesis is Peroxisome proliferator-activated

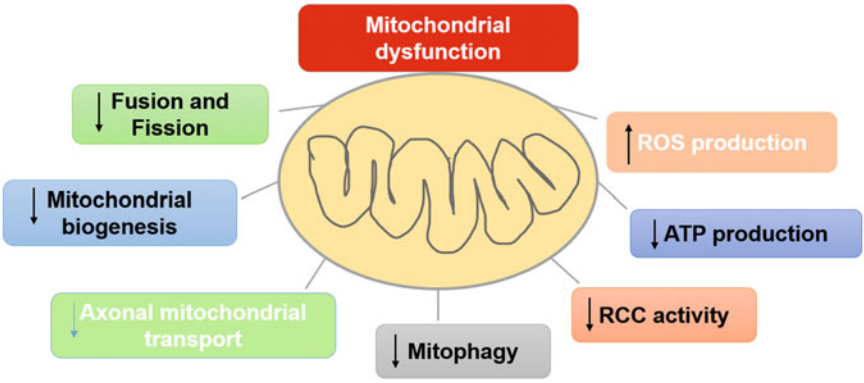

Fig. 3 Mitochondrial dysfunction and its associated pathologies

receptor γ coactivator-1α (PGC-1α) that activates a series of transcriptional factors (Scarpulla 2011).

The enzymatic activity of the mitochondrial RCC results in a leakage of electrons from the RCC, contributing significantly to the formation of ROS (Shariff et al. 2004) (Fig. 3). Therefore, ROS are considered a typical by-product of bioenergetic pathways (Quinlan et al. 2013). However, under normal physiological conditions, ROS production is well balanced by the presence of adequate antioxidant systems, and the damage to the diverse cellular constituents is contained. However, during ageing, as well as during several pathological conditions such as neurodegenerative diseases, this equilibrium becomes unbalanced. Increased ROS concentrations result in molecular damage at the site where they are produced or, through diffusion, in surrounding areas, leading to the generation of oxidative stress condition. The hippocampus region, cortex, and more generally the brain are particularly vulnerable to oxidative stress because of their high consumption of oxygen.

Neurons are strictly dependent on the presence of mitochondria, in particular at the synapses where these organelles produce ATP and buffer Ca^{2+} ion concentration, which are fundamental processes for the implementation of neurotransmission and generation of membrane potential along the axon (Li et al. 2004; Verstreken et al. 2005). This justifies the presence of high amount of mitochondria at the synaptic area, higher than any other part of the neurons. Linked to that, an efficient transport of neuronal mitochondria at the synaptic terminals is fundamental for their correct function.

Mitochondrial dysfunction is one of the factors that may actively contribute to AD onset and progression. In 2004, a new hypothesis called mitochondrial cascade hypothesis (apart from Amyloid cascade hypothesis) was proposed to explain the onset of sporadic AD, which explains that the mitochondrial dysfunction is the primary process to trigger a cascade of events that lead to sporadic late-onset AD (Swerdlow and Khan 2004) (Fig. 3).

The analysis of the samples from different AD experimental models and AD patients showed a strong link between the oxidative stress and mitochondrial

dysfunction. In the transgenic mice over-expressing human APP (Tg mAPP mice), an early and progressive accumulation of Aβ peptide in synaptic mitochondria led to a mitochondrial synaptic dysfunction such as damaged mitochondrial respiratory activity, oxidative stress, and impaired mitochondrial axonal transport (Du et al. 2010). In another study, it is reported that the compromised mitochondria bioenergetics together with elevated oxidative stress levels are early phenomena appearing before the development of observable Aβ plaques in 3xTg-AD mice (Hauptmann et al. 2009; Yao et al. 2009). The mitochondrial dynamics such as fusion and fission processes were found unbalanced in AD, potentially leading to compromised distribution and morphology of mitochondria in the neurons (Hirai et al. 2001) and fragmented mitochondria brains from AD patients (Wang et al. 2008a, 2009). Furthermore, the level of proteins regulating the mitochondrial biogenesis such as PGC-1α, NRF1 and 2, and TFAM was significantly reduced in hippocampus and cellular models overexpressing APP Swedish mutation (Qin et al. 2009; Sheng and Cai 2012). In the AD mouse model of mutant human transgenes of APP and Presenilin-1 (PS1), the mitochondrial biogenesis markers were found declined in the hippocampus region (Song et al. 2018).

The two major and typical histopathological markers of AD, Aβ peptide and tau, harmfully accumulate in mitochondria (Eckert et al. 2010). Aβ peptide and abnormal tau negatively affect axonal transport and consequently the transport of mitochondria along the axon from the neuronal soma to synapses. Several AD models such as transgenic models (APP overexpression) or Aβ-induced AD are characterized by mitochondrial fragmentation and abnormal mitochondrial distribution along the neurons due to alteration of mitochondrial fusion and fission proteins levels (Wang et al. 2008b; Zhao et al. 2010; Calkins and Reddy 2011). All these results lead to two critical remarks: (a) Altered balance between fusion and fission that interferes with mitochondrial transport contributes actively to AD pathogenesis and (b) Mitochondrial dynamics impairment could be a new therapeutic target in AD.

Mitochondria could be targeted through two ways: (a) by pharmacologic approaches acting on mitochondria directly or (b) by action on the lifestyle that indirectly hits this organelle. Pharmacological approaches include Antioxidants, Phenylpropanoids, Mitophagy stimulators, and some miscellaneous compounds such as Oxaloacetate, Nicotinamide adenine dinucleotide (NAD), Pioglitazone, Dimebon (Table 2). Second approach, i.e. Action on Life style, includes calorie restriction, diet, and exercises.

2.4 Peroxisome Proliferator-Activated Receptor Alpha (PPARα)

The first PPAR currently known as PPAR-α was discovered in 1990 (Issemann and Green 1990). PPAR-α regulates oxidative stress, energy homeostasis, andbmitochondrial fatty acids metabolism including fatty acids β oxidation pathway and is the only receptor belonging to PPAR family which influences excitatory

Table 2 Effect of pharmacological approaches on mitochondria in experimental models of AD

Pharmacological approach	Observed effects	AD model
Antioxidants		
Selenium	• Inhibition of ROS production and oxidative damage	• In vitro Aβ42-CFP-overexpressed HEK293 cell line (Chen et al. 2013)
	• Reduction of mitochondrial membrane depolarization	
Coenzyme Q10	• Attenuation of decreased oxidative phosphorylation efficiency and increased H_2O_2 production	• Isolated mitochondria from Aβ1-40 peptide-treated diabetic Goto–Kakizaki aged rats (Moreira et al. 2005)
	• Decreased levels of Aβ and improved cognitive performance	• In vivo Tg19959 mice (Dumont et al. 2011)
	• Reduction of mitochondrial accumulation of Aβ peptide	
Catalase	• Reduction of abnormal APP process, oligomeric Aβ peptides, and BACE1 activity and levels, and oxidative damage	• In vivo MCAT/APP mice (Mao et al. 2012)
	• Increase of protective soluble APPα and CTF83 fragments	
α-Lipoic acid	• Decrease of mitochondrial-related oxidative stress and apoptotic markers	• In vitro AD fibroblast (Moreira et al. 2007)
	• Preservation of COX assembly elevation of ATP levels, Krebs cycle dehydrogenase, complex I, and COX activities	• In vivo aged Wistar rats (Ajith et al. 2014)
Phenylpropanoids		
Resveratrol	• Attenuated Aβ induced cytotoxicity, apoptosis, and intracellular ROS accumulation	• In vitro Aβ peptide-treated PC12 cell line (Jang and Surh 2003)
	• Prevents memory loss	• In vivo APP/PS1 mice (Porquet et al. 2014)
Wogonin	• Rescue the mtΔΨ loss	• In vitro Tet-On Aβ42-GFP-overexpressed SH-SY5Y cell line and
	• Attenuation of mitochondria-mediated apoptosis	• In vivo 3xTg-AD mice (Huang et al. 2017a)
Epigallocatechin-3gallate (EGCG)	• Restored mitochondrial respiratory rates, MMP, ROS production, and ATP levels	• Neuroblastoma cells expressing mutant APP and APP/PS-1 transgenic mice (Dragicevic et al. 2011)

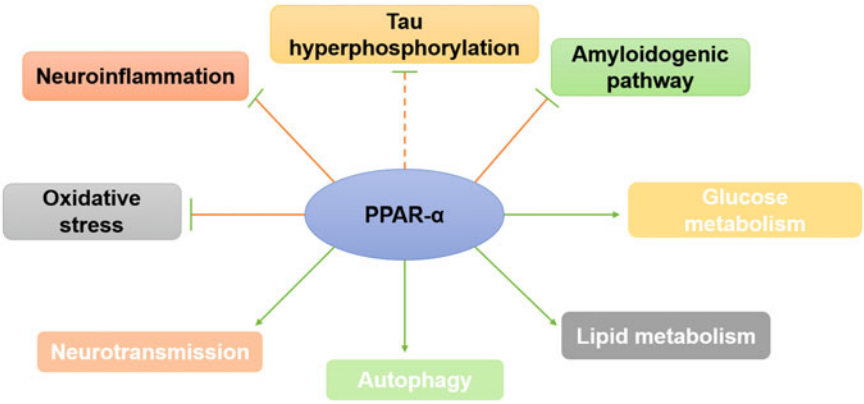

Fig. 4 PPAR-α and its associated pathologies in Alzheimer's disease

glutamatergic neurotransmission and also cholinergic/dopaminergic signaling in the brain. Additionally, PPAR-α is engaged in metabolism of APP in the brain, and directly or indirectly through Aβ, it may also influence tau protein phosphorylation (Fig. 4) (Wójtowicz et al. 2020). PPAR-γ, PPAR-α, and their coactivator PGC-1α play an important role in cell differentiation and mitochondria biogenesis in neurodegeneration and neuroinflammation (Austin and St-Pierre 2012; Scarpulla 2011).

Roy et al. determined the distribution of PPAR-α in different regions of hippocampus and observed that PPAR-α protein was localized in CA1, CA2, and CA3 and in dentate gyrus (DG) of mice brain (Roy et al. 2013). It was found that PPAR-α controls calcium influx and the expression of several genes encoding hippocampal proteins involved in the regulation of synaptic plasticity. PPAR-α is also engaged in expression of NMDA receptor subunit NR2A and NR2B genes, AMPA receptor [2-amino-3(3-hydroxy-5-methyl-isoxasol-4-yl) propanoic acid] associated subunit GluR1, and also AMPA-receptor associated activity-related cytoskeleton proteins (Sakimura et al. 1995; Lee et al. 2003; Tzingounis and Nicoll 2006). All these mentioned genes are related to synaptic plasticity and are regulated by PPAR-α via cyclic AMP response element-binding protein (CREB). Further, many studies demonstrated that PPAR-α and its ligands are involved in regulation of glutamatergic and cholinergic-mediated dopaminergic transmission in the brain (Huang et al. 2017b; Zakrocka et al. 2017; Melis et al. 2010, 2013). However, further studies are necessary to understand the role of PPAR-α in glutamatergic and other signaling pathways in physiological conditions and in AD. The above functions indicate that PPAR-α could be promising target for therapy of AD. Further, the mechanism of its action in the brain should be characterized in depth to enable successful application.

Activation of PPAR-α receptor with specific receptor agonist enhanced transcription of GluA1 subunits of the AMPA receptor which further leads to an AMPA response and better synaptic plasticity (Schmitt et al. 2005). In another study, it is reported that under basal physiological conditions, PPAR-α is involved in the

degradation of APP by activation of β and α secretases leading to liberation of non-amyloidogenic peptide (p3) and soluble sAPPα with possible neuroprotective effect (Corbett et al. 2015). Further, Zhang et al. demonstrated that PPAR-α agonist (GW7647) regulates Aβ generation by inhibition of BACE-1 activity (Zhang et al. 2015). The above studies suggest that alteration of PPAR-α signaling may lead to activation of APP metabolism and Aβ liberation/accumulation through amyloidogenic pathway in AD.

The studies of Vallee and Lecarpentier on AD described that PPAR agonists diminish learning and memory deficit in AD patients (Vallée and Lecarpentier 2016). Anti-amyloidogenic action of PPAR-α agonists (fibrates) was observed in clinic in longitudinal treatment of patients (Blasko et al. 2008). PPAR-α receptor stimulation induces synthesis of allopregnanolone in astrocytes (this hormone thought to be involved in neuroprotective mechanism) (Raso et al. 2011). Therapeutic effects of PPAR-α on neuronal death and microvascular impairment were described by Moran and Ma (2015). Gemfibrozil is a PPAR-α agonist that was undergoing Phase II Clinical trial for AD which downregulates the BACE1 expression (Clinical trial identifier: NCT02045056) (NeurologyLive n.d.). The above evidences and clinical studies suggest the therapeutic potential of targeting PPAR-α.

3 Conclusion

Nowadays, AD has been considered a multifactorial disease due to its numerous pathological cascades and their unclear mechanisms. Due to these reasons, therapy of AD remains a difficult challenge for discovery of novel treatments. Till now, only few Food and drug administration (FDA)-approved treatments are available. Yet, they are only symptomatic treatments and there is a further need to identify and explore new therapeutic targets that focus on main pathological hallmarks of the disease. In this chapter, we have discussed about the novel targets in the therapy of AD. These targets majorly focus on neuroinflammation, synaptic dysfunction, mitochondrial dysfunction, Aβ plaques, and tau hyperphosphorylation which are the crucial pathological events in AD.

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Modified Investigation Tools and Techniques Useful in Alzheimer's Disease Research

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Abstract Alzheimer's disease is a predominant cerebral disease of uncertain etiology with distinctive neuropathological and neurochemical characteristics. It leads to cognitive skill impairment due to synaptic loss and progressive neuronal loss that ultimately results in death after a few years. Today, Alzheimer's disease is one of the top eight deadly diseases in the world. Extensive research is going on to find the root cause and to seize progressive neurodegeneration. According to recent studies, detection of early onset of dementia followed by the implementation of drug therapy may change the fate of failed drugs in clinical trials based on targeting late onset of dementia. However, the event of the early onset of dementia and related biomarkers can be captured at the appropriate moment exclusively with the help of modified tools and techniques. The selection of these tools and techniques depends on various factors like cost-effectiveness, advantages, and weakness. The present chapter aims to bring together all the previously mentioned and recently modified in vitro, in vivo, and ex vivo methods and in vivo brain imaging techniques developed for the identification of Alzheimer's disease biomarkers. Apart from this, the chapter has also provided a brief insight into the current status of clinical trials for new drug therapy against Alzheimer's disease and novel targets which are giving promising drug therapies either to slow down neurodegeneration or improve cognitive skills in Alzheimer's disease.

Keywords Alzheimer's disease · Brain imaging techniques · Voxel-Based Morphomery (VBM) · Transgenic models · APP · PSEN1 · MAPT · Acetylcholinestrase

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Abbreviations

5 × FAD	Mice expressing human APP and PSEN1with total five Alzheimer's disease linked mutations
5-HT6	5-Hydroxy tryptophan receptor
AchE	Acetylcholinesterase
AD	Alzheimer's disease
AD–BXD	Refers to a panel of transgenic mouse strains created to model the genetic diversity seen in human population
APOE	Apolipoprotein E
APP	Amyloid precursor protein
ARPE	Human retina pigment epithelial cells
ATCI	Acetylthiocholine iodide
Aβ	Beta amyloid
BACE1	β-secretase 1
CNS	Central nervous system
CP–MAS	Cross polarized magic angle spinning
DTNB	Dithiobisnitrobenzoic acid
FDA	Food drug administration
FTIR	Fourier transform infrared spectroscopy
GK-38	Glycogen synthase kinase-38
GSH/GSSG	Glutathione/glutathione disulfide
GST	Glutathione-*S*-transferase
ICD	International classification of diseases
iPSC	Disease-specific induced pluripotent stem cells
LMTX	Second-generation tau aggregation inhibitor
MAP	Microtubule affinity regulate
MRI	Magnetic Resonance Imaging
MTT	3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide
NADH	Nicotinamide adenine dinucleotide
NFT	Neurofibrillary tangles
NMDA	*N*-methyl D-aspartate receptor
PET	Positron Emission Tomography
PS1, PS2	Presenilin 1, 2
PSEN1	Presenilin 1
SH-SY5Y	Neuroblastoma cells
SPECT	Single Photon Emission Computed Tomography
STEM	Scanning Transmission Electron Microscopy
TBARS	Thiobarbituric acid reactive substances
TEM	Transmission Electron Microscopy
TNF	Tau Neurofibrillary tangle
TREM2	Triggering receptor expressed on myeloid cells 2
WHO	World Health Organization
α7-nAchR	Acetylcholine nicotinic receptor

1 Introduction

Since more than five decades, extensive research is going on Alzheimer's disease (AD) to determine exact etiology and cure. Alzheimer's disease is supposed to occur due to chronic dementia which is characterized by progressive neurodegeneration, cognitive skills impairment, gradual memory loss, and ultimately complete destruction of brain activity. According to WHO ICD 10 version 2019, F00*, Alzheimer's disease is, "a primary degenerative cerebral disease of unknown etiology with characteristic neuropathological and neurochemical features. The disorder is usually insidious in onset and develops slowly but steadily over several years" (https://icd.who.int/browse10/2019/en#/F00). As per ICD 10 guidelines, dementia in Alzheimer's disease is divided into four subtypes, Early-onset before age 65, Late-onset after age 65, Atypical or mixed type, and unspecified onset. Early-onset of dementia in Alzheimer's disease is known as AD type 2 or presenile dementia, whereas late-onset of dementia in AD is known as AD type 2 or senile dementia (ICD 10, version 2019). According to various epidemiological studies, by 2050, more than one billion of worldwide population may suffer from Alzheimer's disease. In the twentieth century, the recorded rate of Alzheimer's disease occurrence found one case per 7 worldwide (Cornutiu 2015).

Although the estimated digit looks small as compared to the total number of the worldwide population, it is a severe problem because neurodegeneration is a nearly irreversible process. Once the neurons degrade, they are hard to regenerate. Within approximately 8–9 years, a person completely loses brain functions and eventually dies due to loss of CNS control on organ functions. It also affects financial aspects of the patient since a long time-costly treatment is required to manage the disease. To date, the FDA has approved one NMDA receptor modulator and four acetylcholinesterase inhibitors. Several clinical trials based on the amyloid hypothesis have been failed that made researchers to investigate more different perspectives to understand the reasons of failure. According to some failed clinical trial reports, even though the beta amyloid plaque is cleared by the drugs, there was no significant improvement observed in Alzheimer's disease condition in patients. It means that preclinical study results based on transgenic or natural animal models are not sufficient. There is need of implementation of modified in vitro and ex vivo strategies along with in vivo study to get the complete knowledge of target site as well as drug activity at targeted site. Researchers have changed their research methods with the aid of sophisticated instruments and techniques to better understand Alzheimer's disease.

This chapter aims to explore modified methods and techniques used in investigation of pathology of Alzheimer's disease and drug development to help researchers to understand other possible symptomatic targets for effective drug therapy against Alzheimer's disease and other neurodegenerative diseases.

2 Pathophysiological Aspects of Alzheimer's Disease

For the development or discovery of a new drug entity for any disease, knowledge about the pathophysiology of that disease or disorder is an essential aspect. The exact pathogenesis of Alzheimer's disease is still unclear. However, extracellular accumulation of insoluble beta-amyloid plaques in the brain and intracellular Tau Neurofibrillary tangle (TNF) formations are hallmark symptoms for the identification of Alzheimer's disease. β amyloid cascade hypothesis is one of the most followed hypotheses by researchers across the world for Alzheimer's disease drug therapy development (Hardy and Higgins 1992). Amyloid Precursor Protein (APP) plays an important role in the regulation of the life cycle of neurons. Overexpression of APP proteins triggers the secretion of β-secretase followed by γ-secretase. Both hydrolyze excess APP to a soluble form of β amyloid that starts accumulating outside neurons. Some researchers claimed that, when this soluble form of β amyloid combines with an acetylcholinesterase enzyme, it starts to form insoluble β amyloid plaques. Another study claims that β amyloid plaque formation also occurs when the soluble form of β amyloid binds to presenilin PS1 and PS2, Apolipoprotein E (APOE). Excess accumulation of these plaques disturbs the nerve cell life cycle and triggers the hyperphosphorylation of Tau protein. This process is mediated by the activation of several protein kinases like glycogen synthase kinase (GK-38), Microtubule affinity regulate (MAP) kinase. Hyperphosphorylated tau starts binding with unassembled microtubules and forms neurofibrillary tangles inside the neurons. This TNF disturbs normal functions of neurons, leading to toxicity and neurodegeneration (Hardy and Higgins 1992; Li et al. 2015). The whole process is summarized as shown in Fig. 1.

Involvement of proteins and enzymes like APP, β secretase, γ-secretase, Presenilin 1 and 2, Apolipoprotein, MAP kinase, and GK-38 in β amyloid cascade suggests that any mutations of these protein-producing genes may lead to Alzheimer's disease. However, there are also reports about the fact that, despite the heavy accumulation of beta-amyloid plaques, the cognitive skills of patients remained unhampered (Aizenstein et al. 2008; Melzer et al. 2019). Thus, alternative hypotheses like neuroinflammation, oxidative stress, and bacterial infection are also under investigation by many researchers. However, a recent long-term trial study conducted by the National Institute of Health and the US government uncovered some facts related to the mystery behind the failure of the number of clinical trials based on the β amyloid cascade hypothesis. This trial study aimed to investigate whether anti-amyloid therapy started before cognitive impairment symptoms appear could slow cognitive decline in Alzheimer's disease. Observations of this study suggested that, if we target β amyloid accumulation in the early onset of dementia in Alzheimer's disease before the appearance of cognitive skill impairment symptoms, the rate of success of clinical trial increases (Sperling et al. 2020). Early onset of dementia can be detected with the help of brain imaging tools. The hallmark symptoms like plaques of β amyloid and TNF are usually visible in late onset of dementia during in vivo studies. Many clinical trials may have failed because they were testing a drug in late-onset of dementia in Alzheimer's disease when those hallmark symptoms became prominent to identify. However, implementation of

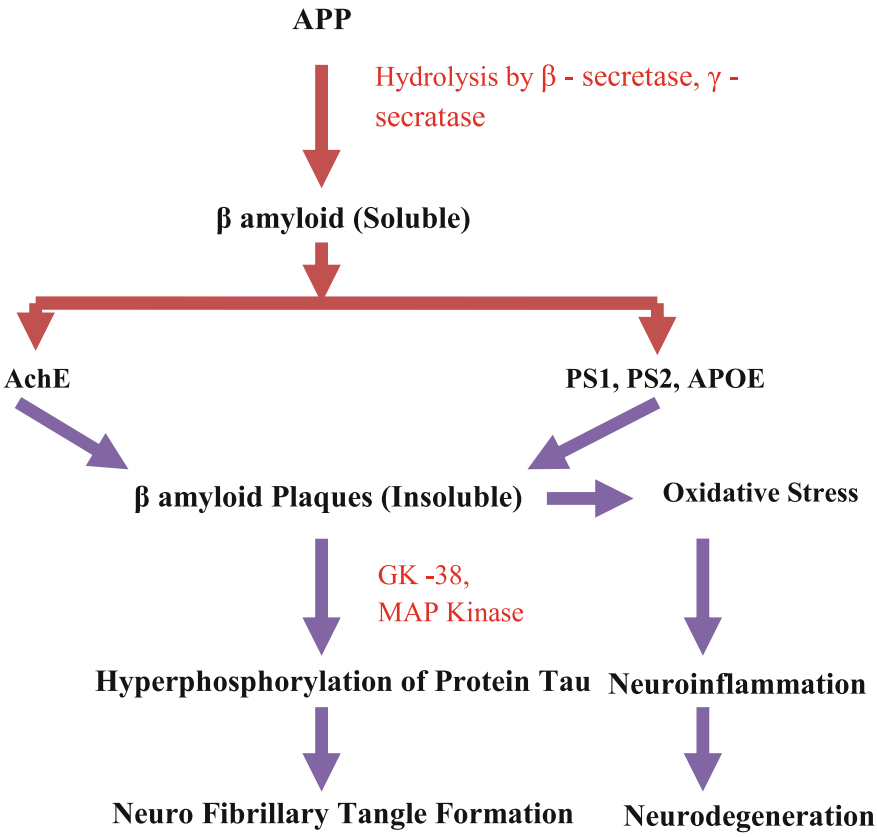

Fig. 1 Schematic representation of "β amyloid cascade"

in vivo brain imaging tools as well as investigation methods for detection of early onset of dementia may be helpful to reanalyze and modify the drugs, especially those used in failed clinical trials.

3 In Vivo and In Vitro Techniques for Pathological Investigation of Alzheimer's Disease

Both in vivo and in vitro techniques are crucial to differentiate between Alzheimer's disease and other dementias. In vivo techniques involve brain imaging techniques like Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), Magnetic Resonance Imaging (MRI), etc. (Tiwari et al. 2015; Zamrini et al. 2004). However, in vitro techniques involve FTIR spectroscopy, Transmission Electron Microscopy (TEM), Scanning Transmission Electron Microscopy (STEM), Circular Dichorism, etc. (Tiwari et al. 2015; Tomaselli et al. 2015). A summary of these techniques is shown in Table 1. PET and SPECT are

Table 1 Ex vivo and in vitro techniques for pathological study and diagnosis of AD

Name of technique	Principle	Advantage	Limitation	References
In vivo techniques				
Positron Emission Tomography (PET)	Involves selective uptake of picomolar quantity of biomolecules attached with radiotracers having high affinity towards β amyloid. ex. ^{18}F labelled fluorodeoxyglucose (FDG), AZD2184, Florbetapir F-18	Noninvasive method, β amyloid-specific radiotracer outlines only β amyloid present in various regions of brain. Presymptomatic diagnosis of β-amyliodosis is possible	Costlier than SCPET, hence rarely used for routine diagnosis	Ziegler (2005), Tiwari et al. (2015)
Single Photon Emission Computed Tomography (SPECT)	Based on uptake of technetium 99m-based lipid soluble radionuclides specific to β amyloid. ex. ethyl cysteine dimer, hexamethylpropylene amine oxime, iodinated (123-I) pyridyl bezofuran derivatives, etc.	Noninvasive, provides precise differential diagnosis. Presymptomatic diagnosis of β-amyliodosis is possible	Less expensive and routinely used for diagnosis	Tiwari et al. (2015), Cheng et al. (2012)
Voxel-Based Morphometry (VBM)	Based on imaging structural differences at every voxel of brain	Possible to quantify brain atrophy in AD		Herholz (2003)
Magnetic resonance imaging (MRI)	In this technique, relaxation patterns of excited protons inside human body due to radiofrequency waves are converted into magnetic resonance image by Fourier transformation. Higher proton density produces stronger FID response signals	Shows high accuracy in AD diagnosis. Detects cerebral volume decrease and hemodynamic changes with high sensitivity and accuracy	Inefficient to detect early onset of dementia in AD	De Carli et al. (1995), Killiany et al. (2002)
In vitro techniques				
Solution state NMR	3D structures of amyloidogenic peptide and monomers studied by using ^{1}H, ^{13}C, ^{15}N markers. Transition of Aβ monomer into oligomers detected by	3D structure of Aβ peptide can be studied in detailed manner	Higher concentration of Aβ needed	Zeeb and Balbach (2004), Shao et al. (1999), Mandal et al. (2006)

(continued)

Table 1 (continued)

Name of technique	Principle	Advantage	Limitation	References
	changes in chemical shift, cross peaks, line width, and NOE (nuclear Overhousner effect)			
Solid state NMR	Insoluble Aβ plaques can be studied	Detailed structural study of AB plaque can be possible	Resolution is poor. For better resolution, CP-MAS techniques need to be used simultaneously	Naito et al. (2004), Lansbury et al. (1995)
X-Ray Diffraction Crystallography	Diffraction signals generated by compartments within crystals are converted into 3D electron density maps	Structures of Aβ plaques can be determined by 3D image	Crystals with high quality needed for good results	Rosano et al. (2005), Sunde et al. (1997)
X-Ray Fiber Diffraction	Diffraction signals generated by compartments within crystals are converted into 3D electron density maps	Reduced size crystals or lower quality crystalline samples can be studied		Sunde et al. (1997)
X-Ray Absorption Spectroscopy	Based on the absorption pattern of high energy X-ray beam by heavy atom and ejection of photoelectrons	Helpful to understand role of metals in Aβ aggregation and redox chemistry	Produces only 2D data	Streltsov (2008)
Circular Dichroism Spectroscopy	Measures differential absorption of circularly polarized light by asymmetric centers as function of wavelength	Useful for qualitative determination of Aβ aggregation	Not suitable for quantitative estimation	Greenfield (2006), Harper et al. (1997)
FTIR	A strong absorption band between 1600 and 1700 cm^{-1} denotes presence of proteins, Aβ protein region observed between 1620 and 1680 cm^{-1}	Provides structural information of Aβ		Tiwari et al. (2015)
Transmission Electron Microscopy	Based on principle of image formation due to passing of electrons through electron transparent specimen	Characterization of Aβ plaques, proteins can be performed		Steven and Belnap (2005), Roher et al. (1996)

(continued)

Table 1 (continued)

Name of technique	Principle	Advantage	Limitation	References
Scanning Transmission Electron Microscopy	Emission gun emits 100 KV beam which scans specimen and image formation occurs due to detector which collects scattered electrons from specimen and produces image	More detailed characterization and structural details of Aβ fibrils, α-synuclein can be obtained		Lashuel and Wall (2005)
Congo red staining	Congo red dye binds to Aβ and produces intense red color. Binding occurs due to interaction between negatively charged sulfonyl group of dye with positively charged N terminals of peptides	Used widely for identification of Aβ in brain slices or tissues		Elghetany and Saleem (1998), Klunk et al. (1989)
Thioflavin T, Thioflavin S fluorescent staining	Thioflavin T,S binds to Aβ structures and produce intense fluorescence	Used to characterize Aβ aggregates		Khurana et al. (2005), Ban and Goto (2006)

highly advantageous for the diagnosis of Alzheimer's disease in the early presymptomatic stage. Other techniques help to study structural changes in the brain during disease progression. In the case of transgenic models and chemically induced animal models for Alzheimer's disease, these techniques help to observe pathogenesis, monitoring of gradual hallmark symptoms development, and their spread in various regions of the brain without scarifying animal.

4 In Vivo and Ex Vivo Animal Models for Alzheimer's Disease

4.1 In Vivo Models

For more understanding of the pathology of Alzheimer's disease, an animal study is an essential resource to test hypotheses practically for the development of prominent therapy. Both vertebrate and invertebrate animals are used by researchers to study the neurodegeneration process closely (Li et al. 2015). The most commonly used

animals are rodents (mice, mouse, rats, both natural as well as transgenic) since they are less expensive, readily available, and easy to handle. The pattern of AD hallmark symptoms development in rodents is nearly similar to humans (LaFerla and Green 2012). Other than rodents, dogs, chicken, zebrafish, rabbits, lemur, cat, primates, Drosophila, guinea pigs, etc. are also preferred as experimental models of Alzheimer's disease (Mullane and Williams 2019).

4.1.1 In Vivo Transgenic Animal Models

As we have seen in Fig. 1, abnormal function of gene proteins like APP, PS1, PS2, β and γ secretase, GK-3β, MAP kinase, etc. mediates the process of extracellular accumulation of insoluble β amyloid plaques and intracellular NFT formations. Researchers developed transgenic models by either injecting or deleting one or more of these genes from rodent genetic material (Knock-In/Knock out) to induce hallmark symptoms of Alzheimer's disease in rodent brains. According to www.alzforum.org https://www.alzforum.org/research-models database, about 198 transgenic rodent models have been developed by researchers for Alzheimer's disease study. Few of them are shown in Table 2. Transgenic models developed in the last 5 years have been discussed in this table. 5 × FAD (C57BL6) model expresses APP (SwedishK670N/M671L, London V71V71, Florida 1716v) and PSEN1 (M146L, L286V) with a total of 5 Alzheimer's disease-linked mutations (Jawhar et al. 2012; Richard et al. 2015). This model shows abundant amyloid accumulation earliest at 2 months age, cognitive skill impairments between 3 and 6 months of age, and neuronal loss at 12 months age. Neurofibrillary tangles were absent. This model is highly suitable to study drug development by applying the β amyloid cascade hypothesis. AD-BXD transgenic mouse models are designed to study genetic factors or heredity and interaction between environmental factors and genetic factors inducing Alzheimer's disease development (Neuner et al. 2019). APOE2 floxed (CureAz) Knock-In models help to investigate the hypothesis of the APOE gene role in the production of insoluble toxic β amyloid plaques (Huynh et al. 2019). BACE1 cKO (Hu Yan) × FAD model is a knock out model in which BACE1 (β-secretase) is deleted from the 5 × FAD mice model to study the influence of BACE1 on Beta-amyloid accumulation in 5 × FAD mice brain (Hu et al. 2018). This model has shown a lesser degree of β amyloid, astrocytes accumulation than the 5 × FAD models that support the hypothesis of β-secretase. It has been reported that TREM2 gene mutation leads to Nasu Hakola disease, an autosomal-recessive disorder characterized by bone fracture and early onset of frontotemporal dementia. TREM2, humanized (common variant) × 5 × FAD models help to study the relationship between the TREM2 gene and Alzheimer's disease (Song et al. 2017; Leyns et al. 2017).

Along with transgenic mice, transgenic rat models also developed since rats are more advantageous than mice in case of well-characterized behavior and sharp cognitive skills and genetically and morphologically more similar to the human brain than mice. Thus, several Knock-In and Knock-out models with APP, MAPT,

Table 2 Selective latest transgenic mouse/rat models

Name of model	Gene	Phenotype characterization	Cognitive deficits	References
Mouse Model				
5 × FAD (C57BL6)	APP, PSEN1, Mouse Thy 1 Promoter	Amyloid plaques in hippocampus, cortex, thalamus, spinal cord, no tangles observed, approximate 40% neuronal loss observed	Spatial working memory impairment develops between 3 and 6 months	Jawhar et al. (2012)[a], Richard et al. (2015)[a]
AD-BXD	APP, PSEN1	Develops amyloid plaques, no tangles formation, no neuronal loss observed, no synaptic loss	Cognitive impairments develops at 14 months	Neuner et al. (2019)[a]
APOE2 floxed (CureAz), APOE3 floxed, APOE4 floxed Knock-in	APOE	APOE2 model shown twofold higher APOE level in brain and serum than APOE3 and APOE4, higher Aβ42 level than remaining two models	Immunoreactivity observed at 4 month old	Huynh et al. (2019)[a]
APP NL-F Knock in	APP	β amyloid plaques observed, tangles absent but elevated phosphorylated tau accumulation around plaques observed	Cognitive impairments observed at 18 months by Y maze test, while by Morris water no signs observed at 18 months	Saito et al. (2014)[a]
APP NL-G-F/ MAPT double knock in	APP, MAPT	Plaques observed at 2 months, NFT absent, no neuronal loss, gliosis observed	Working memory impairments observed in Y maze at 12 months age	Saito et al. (2019)[a], Hashimoto et al. (2019)[a]
APP/PS1/ rTg21221	APP, PSEN1, MAPT	β amyloid plaques observed in cortex region between 8 and 10 months age, NFT absent, neuronal loss present, gliosis observed, decreased synaptic density observed	Cognitive impairments developed lately	Jackson et al. (2016)[a]
BACE1 cKO (Hu Yan) × FAD	Bace 1, APP, PSEN1	β amyloid plaques observed up to 4 month age, NTF absent, neuronal loss absent, gliosis in lesser amount	Cognitive impairments observed lately	Hu et al. (2018)[a]

(continued)

Table 2 (continued)

Name of model	Gene	Phenotype characterization	Cognitive deficits	References
hTau-A152T	MAPT	β amyloid plaques absent, abnormal accumulation of soluble tau observed, tangles absent, neuronal loss in hippocampus at 20 months age, astrocytosis observed, no changes in synaptic density up to 20 months	Cognitive impairments in Morris water maize test observed at 17 months old age, working memory impairments at 10–14 months	Maeda et al. (2016)[a]
MAPT knock-in	MAPT	No amyloid plaques, no NFT, no neuronal loss, no gliosis	Shown same results of Y maze working memory test as wild-type mouse model at 12 months age	Saito et al. (2019)[a], Hashimoto et al. (2019)[a]
TREM2, humanized (common variant) × 5 × FAD	TREM 2, APP, PSEN1	Plaques observed at 8.5 month old age, microgliosis observed at 8.5 months, no NFT observed, no neuronal loss observed, no synaptic loss	No cognitive impairments observed up to 10 months	Song et al. (2017)[a]
TREM2 KO (colonna) × PS19	Trem 2, MAPT	Microgliosis and astrogliosis observed at the age of 9 months	No cognitive impairments observed up to 10 months	Leyns et al. (2017)[a]
Rat models				
AAV-AD	APP, PSEN1	β amyloid plaques and cerebral amyloid angiopathy observed post 30 months of induction, tangles observed, no neuronal loss, deficits of LTP as Schaffer collateral-CA1 synapse at 8 months old	Memory impairment observed in memory test post 3 months of injection	Audrain et al. (2018)[a]
APP + PS1	APP, PSEN1	Abundant plaques in hippocampus and subiculum, scattered plaques in cortex at age of 18+ months, neuronal loss due to necrosis in hippocampus and cortex, gliosis not observed, no tangles observed	Cognitive impairments observed at age of 10 months	Agca et al. (2016)[a], Klakotskaia et al. (2018)[a]

(continued)

Table 2 (continued)

Name of model	Gene	Phenotype characterization	Cognitive deficits	References
SHR24	MAPT	Amyloid plaques absent, Abundant neurofibrillary plaques in cortex region observed, synaptic loss found in animals near to death	Sensory motor deficits and abnormal reflexes observed at age of 3.5 months	Filipcik et al. (2012)[a], Valachova et al. (2018)[a]

[a] Table was prepared from data base, www.Alzforum.org and reference articles. Only selected latest models included in database have been presented in table

PSEN1 with single or multiple mutated genes for β amyloid aggregation study (APP, PSEN1 mutated gene) and Tau hyperphosphorylation (mutated MAPT gene) have been developed. According to the www.Alzforum.org database, more than 13 transgenic rat models have been reported in the literature (Audrain et al. 2018; Agca et al. 2016; Klakotskaia et al. 2018; Filipcik et al. 2012; Valachova et al. 2018). However, we can't completely rely on transgenic models regarding any conclusion of Alzheimer's disease study since the pattern of Alzheimer's disease development in transgenic mice may be different than humans. Hence, many drugs which have shown positive results in transgenic models failed in phase 3 clinical trials (Li et al. 2015).

4.1.2 In Vivo Non-transgenic Animal Models

This category involves animals in which senile dementia or Alzheimer's disease occurs naturally due to age/genetic or environmental factors (Mullane and Williams 2019; LaFerla and Green 2012). These animal models serve as the best tools to study the natural pathophysiology of Alzheimer's disease since they are not chemically or genetically manipulated. But there is less certainty with these models regarding the development of desired Alzheimer's disease symptoms as well as cognitive impairment since in the case of many natural animal models it is observed with no cognitive impairment or behavioral changes despite the accumulation of β amyloid plaques (Aizenstein et al. 2008). Hence, for target-specific studies, transgenic models are mostly preferred by researchers for getting desirable phenotype (Mullane and Williams 2019).

4.1.3 In Vivo Chemically Induced Animal Models

These kinds of animal models are preferred for investigation of the effect of a particular neurotransmitter on cognitive impairment and behavior or to test the

cholinergic hypothesis (Li et al. 2015). In this category, neurotoxicity is induced in animals by intracranial injections of specific chemicals like Acetylcholine inhibitor Scopolamine (Winslow and Camacho 1995) to study the influence of acetylcholine inhibition on cognitive skills and pathological changes, Okadaic acid to induce tau phosphorylation (Kamat et al. 2013), Sodium azide for mitochondrial dysfunction (Zhang et al. 2013), L-methionine for NMDA receptors hyperactivity (Sain), β amyloid (1-42) to induce amyloid cascade (Gouras et al. 2015), heavy metals like Aluminium, lead, mercury for enhancement of reactive oxygen species, etc. (Bonda et al. 2011).

4.1.4 Ex Vivo Animal Models

Ex-vivo animal models comprise of isolation of specific anatomical sections from a diseased brain or healthy brain and preserving them in a similar physiological milieu. This method is also called the brain slice technique (Cho et al. 2007). This technique is applied widely by researchers for many years because of its advantages. This model provides an opportunity to study according to site selectivity and physiological milieu in the living brain. Slices can be isolated from the desired sights of the brain as per the need of the experiment (Li et al. 2015). One can perform multiple experiments by obtaining different anatomical areas through brain slicing. In this way, this model serves as an ideal model for multiple drug screening. The impact of drug molecules or phytoconstituents on insoluble beta-amyloid plaques, neurofibrillary tangles induced by injecting neurotoxic chemicals can be studied in a more precise manner. For testing a novel hypothesis, this model is the best choice. Brai et al. (2017) successfully tested a novel hypothesis of the role of interaction between α7-nAchR and cleaved peptide of Acetylcholinesterase enzyme in the pathogenesis of Alzheimer's disease by using brain slice technique. Despite these advantages, maintenance of the integrity of sliced brain tissues and error in the thickness of section can hamper study output (Li et al. 2015; Brai et al. 2017; Jang et al. 2018).

5 In Vitro Animal Models

In vivo models are insufficient to study Alzheimer's disease at the cellular or molecular level. An in vitro model provides a good opportunity to study neurodegeneration pathway in a detailed manner. The effect of a phytoconstituent or a synthetic drug molecule can be studied at the neuron level by manipulating pathways for beta-amyloid cascade generation. With technical advances, various potential in vitro models have been developed.

5.1 Cell Lines

5.1.1 Primary Rat and Mouse Hippocampus Neurons

Primary neuron cultures are developed from pyramidal or principle neurons obtained from the late-stage embryo of transgenic models of rats/mice. Primary rat/mouse neurons in the hippocampus region have dense and extended networks similar to human neurons, thus both are considered phenotypically the same. Because of its simplicity and cost-effectiveness, this model is mostly used by researchers. This model provides feasibility to study the beta-amyloid aggregation pathway, the effect of drug molecules, or phytochemicals on beta-amyloid plaque formation pathways. Generally, fluorescence technique or MTT assays are used to study the viability of cells (Kaech and Banker 2006).

5.1.2 Primary Chicken Neurons

These are developed by isolating principal neurons from post-fertilized early chick embryo brain. Primary chicken neurons secrete endogenous β amyloid peptides. The sequence of both human and chicken beta-amyloid is identical hence preferred for in vitro analysis. This model is more advantageous for the study of developmental stages of neurons than primary rat/mouse hippocampus neurons (Kumar and Mallick 2016; Andermatt and Stoeckli 2014).

5.1.3 SH-SY5Y Cell Line

This cell line is three times subcloned and developed from SK-N-SH neuroblastoma cells isolated from 4-year-old child bone marrow (Kovalevich and Langford 2013). It is widely used as a model for neurodegenerative disorders as these cells can be transformed into any functional neurons by manipulations. Several advantages of this model include—large expansion of cells before division, expression of all human-specific proteins which are absent in rodent cell lines, less expensive than primary neurons, and ethical procedures not required. However, they are not suitable for Alzheimer's disease study since they are dopaminergic neurons in nature and produce a negligible amount of Beta-amyloid and tau fibrils (Xicoy et al. 2017). For Alzheimer's disease study, this cell line is treated with an APP mutated gene to develop a significant amount of β amyloid plaques (Stockburger et al. 2014). This cell line is highly suitable for Parkinson's disease.

5.1.4 Human Glioblastoma Cell Line H4-sw

Glioblastoma cells are isolated from grade IV neuroglia aggressive tumors (Lathia et al. 2015). When these cells are manipulated with the APP gene, they start to produce a high amount of toxic β amyloid accumulations and tau fibrils (Shin et al.

2010). Human glioblastoma Cell line H4-sw is produced by combining APP695—Swedish mutated gene which produces a high quantity of Aβ1-40 and Aβ1-42. This model is highly suitable to test the β amyloid cascade hypothesis for Alzheimer's disease than the 5H-SY5Y cell line (Sung et al. 2014).

5.1.5 ARPE-19 Cells (Human Retina Pigment Epithelial Cells)

Human Retinal Pigment Epithelial Cells (ARPE-19 Cells) express αβ-Crystallin, a small heat shock protein that plays an important role in inhibiting β amyloid protein aggregation in the retina (Bakthisaran et al. 2015). It is reported that overexpression of αβ-Crystallin is related to neurodegenerative diseases like Parkinson's disease and Alzheimer's disease (Bhat et al. 2009). During Alzheimer's disease studies on transgenic models, finding of β amyloid plaque deposition in retinas was reported due to which eyesight of the transgenic model was found hampered. While in the case of natural models, such deposition was absent. Thus, it can be considered that neuroinflammation due to overactivity of microglia cells to clear excess of β amyloid may be responsible for the degeneration of RPE. It is now considered as additional symptoms by many researchers apart from hallmark symptoms of Alzheimer's disease. Thus, ARPE-19 Cell lines are used to study to elucidate the mystery behind β amyloid deposition in RPE and Alzheimer's disease that can provide a new insight for targeted drug development (Dong et al. 2018).

5.1.6 Disease-Specific Induced Pluripotent Stem Cells (iPSC)

Stem cells offer an opportunity to develop an undifferentiated cell into specific cell-like neurons or brain cells. For a further detailed study of Alzheimer's disease, researchers have developed disease-specific induced pluripotent stem cells by using fibroblasts of patients having the disease. Neural stem cells from Alzheimer's disease patients having mutations in genes of presenilin 1 and presenilin 2 have been isolated and developed as a model to study drug therapy. This model showed significant enhancement of Aβ42 that supports the hypothesis. Similarly, neural stem cells for GK-38, APOE-2, etc. have also been isolated from Alzheimer's disease patients and developed into models. In this way, this model is highly helpful for testing hypotheses, develop drug therapies, and more understanding of pathological events in Alzheimer's disease (Li et al. 2015; Mullane and Williams 2019).

6 In Vitro Enzyme Inhibition Assays for Alzheimer's Disease Study

In many preclinical trials, it is observed that β amyloid may induce neurotoxicity through stimulation of intracellular reactive oxygen species, lipid peroxidation, and neuroinflammation. Many phytochemicals have shown positive effects on β amyloid

plaque clearance or inhibition through antioxidant activities that support this hypothesis. Thus, in vitro enzyme assays provide valuable insight regarding antioxidant and β amyloid inhibition potential of phytochemicals or drug molecules. Along with in vivo and cell line studies, the in vitro enzymes inhibition assay is a rational step for gathering more knowledge about drug moiety's potency against Alzheimer's disease.

6.1 Acetyl Cholinesterase Inhibition Assay

The simple colorimetric method proposed by Ellman et al. (1961) is widely followed by many researchers in the twentieth century. This method is based on the measurement of the intensity of yellow color produced when thiocholine reacts with dithiobisnitrobenzoate ion. High intensity indicates a high quantity of acetylcholine, while lower intensity value indicates inhibition of acetylcholine. This method is widely accepted due to its simplicity, cost-effectiveness, sensitivity, and low amount of sample required and can be used for brain tissue homogenates and blood samples. The protocol by Ellman et al. (1961) is as: 3 mL of 0.1 M phosphate buffer pH 8.0, 20 μL brain homogenate as substrate, 100 μL Dithiobisnitrobenzoic acid (DTNB) reagent, and 50 μL Acetylthiocholine iodide (ATCI) mixed and absorbance to be read at 405 nm after 5 min incubation of temperature. A calibration curve should be drawn of values obtained by absorbance for quantitative estimation (Ellman et al. 1961).

6.2 Catalase Assay (CAT)

This assay demonstrates the amount of peroxide present in tissue homogenates with the help of a UV spectrophotometer. This is based on the principle of measurement of the intensity of color produced due to oxidation products present in tissue homogenate; greater absorbance indicates a high amount of oxidation products present. This assay is widely used to determine the antioxidant properties of phytochemicals. This method is simple, reproducible, and cost-effective. The protocol is as follows: The reaction mixture contains 2.5 mL of 50 mmol phosphate buffer pH 5, 0.4 mL 0f 5.9 mmol hydrogen peroxide as control, and 0.1 mL tissue homogenate. Absorbance should be recorded at 240 nm after 1 min of mixing all components of the protocol (Khan and Khan 2012).

6.3 Superoxide Dismutase Assay (SOD)

This assay is based on the principle of quantification of superoxide anion radicals generated by the oxidation process in tissue homogenate. The absorbance is

measured by using either a spectrophotometer or a colorimeter. Superoxide dismutase is an enzyme that protects cells and tissues from harmful superoxide radicals. There are two protocols which are widely followed by researchers according to experimental need. The first protocol is proposed by Fridovich et al. in 1969 (McCord and Fridovich 1969). According to this protocol, Xanthine and Xanthine oxidase is used to generate superoxide anion radicals in tissue homogenate. Generated superoxide anion radicals react with 2-(4-iodophenyl)-3-(-4 nitrophenol)-5-phenyltetrazolium chloride to form red formazan dye. Quantification of red formazan dye is performed by using a spectrophotometer at 560 nm. The second one is the modified SOD assay by Kakkar et al. (1984). As per the protocol, the assay mixture containing sodium pyrophosphate buffer pH 8.3, Phenazine methosulphate, Nitroblue tetrazolium, and SOD enzyme should be diluted in proper concentration. The reaction starts with the addition of NADH. The incubation period is 90 s at 30 °C. The reaction is stopped by glacial acetic acid addition and the reaction mixture is extracted with *n* butanol. After 10 min, butanol containing reaction mixture is centrifuged and the supernatant is subjected to measure absorbance at 560 nm by a spectrophotometer. During the incubation period, the dismutase enzyme inhibits the NADH-phenazine methosulphate and the TNB reduction process and color develop. Color intensity is directly proportional to the quantity of enzyme. In this way, both protocols are reproducible and widely used for the study of the antioxidant activity of phytochemicals and drug molecules (Assady et al. 2011).

6.4 *Glutathione-S-Transferase Assay*

Glutathione *S* transferase performs cell-protective functions. In Alzheimer's disease, the GST level decreases in the hippocampus, cortex region of the diseased brain. This assay is helpful to study the potential of phytochemicals or synthetic drug molecules to restore the Glutathione *S* transferase level in homogenized brain tissue. The assay was first introduced by Habig et al. in 1974 that later got modified by various researchers. In this assay, Glutathione *S* transferase mediates reaction between L glutathione and 1-chloro 2,4-dinitrobenzene (CDNB) which results in the formation of Glutathione-DNB conjugate which absorbs wavelength at 340 nm. The rate of increase in absorbance is directly proportional to GST activity in tissue homogenate (Habig et al. 1974).

6.5 *Glutathione Reductase Assay*

Glutathione (GSH) is abundantly present in the mitochondrial matrix and exerts the function of maintaining the GSH/GSSG ratio by reducing excess GSSG to GSH that plays a key role in antioxidant activity inside the cell. However, in the case of

Alzheimer's disease, it is observed that the level of Glutathione reductase decreases with age in the cortex that leads to an imbalance of the GSH/GSSG ratio. Excess Glutathione disulfide GSSG causes oxidative stress due to peroxidation that finally results in neurodegeneration (Ansari and Scheff 2010). Apart from age, other causative factors for the reduction of GSH levels are under investigation. Initially, this assessment method was practiced by Mannervick et al. in 1975 after which it was modified several times (Carlberg and Mannervik 1975). In this colorimetric assay, Glutathione reductase reduces GSSG to GSH. Later GSH reacts with 5-5′--dithiobis(2-nitrobenzoic acid) (DTNB) and forms yellow-colored chromophore with GS-TNB. GS-TNB again gets reduced to GSH. The absorbance of the chromophore is measured at 415 nm for quantification of GSH. In another method, Glutathione reductase catalyzes the reduction of GSSG in the presence of NADPH, which gets oxidized to NADP+. The reduction of GSSG causes a decrease in absorption and is measured at 340 nm. In this way, both methods can be employed for assay (Carlberg and Mannervik 1975).

6.6 Lipid Peroxidation (TBARS) Assay

The brain is mainly composed of lipids. Hence, it is highly prone to lipid peroxidation due to free radicals. The study has established a close relationship between AD pathogenesis and lipid peroxidation (Bradley-Whitman and Lovell 2015). Hence, it is a key aspect to be investigated while developing drug therapy. This protocol consists of a reaction mixture of phosphate buffer, the sample of brain tissue homogenate, Ascorbic acid, and Ferric chloride. The reaction mixture incubation period is 1 h at body temperature. The reaction is seized by the addition of 10% Trichloroacetic acid (TCA) followed by Thiobarbituric Acid and boiling for 20 min in a water bath. After the boiling reaction, mixtures are shifted to an ice bath for 10 min and centrifuged. The amount of TBARS formed is measured at 535 nm by using a spectrophotometer (Khan and Khan 2012).

6.7 BACE (β and γ-Secretase) Inhibition Assay

β and γ-secretase peptide inhibition significantly decreases or inhibits β amyloid accumulation (Bradley-Whitman and Lovell 2015; Pietrak et al. 2005). It has become a proven hypothesis through transgenic mice/rat models (Jawhar et al. 2012). Hence, today it is a primary therapeutic target for drug development. It has become a convenient tool for screening of phytochemicals and drug molecules as BACE inhibitors. In this assay, a synthetic mutant APP peptide linked with a fluorophore at one end and a quenching agent at another end is used. When this APP peptide is cleaved by BACE, the bright fluorophore can be easily analyzed with the help of a fluorometer with excitation wavelengths 335–345 nm and emission

wavelengths of 485–510 nm. This novel assay has become a popular tool for screening of several phytochemicals and synthetic drugs (Lee and Byun 2018; Ben Halima et al. 2016).

6.8 β Amyloid Aggregation Inhibition Assay

β amyloid aggregation is a hallmark symptom of Alzheimer's disease as well as a prime target for drug development. It is widely studied with many in vivo transgenic/wild animal models. But researchers have developed novel assays to study β amyloid aggregation in vitro. Guo et al. (2010) developed two simple in vitro assays to evaluate the relative potency of drug molecules (Guo et al. 2010). One assay is based on the principle of binding fluorescence-tagged Aβ1-42 to synthetic Aβ1-42 kept in wells of fluorescent black wall microplates. A solution containing fluorescence-tagged Aβ1-42 added with or without inhibitor or drug candidate added to the plates and fluorescence is measured. Another assay utilizes brain tissue isolated from Alzheimer's disease transgenic mouse brain. A solution containing fluorescence-tagged Aβ1-42 added along with or without drug candidate added over the tissue and binding mechanism is observed through a fluorescence microscope. Lee et al. (2009) developed an engineered assay for screening of β amyloid aggregation. In this assay, they used genetically engineered *E. coli* co-expressing cutinase and ssTorA-Aβ42-Bla. This assay depends on the hypothesis that tiny cell-permeable molecules capable of interfering with wild-type Aβ1-42 aggregation would promote Tat-dependent export of ssTorA-Aβ42-Bla reporter to the periplasm that can act as Aβ1-42 inhibitors. The mechanism is identified with the help of fluorescence (Lee et al. 2009).

7 Behavioral Study of Animal Models

Cognitive skill impairments in association with behavioral changes are predominant symptoms noticed during the diagnosis of Senile dementia in Alzheimer's disease. Thus, at the time of investigation of Alzheimer's disease in animal models, along with β amyloid insoluble oligomers and neurofibrillary tangle formations, behavioral aspects study is a rational part. The hippocampus and cortex region of the brain is full of a dense network of neurons and also the center of cognitive skills in mammals. In Alzheimer's disease, because of neurodegeneration, a dense network becomes thin that directly hampers the cognitive skills and behavior of a person or mammal. While developing a transgenic animal model this aspect is taken into consideration and to check whether the developed model is mimicking the phenotype of Alzheimer's disease in the human brain, the behavioral assay is important. In many transgenic rodent models even if pathological symptoms appear, there were no signs of behavioral changes or cognitive impairments. Transgenic models may

fail to mimic the human brain in AD. Hence, behavioral study is an integral part of Alzheimer's disease study for drug development.

In behavioral tests, spatial memory related to the operational task and contextual memory related to specific responses to environmental changes by transgenic animal models are studied. For spatial memory tasks, Morris Water Maze (MWM), Radial Arm Maze (RAM), and Radial Arm Water Maze (RAWM) models are used (Bryan et al. 2009). The Radial Arm Water Maze model is a combination of the previous two models and designed by eliminating lacunas in those two models. In Radial Arm Water Maze, the submerged platform is located in one of the several arms that make the animal use his spatial as well as working memory to reach up to the platform (Vloeberghs et al. 2006). Working memory models include models based on cognitive behavior analysis. Y maze model is a popular model to study the sustained cognition of mice (Jackson 1943). This model is used to measure the degree of nonrepetitive arm entries of mice. T-Maze model is suitable for both mice and rats (Deacon and Rawlins 2006). This model is used to analyze the spontaneous alteration tendency of rodents. An object recognition test is used to analyze the natural tendency to differentiate between the novel and familiar objects (Janus et al. 2000; Shukitt-Hale et al. 2001). An open field locomotion test is useful to analyze motor behaviors like crossing lines, fecal movements, anxiety, etc. of rodents (Hall 1934). Contextual memory models are based on response analysis given by rodents to a sudden change in the environment around them like fear conditioning and passive avoidance (Fanselow 1980). In fear conditioning, an animal placed in a box is exposed to two different stimuli to measure freezing response (Fanselow 1980; Hamann et al. 2002). While in passive avoidance learning, the ability to avoid mild aversions by an animal is studied (Senechal et al. 2008). In this way, behavioral tests provide knowledge about every aspect of cognitive and behavioral changes in Alzheimer's disease in transgenic models.

8 Current Status of Clinical Trials for Alzheimer's Disease Drug Therapy

Apart from FDA-approved acetylcholinesterase inhibitors and NMDA receptor modulators, many clinical trials failed based on targeting phenotypes β amyloid plaques and neurofibrillary tangles. As a result, in 2019, it is observed that clinical trials based on the β amyloid hypothesis lowered significantly. Now researchers are focusing on symptomatic targets and Behavioral Psychological Symptoms of Dementia (BPSD). Based on these alternate hypotheses, recently few candidates have cleared clinical trials and waiting for the further process (Huang et al. 2020). A ketosis inducer and symptomatic cognitive enhancer, AC-1204, competed for the clinical trial successfully, and the supported hypothesis of ketosis plays important role in β amyloid oligomer clearance (Huang et al. 2020). Idalopirdine 5-HT6 receptor antagonist that targets symptomatic cognitive behavior cleared the clinical

trial phase and supported serotonergic receptor modulation and can enhance cognitive skills in Alzheimer's disease (Huang et al. 2020). Insulin as a metabolism regulator (Huang et al. 2020), Suvorexant (MK-4305) an Orexin antagonist (Herring et al. 2019) which targets Behavioral psychological symptomatic dementia, Nabilone-Cannabinoid receptors 1 and 2 agonists (Huang et al. 2020), TRx0237 (LMTX) Tau stabilizer and aggregation inhibitor (Cummings et al. 2019), and Cholecalciferol-membrane-based receptor agonist have cleared clinical trials successfully and next steps are awaited (Huang et al. 2020). Last year, GV-971 (Sodium Oligomannurarate) was approved by the China National Medical Product Administration (NMPA) for the treatment of mild to moderate Alzheimer's disease (GreenValleypharma). This drug enhances β amyloid clearance by binding to multiple sites of amyloid and inhibits aggregation (Wang et al. 2019). Thus, outcomes of these nonamyloid clinical trials show that drugs acting on symptomatic targets are promising to slow down the progression of Alzheimer's disease and cross the hurdle of clinical trials.

9 Conclusion

Modifications in methods and techniques like In vivo, Ex vivo animal models, Brain imaging techniques, and Behavioral studies of experimental transgenic and non-transgenic rodent models helped researchers to study the pathology of Alzheimer's disease in a more detailed manner and design drug therapy accordingly. As a result, many symptomatic targets like neurotransmitter inhibitors that play important role in amyloid aggregation and neurofibrillary tangle formations in abnormal conditions came into limelight. These tools and techniques also helped to realize that, only targeting the traditional phenotype of Alzheimer's disease is not sufficient to generate strong drug therapy. It's a need to explore extensively other targets like neuroinflammation, oxidative stress, and genetic mutations causing factors. The research trend is now focused on studying the effects of drug therapies on presymptomatic Alzheimer's disease rather than late-onset of dementia in Alzheimer's disease. As a result, many drug molecules are clearing clinical trial phases. Still, more disease-specific drug therapy is lacking that needs more effort in the future.

Highlights

- Identification of early onset of dementia by using modified techniques and tools may be helpful for development of successful drug therapy based on β amyloid hypothesis
- In vivo imaging tools are highly advantageous for detection of early onset of dementia
- Ex vivo animal models or brain slicing technique serves as ideal for multiple drug screening

- In transgenic models, β amyloid plaque deposition in retinas leads to eyesight impairment
- Overexpression of αβ-Crystallin is related to neurodegenerative diseases like Parkinson's disease and Alzheimer's disease

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Dual Specificity Tyrosine Phosphorylation-Regulated Kinase 1A (DYRK1A) Inhibitors: The Quest for a Disease-Modifying Treatment for Alzheimer's Disease

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Abstract Alzheimer's disease (AD) is a multifactor disease that is a most familiar form of dementia. Neurofibrillary tangles (NFTs) and amyloid-β (Aβ) are the neuropathological indicators of Alzheimer's disease. DYRK1A is involved in both NFTs and Aβ-governed neurodegenerative processes of AD. The DYRK1A-mediated tau phosphorylation is also competent in facilitating additional phosphorylation of tau protein by GSK-3β and leads to an increased deposition of NFTs. The role of aberrant DYRK1A has been found in facilitating the accumulation of *p*-tau protein and in the formation of neurotoxic amyloid plaques, therefore making DYRK1A a fascinating disease-modifying target for AD treatment due to its multifunctional role, i.e., serine/threonine and tyrosine kinase activities, self-phosphorylating activity, neuronal proliferation, and neurogenesis, tau phosphorylation, etc.

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At present, no disease-modifying approach or strategy is altering the progression of AD available; the current FDA-approved drugs provide only symptomatic relief. Different DYRK1A inhibitors have been reported in past years. These inhibitors have initially been successful to some extent in developing disease-modifying approaches for the treatment of diseases. Recent findings reveal that the focus of clinical trials in AD for the last 25 years has revolved around AD's Aβ hypothesis. They were ineffective in retrieving cognitive function or decelerating cognitive reduction. Therefore, lowering Aβ is an unjustified strategy, and the clinical trials in AD should sharpen the attention on other AD targets comprising pathological forms of tau. Targeting DYRK1A inhibition could therefore represent an intriguing approach toward disease-modifying approach for treatment of AD. This book chapter will focus on various synthetic, natural, and natural-inspired DYRK1A inhibitors as potential therapeutics for the disease-modifying treatment of AD.

Keywords Alzheimer's disease · DYRK1A · DYRK1A inhibitors · Tau phosphorylation

Abbreviations

AD	Alzheimer's disease
APP	Amyloid precursor protein
Aβ	Amyloid β
c-Abl	c-Abelson
CK	Casein kinase
CNS	Central nervous system
DYRK1A	Dual specificity tyrosine phosphorylation-regulated kinase 1A
GSK3	Glycogen synthase kinase
MARK	Microtubule affinity regulating kinase
NFTs	Neurofibrillary tangles
PhK	Phosphorylase kinase
PKB	Protein kinase B
PKC	Protein kinase C
PKN	Protein kinase N
SFK	Src family kinase

1 Introduction

Globally, there are roughly 55 million people living with dementia; this figure slowly rises by ten million each year. Around the world, 75% of dementia cases go undiagnosed, reaching 90% in some low- and middle-income nations. In 2050, there will be 139 million individuals living with dementia, up from the predicted 55 million in 2019 (Abubakar et al. 2022). Alzheimer's disease (AD) is a neurodegenerative disease that primarily affects older people and accounts for 60–70% of

dementia cases. AD is distinguished by a set of symptoms, including cognitive impairment and noncognitive dysfunctions. Memory loss, language problems, and executive dysfunction are among the cognitive dysfunctions. Noncognitive dysfunctions include behavioral disorders, psychiatric symptoms, depression, hallucinations, delusion, and agitation (Burns et al. 1990). Although the exact cause of AD is unknown, several processes characterizing its pathophysiology have been identified, including oxidative stress (Su et al. 2008), hyperphosphorylated tau proteins, the amyloid β (Aβ) cascade (Bloom 2014), lack of central cholinergic neurotransmitters (Craig et al. 2011), inflammation (Holmes 2013), and toxic metal ions (Greenough et al. 2013). The key factors contributing to cognitive deterioration and AD course are likely amyloid plaque and tau aggregation. The majority of the amyloid plaque is made up of extraneuronal Aβ deposits. A transmembrane protein called amyloid precursor protein (APP) is connected to axonal transport, neurite outgrowth, and neuronal development (Kang et al. 1987). The α-secretase enzyme cleaves the Aβ peptide sequence region of APP after lys-16, roughly in the center of the protein, and prevents the production of Aβ. β and γ-secretase are two intracellular proteases that sequentially cleave the APP to create the Aβ deposits (Sisodia and St George-Hyslop 2002). Aβ plaque buildup serves as a pathogenic catalyst for a cascade that includes neuritic damage, neurofibrillary tangle development via tau protein, and neuronal dysfunction and cell death in AD (Barage and Sonawane 2015). By stabilizing the microtubule and enabling axonal transit, tau, a microtubule-associated protein, keeps the neuron's structural integrity. The aberrant phosphorylation of the tau protein causes it to aggregate in AD, which causes intracellular neurofibrillary tangles (NFTs) to develop, which worsens cognitive function and causes neuronal death. Tau is phosphorylated at 40 locations in the AD brain, most of which are ser/thr-pro motifs (Kumar et al. 2018a). Studies on the pathophysiology of AD have mostly concentrated on the formation of senile plaques and NFTs by Aβ and tau, as well as how these aberrant structures lead to neurodegeneration and neuronal death. The prevalent idea guiding AD research for nearly 25 years has been the amyloid hypothesis, which states that the buildup and deposition of oligomeric or fibrillar amyloid (Aβ) peptide is the main cause of AD. The development of drugs that specifically target Aβ to treat AD has never succeeded. In light of recent research, tau, not Aβ, is thought to be the primary component behind the onset and progression of AD. As a result, rather than being caused by Aβ amyloid, AD is a condition that is brought on by dysfunction in APP metabolism and proceeds through tau pathology (Kametani and Hasegawa 2018).

Protein phosphorylation is perhaps one of the most significant and well-studied mechanisms by which cells control the amounts of proteins. Signal transmission within and between cells is mostly accomplished by protein phosphorylation (Wilson et al. 2018). Since protein phosphorylation plays a role in almost all physiological processes, aberrant phosphorylation is linked to several human disorders. Inhibiting disease-relevant kinases or regulating their activities is thus a sensible strategy for treating many disorders. Protein kinases have thus become the therapeutic focus for novel drug candidates in the few decades since their first discovery (Ferguson and Gray 2018; Zhang et al. 2021). There are 85 potential

phosphorylation sites in the tau protein, including 26 of them in the acidic area, 26 of them in the proline-rich region, 16 of them in the repeat domain region, and 17 of them at the C-terminal end. Different kinases, such as glycogen synthase kinase (GSK3), casein kinase (CK1/2), microtubule affinity regulating kinase (MARK1-4), protein kinase C (PKC), phosphorylase kinase (PhK), protein kinase N (PKN), dual specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A/2), protein kinase B (PKB), Src family kinase (SFK), or c-Abelson (c-Abl), phosphorylate tau at various sites. Although some kinases, such as GSK3 and CK1/2, have a greater impact on tau phosphorylation sites than others, this does not negate the qualitative significance of each kinase in controlling tau phosphorylation (Martin et al. 2013).

2 DYRK-1A

The DYRK family of proteins, a subgroup of the evolutionarily conserved CMGC protein kinase superfamily, contains the dual specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A). Four other mammalian subtypes, DYRK1B, DYRK2, DYRK3, and DYRK4, are part of the DYRK family. Although highly conserved across species, DYRK proteins have little sequence homology with other kinases outside of their catalytic regions (Becker et al. 1998). It carries both serine/threonine and tyrosine kinase activities (Rammohan et al. 2022). The crystal structure of DYRK-1A revealed that it contains an N-terminal lobe with five antiparallel β-strands and a conserved regulatory αC-helix, as well as a bigger C-terminal made up of α-helices. An essential component of the ATP pocket, the hinge region, connects the N- and C terminals of protein. The gatekeeper residue is present in the ATP-binding pocket of the enzyme, and it is responsible for the selective inhibition of the DYRK1A enzyme (Alexeeva et al. 2015; Rothweiler et al. 2016). DYRK1A is 89.4% identical to DYRK-1B, sharing 95.6% resemblance in their kinase domains. No resemblance has been observed in the C-terminal domain of DYRK1A and DYRK1B. The functional specificity of each DYRK family member likely arises from the distinct C-terminal domain of that member (Arbones et al. 2019). DYRK1A possesses self-phosphorylating activity and phosphorylates various targets involved in numerous signaling pathways (Soundararajan et al. 2013). On tyrosine, serine, and threonine residues, DYRK1A autophosphorylates, although only serine and threonine residues are used by the protein to phosphorylate its substrates. The complete catalytic activity of DYRK1A depends on the autophosphorylation of Tyr 312/321 in the activation loop of the enzyme (Himpel et al. 2001) According to a comparative investigation of inhibitors, tyrosine autophosphorylation in the activation loop during DYRK1A autoactivation stabilizes a conformation of the catalytic domain with increased serine/threonine kinase activity without impeding tyrosine phosphorylation (Walte et al. 2013). Nearly 80% of the DYRK1A protein is discovered associated with the cytoskeletal fraction in the human and mouse brain, with the remaining 20% identified in the cytosolic and nuclear fractions (Kaczmarski et al. 2014). DYRK1A is expressed in a number of

brain areas of the adult mouse, both in the cytoplasm and the nucleus. Depending on where it is located inside the cell, DYRK1A phosphorylates several targets. There are more than 20 proteins that DYRK1A can bind to, namely cytoskeletal targets (like Tau and MAP1B), transcriptional regulators, splicing regulators, random proteins (like GS, Notch), and synaptic proteins; these targets could facilitate DYRK1A's function in neural synaptic plasticity (Martí et al. 2003; Murakami et al. 2009, 2012). Central nervous system (CNS) exhibits higher reactivity in DYRK1A-expressing regions while being widely expressed throughout rat development according to its RNA expression profile (Okui et al. 1999). In addition to being present in the cytoplasm and nuclei of different types of neurons in the human brain, DYRK1A is also present in astrocytes, ependymal, and endothelial cells. The location of DYRK1A in these cells suggests that DYRK1A trafficking and usage are cell type- and brain structure-specific (Wegiel et al. 2004). DYRK1A is mostly located in the expanding dendrites and increasing neuropil of differentiating neurons (pyramidal neocortical neurons) (Hämmerle et al. 2003). The DYRK1A protein is found in cycling neurogenic progenitors and developed neurons, and expression of the DYRK1A gene in the developing CNS displays a dynamic spatiotemporal pattern (Hämmerle et al. 2008). DYRK1A is among the 33 genes present in (Down Syndrome chromosomal region) DCR which have been found to be overexpressed in Down Syndrome (DS) (Dowjat et al. 2007). The developmental deviations and age-related pathologies seen in DS are influenced by the overexpression of DYRK1A. There is strong genetic and pharmacological evidences that the majority of cognitive abnormalities seen in DS patients are caused by just 1.5-fold overexpression of DYRK1A (Arbones et al. 2019). Due to its participation in altered brain activities and processes in DS as well as in the early start of neurofibrillary degeneration, amyloidosis, neuronal loss, and AD-like phenotypes in DS, DYRK1A has attracted a lot of interest (Wegiel et al. 2004).

2.1 DYRK1A and AD

DYRK1A participates in a number of biological processes, including neurodegenerative, vertebrate development, synaptic function, and cell cycle regulation (Park et al. 2009). Proteins involved in the synthesis and degradation of other proteins also interact with DYRK1A. Presenilin, a component of the γ-secretase complex that produces the Aβ peptide, is phosphorylated by DYRK1A (Ryu et al. 2010). DYRK1A also alters neprilysin (NEP), which breaks down the Aβ peptide by cleaving peptides on the amino side of hydrophobic residues (Kawakubo et al. 2017). GluN2A, a component of the *N*-methyl D-aspartate glutamate receptor, is phosphorylated at Ser1048 by DYRK1A, which controls synaptic plasticity and neuronal development (NMDARs) (Grau et al. 2014). In vitro, DYRK1A phosphorylates the amyloid precursor protein (APP), and overexpression of DYRK1A results in an increase in the phosphorylation of APP on Thr668, which has been discovered to affect APP conformation, may alter how APP performs in axonal transport, and is

processed by γ-secretases. In the adult human brain, six distinct tau protein isoforms produced by alternative splicing are expressed. The tau protein's functionality can be changed by the DYRK1A protein through tau splicing and tau phosphorylation. Through a variety of splicing factors, DYRK1A plays a very important part in the multimodal regulation of tau isoforms. Aβ protein can influence 3R-tau expression, and levels of 3R-tau rise as AD worsens. This rise could possibly be the result of abnormal DYRK1A levels (Shi et al. 2008). DYRK1A also phosphorylates the splicing factor (ASF), ultimately leading to increasing 3R-tau levels and causing an imbalance of the 3R-4R tau isoforms (Wegiel et al. 2011b). Increased alternative splicing of tau exon 10 and an increase in the 3R-to-4R-tau ratio may be caused by upregulation of DYRK1A, as in DS, leading to neurofibrillary degeneration (Yin et al. 2012). DYRK1A phosphorylates tau at 11 different serine/threonine sites. Human tau is phosphorylated at Thr212 by DYRK1A in vitro; fetal tau is also phosphorylated at this residue, and filamentous tau from AD brain is hyperphosphorylated at this site. In vitro, tau is prepared for phosphorylation by GSK3 at Ser208 via Thr212 phosphorylation (Woods et al. 2001). Tau phosphorylation is similarly correlated with increased DYRK1A expression in Ts65Dn mice (Liu et al. 2008). During brain development, DYRK1A is involved in neuronal proliferation, and neurogenesis, therefore, plays an important part in managing brain growth. DYRK1A escalation has been observed in various areas of AD patients' brains (Wegiel et al. 2011a). DYRK1A is involved in both NFTs and AP-governed neurodegenerative processes of AD. In mouse models of AD, DYRK1A suppression decreased amyloid and tau pathology (Branca et al. 2017). The DYRK1A-mediated tau phosphorylation is also competent in facilitating additional tau phosphorylation by GSK-3β and resulting in an increased deposition of NFTs (Ryoo et al. 2007; Wegiel et al. 2011a). The role of aberrant DYRK1A has been found in facilitating the accumulation of p-tau protein and in the formation of neurotoxic amyloid plaques, therefore, making DYRK1A a fascinating disease-modifying target for AD treatment (Fig. 1).

3 DYRK-1A Inhibitors

3.1 Natural and Natural-Inspired Inhibitors of DYRK-1A

3.1.1 Epigallotechin Gallate

The structural chemical diversity of compounds described as DYRK1A inhibitors has increased over the past 20 years, and a number of chemically diverse families of DYRK1A inhibitors have been identified; Bain and coworkers (2003) reported compound **1** (IC_{50} = 330 nM) as the first natural DYRK1A inhibitor. This compound was found to be selective for DYRK1A over 28 other kinases. Adayev et al. performed kinetic experiments that showed the binding pattern of compound **1** and revealed compound **1** to be an ATP-noncompetitive inhibitor of the DYRK1A enzyme (Adayev et al. 2006).

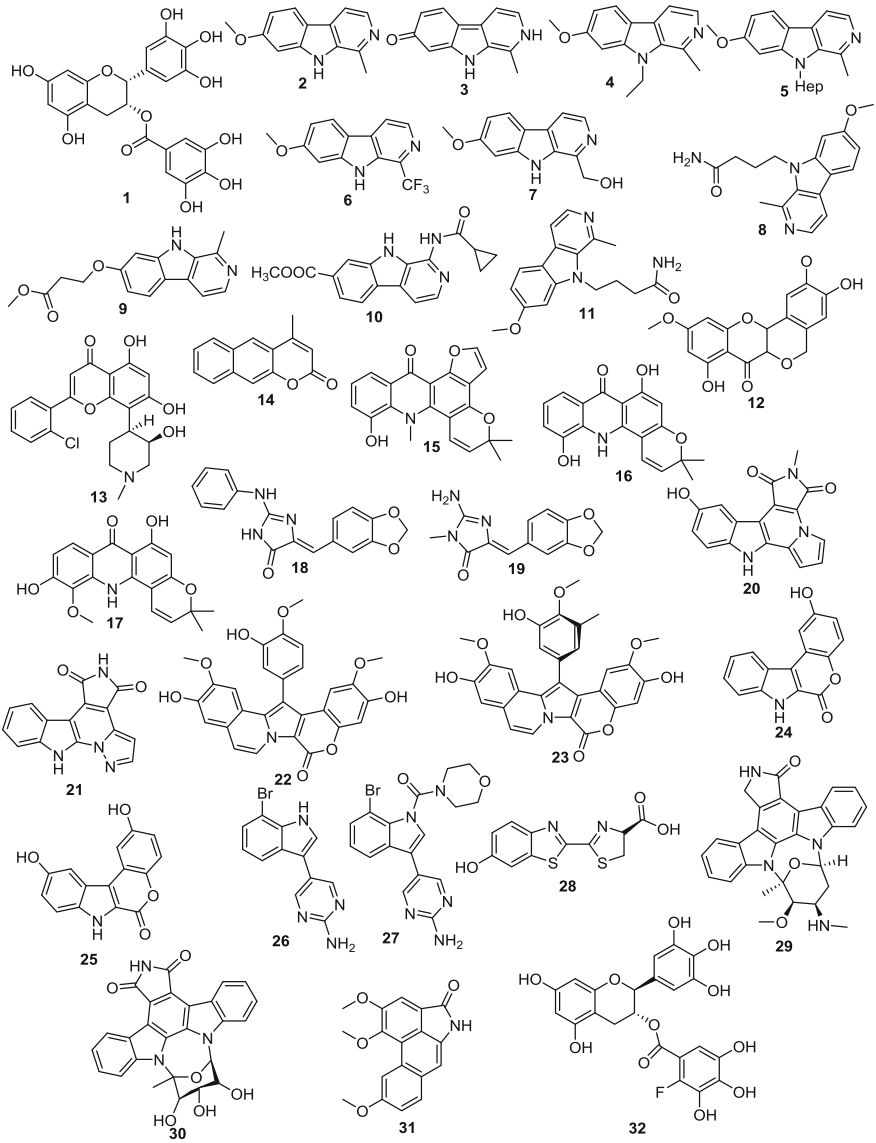

Fig. 1 Natural and naturally inspired inhibitors of DYRK1A

3.1.2 Harmine and Its Derivatives

Bain et al. reported harmine, a beta-carboline alkaloid (compound **2**), as DYRK1A inhibitor with $IC_{50} = 80$ nM) (Bain et al. 2007). Göckler et al. reported that Harmine **2** showed a stronger inhibitory effect on DYRK1A than on other DYRK family

members, with an IC_{50} = 166 nM for the closely related isoform DYRK1B and IC_{50} = 1.9 μM for DYRK2 and IC_{50} = 79.8 μM for DYRK4 (Göckler et al. 2009). Adayev et al. performed kinetic experiments that showed the binding pattern of compound **2** and revealed compound **2** to be an ATP-competitive inhibitor of the DYRK1A enzyme (Adayev et al. 2011). Over the years, various harmine derivatives have been synthesized and reported for DYRK1A inhibition. Balint et al. reported harmol **3** as DYRK1A inhibitor having IC_{50} = 90 nM (Bálint et al. 2017). Tarpley et al. also reported harmol as selective DYRK1A inhibitor over MAO-A (Tarpley et al. 2021). Frost et al. reported harmine derivative 9-ethyl harmine **4** having IC_{50} = 400 nM for DYRK-1A enzyme (Frost et al. 2011). Compound **5** was identified by Drung et al. as a DYRK-1A inhibitor with an IC_{50} of 130 nM after performing synthesis and in vitro investigations (Drung et al. 2014). Ruben et al. synthesized harmine derivatives and reported compound **6** as the most potent compound in the series with IC_{50} = 77.1 nM for DYRK-1A (Rüben et al. 2015). Kumar et al. reported compound **7** with improved selectivity compared to harmine IC_{50} = 54.8 nM (Kumar et al. 2018b). Further, Kumar et al. synthesized and performed biological evaluation on a series of 29 harmine derivatives and reported compound **8** having IC_{50} = 25 nM for DYRK-1A and improved selectivity over other kinases (Kumar et al. 2020a). They also reported compound **9** as potential DYRK-1A inhibitor with IC_{50} = 89.7 nM. Recently, Liu et al. designed, synthesized, and evaluated a new series of harmine derivatives as dual inhibitors of DYRK1A/GSK-3β (Liu et al. 2021). The in vitro kinase profiling revealed compound **10** as a potential DYRK1A inhibitor with IC_{50} = 103 nM while inhibiting GSK-3β enzyme with IC_{50} = 71 nM. Although harmine and several of its derivatives are effective oral inhibitors of the DYRK1A enzyme, they also demonstrate inhibition for monoamine oxidase (MAO)-A, which results in adverse effects and severely restricts their potential therapeutic use. It has been discovered that compound **6** retains activity against DYRK1A enzyme while completely losing activity against MAO-A (Rüben et al. 2015). In order to create harmine-based derivatives, Kumar et al. optimized the harmine's 9-*N*-position in order to increase selectivity for off targets (Kumar et al. 2020b). According to their findings, compound **11** is a unique, effective in vivo lead candidate that exhibits better selectivity for kinases and CNS off targets, as well as in vivo efficacy for cell proliferation and regeneration at lower dosages than those of harmine. In order to investigate the effects of pharmacological suppression of DYRK1A activity, Huizar et al. tested a small library of compounds derived from six different heterocyclic scaffolds (Huizar et al. 2022). They stated that a preliminary in vitro analysis of the gathered small molecule collection revealed that the main heterocyclic analogs, benzofuranones, oxindoles, and pyrrolones, demonstrated statistically substantial DYRK1A inhibition. In vivo phenotypic assays were used for in vivo research utilizing transgenic Drosophila.

3.1.3 Flavanoids

3.1.3.1 Peltogynoids

This Bougainvillea species has the first known report of peltogynoids, a rare form of modified flavonoids (Do et al. 2016). Acaninols are peltogynoids from the family Leguminaceae that Ahmadu et al. identified from the stem bark of the Acacia nilotica (L.) Delile along with the triterpene lupenone. This group established the structures of Acaninol A and B and tested them in vitro over a panel of AD-related kinases. They reported acanilol B (compound **12**) as a DYRK1A inhibitor, with an $IC_{50} = 19$ μM, while being selective over other kinases in the panel (Ahmadu et al. 2010).

3.1.3.2 Flavopiridol

Nguyen et al. identified flavopiridol **13**, a natural flavonoid, as DYRK-1A inhibitor having $IC_{50} = 0.3$ μM. Flavopiridol was discovered to inhibit various kinases examined in vitro, including DYRK1A, CDK1, CDK5, GSK-3, and CLK1 (Nguyen et al. 2012).

3.1.4 Chromene Derivative

Sarno et al. reported 8-hydroxy-4-methyl-benzo(g)chromen-2-one **14** as potential DYRK1A inhibitor with $IC_{50} = 0.6$ μM. While being selective for the CK2 enzyme, they also report inhibition of PIM1 and PIM2 enzymes by compound **14** (Sarno et al. 2012).

3.1.5 Alkaloids

3.1.5.1 Acridone Alkaloids

The two acridone alkaloids, known as chlorospermine A and chlorospermine B **15**, were discovered in the stem bark of the Rutaceae species *Glycosmis chlorosperma* by Beniddir et al. by means of bio-guided isolation. The group also isolated previously known acridone alkaloids atalaphyllidine **16** and acrifoline **17**. These four substances were examined for their ability to inhibit the DYRK1A enzyme, and compounds **15**, **16**, and **17** demonstrated notable inhibition of this enzyme with IC_{50} values of 5.7, 2.2, and 0.075 μM, respectively. However, neither DYRK1A nor any of the other kinases examined in the panel are inhibited by chlorospermine A (Beniddir et al. 2014).

3.1.5.2 Leucettine Alkaloids

In order to assess their effectiveness against DYRK1A and CLKs kinases, Debdab et al. synthesized more than 450 leucettine derivatives (a naturally occurring alkaloid derived from *Leucetta microraphis*, a sea sponge) (Debdab et al. 2010, 2011). DYRK1A/CLK1 was discovered to be inhibited by the compound **18**, with IC_{50} values of 0.041 and 0.071 μM, respectively. By synthesizing a group of analogues known as leucettines, Loaec et al. were able to produce leucetamine B-related polyandrocarpamines molecules that were powerful DYRK inhibitors. They reported compound **19** as DYRK1A inhibitor with IC50 = 420 nM. The compound also inhibited other DYRKs and CKKs (Loaëc et al. 2017).

3.1.5.3 Marine Alkaloids Analogues

The marine alkaloids, granulatimide and isogranulatimide, were converted into a series of pyrrolic analogues and two series of regioisomeric pyrazolic analogues by Deslandes et al. The studies have demonstrated the potency of two granulatimide analogues (**20** and **21**), with IC_{50} values of 0.26 and 0.09 μM, respectively, as DYRK1A inhibitors (Deslandes et al. 2012).

3.1.5.4 Lamellarin Alkaloids

Originally obtained from marine invertebrates, lamellarins are a class of hexacyclic pyrrole alkaloids. Lamellarins reportedly inhibit a number of protein kinases, including CDKs, DYRK1A, CK1, GSK-3, and PIM-1, according to Baunbaek et al. (2008). Lamellarin N, compound **22** with an IC_{50} value of 35 nM, was found to be a potent DYRK1A inhibitor. The 16-methyllamellarin N produced by Yoshida et al. was found to be optically active, and its *(aS)-enantiomer* **23** inhibited DYRK1A (IC_{50} = 0.44 μM), GSK-3, and PIM1 enzyme (Yoshida et al. 2013). Using substituted chromeno[3,4-b]indoles as Lamellarin isosteres, Neagoie et al. built a library of nearly 25 compounds and reported compounds **24** and **25** as potent and selective DYRK-1A inhibitors having IC_{50} values 74 and 67 nM, respectively (Neagoie et al. 2012).

3.1.5.5 3-(Pyrimidyl)indole Alkaloids

The brominated 3-(2-aminopyrimidine)-indoles known as meridianins were first isolated from the tunicate *Aplidium meridianum*, 100 m below sea level, close to the South Georgia Islands (Franco et al. 1998). An assortment of indole alkaloids with marine origins known as meridianins are said to have kinase-inhibitory properties. Meridianins derivatives were synthesized and tested in vitro over a panel of kinases by Giraud et al. (2011). Comparing compound **26** to the other kinases on the

panel, the group claimed it did, in fact, exhibit a 45-fold selectivity for DYRK1A with $IC_{50} = 68$ nM. N1-substituted and C-ring modified derivatives of meridianin were synthesized and their effectiveness as DYRK1A inhibitors and neuroprotective drugs was reported by Yadav et al. This group reported compound **27** as a potent and selective DYRK1A inhibitor being selective over 15 other kinases (Yadav et al. 2015).

3.1.6 Luciferin Derivatives

A common substrate in luciferase-catalyzed bioluminescence tests for in vitro research is D-luciferin **28**. In order to test their effectiveness against the 103-kinases panel, Rothweiler et al. developed and synthesized a small library of 15 D-luciferin analogs The outcomes showed inhibition of DYRK family enzymes as well as additional CMGC-group members, such as ERK8 and CK2. The 16-member focused library formed from D-luciferin underwent inhibition profiling to establish that D-luciferin is a DYRK-selective chemotype (Rothweiler et al. 2015).

3.1.7 Staurosporine

By using solvent extraction and silica gel chromatography, the indolecarbazole staurosporine **29** was purified from cultures of Streptomyces species by Omura et al. (1977). Staurosporine has been described as a strong DYRK1A inhibitor with an IC_{50} value of 29 nM (Rüegg and Gillian 1989) whose lack of selectivity results in unacceptable toxicity, explaining its lack of potential for use in pharmaceutical research. According to Sanchez et al., compound **30** contains an L-rhamnulose moiety and is nanomolar inhibitory to ten other kinases in addition to being considerably active against DYRK1A ($IC_{50} = 4$ nM) (Sánchez et al. 2009).

3.1.8 Aristolactam BIII

Choi et al. identified compound **31** by structure-based virtual screening as a potent DYRK1A inhibitor with $IC_{50} = 9.67$ nM. In the brains of DYRK1A transgenic mice, oral administration of compound 30 immediately decreased tau hyperphosphorylation, according to the research group (Choi et al. 2021).

3.1.9 Polyphenols

Araldi et al. reported the design, synthesis, and biological evaluation of polyphenol derivatives as DYRK1A inhibitors (Araldi and Hwang 2022). Through this research, the more potent and stable trans-fluoro-catechin derivative (compound **32**) has been found to possess $IC_{50} = 35$ nM.

3.2 Synthetic DYRK1A Inhibitors

3.2.1 Imidazole-Based DYRK1A Inhibitors

Pango et al. optimized 4,5,6,7-tetrabromobenzimidazole derivatives and reported compound **33** as a CK2 inhibitor (Pagano et al. 2004). With an IC_{50} of 0.41 μM, the compound inhibits the DYRK1A enzyme. The compound was found to be selective for more than 30 kinases tested in the panel. Different 3,6-disubstituted imidazo [1,2-*b*]pyridazines were evaluated for inhibitory efficacy against CLKs, CDKs, and DYRKs by Bendjeddou et al. The group reported compound **34** as a potent DYRK1A inhibitor having $IC_{50} = 33$ nM with inhibitory activity also for other similar kinases like CDK1 and CLK2 (Bendjeddou et al. 2017).

3.2.2 Pyrazolidine Derivatives

Koo and coworkers isolated a synthetic hit compound through a combination of in silico, in vitro, and the cell-based assay; the obtained hit compound was further investigated and served as a hit for synthesizing 34 compounds library. The study resulted in the identification of compound **35** as DYRK1A inhibitor with $IC_{50} = 0.6$ μM (Koo et al. 2009).

3.2.3 Quinazoline-Based DYRK1A Inhibitors

A series of substituted 6-arylquinazolin-4-amines was made by Mott et al. and tested as CLK4 inhibitors (Mott et al. 2009). The potent analogue's selectivity was investigated using a panel of 402 kinases. They reported compound **36** as the most potent compound of the series with $IC_{50} = 27$ nM. Rosenthal et al. carried out further research on substituted 6-arylquinazolin-4-amines as Clk4 inhibitors. With an IC_{50} value of 13 nM, compound **37** from the series was the most effective at inhibiting DYRK1A (Rosenthal et al. 2011). According to Foucourt et al., a small library of 6,6,5-tricyclic thiazolo[5,4-*f*]quinazolines was synthesized and this process led to the identification of powerful DYRK1A inhibitors with selectivity against the enzymes CK1, CDK5, and GSK3. Compound **38** was reported as the most potent compound of the series with $IC_{50} = 0.22$ nM (Foucourte et al. 2014a, b). Compound **38,** to date, is the most potent DYRK1A inhibitor**.** Hedou et al. prepared a library of 30 new thiazolo[5,4-*f*]quinazolin-9(8*H*)-one derivative using microwave irradiation (Hédou et al. 2016). They reported compound **39** as a multi-AD kinase inhibitor possessing IC_{50} for DYRK1A = 91 nM. New pyrido[3,4-*g*]quinazoline derivatives were designed and synthesized by Esvan et al. and their ability to inhibit kinases was assessed. They reported compound **40** as CLK1 and DYRK1A inhibitor with IC_{50} for DYRK1A = 28 nM (Esvan et al. 2016).

3.2.4 Benzothiazole Derivatives

Ogawa et al., for the first time, reported benzothiazole-based DYRK inhibitors. The group reported compound **41** (also known as INDY) as an ATP-competitive inhibitor of the DYRK1A enzyme with $IC_{50} = 0.24$ μM (Ogawa et al. 2010). On the basis of the DYRK1A/INDY complex's crystal structure, Masaki et al. developed and synthesized a new inhibitor of DYRK family kinases (Masaki et al. 2015). The group reported compound **42** (BINDY) as a potent and selective inhibitor of DYRK1A having $IC_{50} = 25.1$ nM, and this compound displayed potent and selective inhibition of DYRK family kinases. Based on the discovery that firefly D-luciferin selectively inhibits DYRK1A, Rothweiler et al. have investigated the static and dynamic structural characteristics of fragment-sized molecules of the benzothiazole scaffold with respect to DYRK1A (Rothweiler et al. 2016). They reported compound **43** with $IC_{50} = 0.4$ μM as a potential DYRK1A inhibitor. A novel DYRK1A inhibitor, compound **44**, has been described by Stensen et al. (2021). It is a very selective inhibitor of DYRK1A, nontoxic, orally accessible, and has good pharmacokinetic features, including the ability to cross the blood-brain barrier. According to Salah et al., conformationally constrained Dyrk1A inhibitors were designed and synthesized by forming an intramolecular H-bond with a benzothiazole core (Salah et al. 2018). The group reported compound **45** as a potential and selective DYRK1A inhibitor with $IC_{50} = 95$ nM. Inhibitors of DYRK1A with high selectivity were created using 6-hydroxybenzothiazoles by AlNajjar et al. recently (AlNajjar et al. 2022). They reported dual inhibitors of DYRK1A/aggregation of α-synuclein oligomers; the most active compound of the series (compound **46**) was reported to suppress the aggregation of α-synuclein with $IC_{50} = 7.8$ μM.

3.2.5 Indirubin-Based DYRK1A Inhibitor

Indirubins were designed, synthesized, and biologically tested by Myrianthopoulos et al. as increased selectivity DYRK inhibitors (Myrianthopoulos et al. 2013). They stated that the enhanced selectivity is the result of a unique inverse binding mode that was predicted by molecular modeling and crystal structure studies. Compound **47** was reported as the most potent compound of the series with $IC_{50} = 210$ nM.

3.2.6 Azaindole Derivatives

Gourdain et al. synthesized azaindoles derivatives and evaluated them in vitro for inhibition of DYRK1A enzyme. (Gourdain et al. 2013) The most potent compound of series **48** (named DANDY) was reported to depict $IC_{50} = 3.0$ nM. Neumann et al. (2018) describe the synthesis of fluoro derivatives of 3,5-di(polyhydroxyaryl)-7-azaindoles (F-DANDYs) and demonstrate their significant DYRK1A inhibitory activity. They reported compound **49** as a DYRK1A inhibitor with $IC_{50} = 20.7$ nM.

Azaindole derivatives have been identified by Shaw et al. as effective DYRK1A inhibitors with effects on the nuclear transcription factor NFAT (nuclear factor of activated T cells). (Shaw et al. 2017). The group reported compound **50** as the most potent inhibitor of DYRK1A in the series with $IC_{50} = 1$ nM. Powell et al. describe the development of the first DYRK1A macrocyclic inhibitors (Powell et al. 2022). They reported compound **51** (azaindole macrocyclic compound) as a potent and selective DYRK1A inhibitor with $IC_{50} = 3$ nM. Liu et al. reported 6-azaindole derivative (compound **52**) as selective DYRK1A inhibitor having $IC_{50} = 6$ nM (Liu et al. 2020).

3.2.7 Pyridine Analogs

Weber et al. discovered strong and specific DYRK1A inhibitors using structure-guided drug discovery (Weber et al. 2021). The top compounds were reported for their ability to inhibit DYRK1A both in vivo and in cell culture, as well as their drug-like qualities. They reported compound **53** as a potent and selective DYRK1A inhibitor with $IC_{50} = 5$ nM in vitro.

3.2.8 Pyrimidine Derivatives

Coombs et al. developed substituted pyrimidine analogs as inhibitors of the DYRK and CLK kinases (Coombs et al. 2013). The most potent compound of series **54** was in vitro screened over a panel of 442 kinases and found to be an inhibitor of DYRK and CLK kinases. Compound **53** was found to possess $IC_{50} = 60$ nM for the DYRK1A enzyme. Andreson et al. discovered derivatives of pyrido[2,3-*d*]pyrimidines as effective enantio-selective inhibitors of DYRK1B (Anderson et al. 2013). The group reported compound **55** as the most potent compound of the series, with $IC_{50} = 5$ and 8 nM for DYRK1A and DYRK1B, respectively. The synthesis and biological assessment of an original group of 4,7-disubstituted pyrido[3,2-*d*]pyrimidines intended as possible kinase inhibitors were described by Dehbi et al. (2014). The group reported compound **56** as the most potent DYRK1A inhibitor possessing $IC_{50} = 60$ nM. Compound **56** was found to be inhibiting GSK3 and CD5 enzymes along with DYRK1A while being selective over other kinases tested. In their study, Demuro et al. described the discovery and characterization of compound **57**, a dual GSK-3 and FYN inhibitor that also inhibits DYRK1A, a newly discovered target in AD and tauopathies (Demuro et al. 2022). The reported compound, **57**, possesses IC_{50} for DYRK1A 887 nM while inhibiting the DYRK1A enzyme in an ATP-competitive manner. Recently, fragment-derived, selective DYRK1A/1B inhibitors were reported by Lee Walmsley et al. (2021). To find a highly selective, well-tolerated, and brain-penetrant DYRK1A inhibitor, the team used fragment and structure-based discovery techniques. They reported compound **58** as most potent DYRK1A inhibitor in the series ($IC_{50} = 7$ nM) (Fig. 2).

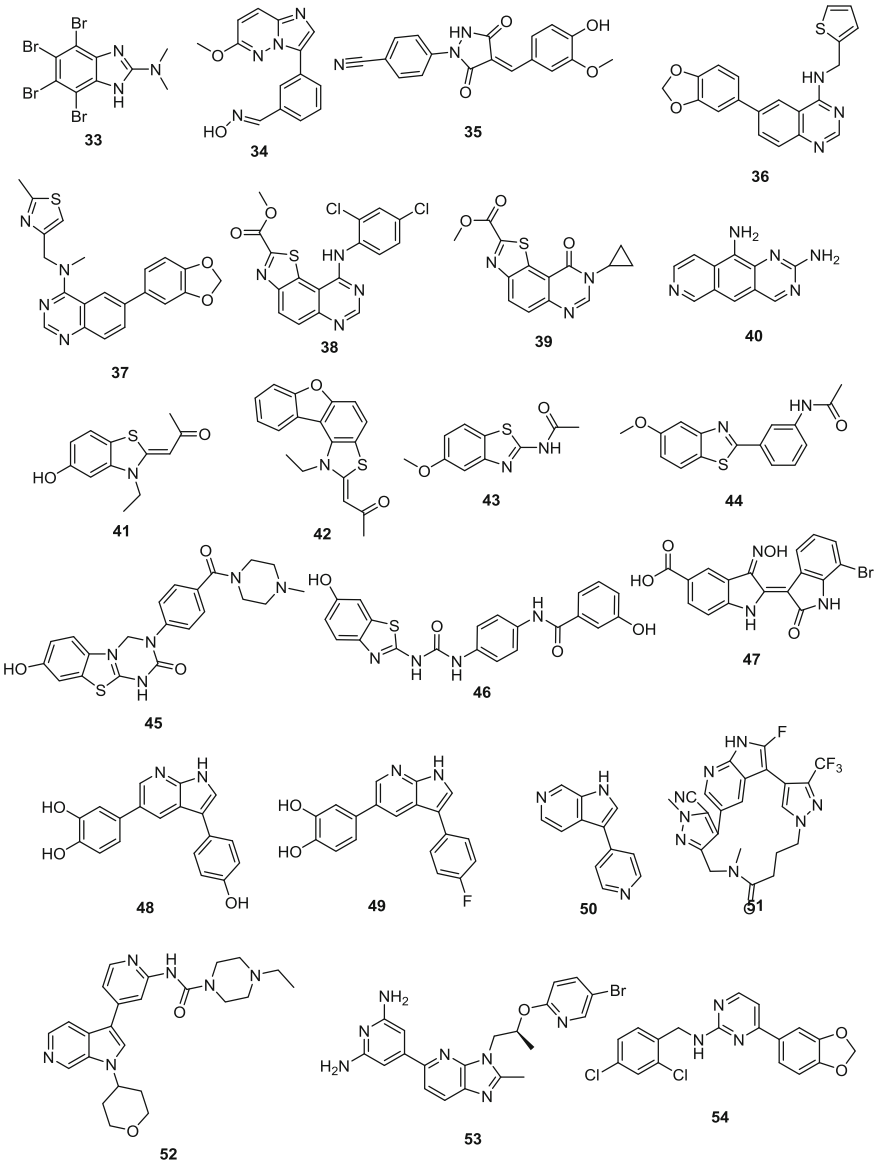

Fig. 2 Synthetic inhibitors of DYRK1A

3.2.9 Pyridazine Derivatives

New pyridazino[4,5-*b*]indol-4-ones and pyridazin-3(2*H*)-one derivatives were developed by Bruel et al. They next examined the inhibitory effects of these compounds on the DYRK1A, CDK5, GSK3, and PI3K kinases (Bruel et al.

2014). They reported compound **59** as a potent and selective DYRK1A inhibitor with IC_{50} = 0.22 μM. Bendjeddou et al. synthesized 3,6-disubstituted imidazo [1,2-*b*]pyridazine derivatives to recognize new inhibitors of several eukaryotic kinases. They reported compound **60** as a potent DYRK1A inhibitor having IC_{50} = 33 nM, while showing inhibition of DYRK1B, ClK2, and CDK1 (Bendjeddou et al. 2017).

3.2.10 Thiophene Derivatives

As a novel family of DYRK1A inhibitors, Schmitt et al. reported the development and optimization of 2,4-bisheterocyclic-substituted thiophenes (Schmitt et al. 2014a). They reported compound **61** as a potent DYRK1A inhibitor with IC_{50} = 100 nM. The compound was found to also inhibit other DYRK family enzymes like DYRK1B and DYRK2. Marino et al. (2014) reported thiophene-based derivative, compound **62,** as DYRK1A inhibitor having IC_{50} = 5 μM; the compound was also found to inhibit Aβ peptide aggregation and tau-phosphorylation. The compound thus was found to be a multi-target compound to target neurodegeneration. Hydroxybenzothiophene ketones have been identified by Schmitt et al. (2014b) as novel, selective dual CLK/DYRK1A/1B inhibitors that can control the alternative pre-mRNA splicing of model gene transcripts in living cells. They reported compound **63** as DYRK1A inhibitor having IC_{50} = 200 nM, while inhibiting DYRK1B and CLK enzymes. Darwish et al. synthesized 2,4-bispyridyl thiophene aryl and aralkyl amides derivatives from previously reported 2,4-bispyridinyl thiophene moiety (Darwish et al. 2018b). They reported compound **64**, a benzyl amide derivative, as potential DYRK1A inhibitor (IC_{50} = 14.3 nM) with excellent selectivity over DYRK1B. Additionally, they stated that compound **64** could effectively inhibit Dyrk1A in HeLa cells, an IC_{50} of 79 nM. Darwish et al. again reported synthetic derivatives of 2,4-bispyridyl thiophene moiety, this time playing around cyclopropylamide ring (Darwish et al. 2018a). They reported compound **65** as a potent DYRK1A inhibitor with IC_{50} = 3.2 nM. The reported compound demonstrated improved metabolic stability as well as a high likelihood of penetrating the CNS.

3.2.11 Quinoline Derivatives

Falke et al. developed 10-iodo-substituted quinoline derivatives as highly effective DYRK1A inhibitors with significant selectivity against CLKs (Falke et al. 2015). They reported compound **66** as the most potent with IC_{50} for DYRK1A 6 nM while being highly selective over CLK kinases.

3.2.12 Thiazolidinones

Sonamato et al. discovered CaNDY (imino-thiazolidinone analogue, compound **67**) as a powerful DYRK1A inhibitor (Sonamoto et al. 2015). By competing with ATP, it caused the selective degradation of DYRK1A and decreased the catalytic activity of recombinant DYRK1A with an IC_{50} value of 7.9 nM. Kii et al. targeted the folding mechanism of the DYRK1A kinase to selectively suppress it (Kii et al. 2016). In contrast to other DYRK family members including DYRK1B and DYRK2, they described compound **68** (FINDY) as a highly selective DYRK1A inhibitor with $IC_{50} = 35$ μM. According to the expected binding mechanism in the ATP pocket of DYRK1A, Miyazaki et al. created and synthesized structural variants of FINDY (Miyazaki et al. 2022). They reported compound **69** (*dp*-FINDY) as DYRK1A inhibitor with $IC_{50} = 0.53$ μM which is more potent than its analog FINDY.

3.2.13 Indole Derivatives

Fedorov et al. reported dichloroindolylenaminonitril as DYRK1A inhibitor (compound **70**) with $IC_{50} = 55$ nM (Fedorov et al. 2011). Compound **70** was found to be a potent and non-ATP-competitive inhibitor of CLK. A group of oxindole-containing ferrocenes has been developed, examined by X-ray crystallography in the solid state, and tested for in vitro kinase inhibition by Amin et al. (2013). They reported compound **71** as DYRK1A inhibitor having $IC_{50} = 6.8$ μM and selectivity over some DYRK enzymes and VEGFR2. Labriere et al. reported the synthesis of indole-containing pyridocarbazoles (Labrière et al. 2016). Compound **72** was identified as a DYRK1A inhibitor with an IC_{50} value of 18 nM, as well as an inhibitor of the DYRK1B, GSK3, and CDK5 enzymes. Molecular docking studies were used by Meine et al. to assess a series of indole-3-carbonitriles as putative DYRK1A ligands (Meine et al. 2018). They reported compound **73** as a small and less lipophilic DYRK1A inhibitor with $IC_{50} = 3.30$ μM. Lechner et al. reported the design, synthesis, and biological evaluation of a series of new derivatives of [*b*]-annulated halogenated indoles (Lechner et al. 2019). They reported compound **74** as potent DYRK1A inhibitor with $IC_{50} = 6$ nM, and this compound also inhibited the CLK1 enzyme showing $IC_{50} = 0.5$ μM while being selective over other kinases tested in the panel.

3.2.14 Purine Derivatives

Davies et al. reported compound **75** as a potent DYRK1A inhibitor ($IC_{50} = 0.9$ μM) while searching for CLKs inhibitors (Davies et al. 2002). Lamor-theys et al. reported

compound **76** (purvalanol A) and compound **77** (roscovitine) as DYRK1A inhibitors having IC_{50} values 0.3 and 3.1 μM, respectively (Lamoral-Theys et al. 2010). Bettayeb et al. reported compound **78**, purine derivative as a DYRK1A inhibitor ($IC_{50} = 0.9$ μM) (Bettayeb et al. 2008). The inhibition study was performed over a panel of more than 100 kinases, and compound **78** was found to inhibit CDKs more potentially.

3.2.15 Pyrazolopyrimidine Analogs

Lin et al. discovered and investigated DYRK1A inhibitors. They performed structure-based virtual screening and identified synthetic compound **79** as a DYRK1A inhibitor ($IC_{50} = 2.99$ μM) (Lin et al. 2022). The group performed selectivity studies over a panel of 97 kinases and concluded that it was selective over CMCG family kinases with promising activity towards DYRK1A.

3.2.16 Pyrazolopyridine Analogs

Park et al. reported the synthesis of novel pyrazolopyridine derivatives as potential DYRK1A/1B inhibitors (Park et al. 2021). They reported compound **80** as a potential DYRK1A/1B inhibitor with $IC_{50} = 5$ nM/3 nM.

3.2.17 Pyrazolopyridazine Analogs

Henderson et al. repurposed compounds utilized in earlier kinase inhibitor research studies by studying data in the public domain (Henderson et al. 2020). By the addition of methyl groups to the chosen starting compound (CDK and GSK-3β inhibitor), they reported compound **81**, a highly selective DYRK1A inhibitor possessing $IC_{50} = 76$ nM. Henderson et al. again reported the discovery and characterization of pyrazolopyridazine derivatives as ligand-efficient and potent DYRK1A inhibitors (Henderson et al. 2021). They reported compound **82** as a potential DYRK1A inhibitor with $IC_{50} = 220$ nM (Figs. 3 and 4).

3.2.18 Pyrrole-2,5-Dione Analogs

Small molecule inhibitors of DYRK1A were found by computational and experimental methods by Yoon et al.; they reported compound **83** (which is a known GSK3 inhibitor) as a DYRK1A inhibitor with $IC_{50} = 445$ nM (Yoon et al. 2020).

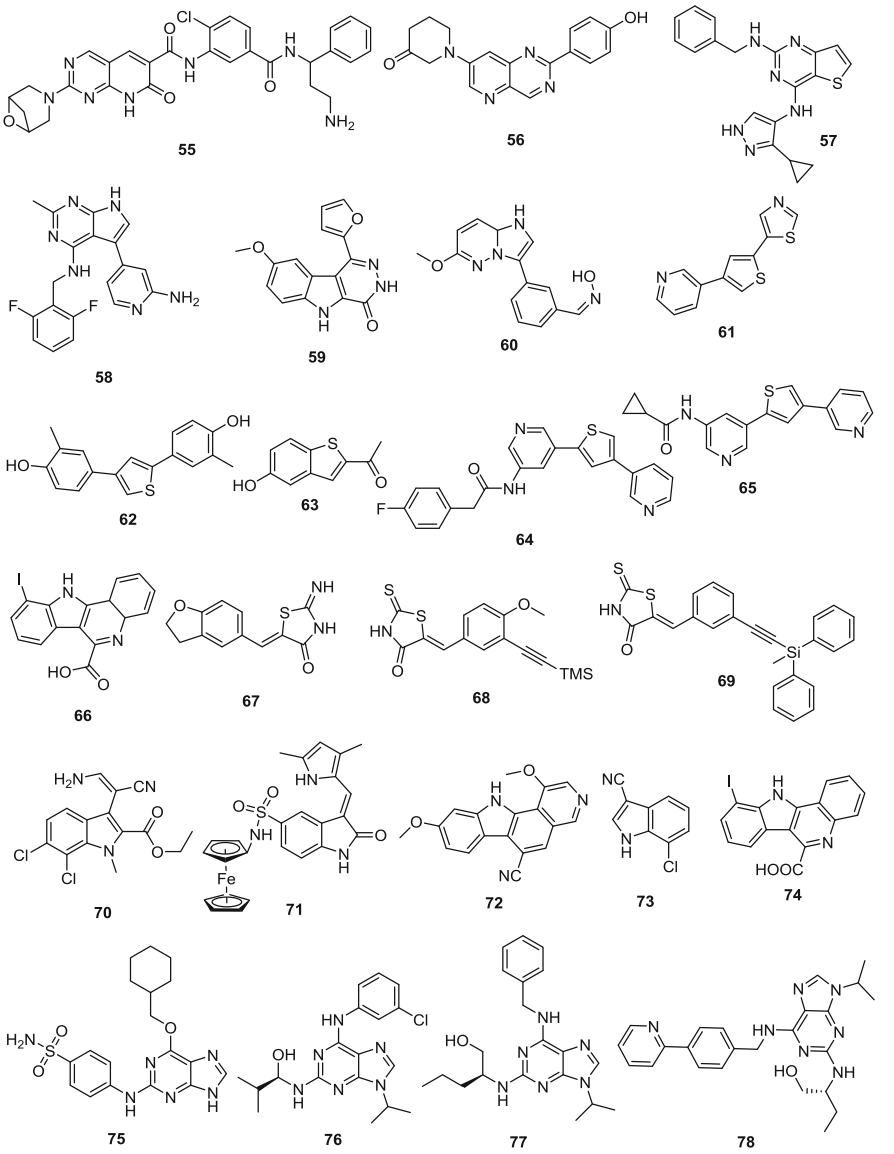

Fig. 3 Synthetic inhibitors of DYRK1A

4 Conclusion

This chapter has provided a systematic insight into the natural, naturally inspired as well as synthetic inhibitors of the DYRK1A enzyme. The natural compounds comprise almost half of the discovered DYRK1A inhibitors depicting the therapeutic

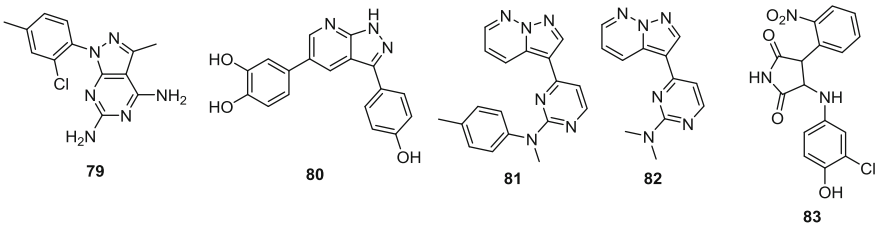

Fig. 4 Synthetic inhibitors of DYRK1A

capability of compounds of natural origin. The various synthetic classes discussed here provide insight into the variety of chemical moieties capable of interacting with DYRK1A protein and inhibiting it. The literature covered in this chapter depicts that the development of innovative, powerful, and selective DYRK1A inhibitors is still restricted to the early stages of lead identification. The majority of DYRK1A inhibitors that have been reported compete for ATP and inhibit DYRK1A enzyme in an ATP-competitive manner; as a result, they are not selective for DYRK1A and show inhibition of other similar kinases. DYRK1A is a promising target for disease-modifying treatment for AD; therefore, small molecule DYRK1A inhibitors may be a therapeutic option for AD-related cognitive impairment. Modulating DYRK1A's activity to levels typically seen in healthy people, however, requires caution due to the documented involvement of DYRK1A in regulating a number of signaling pathways important for brain development and function. We can confidently predict that as information about DYRK1A increases, more applied research will produce distinct, effective, and selective DYRK1A inhibitors that significantly enhance clinical outcomes.

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β-Secretase as a Primary Drug Target of Alzheimer Disease: Function, Structure, and Inhibition

Saroj Verma and Debashish Paramanick

Abstract β-secretase (BACE1) is responsible for regulating different biological functions. Several biochemical studies reported that BACE1 enzyme plays a key role in Alzheimer disease (AD). During the past decade, exploration has been made to understand the structure and function of β-secretase in AD. This chapter covers recent research reports made in understanding the biological structure and functions of BACE1 and its role in AD. In addition, efforts are also made to report critical features of pharmacophore to design selective BACE1 inhibitors.

Keywords β-secretase · BACE1/BACE2 function · BACE1/BACE2 structure · BACE1 inhibitors

Abbreviations

AD	Alzheimer's disease
ADAM	A disintegrin and metalloprotease domain protein
BACE	β-site amyloid precursor protein-cleaving enzyme
CatD	Cathepsin D
cMD	Classical molecular dynamics
CTF	Bound carboxyl terminal fragment
GGA	Golgi associated, gamma adaptin
MM-GBSA	Molecular mechanics generalized Born surface area
MR-aMD	Multiple replica accelerated molecular dynamics
MR-GaMD	Multiple replica Gaussian accelerated molecular dynamics
MSMD	Multiple short molecular dynamics
NCSTS	Nicastrin
PC	Principal component
PSEN1	Presenilin-1

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RTN	Reticulon
SSBs	Disulfide bonds
TACE	TNF-converting enzyme
WHO	World Health Organization

1 Introduction

Over 55 million of the population live with dementia worldwide (WHO 2022). Alzheimer's disease (AD) is the most common cause of dementia (loss of cognitive functioning). In dementia, brain and nerves cells are blocked by abnormal level of misfolded proteins. Misfolding of proteins results in signal disturbance of the transmitters which are predominantly responsible for storing memories (Soto and Estrada 2008; Soto and Pritzkow 2018). Different isoforms of the membrane protein amyloid precursor protein (APP) exist. The common isoforms comprise, respectively, 695 (APP695), 751 (APP751), and 770 (APP771) amino acids (aa). Among these, APP695 is the most abundant and is only expressed in neurons. APP751 and APP770 are expressed in both neural and non-neural cells (Min et al. 2017; Bayer et al. 1999). APP is divided into different domains, namely N-terminal extracellular region (similar to cysteine-rich growth factors), acidic domain, Kunitz domain, glycosylated domain, hydrophobic transmembrane domain, and C-terminal intracellular domain (cytoplasmic domain) (O'Brien and Wong 2011; Lee et al. 2021). The APP is processed by two different pathways, i.e. major non-amyloidogenic and minor amyloidogenic. The α-, β-, and γ-secretases are involved in proteolytic processing of the APP (Chow et al. 2010; Murphy and LeVine 2010). Amyloidogenic pathway is associated with AD. In amyloidogenic pathway, β- and γ-secretases are involved in sequential cleaving of APP that produces abnormal and toxic amyloid β (Aβ, 42 amino acids). Furthermore, Aβ undergoes oligomerization, fibrilization, and clumping that result in formation of insoluble plaques. Plaques are collections of misfolded proteins (Aβ), thought to play central role in AD pathogenesis (Fig. 1). Environmental variables and genetic abnormalities/mutations may be associated with the production of toxic Aβ.

As β-secretase is one of the two enzymes that cleave the APP to produce the 40–42 residue Aβ, a key indicator of the progression of AD (Zhao et al. 2020). β-secretase is popularly known as β-site amyloid precursor protein-cleaving enzyme 1 (BACE1), membrane-associated aspartic protease 2, memapsin 2, aspartyl protease 2, etc. (Dislich and Lichtenthaler 2012; Hong et al. 2000). Researchers selected β-secretase as a leading drug target for design and exploration of inhibitors against AD. In 2000, the co-crystal (PDB; 1FKN) structure of β-secretase-peptide inhibitor was introduced that works as roadmap for exploration of inhibitors against AD (Hong et al. 2000). Subsequently, number of crystal/co-crystal structure of β-secretase was developed for exploration of 3D structure, function, and inhibition of target (Hong and Tang 2004; Ghosh et al. 2008; Evin and Hince 2013; Li et al. 2013).

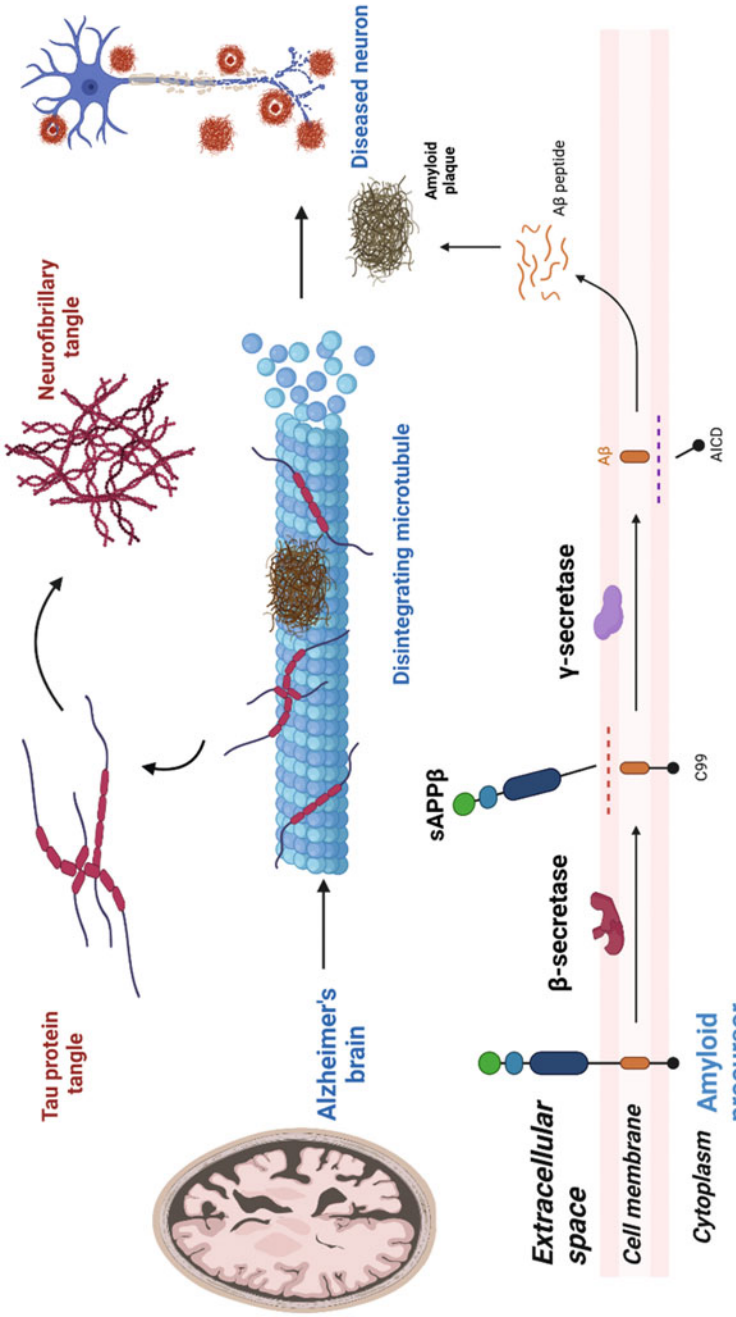

Fig. 1 Pictorial representation of pathophysiology of AD

In this backdrop, this chapter reviews function, structure, and inhibition of target to advance the understanding and knowledge of β-secretase for the exploration of inhibitors against AD. Earlier review report discusses the general aspects of the topic (Vassar 2002; Yan and Vassar 2014; Venugopal et al. 2008). This chapter advances the topic in direction of deep knowledge of β-secretase enzyme and metabolic pathway at structural level. In this chapter, we attempt to summarize function, different levels of β-secretase structure, and its inhibition.

2 Function of β-Secretase

β-secretase is a type-I integral membrane glycoprotein that is generated as a pre-proenzyme and has a brief prodomain of 24 amino acids and consists of extracellular catalytic domain, a single-transmembrane domain, and a cytoplasmic tail. The leader sequence of the enzyme has 21 amino acids and is deleted during co-translation. β-secretase is processed by proprotein convertases, to remove the prodomain in the secretory pathway (Vassar 2002). In comparison to other aspartyl proteases, the prodomain of β-secretase is quite small. Additionally, it is known that the pro-β-secretase protein retains around 30% of the activity of the mature protein. β-secretase function is regulated by important factors, such as the target organelle's pH and other associated proteins. The protein can cleave its recognized substrate APP. It produces toxic amyloid β (Aβ), which is a key early player in the aetiology of AD (Yan and Vassar 2014; Venugopal et al. 2008; Yan 2017).

The basic model of Alzheimer's disease, the amyloidogenic pathway, is still controversial. Lack of a mechanistic link between the detrimental effects on synapse structure and function and the cleavage products of the APP, such as soluble Aβ monomer, fibril, oligomers, and molecular fragments, is one potential reason (Liu et al. 2021; Hampel et al. 2021a, b). Researchers reported that β-secretase is known to be β-site APP-cleaving enzyme (Fig. 2). The γ-secretase appears to be a protein complex composed of PS1 or PS2, nicastrin, Aph1, and Pen2. However, three potential α-secretases were identified as TACE (TNF-converting enzyme), ADAM (a disintegrin and metalloprotease domain protein), and ADAM-10. These secretases are interlinked and participate in production of APP (Nhan et al. 2015; Cole and Vassar 2007).

APP production is dependent upon BACE1 cleavage. BACE1 initiates Aβ synthesis by cleaving APP at the Asp + 1 residue of the Aβ sequence to generate the N-terminus of the peptide. This scission releases two cleavage fragments: a membrane-bound carboxyl terminal fragment (CTF), C99, and a secreted APP ectodomain, APPsβ. γ-secretase then cleaves C99 to produce the C-terminus of the Aβ peptide and an APP intracellular domain (AICD). It has been demonstrated that the AICD may play a role in transcriptional transactivation. Cleavage by the γ-secretase complex is not precise; while the majority of Aβ peptides liberated by γ-secretase activity end at amino acid 40 (Aβ40), a small proportion end at amino acid 42 (Aβ42). It is γ-secretase-dependent cleavage that is affected by most familial

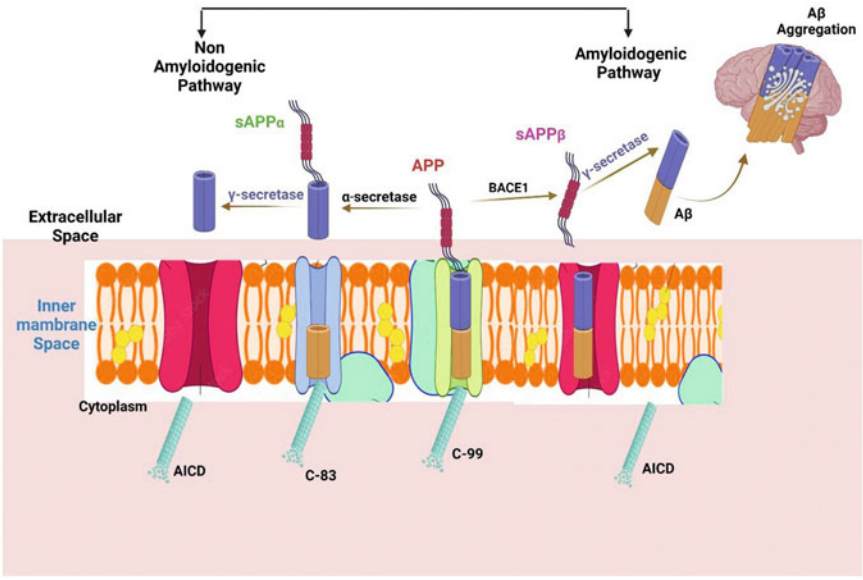

Fig. 2 APP metabolic pathway: amylidogenic and non-amylidogenic

AD (FAD) mutations to cause excess generation of Aβ42 in FAD (Cole and Vassar 2007).

In the non-amyloidogenic alternative pathway, α-secretase cleaves APP at Leu + 17 within the Aβ domain. α-Secretase cleavage generates the secreted APPα ectodomain and a CTF, C83, which is then cleaved by γ-secretase to generate the non-amyloidogenic 3 kDa fragment, p3. As the α- and β-secretase moieties compete for APP substrate, an increase in non-amyloidogenic APP metabolism is frequently accompanied by a decrease in the amyloidogenic processing pathway, and vice versa (Nhan et al. 2015; Cole and Vassar 2007).

Experimental and theoretical data suggested various interacting proteins directly and indirectly are associated with the pathway of APP and catalytic subunits of gamma secretase-NCSTS (Nicastrin) and PSEN1 (Presenilin-1), GGA1/2/3 (Golgi associated, gamma adaptin ear containing, arf binding protein 1) and RTN3/4 (Reticulon-4), Furin, RHBDL4, etc. (Fig. 3) (Pasternak et al. 2003; Murayama et al. 2006; Kosicek et al. 2014; Zhang et al. 2022; Recinto et al. 2018).

Experimental work revealed the presence of presenilin-1, nicastrin, and APP as resident lysosomal membrane proteins as well as the fact that secretase is a lysosomal protease. These findings support the significance of the lysosomal/endosomal system in the production of Aβ and point to lysosomes' potential participation in the pathophysiology of AD. The presenilin complex is a bilobed structure. The ectodomain of nicastrin is located in the head domain. Numerous point mutations in the PSEN genes have been associated to familial AD, according to genetic studies (Pasternak et al. 2003). RTN4 is more concentrated in oligodendrocytes in the brain

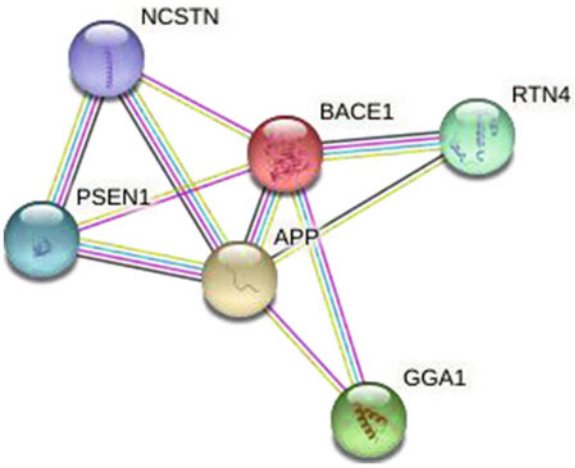

Fig. 3 BACE1 protein interaction map displaying key interacting proteins in pathway. The pathway created from String 10.5 uses connecting lines to display the various types of evidence for linkage between proteins, including fusion evidence, neighbourhood evidence, co-occurrence evidence, experimental evidence, text mining evidence, database evidence, and co-expression evidence

than RTN3, where BACE1 mostly colocalizes with neurons. Any reticulon protein with increased expression greatly lowers Aβ synthesis (Murayama et al. 2006). Increased localization of APP at the cell surface brought about a shift in APP processing toward the non-amyloidogenic pathway as a result of GGA1 overexpression (Kosicek et al. 2014). In the secretory route, the crucial mammalian proprotein convertase furin catalyses the proteolytic maturation of several prohormones and proproteins. Growth factor, receptor, and enzyme proproteins are among the substrates of furin in the brain. A growing body of evidence suggests that furin plays a critical role in the pathogenesis of neurodegenerative and neuropsychiatric illnesses, such as decreased expression of FURIN mRNA in the brains of people with schizophrenia or AD (Zhang et al. 2022).

Some researches suggested that RHBDL4 is a mammalian intramembrane rhomboid protease that can process APP in a different way. Reduced Aβ levels result from RHBDL4's effective cleavage of APP inside the cell, which avoids APP's participation in amyloidogenic processing. RHBDL4 repeatedly cleaves APP in the ectodomain, leaving behind a number of N- and C-terminal fragments that cannot be further broken down by conventional APP secretases. C-terminal fragments made from endogenous APP are produced at lower quantities when endogenous RHBDL4 is knocked down. Similarly, it was discovered that RHBDL4 is a substrate for the members of the APP family APLP1 and APLP2. Insight into APP and rhomboid physiology is provided by RHBDL4-mediated APP processing, which makes future research into its effects on AD disease pathogenesis possible (Recinto et al. 2018).

Interindividual variation in BACE1-related pathophysiological processes may be explained by a number of significant genetic, epigenetic, and posttranslational variables that have been shown to affect the levels of BACE1 gene expression and enzyme activity (Hampel et al. 2021a, b; Vassar 2004). The most likely biological and pharmacological explanations for BACE1 activity are still up for debate in the scientific community. The science still needs to learn more about the enzyme's physiological significance, as well as the physiological activities of BACE1 substrates involved in synaptic homeostasis.

3 Structure of β-Secretase

In 1999, β-secretase enzyme (BACE) was identified. It was classified as BACE1 (501 amino acids) and BACE2 (518 amino acids) (Vassar et al. 1999). The structure of both enzymes is ~75% homologous. According to research, BACE1 is the key participant in the AD process in the brain, whereas BACE2 is still poorly understood (Hrabinova et al. 2021).

Human β-secretase encoded by gene BACE1 (BACE/KIAA1149) is localized mainly in Golgi network, endoplasmic reticulum, endosomes, cell surface, etc. (Zhang and Song 2013). In human genome, β-secretase is located at chromosome position 11 (NC_000011.10). It includes 501 amino acids with molecular weight and isoelectric point (PI) about 55710.28 Da and 5.19, respectively. It is a transmembrane protein that includes 44.31% hydrophobic, 10.98% acidic, 10.38% basic, and 34.33% neutral amino acids. β-secretase is broadly classified as hydrolase, responsible for breaking peptide (CONH) bond, and therefore it is also named as peptidase/aspartic acid endopeptidases (Vassar et al. 1999). Several 3D structures of β-secretase have been explored for defining their enzymatic and inhibitory activity. The 1FKN was the first complexed structure of β-secretase and inhibitor OM99-2 (Glu-Val-Asn-LeuAla-Ala-Glu-Phe, $K(i) = 1$ nM). The structure represents protease domain (consisting of 391 amino acids) with open and less hydrophobic active site. Subsequently, new complexed structure (1M4H) of β-secretase and inhibitor OM00-3 (Glu-Leu-Asp-LeuAla-Val-Glu-Phe, $K(i) = 0.3$ nM) was generated. OM00-3 showed better interaction and is located at different position S(3)′ and S(4)′ subsites in comparison to the previous one. Dissimilar binding conformation for the P(2) and P(4) side chains was also observed. The structural and kinetic data showed that the substitution of an alanine P(2)′ in place of valine in OM99-2 stabilizes the binding interaction of P(3)′ and P(4)′ (Hong et al. 2000).

Shimizu et al. reported the catalytically active form of BACE1 crystal structure: 2ZHS, pH 4.0; 2ZHT, pH 4.5; 2ZHU, 5.0; and 2ZHV, pH 7.0. The shape of the flap and subsites in the active form are novel structural elements that facilitate substrate binding. The well-defined functionally necessary residues and water molecules are crucial to the repeated activation of BACE1. They clarified the dehydrated form of BACE1's crystal structure, showing how the dynamics of an Asp-bound water molecule, which is required for the enzyme's activity, directly affect the enzyme's catalytic capabilities. These discoveries give insight on a particular mode of control of BACE1 activity and explain how BACE1 adjusts its activity during cellular trafficking (Shimizu et al. 2008).

To explore conformational changes in the enzyme's active-site area, molecular dynamics (MD) simulations of monomeric and "dimeric" BACE1 were performed by Xu et al. It was shown that the most flexible area, adopting several conformational states in the different crystal structures, is a flap able to cover the active site. It has been demonstrated that the conformation of the flap is affected by both the presence or absence of an inhibitor within the active site and the crystal packing. Most of the time, the flap is seen in an open conformation in apo structures, but in the crystal

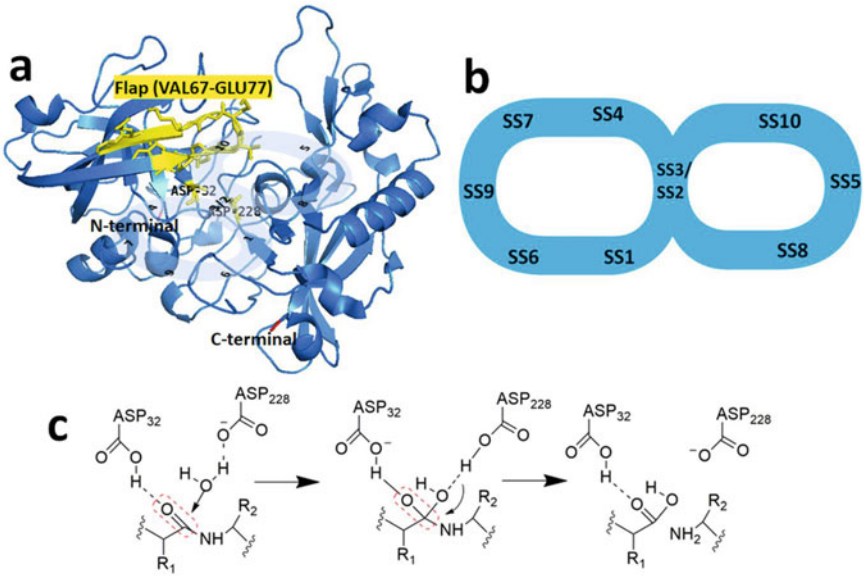

Fig. 4 Representation of BACE1 structure and catalytic activity. (**a**) 3D structure of BACE1. (**b**) Probable subsites of binding pocket for binding interaction of different inhibitors. (**c**) Catalytic activity of BACE1 involving aspartic acids 32 and 228

structures of complexes, a direct hydrogen-bonding connection between the flap's main chain atoms and the inhibitor is required for the flap to adopt a closed conformation (Xu et al. 2012). Therefore, a systematic investigation of the enzyme's conformational flexibility may help in the development of BACE1 inhibitors.

Through 3D structure analysis, several binding pocket subsites of BACE1 are investigated for the development of potential and selective inhibitors. Hu et al. used 354 ligand-bound crystal structures to systematically explain the binding location of BACE1 (Hu et al. 2019). The ligand binding pocket may be divided into ten subsites to a detailed investigation of the frequency and patterns of interactions between residues and ligands, which also makes it possible to pinpoint the best ligand substructures for each subsite. The study revealed cluster of 8-like structural shape important for binding interaction of BACE1 inhibitors. In addition, they also revealed four main ligand binding mechanisms (Fig. 4). The binding pocket includes two conserved amino acids, ASP32 and ASP 228, and is located between interface of N- and C-terminal and covered by flap (VAL67-GLU77). In active form, it represents different structural features that promote substrate binding. Experimental study revealed that the flexible antiparallel-hairpin known as a flap covers the active site of BACE1. It is thought that this flap controls substrate access to the active site and positions the substrate in the proper shape for the catalytic activity. However, the substrate-free (apo) structure of BACE1 represents that the flap was in an open conformation, contrary to reports that the flap of the inhibitor-bound form is firmly packed in a closed shape. According to the study, a conformational change must

occur when an inhibitor or substrate binds to the active site and may kinetically contribute to substrate binding in the closed conformation and product release in the open conformation (Hu et al. 2019).

4 Rational Design of Selective Inhibitors of BACE1

BACE1 is a main therapeutic target for AD, despite the known adverse effects of BACE1 inhibitors. Depigmentation is the most persistent adverse effect observed in preclinical studies of β-secretase (BACE1 and BACE2) inhibition; it is generated by BACE2 inhibition. Therefore, the rational design of selective BACE1 inhibitors is a pressing need for the treatment of AD. BACE1 and BACE2 share numerous structural similarities, making the development of highly selective BACE1 inhibitors particularly difficult. Given the significance of the BACE1 target, researchers/pharmaceutical companies strive to develop selective inhibitors (Fig. 5) (Imbimbo and Watling 2019; Das and Yan 2019; Rombouts et al. 2021; Fujimoto et al. 2019; Ueno et al. 2021).

Among BACE1 inhibitors, Amidine-based pharmacophore is the most potent inhibitor (Fig. 6) (Das and Yan 2019; Rombouts et al. 2021). Several sub-pockets of

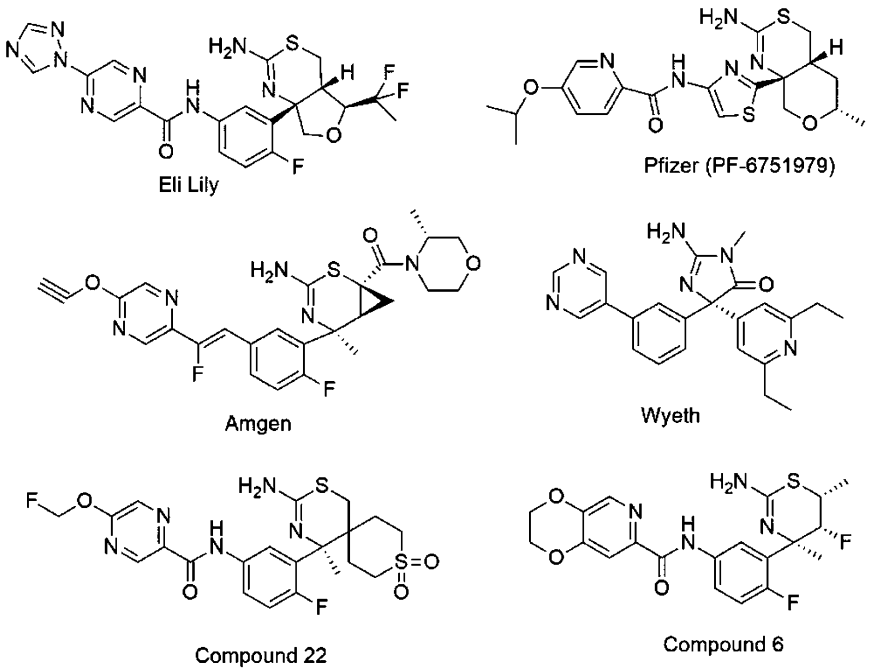

Fig. 5 BACE1 inhibitors with high selectivity (Rombouts et al. 2021; Fujimoto et al. 2019; Ueno et al. 2021)

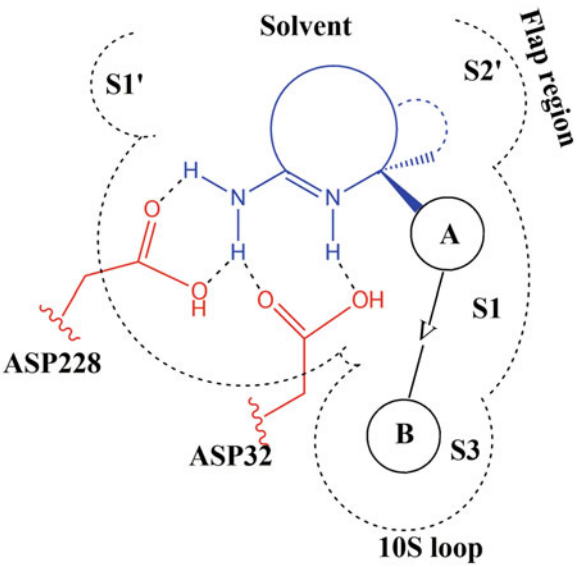

Fig. 6 Representation of the BACE1 pharmacophore and the position of sub-pockets S1, S3, S1′, and S2′. (Figure adopted from Rombouts et al. 2021)

BACE1 can be targeted in addition to the two catalytic residues, Asp32 and Asp228, that form a strong salt bridge with the amidine. The flap, a flexible antiparallel β-hairpin that controls substrate access lining the S2′ pocket, has proven to be very important in the development of BACE1 selective inhibitors. The rear of the S2′ pocket also has a residue difference with BACE2 that has been targeted by different pharmaceutical companies with comparable spirocyclic series. The S1 pocket is usually occupied by a (hetero)aromatic "A"-ring and can be accessible via a quaternary centre alpha to the amidine. The S3 pocket can be reached from this, usually via an amide linker ("L") in which the NH makes a hydrogen bond with the enzyme's Gly230. In azaxanthene inhibitor series, S3 pocket is typically occupied by a heteroaromatic "B"-ring, which confers potency and selectivity over aspartyl proteases cathepsin D (CatD). Furthermore, the S3 pocket is surrounded by the 10S loop, which has been exploited by research groups to obtain BACE1 selectivity over BACE2 (Rombouts et al. 2021).

Fujimoto et al. used structure/target-based drug design to develop effective and selective BACE1 inhibitors. They focused on the flap region, where the shape and flexibility of these enzymes vary. Analysis of the cocrystal structures of an early lead molecule inspired the incorporation of spirocycles, which resulted in extremely selective molecule 22 (BACE1, IC_{50}: 3.8A nM, BACE2, IC_{50}: 2094 nM) toward BACE1. The structures of 22 bound to BACE1 and BACE2 revealed that a relatively significant energetic penalty in the flap of the 22-bound BACE2 structure could result in a decrease of BACE2 potency, resulting in its high selectivity (Fujimoto et al. 2019). Recently, Ueno et al. discovered extremely selective fused pyridine-derived amyloid precursor protein-cleaving enzyme (BACE1) inhibitors with high in vivo efficacy through 10s loop interactions. They focused on the fact that the 10s

loop lining of the S3 pocket in BACE1 can form both "open (up)" and "closed (down)" conformations, whereas in BACE2, it prefers to adopt a "closed" form; thus, more space is available in BACE1. The cocrystal structures indicated that BACE2 has designed compound (6) considerably higher B-factors in the 10s loop than BACE1. Thus, BACE2 destabilization appears to provide structural insights into the decreased BACE2 potency of 6, explaining the considerable improvement in BACE1 selectivity (Ueno et al. 2021).

The crystal/co-crystal structures of BACE1/2 and their inhibitors were analysed to offer a broad description of the structural differences between BACE1 and BACE2. X-ray crystallography produces nearly static images of structures. Nonetheless, molecules in solution are constantly moving. In this situation, the only way to explore the conformational flexibility of a molecular system at the physiological level is molecular dynamic simulation. Considering this, researchers investigated the mechanism underlying inhibitor selectivity against BACE1 and BACE2 (Verma and Prabhakar 2015; Kumalo and Soliman 2016; Gueto-Tettay et al. 2017; Chen et al. 2019, 2020, 2021; Li et al. 2021).

Chen et al. utilized multiple short molecular dynamics (MSMD) simulations in conjunction with the molecular mechanics generalized Born surface area (MM-GBSA) approach in order to examine the binding selectivity of the three inhibitors, DBO, CS9, and SC7, on BACE1 over BACE2 (Chen et al. 2019). The findings demonstrated that the entropy effect is crucial in determining the selectivity of inhibitors toward BACE1 and BACE2, which establishes that DBO has higher selectivity toward BACE2 than BACE1, while CS9 and CS7 can more preferentially bind to BACE1 than BACE2. BACE1 and BACE2 may share the same hot interaction sites, according to a hierarchical clustering analysis based on the energetic contributions of residues. The inhibitor-residue interaction spectrum was computed using the residue-based free-energy decomposition method. The results show four common binding sub-pockets corresponding to the various inhibitor groups, which can be used as effective targets for creating highly selective inhibitors for BACE1 and BACE2 (Chen et al. 2019).

Multiple replica accelerated molecular dynamics (MR-aMD) simulations, principal component (PC) analysis, and free energy landscapes that were used to decipher the effect of disulfide bonds (SSBs) in BACE1 on the bindings of three inhibitors (3KO, 3KT, and 779) to BACE1. Cross-correlation analysis results indicate that SSB breaking has a major impact on the structural flexibility and internal dynamics of inhibitor-bound BACE1. PC analysis and free energy landscapes show that breaking SSBs not only clearly induces BACE1 conformational rearrangement, but also significantly changes binding poses of these inhibitors in BACE1. This mechanism leads to more disordered binding of inhibitors to BACE1, which is supported by an increase in binding entropy of inhibitors to BACE1. The free energy decomposition method based on amino acid residues was used to assess the contributions of individual amino acid residues to inhibitor-BACE1 binding. Amino acid residue-based free energy decomposition shows that residues L91, S96, V130, Y132, Q134, W137, F169, I171, and I179 can be employed as binding pockets for BACE1 drug candidates (Chen et al. 2020).

A possible BACE1 inhibitor known as C28 has recently been reported to have more selectivity to BACE1 over BACE2 than the previously reported inhibitors AZD3293 and AZD3839 (∼1.5-fold and 14-fold selectivity), which were both identified as BACE1 inhibitors (Li et al. 2021). Several molecular modelling techniques were used in this study to identify the selective mechanisms. Classical molecular dynamics (cMD) revealed significant fluctuations appeared in the protein structure. Additionally, according to free energy estimates, AZD3293 to BACE1 and BACE2 have equal binding affinities, whereas AZD3839 and C28 have substantially higher binding affinities to BACE1. Electrostatic interactions are thought to be the main cause of these differences. Accelerated molecular dynamics simulations were used to further observe the protein dynamics and energy disparities. The inhibitors' distinct patterns of dissociation from the binding pockets of BACE1 and BACE2 were also revealed by the umbrella sampling simulations, as well as the fact that various energy barriers were responsible for the selectivity. The rational creation of more effective BACE1 selective inhibitors may be designed easily by the information generated by this study (Li et al. 2021).

Multiple replica Gaussian accelerated molecular dynamics (MR-GaMD) simulations and the molecular mechanics general Born surface area (MM-GBSA) approach were recently used to study the influence of pH-dependent protonation on the binding of the inhibitors CS9, C6U, and 6WE to BACE1 (Chen et al. 2021). Dynamics simulations investigations based on the MR-GaMD trajectory demonstrate that pH-dependent protonation has a substantial impact on the structural flexibility, coupled movements, and dynamic behaviour of inhibitor-bound BACE1. According to the generated free energy profiles, inhibitor-bound BACE1 tends to occupy more conformations in the protonated state at low pH than at high pH. The binding free energies computed by MM-GBSA indicate that inhibitors have better binding capacities under conditions of protonation at high pH than under situations of protonation at low pH. In addition, pH-dependent protonation has a substantial effect on the hydrogen bonding interactions between CS9, C6U, and 6WE and BACE1, hence altering the binding capacities of the three inhibitors to BACE1. Moreover, in various protonated settings, three inhibitors share common interaction clusters and similar binding locations in BACE1, which are dependable targets for the construction of powerful BACE1 inhibitors (Chen et al. 2021).

5 Conclusion

β-secretase enzyme is a potential target for AD. There are two major forms of enzyme, BACE1 and BACE2. The APP metabolic pathway clearly indicates that BACE1 plays an important role in the development of abnormal and toxic amyloid β (Aβ, 42 amino acids) responsible for AD. However, the high homology surrounding the catalytic region of BACE1 and BACE2 constitutes a tremendous obstacle to the development of selective BACE1 inhibitors. Functional and structural (3D) understanding of the target site will lead to the development of BACE1

selective inhibitors. Experimental studies revealed that the cluster of eight-like structural shape, participation of aspartic acid residues (ASP 32, ASP228), flap region, and 10s loop are critical for BACE1 inhibitor binding. An advanced level of molecular dynamic simulation analysis of target and inhibitor co-crystal/docked conformation can lead to rational design of selective inhibitors.

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Endocrine Receptors: The Potential Therapeutic Targets for Alzheimer's

Tabassum Zafar, Ab Qayoom Naik, and Bashirulla Shaik

Abstract Alzheimer is characterized by the gradual loss of memory, thinking, brain functions, and behavioral alterations that ultimately interfere with the patient's capability to live a well-balanced comfortable and intellectual life. Memory, communication, and thinking are integral outcomes of the nervous system, especially brain's electrical and biochemical functionality. The link between dementia and various hormones, neurohormones, and endocrine receptors is a less explored domain, which has potential possibilities of novel therapeutic and preventive outcomes. Present chapter will discuss the biochemical pathways and endocrine modulations with an emphasis on role of estrogen receptors in Alzheimer treatment, prevention, and care.

Keywords Alzheimer · Dementia · Estrogen · Endocrine receptors · Therapeutics

1 Introduction

Alzheimer's disease (AD) is one of the widely known neurodegenerative disorders that is characterized by progressive and gradual impairment of cognitive functions that alter behavior and affect the functions related to comprehension, language, learning, attention, and memory. The severity of Alzheimer's symptoms is related to the stage of the disease. Alzheimer's symptoms can be categorized as presymptomatic/preclinical, mild, and dementia-stage. Dementia is referred to as memory loss which is a prime symptom of Alzheimer's disease.

Alzheimer's disease causes progressive episodic short-term memory loss, which leads to long-term memory impairment over time. Changes in decision-making

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abilities, problem-solving skills, a lack of motivation, depression, and lack of organization result in difficulties with multitasking and abstract thinking. In the later stages, loss of visuospatial abilities results in language disorder, dyspraxia, sleep deprivation, social withdrawal, and dystonia.

All the neuropsychiatric symptoms are the ultimate result of the involvement of various receptor-based mechanisms. The implications involved in disease activation and progression involve NMDA glutamatergic receptors of both ionotropic and metabotropic type, AMPA and, GABA receptors, muscarinic M1 or M2, β2 noradrenergic receptors, D2 dopaminergic receptors, serotonergic 5-HT6 receptors, and the cholinergic nicotinic α7 receptor.

2 Pathophysiology of Alzheimer's Disease

Alzheimer's disease is a chronic, progressive neurological disorder associated with memory loss, behavioral and emotional abnormalities, and thinking and language complications. The beginning of this disease is very tricky with no presence of a significant synchronous cerebrovascular illness, but rather a history of cognitive degeneration. Alzheimer's disease, the most common cause of dementia in aged people, is identified by huge plaque buildup and neurofibrillary pathology along with synaptic loss (Serrano-Pozo et al. 2011).

The pathophysiology of Alzheimer's disease is credited to a number of factors such as cholinergic dysfunction, amyloid/tau toxicity, and oxidative stress/mitochondrial dysfunctions.

2.1 Amyloid β Hypothesis

Amyloid beta (Aβ) progression and senile plaques (SP) deposition are two of the classical features of AD progression. Various secretase enzymes split the amyloid precursor protein (APP) and form a soluble small protein named amyloid beta. High production of Aβ results in less clearance and high deposition of oligomeric toxic substances named protofibrils, fibrils, and plaques (Shaik et al. 2018).

2.2 Oxidative Stress Hypothesis

The formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is one of the cellular signaling pathways that causes cellular damage. Brain neurons are vulnerable to oxidative stress injuries. The brain is the organ that utilizes 20% more oxygen than other mitochondrial respiratory tissues. In AD progression, it

is assumed that polyunsaturated fatty acids interact with ROS and cause molecular apoptosis by altering oxidative stress markers.

2.3 Metal Ion Hypothesis

Ionosphere and metal chelators are also considered to be causative agents of the development and pathogenesis of AD. Current research findings suggest that altered equilibrium of redox transition metals is involved. High concentrations of copper (Cu) and iron (Fe) are known to have an association with AD progression.

2.4 Cholinergic Hypothesis

According to the "Cholinergic Hypothesis" of AD, low cholinergic receptor binding and decline in acetylcholine-mediated neurotransmission are considered the prime causes of AD progression. This hypothesis allows the class of acetyl-cholinesterase inhibitors to be one of the treatment strategies for AD patients.

The mononuclear phagocyte system including microglia is characterized by plasticity and functional polarization. Macrophage activation and polarization in varied forms including m1, M2a, M2b, and M2c constitute a sequence, though these varied forms are strikingly different. An alternate activation phenotype known as M2a is connected to the Th2 response, type 2 inflammation, allergies, and parasite encapsulation. The M2a phenotype of microglia, which is necessary for the inflammatory phase to resolve, is induced by IL-4 and IL-13 (Mantovani et al. 2004; Villa et al. 2015).

Current AD diagnosis involves the use of noninvasive imaging tools such as positron emission tomography (PET), fludeoxyglucose PET, and Magnetic resonance imaging (MRI) in a combination of neuropsychological testing. A clinical diagnosis of AD requires neuroimaging and monitoring accepted biomarkers. Apart from the presence of amyloid oligomers and plaque accumulation, the concentrations of Aβ peptides (Aβ1-42:Aβ1-40 ratio), as well as total and hyperphosphorylated τ (Thr181 and Thr231) proteins in cerebrospinal fluid, are used for detection of AD (Wadghiri et al. 2003). The current therapeutic options such as rivastigmine, galantamine, and donepezil are not completely effective against the disorder because of the unfavorable pharmacokinetics and pharmacodynamics. The inadequate physicochemical properties, unstable pharmacokinetics, unfavorable absorption parameters, and related ADMET properties of drugs are responsible for the poor therapeutic management of AD. The selective transport of semipermeable blood-brain barrier also serves as an obstacle to the transmigration of neurotherapeutic molecules across the central nervous system (Tiwari et al. 2019).

3 Scope of Therapeutic Approaches

The deleterious biological responses that are reported in AD involve protein misfolding, which distorts cellular systems and neuronal death. In silico tools such as QSAR models, molecular docking, and molecular dynamics simulations are widely used in drug molecule prediction and disease progression studies. QSAR and molecular docking studies are used in AD interventions to predict amino acid residues of various types, including catalytic anionic site (CAS) and peripheral anionic site (PAS), and to assess the interaction potencies of various drug molecules based on ligand affinity (Shaik et al. 2018).

Medications including cholinesterase inhibitors and memantine are used to treat the symptoms of the disease to manage it. These medications have no impact on the disease progression or its cure. Several clinical trials are being used to analyze different therapeutics with mixed outcomes including nonsteroidal anti-inflammatory medications (NSAIDs), especially coxibs, selegiline, and vitamin E. Besides, selective estrogen receptor modulators (SERM) or estrogens are also tested because of their use as hormone replacement therapeutics in both early and late-menopause (Anand et al. 2014). The pathogenesis of Alzheimer's disease was not investigated until recently. A number of factors including mitochondrial dysfunction, tau proteins, inflammation, and its determination, oxidative stress, microglial dysfunction, autoimmune illness, infections, and amyloid (A) buildup are collectively involved in the development of Alzheimer's disease. There are a very small number of cases having early-onset of Alzheimer's disease (AD) related to autosomal dominant genetic abnormality due to mutations in presenilin-1, presenilin-2, or amyloid precursor protein genes. Such mutations augment the amyloid-neurotoxic peptide formation resulting in an unknown sequence of ill-timed events, leading to neuronal death. Further, the E4 allele of the apolipoprotein E (APOE) gene is associated with an increased risk of developing Alzheimer's disease (AD), though the origin of most of the AD cases, late-onset AD in particular, is still unknown (Finch and Morgan 2007). Common brain areas affected by AD have frequent inflammation which is also associated with the pathophysiology of AD disorder. Microglia which play a significant physiological role in synaptic maintenance, growth factor release, immunological surveillance, and neurogenesis is the key cell in affected in AD pathogenesis. The release of pro-inflammatory cytokines from activated microglia brings about the inflammatory response including tumor necrosis factor (TNF), IL-23, IL-12, nitric oxide, and other mediators. The buildup of plaques and associated inflammation in AD is associated with the dysfunction of pro-inflammatory cytokines. Besides, the elevated risk of AD and other neurodegenerative diseases may be due to genetic abnormalities affecting microglia activity (Loane and Byrnes 2010; Zhang et al. 2013; Ransohoff 2016).

Initially, the role of reversible inhibition of acetylcholinesterase (AChE), a serine hydrolase, was considered a potential therapeutic tool for Alzheimer's disease (AD). However, recently many researchers reported the potential for estrogen receptor modulation to improve cognitive function by reducing the formation of senile

plaques (Abdizadeh et al. 2020). AD involves β-site APP (amyloid precursor protein) cleaving enzyme-1 (BACE-1) which is also revealed to be a potential therapeutic option for the management of AD (Shaik et al. 2022).

Dementia, which is defined as a deterioration in thinking and independence in daily tasks, is caused by AD-dependent degradation of brain cells. The cholinergic and amyloid hypotheses were put up as two key causes of AD, and AD is thought to be a complex illness. In addition to age dependence, other factors that can contribute to the advancement of AD include genetics, head injuries, vascular infections, excessive stress levels, and environmental factors (Barnes et al. 2012). The pathogenesis of AD is being studied globally in the twenty-first century by focusing on free radical damage, aberrant tau protein metabolism, amyloid-cholinergic pathway investigation, and inflammatory responses. Two primary medicines are frequently used in fashion as AD treatments. These work as cholinesterase enzyme inhibitors and *N*-methyl D-aspartate antagonists (NMDA). However, professionals continue to find the prevention of disease to be a perplexing and unresolved riddle. The complexity of AD patterns highlights the need for new disease-modifying therapies (DMT), including chaperones, herbal remedies, endocrine checkpoints, and natural pharmacological molecules for better management and efficient disease prevention. In times of epidemic type of infectious diseases, the role of herbal alternatives and phytoestrogens is interestingly explored for medicinal applications (Zafar 2020; Naik et al. 2021). The high efficacy and safer usage create the possibility of classical neurodegenerative disease management using a similar set of herbal components (Zafar 2022).

Research studies investigating the origin of AD have put forward different ideas regarding the pathophysiology of AD. One of the most known ideas describing the progression of AD disease is amyloid hypothesis. The initiation of changes in AD is associated with the creation of amyloid plaque (insoluble protein and other cellular material deposits outside the neurons) due to elevated beta-amyloid peptide formation. The altered metabolism of APP (amyloid precursor protein (APP) breakdown produces 38–42 amino acid peptides known as Aβ) is believed to be responsible for elevated A42 synthesis and successive plaque development. Besides, the development of AD is associated with another significant protein known as tau protein (regular microtubule component) which stabilizes the microtubule structure. The tau protein found in medium and large pyramidal neurons forms neurofibrillary tangles (NFTs) due to hyperphosphorylation in AD, resulting in altered microtubule integrity and subsequent neuronal death. The early presence of NFTs in the hippocampus and temporal lobes of such patients demonstrates the pathological characteristic of AD (Hardy and Allsop 1991; Morris et al. 2011).

Among the several significant factors responsible for the development of AD, increased free reactive oxygen species (ROS) formed due to overproduction and poor clearance are believed to be one of the contributing factors. Excessive ROS species production affects regular cell function. The presence of elevated ROS markers including 3-nitrotyrosine and reactive aldehydes, by-products of lipid peroxidation (protein oxidation markers) in the central nervous system (hippocampus and cerebrospinal fluid) in AD patients, has been reported by many studies.

Nucleic acids are also affected by ROS in such AD patients as shown by increased DNA breakage in the cerebral cortex and hippocampus and mRNA or rRNA. Besides, a decrease in antioxidant molecules and their functions including glutathione peroxidase, superoxide dismutase, etc. was also reported in AD patients (Venkateshappa et al. 2012; Wang et al. 2014).

There were structural modifications reported in mitochondria in neuron biopsy specimens in AD patients. Mitochondrial dysfunction due to DNA damage, dysfunctional energy metabolism, and improper calcium handling are some of the associated effects responsible for the development of AD (Kruman et al. 1998; Hirai et al. 2001).

The therapeutic role of various enzymes and endocrine receptors in the development and therapeutic management of multiple diseases is evident in various studies. In contrast to all the recent advancement in point-of-care devises, there is still no handy testing devices available in the market to detect neurological abnormalities. However, a set of cognitive test batteries can help to detect behavioral dysfunction and memory impairments in neurologically abnormal patients (Zafar 2019).

4 Endocrine Receptors and Alzheimer's Disease

Age-related loss of sex steroid hormones is considered a direct implication of AD. The precipitous estrogens and progestogens decrease in postmenopausal women are thought to have enhanced susceptibility to AD pathogenesis. This concept is supported by widespread epidemiological studies, though negated by some clinical studies. A number of neuroprotective effects related to AD prevention, especially the promotion of neuron viability and β-amyloid accumulation reduction, are supported by several experimental studies. The promotion of neuron viability and β-amyloid accumulation reduction serve as critical factors in the initiation and progression of AD. Recent findings suggest neural responsiveness to estrogen can diminish with age, reducing the neuroprotective actions of estrogen and, consequently, potentially limiting the utility of hormone therapies in aged women. Further, progestogens also modulate estrogen neuroprotective effects, particularly continuous progestogen exposure is associated with the inhibition of estrogen actions, whereas cyclic delivery of progestogens may enhance the neural benefits of estrogen. Recent studies are focused on elucidating a parallel relationship between sex steroid hormones and AD risk in men (Kwakowsky et al. 2016).

Hormone receptors other than estrogens also play a significant role in the development of Alzheimer's disease. Luteinizing hormone (LH) and its receptor have been reported to modulate neuronal plasticity and cognition. The most important thing about the role of LH in AD is that the levels of brain LH are inversely related to levels in the periphery, thus low upon menopause-related upregulation of peripheral LH. It was believed that the downregulation of peripheral LH improves function and plasticity in AD mouse models which were associated with brain LH. However, it is still unclear if there is any involvement of central LHR signaling

in these LH-associated improvements. Several research studies report that hCG treatment differentially modulates cognition, pathology, and plasticity markers, suggesting involvement of LHR in CNS function. This opens a new window for the study of LHR as a possible contributor associated with menopause and increased AD risk (Mey and Casadesus 2021).

The fact that development of AD in postmenopausal women due to estrogen level decline proved to be inconclusive as the Women's Health Research Initiative Memory Study and the population-based nested case-control study failed to demonstrate that estrogen/progesterone (hormone replacement therapy [HRT]) or estrogen replacement therapy could prevent the cognitive decline or reduce the risk of AD. These observations led to the conclusion that AD development could be due to a progressive increase in luteinizing hormone (LH) levels in postmenopausal women. Therefore, several studies have reported that increased LH level is positively associated with neuropathological, behavioral, and cognitive changes in AD. Besides, a potential role of LH in furthering the amyloidogenic pathway of precursor protein metabolism and deposition of amyloid β plaques in the hippocampus, a region involved in AD, is also reported. Hippocampus abundantly expresses that cognate receptors that mediate LH effects and thus reduction in LH levels by treatment with gonadotropin-releasing hormone agonists could provide therapeutic benefits (Rao 2017).

The presence of functional LH/hCG receptors in the central and peripheral nervous systems, besides other nongonadal tissues, has changed the old paradigm that these receptors are only found in gonads and regulate only gonadal functions (Lei et al. 1993; Rao et al. 2003; Meng et al. 2007). There are several reports that have demonstrated the presence of LH/hCG receptors in fetal rat brains and in adult rats, mice, bovine, and human brains (al-Hader et al. 1997; Apaja et al. 2004). In addition, LH/hCG receptors were also found in primary as well as immortalized cells, such as hypothalamic GT1-7 neurons, α-T3 anterior pituitary gonadotropins, HN33p hippocampal neurons, and so on (Huang et al. 1995; Mores et al. 1996; Li et al. 1996).

Regarding the second hormonal factor that contributes to the onset of AD, it is well known that as ovarian function declines with aging, estrogen levels fall and LH levels rise as a result of estrogens' loss of their protective negative feedback mechanism. Therefore, an increase in LH and/or follicle-stimulating hormone (FSH) levels is the result of estrogen levels declining. Before the identification of hippocampal LH/hCG receptors, increased elevation of LH levels in AD-affected women, and direct LH effects on cognition and biochemical abnormalities typical of AD, this theory, however, garnered surprisingly little attention. FSH's function is uncertain, despite evidence of its production and receptors in the rat hippocampus. Some other reports also support the fact that LH is involved in AD development. The elevated LH levels are associated with increased AD risk as it was found that circulating LH levels are more than double in postmenopausal women who have developed AD as compared to those who did not develop AD or non-AD patients with cognitive deficiency or those with AD (Payami et al. 1996; Bowen et al. 2000). In the mouse brain, the LH level lowering enhances cognition, lowers A levels, and

protects memory loss in an AD model produced by neurotoxic. Compared to control brains, Alzheimer's disease brains exhibit a more immunoreactive LH. In addition, compared to age-matched normal brains, LH builds up in pyramidal neurons of AD brains (Bowen et al. 2002).

Men who experience normal age-related testosterone decline are more likely to develop numerous disorders, including AD. Similar to estrogen, testosterone has been shown to be an endogenous neuroprotective agent that lowers β-amyloid deposition while also increasing neuronal resilience against AD-related stressors. The activation of androgen pathways and the subsequent beginning of protective estrogen signaling processes are two ways by which androgen neuroprotective effects are mediated (Pike et al. 2009). Neuron survival in multiple CNS regions, normal neuron development, and neural injury are regulated by sex steroid hormones. A potential function of sex steroids is the translation of their neuroprotective activities into efficacious strategies against age-related neurodegenerative diseases including AD.

5 Mechanism of Action of Estrogen Receptors in Alzheimer's Disease

The pathophysiology of Alzheimer's disease is associated with two types of estrogen receptors, i.e., nuclear receptor-ERα and ERβ. Of the ERβ, six splice variants have been elucidated in the brain and other tissues, wherein ERβ1 isoform has a proven neuroprotective effect and ERβ2 isoform possesses a tumoral suppressor effect (Bottner et al. 2014; Dey et al. 2015; Shaik et al. 2021). G-Protein-coupled receptors ER1 (GPER1) are membrane receptors present in the brain and at the periphery and mediate rapid non-genomic signaling of estrogens. In addition, the GPER1 receptor coupled with Gs-protein increases the intracellular concentration of cAMP, and the presence of ERα is believed to be vital for its effect and both of these receptors might be coupled (Alexander et al. 2017).

Recently, a new SERM, known as diphenylacrylamide compound STX (2-(4-hydroxyphenyl)-3-phenylpent-2-enoic acid [4-(2-dimethylaminoethoxy)phenyl] amide, *E*-enantiomer), was identified exhibiting neuroprotective effects of estrogens except inducing oncogenic and thrombotic effects. STX binds G protein-coupled estrogen receptor GqMER located in the plasma membrane. However, unlike E2 (17β-estradiol) and its analogs, STX lacks affinity to nuclear estrogen receptors ERα and ERβ. The presence of two types of receptors facilitates estrogens with complex genomic and non-genomic and some pleiotropic activities due to the central and peripheral distribution of receptors. The presence of estrogen receptors in the brain is found in cognitive zones including amygdala, cerebral cortex, hippocampus, and basal forebrain (Gillies and McArthur 2010; Gray et al. 2016). The neuroprotective effect of estrogens may be a multifactorial combination of neurobiological and signaling actions that play a vital role in the prevention of cognitive

impairment in AD and the improvement of cognitive functions of AD patients (Zhang et al. 2014; Arevalo et al. 2015).

AD is characterized by the extensive build-up of plaque and neurofibrillary pathology. Estrogens and SERMs are believed to be closely associated with inflammation, resolution, and pathogenesis of Alzheimer's disease. The impact of pharmacological interventions on the membrane or intracellular signaling pathways associated with estrogens and believed to be potential clinical treatments in preventing Alzheimer's disease is yet to be investigated. The use of estrogens may influence the determination of the inflammation process, specifically ER or G protein-coupled estrogen receptors (GPER1 or GqMER) actions, which may have positive consequences on Alzheimer's disease.

The essential factors implicated in the neuroprotective effect of estrogens are their anti-inflammatory activities. Besides, E2 plays a vital role in attenuating endothelial dysfunction and modulates the permeability of the blood-brain barrier by acting on ERα and ERβ. In addition, activation of nuclear factor kappaB (NFκB), which induces the transcription of certain cytokines (tumor necrosis factor-alpha, chemokine ligand 2-CCL2, interleukine 6), is also inhibited by E2. It also suppresses the mRNA expression of pro-inflammatory endothelial molecules: e-selectin, ICAM-1, and VCAM-1 (Vegeto et al. 2008; Nakagami et al. 2010).

Due to a potential change in the microglia's phenotype to M2a, which could start the resolutive phase in brain tissue, ERα selective agonists may be an alternative to hormone replacement therapy (HRT) in AD patients. The genetic deletion of ERα, which is linked to a spontaneous reactive phenotype of microglia, suggests this mechanism (Vegeto et al. 2003). Several other mechanisms involved in the neuroprotective role of estrogens are as follows; mitochondrial function repairing due to inhibition of beta-amyloid oligomers; antioxidant effect due to enhancement of reduced glutathione concentrations and decreased oxidative DNA damage in mitochondria; enhanced amnesic function due to NMDA-dependent mechanism and long-term potentiation; neurogenesis particularly in comu ammonis 1 (CA1) and hippocampal region; etc. (Woolley et al. 1990; Brinton 2001; Bimonte-Nelson et al. 2004; Sarkar et al. 2015).

6 Selective Estrogen Receptor Modulators (SERM) Mechanisms of Action in AD

A vast group of compounds is included, SERM, which act both as partial agonists by selectively influencing a certain type of estrogen receptors and also as antagonists on other types of signaling systems (membranous/intracellular) associated normally with natural estrogens. Clomiphene, tamoxifen, bazedoxifene, and ospemifene are some of the partial agonists of ERβ. It also includes ERα receptor agonists, agonists of membrane receptors GPR30, and agonists of GqMER receptors (e.g., STX) (Hajjo et al. 2012; Maximov et al. 2013; Gray et al. 2016). During recent years,

several experimental and clinical studies have been investigating the possible useful effects of SERM against the risk of progression and development of AD compared to those of estrogens (E2 and others).

In addition to the common effects of SERMs and estrogens, some of the off target SERM effects include; the action of SERMs on the glutamatergic system by activating the glutamate/aspartate transporter (GLAST), increased expression of transforming growth factor beta 1 (modulator of the stimulatory effects of estrogens (E2) and SERMs (tamoxifen) on the dysfunctional transport of glutamate-mediated process through activation of signaling pathways including mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and phosphoinositide 3-kinase (PI3K)/Akt). These pathways are active in neurodegenerative disorders characterized by altered glutamate homeostasis (Lee et al. 2009), (b) Tamoxifen's ability to suppress CDK5 and P2 protein provides a window for treating tauopathies like AD. Tamoxifen and raloxifene have been shown in studies to have neuroprotective effects against beta-amyloid hydrogen peroxide and glutamate-induced toxicity without significantly improving estrogen-dependent memory at sub-lethal levels. Additionally, telomerase catalytic subunit (TERT) phosphorylation at the transcriptional and posttranscriptional levels and the association of NF-B were both enhanced by raloxifene and E2, contributing to the neuroprotective effects of these drugs. Additionally, raloxifene exhibits estrogen-like properties on the hippocampus choline acetyltransferase (ChAT), a known estrogen-responsive gene product, in vivo, suggesting that SERMs may improve cholinergic transmission in the brain (Wu et al. 1999; O'Neill et al. 2004; Du et al. 2004; Corbel et al. 2015).

7 Conclusion

The high incidence possibility of AD is increasing with the growing population. The gender disparities in AD occurrence should be taken into account for a better understanding of endocrine receptor involvement in dementia and Alzheimer's. The role of sex steroids on brain function and neurological diseases should be taken into consideration to explore the research possibilities and therapeutic potential. The existing data about the involvement of estrogen and other endocrine receptors with AD and similar health issues show many research gaps that need to be addressed by exploring the epidemiological, neuropsychological, and biological effects of these receptors with neuronal networks in various settings to narrow down the specific therapeutic outcomes. The limitations of delayed identification of recognizable symptoms of AD are one of the prime culprits for poor identification of the Alzheimer's patient at clinical front. The enzyme inhibitors are also difficult to get approved due to their side effective natures and overwhelming interactions with other physiological process. The difficulty of various therapeutic formulations to cross the blood-brain barrier is also another vital cause for poor clinical improvement in AD patients.

All these limitations support the need of investigation of new generic in vivo synthesizing molecules which can prevent the AD progression at significant extend. The use of combination therapies along with multitarget-directed ligands approach to deal with multifactorial onset of AD can result in better prevention and effective management of disease. Authors also suggest the use of synthetic neuropeptides and neuromodulator substances as a potential drug candidate against AD.

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Calcium Channels as a Potential Therapeutic Target for Alzheimer's Disease

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Abstract Alzheimer's disease (AD) is the most common type of dementia that characterizes the accumulation of amyloid-β plaques and neurofibrillary tangles in the brain. AD is the most common cause of death and accounts for approximately 60–70% of the total cases imposing greater socio-economic burden across the globe. Despite extensive research, the mechanisms underlying AD are unknown. There are many hypotheses that define the pathogenesis of Alzheimer's disease among which the amyloid cascade hypothesis is widely accepted. The hypothesis discusses the role of amyloid-β in causing alteration in calcium homeostasis. Amyloid-β-mediated dysregulation of calcium ions homeostasis has been extensively studied to understand the underlying mechanisms of neurodegeneration observed in AD. Amyloid-β and tau interact with the calcium and calcium ion channels on the plasma membrane, endoplasmic reticulum, and mitochondria of the neuronal cells to form the vicious circle of calcium load and amyloid-β accumulation. The disruption in calcium homeostasis accompanies changes as observed in the whole brain pathology of AD. This includes synaptic dysfunction, impaired cognition, mitochondrial dysfunction, oxidative stress, inflammation, and cell apoptosis. This presents an immense need for a variety of potential therapeutic targets that could prevent or slow the progression of AD. This book chapter deals with the comprehensive source of information about mechanisms through which amyloid-β and tau interact with

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various calcium ion channels and the beneficial effect of calcium ion channel modulator in AD.

Keywords Alzheimer's disease · Calcium dyshomeostasis · Amyloid-β · Tau · Cognitive dysfunction

Abbreviations

3XTg-AD	Triple-transgenic mouse model of Alzheimer's disease
AChE	Acetylcholinesterase
AMPAR	α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor
AMPK	5′ adenosine monophosphate-activated protein kinase
Aβ	Amyloid beta
Ca^{2+}	Calcium
NCX	Na^{+}/Ca^{2+} exchanger
NMDAR	N-methyl D-aspartate receptor
PMCA	Plasma membrane Ca^{2+}-ATPase
ROS	Reactive oxygen species
RYR	Ryanodine receptor
SOCE	Store-operated Ca^{2+} entry channels
STIM	Stromal interaction molecules
ULK1	Unc-51 like autophagy activating kinase 1

1 Introduction

Alzheimer's disease (AD) is a neurodevelopment disease that characterizes deterioration in cognitive function, progressive memory loss, language disorders, and personality changes. Although it mainly affects individuals in older age, however, it is not an inevitable consequence of a usual process of ageing (Alzheimer's Association 2016). Alzheimer's disease is the most common cause of death that accounts for approximately 60–70% of the total cases. Recent data suggest that more than 55 million people are suffering from Alzheimer's with approximately 10 million new cases globally every year (World Health Organization 2022). Among these, the majority are sporadic and the minority, accounting for 1–2% of cases are familial (due to genetic mutation) Alzheimer's disease. There are many hypotheses that define the pathogenesis of Alzheimer's disease. These include amyloid, tau protein, excitatory amino acid, genetic, chronic inflammatory, vascular, cholesterol, neuronal apoptosis, and oxygen-free radicals' hypothesis. Among these hypotheses, the amyloid cascade hypothesis is the widely presumed hypothesis to cause Alzheimer's disease pathophysiology (Mohandas et al. 2009). Alzheimer's disease is marked by the presence of massive extracellular deposition of amyloid-β (Aβ) aggregates

(senile plaques) and intracellular accumulation of neurofibrillary tangles (NFTs) (hyperphosphorylated tau proteins deposits) in the brain (Elahi and Miller 2017; Jayant et al. 2016a, 2016b; Singh et al. 2013). Aβ and NFT cause axonal disintegration, synaptic dysfunction, and degeneration that impair cognitive function. Furthermore, Aβ activates cerebral immune responses and inhibits brain metabolic function that causes upregulation of various pro-inflammatory markers and oxygen-free radical species generation (He et al. 2013; Olsson et al. 2016; Lista and Hampel 2017; Baldacci et al. 2017; Heneka et al. 2015; Iturria-Medina et al. 2016; de la Monte and Tong 2014; Steinman et al. 2021). The increase in inflammation and oxidative stress is known to cognitive dysfunction, microvascular dysfunction, and neuronal apoptosis in various experimental models (Sharma et al. 2021a, 2021b, 2022; Aggarwal et al. 2022). Despite decades of efforts and research, till date there is no effective treatment strategy that could stop or slow the disease progression. However, there are several drugs that may help in alleviating symptoms. These include FDA-approved drugs such as acetylcholinesterase inhibitors (AChE) and N-methyl D-aspartate receptor blockers (NMDAR), which also possess a variety of side effects (Cacabelos 2007; Olin and Schneider 2002). Hence, there is an enormous and urgent medical need for the development of novel therapeutic strategies that could target the underlying pathogenic mechanisms associated with Alzheimer's disease.

The calcium (Ca^{2+}) channel is a transmembrane ion channel that is permeable to Ca^{2+} ions and can be gated by either voltage or ligand binding. Ca^{2+} channels play an important role in several physiological functions of the cells. Ca^{2+} signalling (Ca^{2+}) regulates neuronal growth and differentiation, action potential properties, synaptic plasticity, exocytosis, learning, memory, and cognitive functions inside the brain (Brini et al. 2014). The concentration of Ca^{2+} needs to be regulated at optimal concentrations for normal neuronal functions. This can be achieved by the channel proteins of the neuronal plasma membrane, endoplasmic reticulum, and mitochondria. Alterations in the Ca^{2+} homeostasis can disrupt normal neuronal structure and function. Evidence suggests that disruption in the Ca^{2+} homeostasis plays an important role in the neuropathology of Alzheimer's disease and is associated with oxidative stress, neuroinflammation, autophagy, memory loss, and cognitive dysfunction (Jain and Sharma 2015; LaFerla 2002; Jain et al. 2016; Singh and Sharma 2016; Bobich et al. 2004; Zhang et al. 2023). Disruption in the Ca^{2+} homeostasis can cause Aβ plaque formation, NFT accumulation, and disruption in the synaptic plasticity, which further affects the Ca^{2+} homeostasis, thus forming a vicious cycle (Bezprozvanny 2009). Ca^{2+} dysregulation in the plasma membrane, endoplasmic reticulum, mitochondria, and cytosolic space has been reported in various Alzheimer's disease models (Wang et al. 2017; Liang et al. 2015; Lee et al. 2020). Ca^{2+} channel blockers have demonstrated to show beneficial effects against memory impairment, cognition, inflammation, and oxidative stress in various experimental models of Alzheimer's disease (Bobich et al. 2004; Chorvat et al. 1998; Sadleir et al. 2022; Lee et al. 2020; Jayant and Sharma 2016). Therefore, in order to interrupt the progression of Alzheimer's disease, little attention is required to be paid to Ca^{2+} channel blocker as the therapeutic target.

In this book chapter, we will be discussing the mechanisms through which amyloid-β and tau interact with various calcium ion channels and the beneficial effect of calcium ion channel modulator in AD.

2 Ca^{2+} Homeostasis

The regulation of Ca^{2+} dynamics is very complex in neurons. Ca^{2+} plays a vital role in various signal transduction mechanisms that regulate cell survival, differentiation, proliferation, and apoptosis. Ca^{2+} is involved in the regulation of various neuronal functions such as neurotransmitter release, synaptic plasticity, gene transcription, membrane excitability, and neuronal death to cite a few (Brini et al. 2014). Ca^{2+} binds with calmodulin (CaM) and causes activation of calcineurin (CaN), Ca^{2+}/calmodulin-dependent protein kinase (CaMKII), and CaMKIV, which are responsible for playing a crucial role in synaptic strengthening and memory formation (Lisman et al. 2002; Kang et al. 2001). Furthermore, Ca^{2+} modulates the function of protein kinase C (PKC) that regulates cell survival and cell division (Brini et al. 2014).

The concentration of Ca^{2+} ions in the cytosol (50–200 nM) is 10,000 folds less than the concentration of Ca^{2+} ions outside the cell (1–2 mM). This concentration gradient is largely maintained via the continuous removal of Ca^{2+} ions by the plasma membrane and endoplasmic reticulum Ca2+-ATPases (SERCA) (Wang et al. 2017). This regulation involves proteins that are localized essentially in all major subcellular structures such as cytosol, plasma membrane, endoplasmic reticulum, and mitochondria. The plasma membrane Ca^{2+} channels include voltage-gated Ca^{2+} ion channels, receptor-operated Ca^{2+} channels, and store-operated Ca^{2+} entry channels (SOCE) that activate when upon the emptying of cellular Ca^{2+} stores (Michaelis 1998). Voltage-gated ion channels allow Ca^{2+} ions influx upon depolarization of the membrane potential. Voltage-gated ion channels are divided into L-type, P-type, N-type, R-type, and T-type Ca^{2+} channels depending upon the physiological and pharmacological properties of the current they carry. Receptor-gated Ca^{2+} channels require the binding of specific ligand to the extracellular domain. L-Glutamate, the main excitatory neurotransmitter, activates two different classes of receptors: the ionotropic receptors (iGluR) and the metabotropic receptors (mGluR). The two principal types of iGluR are NMDAR and α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor (AMPAR), which activates not only upon glutamate binding but also upon membrane depolarization (Striggow and Ehrlich 1996; Brini et al. 2014). Another ionotropic receptor is the purinergic (P2X) receptors that respond to the extracellular ATP to induce membrane depolarization and Ca^{2+} influx. Extracellular ATP is produced via neurons, neuroglia, and via damaged cells during any cerebral insult. P2XR inhibits and facilitates long-term changes in synaptic strength and modulates synaptic plasticity (Pankratov et al. 2009). The mGluR is coupled with the G-protein-coupled receptors that activate downstream signalling pathways to activate phospholipase C. mGluR produces rapid transient depolarization that

produces Ca^{2+} ions from the intracellular stores and a prolonged depolarization via activation of TRP channels (Striggow and Ehrlich 1996). The metabotropic P2Y receptors also initiate Ca^{2+} signals and propagate Ca^{2+} waves across the astroglial networks upon activation of the astrocytic receptors via ATP released during the synaptic transmission. This further diffuses inositol (1,4,5)-trisphosphate (IP3) from the gap junctions (Hamilton and Attwell 2010).

The endoplasmic reticulum and mitochondria serve as the intracellular Ca^{2+} stores (Striggow and Ehrlich 1996). The influx of Ca^{2+} from the plasma membrane enters the cytosol; however, excess Ca^{2+} ions are removed by the endoplasmic reticulum via Ca^{2+} ATPase (SERCA pump). The influx of Ca^{2+} ions activates RYR receptors in the endoplasmic reticulum to release Ca^{2+} ions from the stores. On the other hand, the activation of metabotropic receptor that is coupled to GTP-binding proteins G_{q11} produces IP3, which serves as the ligand for IP3R in the endoplasmic reticulum. RYR and IP3R activation increases the release of Ca^{2+} from the intracellular Ca^{2+} stores of the endoplasmic reticulum. However, the release of Ca^{2+} from the endoplasmic reticulum is regulated by an array of various regulatory proteins such as FK506-binding proteins, calmodulin, calcineurin, cytoskeletal protein ankyrin, and sorcin (Brillantes et al. 1999; Cameron et al. 1996; Yamada et al. 1995; Pickel et al. 1997; Bourguignon and Jin 1995). High levels of endoplasmic reticulum Ca^{2+} pool in the synapse suggest the role of Ca^{2+} in synaptic plasticity. Furthermore, to assure the re-filling of the depleted intracellular Ca^{2+} stores, the endoplasmic reticulum comprises transmembrane stromal interaction molecules (STIM) that detect the depleting Ca^{2+} concentration and communicate with the SOCE channels on the plasma membrane with ORAI subunits (Venkiteswaran and Hasan 2009). Unlike SOCE, TRC channels show high Ca^{2+} sensitivity and are present in many excitable and non-excitable cells. These channels are also localized in the endoplasmic reticulum and Golgi apparatus and are known to interact with ORA1 to act as a regulatory subunit to confer STIM1-mediated Ca^{2+} store depletion sensitivity to SOCE (Lu et al. 2010; Liao et al. 2007). In addition to this, the plasma membrane has ARC channels that are highly selective, small conductance Ca^{2+} ion channels, activated at low agonist concentrations at the appropriate receptors on the plasma membrane. ARC channels are biophysically like SOCE channels whose activation depends upon STIM1 proteins present inside the plasma membrane (Thompson et al. 2013). However, the extrusion of Ca^{2+} ions from the plasma membrane to the extracellular environment occurs via Ca^{2+} ATPase (PMCA) and Na^{+}/Ca^{2+} exchanger (NCX) in the plasma membrane (Brini and Carafoli 2011).

Also, mitochondria play a crucial role in the energy metabolism and regulation of cytoplasmic Ca^{2+} levels under normal and pathological conditions. Mitochondria are abundantly present in the neurons and neuroglia. Mitochondria serve as low-affinity, high-capacity transient Ca^{2+} stores. Changes in the cytosolic concentration of Ca^{2+} ions allow Ca^{2+} to enter inside the mitochondrial membrane (inner) via an electrophoretic uniporter in the mitochondria (MCU) to reach a sub-micromolar threshold (Nicholls 2005). However, during the declining phase of the signals, the mitochondria release Ca^{2+} via Na^{+}/Ca^{2+} exchanger (NCX) in the local environment (Scanlon

et al. 2000). Excessive mitochondrial Ca^{2+} uptake increases reactive oxygen species (ROS) formation, inhibits ATP synthesis, induces mitochondrial permeability transition pore (mPTP) opening, and releases cytochrome c as well as apoptosis-inducing factors (AIF) from the mitochondrial intermembrane space into the local environment (Brustovetsky et al. 2003; Jiang et al. 2001). Furthermore, disruption in the cytosolic Ca^{2+} concentration and Ca^{2+} signalling will impact the essential neurological functions played by Ca^{2+} including the release of neurotransmitter and signalling, LTP, LTD, synaptic plasticity, neuronal plasticity, and cognition. Such alterations have been observed against several neurodegenerative disorders such as Alzheimer's disease.

3 Dysregulation of the Ca^{2+} Homeostasis in AD

The Ca^{2+} hypothesis was first formulated by Khachaturian in 1989 (Khachaturian 1989). The work explored how the activation of the amyloidogenic pathway modulates various Ca^{2+} signalling pathways responsible for memory formation and cognition (Khachaturian 1989). Ca^{2+} dyshomeostasis has been reported in various in vivo and in vitro experimental studies of Alzheimer's disease, influencing both tau hyperphosphorylation and Aβ (Stutzmann et al. 2007; Zhang et al. 2023). Several studies, however, have demonstrated that the irregulation of calcium homeostasis occurs way before the development of Alzheimer's symptomology and pathogenesis (Disterhoft et al. 1994). Furthermore, the increase in the calcium load promotes Aβ formation and production, whereas the increase in Aβ accumulation stimulates more Ca^{2+} production and signalling forming a vicious circle (Wang et al. 2017; Liang et al. 2015; Lee et al. 2020; Bosson et al. 2017; Ferreira et al. 2015). Therefore, Aβ and Ca^{2+} synergistically intensify the cognitive deficits and neurodegeneration in individuals suffering from dementia.

Alzheimer's disease is marked by an increase in Aβ accumulation and tau phosphorylation. Aβ causes Ca^{2+} reflux across the plasma membrane and intracellular store (Jensen et al. 2013). Aβ and Tau have been reported to alter the Ca^{2+} ion receptor present on the plasma membrane, endoplasmic reticulum, and mitochondria. Researchers are working to elucidate the mechanisms of calcium dyshomeostasis in Alzheimer's disease. Tau plays a crucial role in axonal transport. Tau is reported to interact and inhibit PMCA, which is responsible for maintaining the cytosolic Ca^{2+} ions in the cells. However, the major PMCA activator calmodulin was observed to rescue the cells from the tau-mediated Ca^{2+} dyshomeostasis (Berrocal et al. 2017). Ca^{2+}-dependent PMCA activity is found impaired in the brain of dementia patients. Aβ inhibits PMCA activity and impairs PMCA-mediated Ca^{2+} regulation in Alzheimer's disease (Mata et al. 2011). Aβ interaction with NMDA and AMPA receptors is another such mechanism. Aβ downregulates the cell surface AMPA receptors and increases AChE activity contributing to cognitive dysfunction (Small 2012). The increase and decrease in the AChE activity and AMPA receptors, respectively, disrupt the homeostatic mechanism of glutaminergic

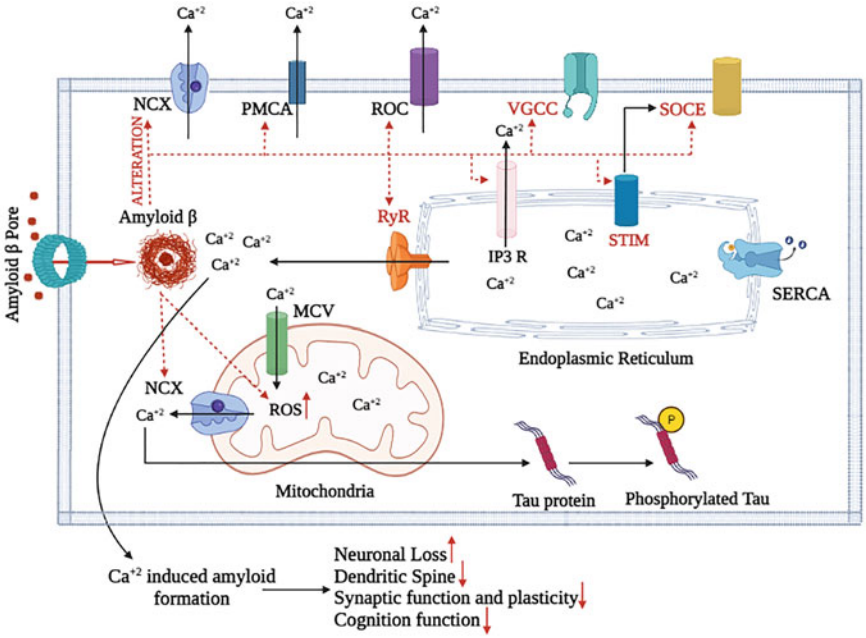

Fig. 1 Dysregulation of the Ca^{2+} homeostasis in AD. *PMCA:* plasma membrane Ca^{2+}-ATPase, *NCX:* Na^{+}/Ca^{2+} exchanger, *RYR:* ryanodine receptor, *Ca^{2+}*: calcium, *ROS:* reactive oxygen species, *STIM:* stromal interaction molecules, *SOCE:* store-operated Ca^{2+} entry channels, *ROC:* receptor-operated Ca^{2+} channels

and cholinergic signalling. Furthermore, the treatment with Glu2A and Glu2B subunit antagonists reported the modulation of NMDA receptors by Aβ and vice versa in the dysregulation of calcium homeostasis (Ferreira et al. 2012). Aβ induces inward currents in the plasma membrane that causes an increase in the intracellular Ca^{2+} load and neuronal cell death via NMDA and AMPA receptor involvement (Alberdi et al. 2010). This increase in intracellular Ca^{2+} causes increased mitochondrial Ca^{2+} uptake, causing excessive reactive oxygen species formation and oxidative stress. Aβ modulates Ca^{2+} stores to impair the intracellular calcium signalling pathways. Aβ-mediated release of Ca^{2+} from the endoplasmic reticulum is via both IP3R-dependent and IP3R-independent pathways (Jensen et al. 2013). Aβ increases the expression levels of RYR2 that are correlated with cognitive dysfunction and neurodegeneration in the later stages (Bruno et al. 2012). Furthermore, Aβ interacts with pCaMKII and inhibits its activity in the synaptic spines of hippocampal neurons in a CaN-dependent manner (Reese et al. 2011). Thus, Ca^{2+} dyshomeostasis becomes an important therapeutic target against observed cognitive and neuronal functional disorders in Alzheimer's disease (Fig. 1).

4 Therapeutic Effect of Ca^{2+} Ion Channel Modulator in AD

Aβ and tau interact with calcium channels present in all major subcellular structures such as cytosol, plasma membrane, endoplasmic reticulum, and mitochondria. The next part of the chapter is going to discuss the mechanisms through which Aβ and tau interact with various calcium ion channels and the beneficial effect of calcium ion channel modulator in AD.

4.1 Voltage-Gated Ca^{2+} Channel

4.1.1 L-Type

L-type voltage-gated Ca^{2+} channel blockers such as verapamil, diltiazem, isradipine, and nimodipine have shown protective effects against amyloid beta protein precursor C-terminal fragment (APP CTF)-induced neurotoxicity in MC65 neuroblastoma cells. Isradipine-mediated cytoprotection involved the downregulation of Cav1.2 Ca^{2+} channels expression and intracellular Ca^{2+} influx from the extracellular environment. In the same experiment, the administration of isradipine (3 μg/g/day) for 60 days provides neuroprotection in a triple-transgenic mouse model of AD. However, isradipine showed no effects against the amyloid beta oligomer formation during in vitro studies (Anekonda et al. 2011).

In Alzheimer's disease, tau aggregation and phosphorylation are known to trigger several cellular and molecular pathways that disrupt cellular homeostasis. Tau aggregation post 1 day in cortical co-cultures of astrocytes and neurons was able to stimulate ROS production via activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Furthermore, late tau aggregates (insoluble) caused a gradual increase in the membrane ionic current, inducing Ca^{2+} signals inside the cell. However, nifedipine, a voltage-gated Ca^{2+} channel blocker, was observed to inhibit the tau-mediated ROS production and increased Ca^{2+} signals (Esteras et al. 2021).

Zheng et al. (2022) in their study utilized A*β*-induced *C. elegans* Alzheimer's disease model to study the neuroprotective effect of ethylene glycol tetraacetic acid and nimodipine against Ca^{2+} acetate-induced increase in intracellular Ca^{2+} content, Aβ 1-42 aggregation, oxidative stress, and neurodegeneration (Zheng et al. 2022). In another study, nimodipine-containing chow when fed for 2–8 months had no effect against Aβ accumulation or neuritic dystrophy in 5XFAD transgenic mice. However, the administration of nimodipine did not exaggerate or worsen the Alzheimer's disease phenotype, suggesting its safer use in patients (Sadleir et al. 2022).

In another study, Ishii et al. (2019) showed that the treatment with nimodipine during early Aβ pathology rescues against Aβ-mediated transition from high- to low-voltage threshold-induced activation of L-type Ca^{2+} channel currents. This protects the arcuate neuropeptide Y neurons against disrupted intracellular Ca^{2+} homeostasis and neuronal dysfunction, which is an important risk factor in Alzheimer's disease (Ishii et al. 2019).

Another novel derivative that has shown the potential ability to block L-type Ca^{2+} channels is 4,7-dihydro-2*H*-pyrazolo[3-*b*] pyridine family compounds. These compounds demonstrate potent anti-inflammatory and antioxidant properties along with their ability to inhibit GSK-3β and L-type Ca^{2+} channels. Hence, they protect against impaired Ca^{2+} homeostasis observed in in vivo and in vitro models of Alzheimer's disease. Michalska et al. (2020) have employed these compounds and have recorded their potential effects against cell death induced by tau hyperphosphorylation in SH-SY5Y neuroblastoma cells (Michalska et al. 2020).

4.1.2 T-type

Research studies have shown the beneficial effect of novel therapeutic compound thyl-8-methyl-2,4-dioxo-2-(piperidin-1-yl)-2H-spiro[cyclopentane-1,3-imidazo [1,2-a] pyridin]-2-ene-3-carboxylate (SAK3) against Alzheimer's induced cognitive dysfunction in experimental mice. SAK3 is a T-type Ca^{2+} channel enhancer that increases the Ca/CaM-dependent protein kinase II and proteasome activity in Alzheimer's experimental model. This promotes Aβ degradation in experimental mice. Furthermore, SAK3 was observed to ameliorate oxidative stress markers in the microglial cells that further provide neuroprotection (Degawa et al. 2021; Yuan et al. 2021). Wang et al. (2018) in their experiment has demonstrated that SAK3 (0.5 mg/kg) promotes acetylcholine release from the hippocampus of the experimental mice, which is significantly responsible for the hippocampal-mediated increase in learning and psychomotor behaviours. However, these effects were antagonized in the presence of T-type (NNC 55-0396), α7 nicotinic ACh receptor (nAChR) (methyllycaconitine), and α4 nAChR (dihydro-β-erythroidine) receptor antagonist in the mouse hippocampus region, suggesting the role of T-type Ca^{2+} channel in the beneficial effect against Alzheimer's disease (Wang et al. 2018). In another study, SAK3 (0.5 mg/kg, p.o.) administration rescued the hippocampal cells from A-β-induced inhibition of acetylcholine release and memory impairment in olfactory bulbectomized mice. The beneficial effect of the T-type Ca^{2+} channel was illustrated in the presence of NNC 55-0396, a specific T-type Ca^{2+} channel blocker and Cav3.1 knockout (KO) mice wherein SAK3 administration showed no effect against the impaired Aβ pathology (Yabuki et al. 2017). Furthermore, downregulation of T-type Ca^{2+} channels or decline in Cav3.1 expressions was observed to increase rapidly the production of Aβ via decreasing the non-amyloidogenic processes in N2a cells and 3xTg-AD mouse model of Alzheimer's disease (Rice et al. 2014). The same effect was observed in the presence of the pharmacological T-type Ca^{2+} channel blocker (NNC-55-0396) (Rice et al. 2014).

Another T-type Ca^{2+} channel activator spiro [imidazo [1,2-a] pyridine-3,2-indan]-2(3H)-one (ST101) is reported to increase cognitive function via CaMKII autophosphorylation and AMPA-type GluR1 phosphorylation in experimental animals. Furthermore, in vitro analysis in cortical slices demonstrated that the administration of 1 μM mibefradil, a selective T-type inhibitor, abolished the long-term potential induced by ST101 treatment. This demonstrated the therapeutic effect of

T-type Ca^{2+} channels in enhancing cognition in Alzheimer's disease model (Moriguchi et al. 2012).

Nogo receptors (NgR1-3) activation is demonstrated to impair the synaptic assembly, plasticity, and learning in experimental studies. Aβ receptors activate NgR1-3 receptors on the dendritic shaft of neurons to cause inhibition of the Ca^{2+} signalling. This disrupts Ca^{2+} signalling and causes impairment in synaptic function and plasticity. Zhao et al. (2017) in their study have utilized T-type Ca^{2+} channels against Aβ-NgR signalling and have reported beneficial effects against impaired Ca^{2+} homeostasis, synapse assembly, learning, memory, and cognitive deficits (Zhao et al. 2017).

4.1.3 P/Q-Type

Aβ oligomers have shown to directly modulate the P/Q-type Ca^{2+} channel in a dose-dependent manner. Aβ interacts with α1A subunit of P/Q-type Ca^{2+} channels and is hypothesized to trigger excitotoxic events in the brain of people suffering from Alzheimer's disease (Mezler et al. 2012).

However, previously in an experiment it was observed that synthetically produced Aβ (1-42) oligomers at 8nM concentration inhibit presynaptic P/Q currents that impair the release of synaptic vesicles at both GABAergic and glutaminergic synapses. Insufficient release of synaptic vesicles impairs synaptic functions. In this experiment, the inhibitory effect of the Aβ on the release of synaptic vessels was reversed via the administration of a specific enhancer of the P/Q currents, roscovitine (Nimmrich et al. 2008).

Aβ causes activation of L-, N-, and P-type Ca^{2+} channels, which cause a significant increase in the levels of Ca^{2+} ions in cortical synaptosomes as recorded in cultured cortical neurons (MacManus et al. 2000). In another experiment conducted by Hermann et al. (2013), similar results were observed by the administration of Aβ in HEK293 cells, where Aβ-mediated increase in Ca^{2+} ions was reversed via administration of P/Q- and N-type Ca^{2+} channel blockers (Hermann et al. 2013) (Table 1).

4.2 Receptor-Gated Ca^{2+} Ion Channels

4.2.1 RYR

RYR is observed to be overactivated in Alzheimer's disease (Stutzmann et al. 2007; Supnet and Bezprozvanny 2010; Paula-Lima et al. 2011; Bruno et al. 2012). Increased RYR-evoked Ca^{2+} signalling within dendritic processes, dendritic spine heads, and soma of pyramidal neurons are observed in 3XTg-AD and TAS/TPM AD mice as compared to normal animals. Impaired RYR functionality disrupts other cellular and molecular pathways that occur in normal homeostasis (Chakroborty

Table 1 Therapeutic effect of voltage-gated Ca^{2+} ion channel modulator in AD

Treatment	Target (type of calcium channel)	Experimental model of AD	Dose	Molecular changes	Major results	References
Nimodipine	L-type voltage-gated Ca^{2+} channels	5XFAD mouse model	Nimodipine incorporated into mouse chow at 300 ppm, doses ranging from Females: 40–51 mg/kg/day Males: 33–41 mg/kg/day	Decrease GFAT, Iba-1	Blocks Aβ42-induced mitochondrial toxicity in microglia Improve cognition. Normalizes learning and memory in both groups	Sadleir et al. (2022)
LiCl	L-type voltage-gated Ca^{2+} channels	3xTg-AD mice	Mixed in Chow diet	IP3R-dependent ER Ca^{2+} and VGCC-mediated Ca^{2+} signalling. Restored nNOS	The LiCl treatment enhanced post-tetanic potentiation (PTP), a form of short-term plasticity in the hippocampus	Wiseman et al. (2023)
Memantine nitrate	L-type voltage-gated Ca^{2+} channels	APP/PS1 transgenic mice and triple-transgenic (3 × Tg-AD) mice models	6 mg/kg by gastric gavage	Inhibits the calcium influx. Reverses dysregulations of ERK and PI3K/Akt/GSK3β pathway, prevents glutamate excitotoxicity	Accelerates Aβ degradation in 3 × Tg-AD mice, restores dendritic spines, reduces plaque deposition, improves behavioural performance	Wu et al. (2021)
Isradipine	L-type voltage-gated Ca^{2+} channels	Triple-transgenic mouse model of AD	Subcutaneous implantation of carrier-bound isradipine (3 mg/kg)	Cav1.2 upregulation	Prevent Aβ-induced Ca^{2+}-influx. Downstream of Aβ oligomer formation	Anekonda et al. (2011)
Urolithin	L-type voltage-gated Ca2+channels	Streptozotocin (STZ)-induced diabetic mouse model	2.5 mg/kg/day I.P.	2.5 mg/kg/day I.P.	Prevention of mitochondrial calcium influx and ROS production	Lee et al. (2020)
Nifedipine			5 μM			

(continued)

Table 1 (continued)

Treatment	Target (type of calcium channel)	Experimental model of AD	Dose	Molecular changes	Major results	References
	L-type voltage-gated Ca^{2+} channels	Co-cultures of cortical neurons and astrocytes		Blocks tau-induced ROS production	Nifedipine inhibits the calcium signals and blocks the ROS production induced by the same tau species	Esteras et al. (2021)
Nimodipine	L-type voltage-gated Ca^{2+} channels	Tg2576 mice transgenic	10 mg/kg (i.p.)	Arcuate NPY neurons have increased cytoplasmic-free Ca^{2+}	Nimodipine decreases cytoplasmic-free Ca^{2+} and blocks L-type Ca^{2+} currents in NPY neurons	Ishii et al. (2019)
4,7-dihydro-2H-pyrazolo[3-b]pyridines derivatives	L-type voltage-gated Ca2+channels	SH-SY5Y neuroblastoma cell cultures and mixed glial cultures of the cerebral cortex of 2- to 5-day-old Sprague Dawley rats	0.1, 1, 3, 10, and 30 μM	GSK-3β inhibition, reduced ROS and RNS, reduced Ca overload	Antioxidant, anti-inflammatory, and neuroprotective effects, reduced tau hyperphosphorylation	Michalska et al. (2020)
Icariin	T-type voltage-gated calcium channels	In vitro	Icariin dissolved in DMSO and final concentration is 0.1% in extracellular solution	Reduce AchE activity in the brain Increase SOD activity	Improves spatial memory	Li et al. (2017)
Spiro[imidazo [1,2-a]pyridine-3,2-indan]-2(3H)-one (ST-101)	T-type voltage-gated calcium channels	In vitro	Rat somatosensory cortical and hippocampal slices treated with 0.01–100 nM ST101	PKC activation, LTP induction phosphorylation of glutamate type AMPA receptor subunit 1. Improves ERK phosphorylation in rat cortex	Improves cognition	Moriguchi et al. (2012)
NNC 55–0396	T-type voltage-gated	APPNL-F KI mice (in vitro)	1 μM in ringer's solution was infused into	The cognitive improvement by SAK3 is closely	Significantly enhanced the T-type calcium	Wang et al. (2018)

	calcium channels		brain regions through a microdialysis probe	associated with enhanced acetylcholine (ACh) release in the hippocampus	channels in the brain region	
1,4-Dihydropyridine derivatives	T-type voltage-gated calcium channels	In vitro	1 μM	Antioxidant, inhibit GSK-3β, a kinase related to tau hyperphosphorylation	Neuroprotection, anti-inflammatory property, reduction in ROS production and cytoprotective property	Michalska et al. (2020)
Roscovitine	P/Q-type calcium channels	In vitro: primary hippocampal cell cultures were prepared	20 μm	Aβ1–42 globulomer strongly impairs presynaptic P/Q-type calcium currents at both glutamatergic and GABAergic synapses	Enhance P/Q-type calcium Improvement in the Aβ oligomer-induced deficits in neurotransmission. Enhancement of cognitive function	Nimmrich et al. (2008)

et al. 2012). One such mechanism in RYR mediated impairment in synaptic function. Young transgenic mice have recorded increased endoplasmic reticulum-evoked Ca^{2+} release at the glutaminergic synapses. This causes loss in synaptic function and impairment in the downstream cognitive pathways (Goussakov et al. 2010). Another mechanism known to be impaired by RYR dysregulation is autophagy. Autophagy clears the damaged cell organelles and protein aggregates in the body. This mechanism was reported to be dysregulated in Alzheimer's disease's clinical and pre-clinical studies. In an experiment by Zhang et al. (2023), it was observed that the increase in the basal RYR2 activity causes inhibition of the autophagy pathways and increases Aβ accumulation in the hippocampus region via activation of calcineurin and inhibition of 5′ adenosine monophosphate-activated protein kinase–Unc-51 like autophagy activating kinase 1 (AMPK-ULK1) pathway on RYR2-E4872Q knock-in mouse model (Zhang et al. 2023).

RYR upregulation and post-translation modification in Alzheimer's disease cause an excessive influx of Ca^{2+} ions in the sarcoplasmic reticulum. A novel potent RYR inhibitor compound 12a (having semicarbazone and imidazolyl moieties) was observed to inhibit Ca^{2+} release and Ca^{2+} load in R614C cells. Furthermore, the administration of compound 12a demonstrated the beneficial effect against cognitive deficits in Alzheimer's disease animal model (Dai et al. 2021).

Aβ 42 is reported to interact with RYR receptors, which lead to Ca^{2+} dysregulation in the GABAergic synapses causing GABAergic synaptic dysfunction. Furthermore, in experimental studies, it was observed that alterations in the RYR receptors exaggerated the dysregulation in the Ca^{2+} homeostasis; however, stabilizing the RYRs and calstabin, the accessory protein by S107 was observed to reverse the Ca^{2+} dysregulation and synaptic dysfunction in the early-stage onset of Alzheimer's disease (Hidisoglu et al. 2022). Depletion in the calstabin 2 in RYR receptor enhances endoplasmic reticulum Ca^{2+} release and activation of Ca^{2+} signalling pathways that lead to the formation of Aβ in the brain. This further impairs synaptic plasticity, learning, memory, and cognitive function in experimental animals (Lacampagne et al. 2017). Bussiere et al. (2017) in their experiment demonstrated a functional interplay between Aβ, β-adrenergic signalling, and RYR2 receptor-mediating altered Ca^{2+} signalling. It was observed that Aβ-mediated post-translation modification in the RYR2 receptor occurs via β2-adrenergic signalling that further activates PKA. This causes increased formation and processing of β-amyloid precursor protein. β-amyloid precursor protein processing and Aβ formation were abolished in the presence of RYR-calstabin stabilizers and β2-adrenergic blockers in SH-SY5Y cells expressing amyloid precursor protein (Bussiere et al. 2017).

In other experiments, it was observed that Aβ promotes mitochondrial fragmentation via stimulating the uptake of mitochondrial Ca^{2+} ions released through A-β-mediated RYR Ca^{2+} leak. This causes reactive oxygen species formation and oxidative stress in the neuronal cells (Abou et al. 2020).

4.2.2 NMDAR

Companys-Alemany et al. (2022) observed the effect of novel NMDAR antagonist UB-ALT-EV in 5XFAD mice model. UB-ALT-EV was observed to decrease astrocyte and microglial activation (GFAP, Iba-1) in experimental mice. Furthermore, UB-ALT-EV downregulated the expression levels of various pro-inflammatory (TREM2, NF-κB, IL-1β, IFN-γ, CCL2, and CCL3) and oxidative stress markers, whereas it upregulated the expression levels of several anti-inflammatory markers (Ym1, Arg1, IL-19, and IL-22) showing a potential therapeutics against Alzheimer's disease (Companys-Alemany et al. 2022).

The administration of a novel memantine nitrate compound, MN-08, for 4 months provides neuroprotection against Aβ accumulation, neuronal loss, dendritic spine loss, and cognitive deficits in 3Xtg-AD mice. Similar neuroprotective effects were observed upon the administration of MN-08 for 6 months in APP/PS1 transgenic mice. In vitro analysis demonstrated that MN-08 antagonizes NMDAR and inhibits Ca^{2+} influx in the cell culture. Furthermore, MN08 treatment prevented glutamate-induced neuronal loss via activation of ERK and PI3K/Akt/GSK3β pathways in primary hippocampal neurons (Wu et al. 2021).

Apolipoproteins (APOE) are an important risk factor for the predisposition of Alzheimer's disease. APOE4 exposure in primary cortical neurons derived from the embryos of Sprague Dawley rats and C57BL/6J mice has demonstrated a significant decrease in the global protein synthesis in the cell culture. In this experiment, APOE4 exposure activates both L-type and NMDAR receptor channels, which increase intracellular Ca^{2+} levels via eukaryotic translation elongation factor 2 (eEF2) gene phosphorylation. APOE4-mediated increase in the Ca^{2+} levels inhibits translation of NMDA activity-mediated protein synthesis in the primary cortical neurons. This inhibits synaptic translation that will lead to cognitive dysfunction (Ramakrishna et al. 2021).

4.2.3 SOCE

PMCA is reported to be functionally impaired in Alzheimer's disease. Furthermore, Aβ and tau inhibit PMCA, which causes impairment in Ca^{2+} homeostasis in a cell. Berrocal et al. (2021) have utilized recombinant sorcin to provide beneficial effects against impaired PMCA in Alzheimer's disease in vivo and in vitro studies. Sorcin, a Ca^{2+} binding protein, is expressed highly in the brain and interacts with NCX, PMCA, SERCA, and RYR in neurons. Sorcin administration abolished the inhibitory effect of Aβ and tau on the activity of PMCA and activated the activity of PMCA in cultured SH-SY5Y human neuroblastoma cells (Berrocal et al. 2021). Furthermore, neuronal deletion of mitochondrial NCX causes an increase in amyloidosis and tau phosphorylation, which further decreases learning, memory, and cognition in experimental mice. Mitochondrial NCX in 3XTg-AD mice was observed to increase the levels of Ca^{2+} in the intra-mitochondrial space. The increase in the mitochondrial Ca^{2+} resulted in metabolic dysfunction, superoxide generation, and neuronal death in experimental mice (Jadiya et al. 2019).

In Alzheimer's disease model, the functionality was SOCE was found to be impaired. The activation of STIM1 is crucial for the activation of SOCE. Poejo et al. (2022) have demonstrated that incubation of 2 μM Aβ (1–42) oligomers for 2 and 5 h in immortalized mouse hippocampal cell line (HT-22) internalized 62 ± 11 nM and 135 ± 15 nM of Aβ (1–42) oligomers, respectively. The internalized Aβ (1–42) oligomers bind with STIM1 at a site located close to the CaM-binding site in STIM1. This caused alterations in the Ca^{2+} homeostasis and SOCE functionality (Poejo et al. 2022).

4.2.4 AMPAR

Aβ is observed to downregulate the expression levels of cell surface AMPA-type glutamate receptor 2 in in vivo and in vitro Alzheimer's disease studies. Furthermore, in an experiment conducted by Li et al. (2017), it was observed that A-β-mediated decrease in the GluR2 receptor is via protein kinase C (PKC)-mediated phosphorylation of Serine-880. PKC antagonist bisindolylmaleimide I was observed to antagonize the effect mediated by Aβ on GluR2 receptors and serine-880 phosphorylation in the experimental model of Alzheimer's disease. This provided beneficial effects against Aβ-induced disturbances in the Ca^{2+} ion homeostasis, synaptic function, and cognitive function in the cytosolic space (Li et al. 2017).

4.2.5 IP3R Ca^{2+} Channels

Aβ-mediated increase in the Ca^{2+} ions in the intracellular space and resulting apoptosis is mediated translocation of Bax in cells. Oseki et al. (2014) have demonstrated that the translocation of Bax in the cells was dependent on the mobilization of Ca^{2+} from the IP3R in the endoplasmic reticulum. Aβ-induced apoptosis was inhibited via administration of BAPTA-AM, a Ca^{2+} chelator, and via the administration of xestospongin C, a selective IP3R inhibitor in the cortical astrocyte culture cells (Oseki et al. 2014) (Table 2).

4.3 Other Therapeutic Agents Modulating Ca^{2+} Channels

Aβ oligomers cause perturbation in mechanical properties of the lipid bilayer membranes, which change the membrane tension and cause activation of several NMDA and AMPA receptors. The activation of these receptors initiates Ca^{2+} influx, which further causes impairment in Ca^{2+} homeostasis. This effect was abolished by the treatment of lysophosphatidylcholine, which inhibited the activation of NMDA and AMPA receptors via oligomer-induced membrane stretch and tension (Fani et al. 2021).

Table 2 Therapeutic effect of receptor-gated Ca^{2+} ion channel modulator in AD

Treatment	Target (type of calcium channel)	Experimental model of AD	Dose	Molecular changes	Major results	References
Dantrolene	RyR2	3xTg and TASTPM double-transgenic mice	10 mg/kg (i.p.)	Aberrant ER Ca+2 signalling was normalized and levels of RyR2 were increased	Dantrolene reversed the Ca +2 signalling dysregulation and restored RyR2 levels, synaptic plasticity, and transmission. Amyloid deposits were also found to be reduced.	Chakroborty et al. (2012)
Rycal S107	RyR2	3xTg and APP +/- PS1 +/-	75 mg/kg/day (oral—3 × Tg; with drinking water—APP ±/PS1 ±)	ER and Ca+2 leak activates Ca+2-dependent signalling pathways	Prevention of stress-induced dissociation of calstabin 2 from the RyR2 complex and improved hippocampal synaptic plasticity (LTP and LTD) and cognitive function	Lacampagne et al. (2017)
12a compound (with semicarbazone and imidazolyl moieties)	RyR1	R614C RyR1 cell lines and FAD mice	0.1, 3, 10 μM and 3.2 mg/kg (i.p.)	AchE inhibition and SOICR inhibition	12a showed similar activity to dantrolene. It improved cognitive behaviour in MWM	Dai et al. (2021)
Recombinant Sorcin	RyR and PMCA	SH-SY5Y human neuroblastoma cells	10 nM and 1 μM	Decrease ROS	Decreased oxidative stress, and apoptotic cells in cultured cells. Increased PCMA activity and maintained calcium homeostasis	Berrocal et al. (2021)
PNU282987	α7 nAChR	Rat pheochromocytoma cell (PC12) lines	10μM	Ca influx through CICR and IICR	Decreased EB3 comet motility in a calcium-dependent manner	King and Kabbani (2018)

(continued)

Table 2 (continued)

Treatment	Target (type of calcium channel)	Experimental model of AD	Dose	Molecular changes	Major results	References
UB-ALT-EV and memantine	NMDA receptors	5XFAD mice	5 mg/kg (orally, through drinking water)	Calcineurin (CaN)/NFAT pathway	UB-ALT-EV decreased astrocyte and microglial activation (GFAP, Iba-1), Downregulated pro-inflammation (TREM2, NF-κB, IL-1β, IFN-γ, CCL2 and CCL3) and oxidative stress markers. Upregulated anti-inflammatory markers (Ym1, Arg1, IL-19, IL-22). No significant effect by memantine	Companys-Alemany et al. (2022)
Xestospongin C	IP3R	Cortical astrocyte culture	1 μM	Aβ induces Ca2+ dysregulation which mobilizes Ca2+ from extra and intracellular stores, primarily through ER via IP3R	Pre-treatment with Xestospongin C abolished Bax translocation mediated by Aβ. Ca2+ from IP3R may be responsible for Aβ induced apoptosis and its removal may induce a protective effect	Oseki et al. (2014)

Lithium chloride was observed to stabilize the abnormalities of Ca^{2+} homeostasis in hippocampal neurons of 3XTg-AD mice. The administration of lithium chloride through diet chow for 30 days restored the neuronal nitric oxide synthetase levels (nNOS) and hyperphosphorylated tau levels to normal control levels in the hippocampal neurons and overlying cortex via reducing the aberrant IP3R and VGCC-mediated Ca^{2+} signalling. The alleviation of the disrupted Ca^{2+} homeostasis was observed to increase short-term synaptic plasticity in experimental animals (Wiseman et al. 2023).

In another experiment, it was observed that Aβ forms channels that allow the entry of Ca^{2+} ions through it causing an increase in the levels of intracellular Ca^{2+} ion concentration. Arispe et al. (2010) in their experiment have demonstrated that administration of NAHis04, a small four-histidine peptide in the micromolar range, potentially blocks the increase in the levels of intracellular Ca^{2+} ions and apoptosis when the culture PC12 cells were exposed to Aβ (Arispe et al. 2010).

Antioxidants such as edaravone have also shown protective effects against A-β-induced disrupted Ca^{2+} signalling, cholinergic signalling, learning and memory impairment, neurotoxicity, and neuronal cell death (He et al. 2014). NE100, a Sig-1R antagonist, enhanced the axonal length via inhibition of voltage-gated channel channels with naïve hippocampal neurons (Li et al. 2017). In AF64A-lesioned rats, dimebolin, a non-selective antihistamine, has demonstrated to interact with acetylcholinesterase, NMDA, AMPA, and voltage-gated Ca^{2+} channel and protect against Alzheimer's disease-induced cognitive dysfunction (Schaffhauser et al. 2009).

5 Conclusion

Targeting Ca^{2+} channel and Ca^{2+} dysregulation mechanisms can prove to be a promising target for treating Alzheimer's disease. The literature is full of evidence that suggests dysregulation of calcium homeostasis in Alzheimer's disease. Targeting Ca^{2+} signalling pathways and restating the Ca^{2+} homeostasis may prevent Aβ-induced neuronal and synaptic loss as in individuals suffering from Alzheimer's disease. Potential targets include NMDAR, AMPAR, PMCA, SOCE, RYR, IP3R, NCX, and additional Ca^{2+} signalling proteins and other small molecular proteins that interact with these targets to provide the neuroprotective effect. However, considering the importance of calcium signalling in the overall body, limitations lie in the acceptable doses of these compounds in the clinical studies.

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Traversing Through the Trajectory of Pathogenic Astrocytes in Alzheimer's Disease

Gadde Shareena, Dileep Kumar, and Dee Wu

Abstract Alzheimer's disease (AD) is a prevalent and debilitating neurological condition with no curable therapies in the pipeline. Alzheimer's disease-related neurodegeneration negatively influences various brain cells, including astrocytes, neurons, and microglia. Interactions between healthy neural cell types and AD hallmarks: Aβ plaques and tau fibril deposits have been shown to produce detrimental cellular stresses and deterioration in brain functions. Astrocytes help to regulate nutritional balance, cerebral blood flow, tissue healing, and the blood–brain barrier. They engage in the CNS's inflammatory/immune responses. Evidence also suggests that astrocytes have neuroprotective and neurotoxic effects based on the disease stage and microenvironment. Changes in astrocyte function have been observed in individuals with early-onset Alzheimer's disease, displaying disproportions in gliotransmission, neurotransmitter homeostasis, astroglial atrophy, disruptions in synaptic associations, neuroinflammation, and neurodegeneration. Moreover, the presence of Aβ plaques appears to impact astrocytes, affecting calcium levels and pro-inflammatory activity via RAGE-NF-kB pathway. The conventional treatment approaches for AD-related neuropathology are not anticipated to be comprehensive. The rationale for this may be that AD treatment involves a therapeutic strategy to minimize its cascading recurrence, and most drugs are inefficient at developing effective responses. Hence, researchers are keen on exploring the roles of astrocytes and their molecular pathways to identify potent therapies in AD. This review provides a broad approach, emphasizing the roles of astrocytes in healthy brains and their pathological changes in AD, as well as potential astrocyte-related therapy

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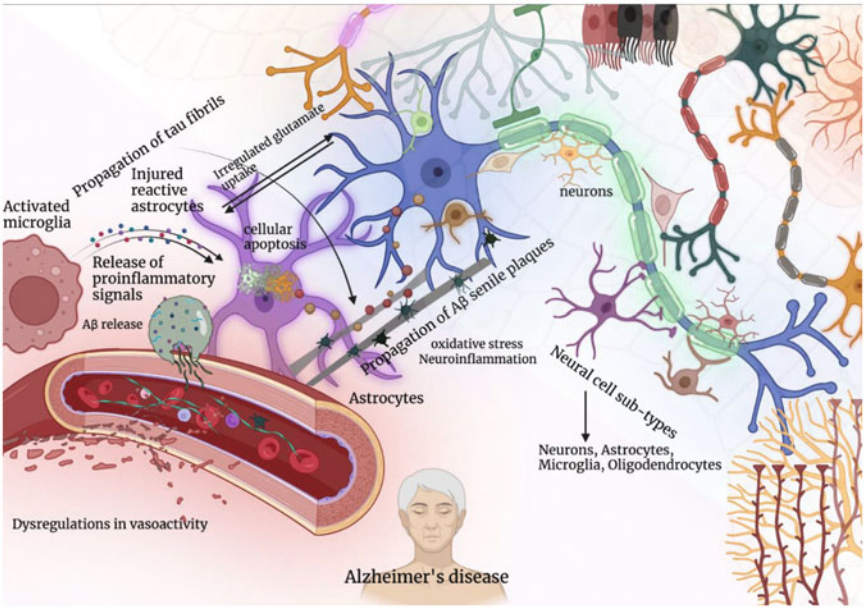
regimens. Novel strategies comprising astrocytes and aimed at alleviating oxidative stress and neuroinflammation in AD have also been presented.

Graphical Abstract



The graphical abstract demonstrates the neuropathological implications of Alzheimer's disease on several neural sub-populations, i.e. neurons, astrocytes, microglia, and oligodendrocytes.

Keywords Alzheimer's disease · Astrocytes · Biomarkers · Gliogenesis · Reactive astrocytes · Treatment regimen

Abbreviations

2 ERK	Extracellular signal-regulated kinase
ABCC1	ATP-binding cassette subfamily C member 1
AD	Alzheimer's disease
AICD	Amyloid precursor protein intracellular domain
ALS	Amyotrophic lateral sclerosis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
Aβ	Amyloid-beta
B CN	Calcineurin

BBB	Blood–brain barrier
BMP	Bone morphogenetic proteins
CBF	Cerebral blood flow
CFB	Complement factor
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
Cx43	Gap junction alpha-1 protein
DAM	Disease-associated macrophages
DG	Dentate gyrate
EAAT2	Excitatory amino acid transporter
EGFR	Epidermal growth factor receptor
GABA	Gamma-aminobutyric acid
GFAP	Glial fibrillary acidic protein
GH	Growth hormone
GLAST1	Glutamate transporter
GLT-1	l-glutamate transporter
GSH	Glutathione
GSK3β	Glycogen synthase kinase-3 beta
HMGB1	High mobility group box 1 protein
IkB-α	Nuclear factor of kappa light polypeptide gene enhancer in B-*cell* inhibitor
ILGF-1	Insulin-like growth factor 1
JAK	Janus kinase
LRPs	Lipoprotein receptor-related protein
MAO-B	Monoamine oxidase B
MAP-2	Microtubule-associated protein 2
MEK1/MAP2K	Mitogen-activated protein kinase
MMP	Mitochondrial membrane potential
MX1S	MX dynamin-like GTPase 1
MyD88	Myeloid differentiation primary response 88
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NFAT	Nuclear factor of activated T cells
NF-kB	Nuclear factor kappa B
NFTs	Interneuronal neurofibrillary tangles
NICD	Notch intracellular domain
NMDAR	N-methyl-D-aspartate receptor
NOTCH	Notch homolog 1, translocation-associated
NOX	NADPH-oxidized
NPCs	Neural precursor cells
NRF2	Nuclear factor-erythroid factor 2-related factor 2
NSCs	Neural stem cells
OXPHOS	Oxidative phosphorylation
PAR	Poly(ADP-ribose) polymers
PARP	Poly (ADP-ribose) polymerase-1

PD	Parkinson's disease
PGC-1α	Peroxisome proliferator-activated receptor-gamma coactivator
PHF	Paired helical fragments
ptau	Phosphorylated tau
RAGE	Receptor for advanced glycation end products
RBP-J	Recombination signal binding protein for immunoglobulin kappa J region
ROCK inhibitor	Rho-kinase inhibitor
ROS	Reactive oxygen species
R-SMADs	Receptor-regulated Smads
S100B	*S100* calcium-binding protein B
sAPP-β	Soluble amyloid precursor protein beta
SGZ	Sub-granular zones
sNMDAR	Synaptic N-methyl-D-aspartate receptor
SOCS3	Suppressor of cytokine signalling 3
SR	Scavenger receptor
STAT	Signal transducer and activator of transcription
TFAM	Mitochondrial transcription factor A
TG	Transgenic mice
TGIF	Transforming growth-interacting factor
TLR4	Toll-like receptor 4
TNF-α	Tumour necrosis factor alpha
TRF	Thyrotropin-releasing factor
TTP	Alpha-tocopherol transfer protein
U.S. FDA	United States Food and Drug Administration
V-SVZ	Ventricular–sub-ventricular zones
α-TCP	Alpha-tocopherol

1 Introduction

Breakthroughs in science and technology have improved our standard of health and extended our life expectancy. Although a robust treatment regimen is present for many severe conditions, individuals suffering from age-related ailments such as Alzheimer's disease (AD) face difficulties due to the body's growing degradation followed by reduced lifespan and ineffective combination of therapies in the current research. Alzheimer's disease (AD) is a progressive neurological condition that entails peculiar neuronal deterioration followed by pathological changes in the brain tissues. They consist of intractable depositions of aberrant Aβ plaques and fibrillary tangles, which are considered two prominent markers (Goate et al. 1991; Braak and Braak 1991; Karran et al. 2011; Huang and Mucke 2012), and AD accounts for approximately 70% of the total number of dementia cases (Small et al. 1997) whilst being the seventh leading cause of mortality worldwide

(Fratiglioni et al. 1999). Despite tremendous advances in contemporary medicine and the amassing of pertinent knowledge over the preceding decades, there remains a continued shortfall in the shortage of effective medicines and therapies for distinct disorders such as Alzheimer's disease. The prevalence of AD-related dementia is predicted to rise over time, inflicting tremendous burdens on the livelihoods of affected individuals and caregivers (Varela et al. 2011) and overtaxing the international health economy.

Astrocytes are critical CNS resident cells that engage in several biochemical processes (Emsley and Macklis 2006). They share standard features with neurons, including their heterogeneity and various physical and functional properties such as trophic regulation, BBB formation, and CBF regulation (Matyash and Kettenmann 2010). Neuronal survival depends on astrocytes, wherein degeneration of astrocytes is one of the leading causes of neurodegenerative diseases. Genetic biomarker of astrocytes, i.e. GFAP, glutamine synthetase, and calcium-binding S100B, differentiates them from other glial and neuronal cell populations. The interconnections among pre-synaptic, the post-synaptic membrane of neurons, and astrocytes constitute neuron–glial transmissions, enabling anchored coordination of neuro/gliotransmission, synaptic plasticity constituting a cellular construction, termed as 'Tripartite synapse' in neural tissues (Araque et al. 1999; Perea et al. 2009). Intercellular communications among innumerable astrocytic cell populations, assembled in the perpetual frame, referred to as *Astroglial syncytia*, a feature that calcium waves and excitatory responses stimulate the release of several neurotransmitters, including glutamate GABA, D-serine, ATP, and taurine in the neural circuitry (Angulo et al. 2008; Bezzi et al. 1998).

Alois Alzheimer observed glial cells densely filled with neuritic plaques in demented brain tissues, which led to the discovery of pathological astrocytes. As a result, reactive astrogliosis has been proven as a morphological hallmark of Alzheimer's disease (Nagele et al. 2004). Reactive astrocytes that produce pro-inflammatory chemicals may increase the production of secretases, promoting the conversion of APP on neuronal membranes to neurotoxic fibrillary Aβ plaques. Additionally, exogenous Aβ may trigger pro-inflammatory astrocyte responses that can sustain neurodegenerative insults such as increased astrogliosis, progressive neuronal death, Aβ, NFT production, tau phosphorylation, and dystrophic neurite development (Tang 2009; Rojo et al. 2008). Furthermore, Aβ induces microglia to create ROS and TNF, which activates astrocytes via IL-1. To increase Aβ-induced astrocyte reactivation, IL-1 binds to IL-1 receptors on the astrocytic membrane. Studies also report that Aβ also induces astrogliosis via ERK phosphorylation in astrocytes of AD brain, promoting further astrogliosis, suggesting that Aβ-activated ERK in astrocytes might be an important event in early-onset AD (Webster et al. 2006).

Since AD's treatment regimens are relatively ineffectual and poorly understood for their complicated underlying mechanisms, bringing in a multi-perspective avenue onto the conventional passageway facilitates the admittance of novel therapeutic targets. Astrocytes expedite the influx of multiple targets aiding the development of disease-attenuating/modifying interventions for their complicated signalling.

Astrocytes are increasingly being researched as a key to solving the mysteries of Alzheimer's disease because they have consistently remained underappreciated.

This chapter aims to provide substantial comprehension on the portrayal of astrocytes in neurodegenerative conditions, AD in specific. Also, we focus on elucidating and illustrating several altered mechanisms compelled at generating pathogenic and reactive astrocytes and briefly highlight its therapeutic unravelment.

2 Structural, Morphological, and Functional Basis of Astrocytes: An Essential Component of AD

The human brain comprises a multitude of neuronal cells and glial cells, including neurons, astrocytes, satellite glia, microglia, oligodendrocytes, Schwann cell, radial glia, and ependymal cells (Jäkel and Dimou 2017). The assemblage of these cells provides a proletarian environment wherein their synergistic action facilitates transmission of electrical and chemical signalling across the body systems, regulates the myelin sheath formation resulting in fast impulse conduction, and plays critical roles in the neural–immune system (Jessen 2004). Astrocytes are viewed as the specialized cell subtypes of glial cells in the central nervous system. They outnumber the neurons to nearly fivefold and are usually implicated in enhancing several complex functions in the healthy CNS possessing synaptic support, axon guidance, excess neurotransmitter clearance, and regulate blood–brain barrier and blood flow. Although astrocytes possess broad similarities with neurons supporting the neural framework, they do not facilitate the transmission of electrical impulses.

Astrocytes, given to their radially organized foot processes, are distinguished by their star-shaped appearance and the presence of glial fibrils. Astrocytes potentially communicate with and modulate the surrounding brain vasculature via the foot processes, marked by the presence of a glial fibrillary acidic protein (GFAP). The networked coordination among several astrocytes is achieved via their coupled responses through gap junctions, produced as a response to external stimuli (Oberheim et al. 2012). Several heterogeneous cell types build upon the astroglial lineage with which the brain develops, functions, and is repaired. As the protocols of developing cell lines in the embryonic stage commence, stem cells are characterized by differentiation into a particular cellular identity and then differentiating into lineage-specific progenitors that perform specific physiological functions. Neural precursor cells (NPCs), present in the telencephalic neuroepithelial cell of foetal embryonic brains, form the earliest manifestations of three fundamental CNS neural cell types: astrocytes, neurons, and oligodendrocytes (Kriegstein and Alvarez-Buylla 2009). Although several alterations and pathways are evidently associated with astrogliosis, such as methylation of DNA of astrocyte-specific gene promoters and JAK-STAT pathway, uncertainty persists as to how neural stem cells are sensitized to become astrocytes during brain development (Takizawa et al. 2001). Proliferative and pluripotent cell types from neuronal stem cells in embryonic brains develop into

terminally differentiated neurons, and glial cells (astrocytes and oligodendrocytes) are termed neuro/gliogenesis. In contrast to neurogenesis, gliogenesis begins during the late stages of embryogenesis and continues throughout the postnatal phases, wherein the ventricular–sub-ventricular zones (V-SVZ) and sub-granular zones (SGZ) of lateral ventricles and dentate gyrus (DG) in the hippocampal regions are considered as the principal neurogenic structures (Lim and Alvarez-Buylla 2016).

2.1 JAK/STAT Signalling

Astrogliosis is distinguished by a plethora of context-dependent mutations, which is fascinating despite maybe unsurprising. STAT3 signalling proceeds through a series of biochemical pathways, meaning that STAT3's concept in neuroprotection and neurodegeneration fluctuates depending on the context (Tyzack et al. 2017). JAK-STAT is regarded as a highly regulated intracellular signalling pathway, prevalently involved in regulating gene expressions (Oyinbo 2011). They involve two diverse families of proteins, i.e. JAK and STAT, comprising cytoplasmic protein tyrosine kinases and transcription factors. JAK encompasses TYK2, JAK1, JAK2, and JAK3 (Laurence et al. 2012), whilst STAT comprises STAT (1-4), STAT5A, STAT5B, and STAT6 (Nicolas et al. 2013). Phosphorylated JAKs produced as an outcome of their tyrosine phosphorylation via cellular membrane receptor binding and activate cytoplasmic STAT through tyrosine phosphorylation and eventually directs STAT dimerization (Darnell Jr et al. 1994), an indispensable event reported in glial scar formation, CNS injury, and neurogenesis (Vahedi et al. 2012; Yao et al. 1997). CNTF receptor-induced activation in the embryonic cortical precursor stem cells actuates the STAT1, STAT3, and JAK1 and subsequently leads to the differentiation of NPCs and NSCs into astrocytes (Xiao et al. 2010). Additionally, Gp130, a receptor complex, is substantially implicated in JAK/STAT through its activation on STAT3 and inducement of astrogliosis (Nakashima et al. 1999) whilst affecting IL-6 cytokine family signal transduction and inducing phosphorylation of STAT3 at Tyr705 promoting glial differentiation (Sriram et al. 2004). Activating cytokines in JAK/STAT signal enacts crucial supervision in monitoring lymphoid and myeloid cell development and differentiation; thus, pathological involvement, such as AD and PD, motives the uncontrolled dysregulation and neuroinflammation in astrocytes. On the other hand, the JAK-STAT signalling is silenced by the suppressor of cytokine signalling (SOCS) family of proteins such as SOCS-2, SOCS-3, and SOCS-6 by limiting a spectrum of JAK/STAT enhancing factors such as ILGF-1 and GH signalling (Gupta et al. 2011). SOCS-3's direct interaction with the Gp130 receptor through its SH2 domain reduces JAK/STAT signalling (Lang et al. 2003). Furthermore, it has been demonstrated that overexpression of SOCS3 restricts NSC differentiation into astrocytes whilst augmenting neurogenesis (Cao et al. 2006).

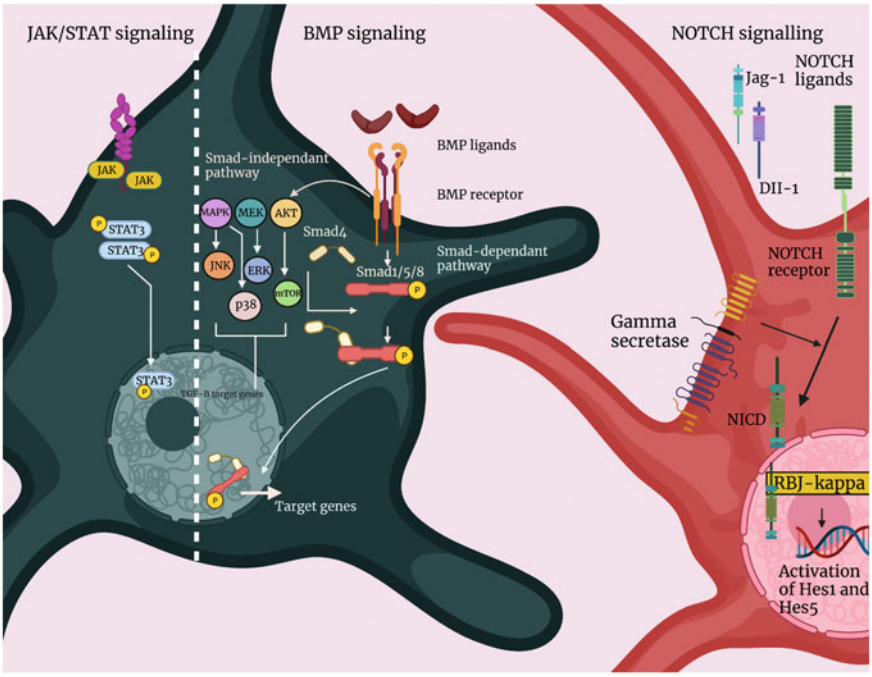


Fig. 1 The above illustration shows the predominant signalling providing transcriptional control framework and is involved in the processes of glial cell formations and astrogliosis. They include JAK/STAT signalling, BMP signalling, and NOTCH signalling

2.2 NOTCH Signalling

Notch signalling constitutes a compendium of conserved signalling pathways via its four receptor variants, i.e. NOTCH-1, NOTCH-2, NOTCH-3, and NOTCH-4 in mammals. The canonical trans-activation of NOTCH signalling initiates with binding the extracellular domain of transmembrane receptor protein to the extracellular domain of notch ligands such as Jaggeg-1 (Jag-1) and delta-like-1 (Dll-1) and determines the outcome of astrocytes. The observed conformational alteration favours the release of Notch intracellular domain (NICD) via enzymatic cleavage via γ-secretase. Released NICD translocated to the cellular nucleus interacts with DNA-binding RBP-J kappa protein, leading to activation of target genes, i.e. Hes1 and Hes5 *(the above signalling pathways are described in* Fig. 1). In vitro studies suggest that amyloid-beta treatments show altered BMP and NOTCH signalling expressions, implying that their levels might be associated with dysregulated cellular homeostasis in neurodegenerative disorders like AD (Acaz-Fonseca et al. 2019).

2.3 *Bone Morphogenetic Protein (BMP) Signalling*

Another group of growth factors, bone morphogenetic proteins (BMPs), constitutes an essential subclass of TGF-β morphogenetic signalling members that orchestrate tissue architecture and physiological function throughout the body. Their significance underlies various roles, including bone function homeostasis and embryonic cell lineage development. BMP's binding to the type I and type II BMP receptors facilitates the tetramerization of the receptor species. Consequently, the type II BMP receptors expedite the activation and phosphorylation of type I BMP, which further phosphorylates receptor-coupled R-SMADs (SMAD1, SMAD5, and SMAD8) (Bragdon et al. 2011). Amplified R-SMADs enable interactions with SMAD4 by forming heteromeric complexes. The 'nouveau' synthesized complex translocates to the nucleus and functions as a transcription factor whilst recruiting, binding with other transcription factors such as p300 and TGIF and mediating translational changes, respectively (Itoh et al. 2000; Morrison et al. 2000). The associations ultimately result in their binding to the Gfap promoter.

3 Tau Fibrils, Amyloid-Beta Plaques, and Their Periodic Rhythms of Neurodegeneration in Astrocytes

As tau proteins lose their ability to append to microtubules, their predicted role in sufficiently maintaining the cytoskeletal orders in the axonal process is ineffectual in AD. Influences of several mistranslated factors, conformational changes, and misfolding in tau's structure are responsible for the initiation of neuro-deteriorating cascade in brain tissues, leading to its accumulation in cytosol of the somatodendritic compartment (Luna-Muñoz et al. 2007). The authenticity of tau protein under physiological homeostasis and pathological conditions is affected by a series of biochemical protein modifications, regarded as 'post-translational modifications'. They include phosphorylation, acetylation, glycosylation, ubiquitinylation, aggregation, and nitration. Among these, a particularly active area of research is hyperphosphorylation for its extensive involvement in neurodegenerative disorders (Arendt et al. 2016). Post-mortem analysis speculates that tau phosphorylation levels were three- to fourfold higher than normal tau levels, i.e. 2–3 moles of phosphate for every mole of tau (Khan et al. 2021). Clinical signs are fundamentally observed decades prior to clinical symptoms, in the forms of neuropil threads and pre-tangles. The presence of NFTs is a late manifestation of AD, yet an important diagnostic marker. The interneuronal fibril lesions constitute several forms of aberrant tau species, including tau monomer, tau oligomers, truncated tau, PHFs, and NFTs. Tau fibrils deposits, initially found in the brain's entorhinal cortex and hippocampal regions, gradually propagate like 'tau seeds' and traverse along the neuro/glio-anatomically connected regions of the brain (Kidd 1963; Meraz-Ríos et al. 2010). Substantial misfolded tau protein build-up is observed in axonal and somatodendritic

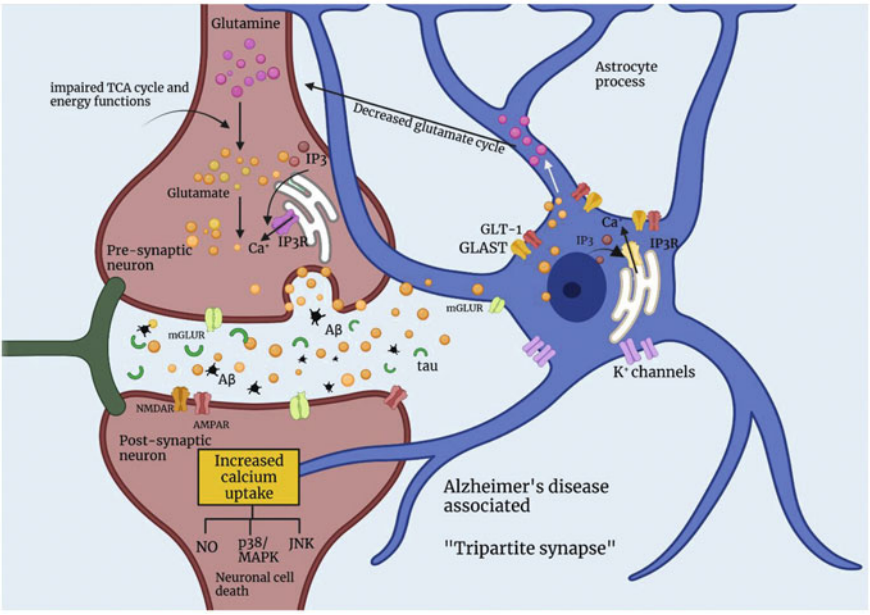


Fig. 2 The above schematic diagram represents the 'tripartite synapse' association, formed by pre- and post-synaptic membrane of neurons and astrocytes. The arrangement promotes the proper signalling transmission across the spaces. AD-associated events stimulate the promotion of impaired glutamate transport and increased calcium uptakes, leading to neurodegeneration

domains of cortical neurons (Ebneth et al. 1998), promoting tau–tau interactions, further exacerbating multimer species development. Upsurge in the NFTs lesions in the brain is associated with neurodegenerative modifications, including potential microtubule disturbances and instabilities, weakened axonal transport, neurotoxicity, synaptic loss (Trushina et al. 2019; Masliah et al. 1989), impaired cognition, increased expression of ROS, and eventually neurodegeneration (Kaniyappan et al. 2017). Many studies have recently focused on annotating the probable intermediary between neuropathological tau and astrocytes. Although astrocytes are regarded as widely considered glial cell types, their reciprocity with aberrant tau is largely marginalized. As one might assume, astrocytes' exposure to Aβ peptides does not elevate tau levels, postulating that increased endogenous astrocytic tau results from the internalization of tau as monomers through injured neuronal cells (*The pathogenic roles of tau fibrils and amyloid-beta plaques are shown in* Fig. 2).

Aβ plaques affect the neuronal regions by themselves whilst also via activating cascades on aberrant tau, several protein kinases, transcription factors, and pathways that, once initiated, proceed independently of Aβ. Overexpressed Aβ contributes to pro-inflammatory immune responses, oxidative stress, and production of reactive oxygen species. The physiological integrity of astrocytes is affected due to the presence of Aβ species. Similar to the neuronal population, astrocytes display immense pathological alteration, modulating their generalized roles in AD. The *β*-

amyloid plaques consist *of β-amyloid* peptides. The peptides are the cleavage product of a precursor protein, the APP protein. In humans, the APP gene on chromosome 21 encodes a type 1 transmembrane protein with three prevalent isoforms, APP695, APP751, and APP770 (695, 751, and 770 amino acids, accordingly) (Kaniyappan et al. 2017). The anomalous mutations on the APP gene, including missense mutations and APP duplications, form the primary causal elements in designating the footings of AD. APP is an integral type I transmembrane protein possessing a sizeable extracellular domain and a relatively shorter cytoplasmic segment. It is favourably defined in several neural and non-neural tissues, including neuronal synapses, brain tissues, spinal cord, glial cells, extra-neuronal tangles, trigeminal ganglia, and adrenal glands (Arai et al. 1991). APP plays a critical regulatory role in several essential cellular processes, including synaptic plasticity, anterograde neuronal transport (Turner et al. 2003), synaptic transmission, neurite outgrowth, synaptogenesis (repair) (Priller et al. 2006), as typically observed in the nervous system. The sequential proteolytic cleavage of APP by β-and γ-secretases, respectively, yields an intermediate product, i.e. a soluble, large N-terminal, fragment, sAPP-β and a C-terminal fragment, β-CTF consequently resulting in the production of AICD and Aβ40-42 peptides. Once released as an upshot to APP's sequential cleavage, the Aβ monomers establish their aggregation into diverse assemblages of protofibrils, amyloid fibrils, and oligomeric species, constitutively found in the senile plaques. Variations in the plaque composition are correlated with the age of the AD individuals. The coexistence of these diverse compositions of Aβ plaques renders a strict approach in anatomizing its pathogenesis. The consequences of Aβ spread signify loss of synapses with progressive neurodegeneration in the neocortical (Arnold et al. 1991) and limbic regions (Terry et al. 1991) whilst also including correlated cognition impairments in AD individuals (DeKosky and Scheff 1990; Larson et al. 1992).

4 Glutamate Transports and Their Pathogenic Alterations in AD Astrocytes

Synaptic release of glutamate through high-affinity glutamate receptors across the astrocyte forms elaborates conformity representing integral neuronal activity and major functioning at removing excess glutamate at synapses, protecting the brain's conservatory from neuronal excitotoxicity due to excess glutamate uptake (Danbolt 2001). Even though about five EAAT subtypes have been described in mammals with diverse expressions throughout the cortex, EAAT1 (GLAST: rodent analogue) and EAAT2 (GLT1: rodent analogue) are discerned as two predominant isoforms of the hippocampal domain (Arriza et al. 1994) facilitating a constant dominion over glutamate homeostasis and maintaining transmembrane ion fluxes of Na^+, K^+, Ca^{+2}, Cl^-, and protons (Araque et al. 2014). Escalation in Aβ expression in AD is principally associated with a significant decline in the activities and demographics of EAAT1/2 in the cortical and hippocampal astrocytes, clearly suggesting that

pathological progression of neurodegenerative diseases facilitates dysfunctional glutamate recycling and ion fluxes (Jacob et al. 2007).

Several AD-associated animal models show alterations in Gfap expression and calcium dysregulations to be frequently observed in pathological conditions associated with heightened Aβ levels. Compared with neurons, the notion of 'cellular excitability' in astrocytes is established due to the characterized variations in cytosolic calcium levels as a response to chemical messages (Verkhratsky 2019). Comparative research works led by Kuchibhotla et al., on APP/PS1/TG and wild-type mice, observed elevated Ca^{+2} concentrations and astrocyte activity independent of neuronal activities (Kuchibhotla et al. 2009). Amyloid species aid in enhancing the calcium signalling in astrocytes, leading to disrupted gliotransmission. Cellular interactions of Aβ with several surface receptors including nicotinic receptors (α7-nAChRs) (Xiu et al. 2005), metabotropic glutamate receptor (mGluR5) (Lim et al. 2013), and purinergic receptor (P2Y1) (Delekate et al. 2014) and with endoplasmic reticulum via inositol triphosphate receptors (IP3Rs) and ryanodine receptors (RyRs) (Alberdi et al. 2013) contribute to uncontrolled influxes of calcium ions in the astrocytes, respectively. Aberrant depositions of Aβ oligomers intend to directly or indirectly affect the L-type calcium channels whilst boosting the recurrence of NMDAR-associated currents along with increased calcium levels via α7-nAChRs in the hippocampal astrocytes (Alves et al. 2019). Families of glutamate receptor proteins, ionotropic glutamate receptors (iGluRs), NMDA receptors, AMPA receptors, kainate receptors, and metabotropic receptors have been related to pathogenic alterations observed in several neurodegenerative models such as AD and ALS. The physiologic function of NMDA receptors, which govern the synaptic transmission, cerebral vasodilation, and neuronal–glial signalling, is reasonably distressed due to upheaved levels of Aβ peptides (Rothstein et al. 1996). They affect NMDAR's expression via their interference with neuronal–glial chemical converse whilst instigating spine loss. Excess glutamate stimulation, a marked primitive neurotransmitter in the brain, results in neuronal and astrocytic excitotoxicity. Although NMDAR expression in non-neuronal cells is not well understood, the astrocytic NMDAR showcases a well-defined framework and function with applicably weak calcium ion permeability. Sodium-dependant transmembrane symporter glutamate transporter-1 (GLT-1) on the astrocyte accounts for nearly 90% uptake of excess extracellular glutamate concentrations in the brain tissues, displaying around four- to six-fold higher expression as compared with glutamate aspartate transporter-1 (GLAST1) (Rothstein et al. 1996; Lehre and Danbolt 1998). After their initial release from the pre-synaptic terminals, glutamate interacts essentially with two principal ionotropic receptors, i.e. AMPA and NMDA receptors on the post-synaptic membrane. Aberrant tau and Aβ species are shown to alter the homeostasis of calcium and glutamate uptake signals with prevalent reduced expression of GLT1, subsequently leading to neurodegeneration in the brain tissues. Ex vivo cultured astrocyte preparations display reduced expressions of GLAST and GLT-1 when strengthened with Aβ1-42 via adenosine A2A receptor, disrupting the physiological clearance of excess Aβ and neurotransmitters. Heterogeneity in activities of eNMDAR and sNMDAR is recurrently ascertained due to elevated levels of Aβ oligomers,

showcasing their engagement to astrocytic α7nAChRs (Canas et al. 2009). It leads to a series of debilitating effects, including the local release of glutamate from astrocytes into the extracellular spaces, leading to activation of eNMDAR, and, subsequently, fated to functional, molecular changes, dendrites, and spine loss along with synaptic damage. Studies on APP/PS1 TG mice using in vivo glutamate sensor iGluSn and two-photon imaging spectacled that anomalous fluctuations of glutamate levels were eminently analogous to varied expressions of senile plaques (Hefendehl et al. 2016).

5 Oxidative Stress and Their Uncontrollable Outgrowths in Astrocytes

Age-related cognitive impairments are often associated with manifestations of oxidative stress and reduced antioxidant defence mechanisms during the course of the pathological condition in brain tissues (Perrig et al. 1997). The NADPH-oxidized (NOX) pathway and mitochondrial (mROS) pathways are regarded as two fundamental sources of endogenous ROS species in the astrocytes. The presence of Aβ moderately stimulates and promotes the astrocytic NOX2 isoforms leading to astrogliosis (Chay et al. 2017). Overexpression and imbalances among free radicals and antioxidants supplement pernicious penalties to the biomolecules, eventually leading to rising of several chronic disease conditions, including neurodegenerative diseases, cardiovascular conditions, and chronic inflammation (Fridovich 1999). Neurodegeneration has been anticipated to be a synergistic effect of several factors such as genetic predisposition, environmental, and abnormal metabolism. ROS are regarded as an assemblage of small, oxygen-deprived molecules produced primarily as a result of aerobic metabolism in the body via various chemical reactions. They include hydrogen peroxide (H2O2), nitric oxide (NO), superoxide, and hydroxide radicals. Metabolism of excitatory neurotransmitters and amino acids in the brain and neuronal tissues act as sources of active ROS and render its predisposition towards oxidative stress (Floyd and Carney 1992). The neurobiochemical constitution of brain tissue is predominantly prone to ROS since it encompasses an elevated reservoir of iron, ascorbate, fatty acids, and unsaturated lipids that are predisposed to peroxidation and oxidative alterations. NOX, a family of membrane-bound integral enzyme complexes, are accounted as primary sources of cellular ROS, prevalent throughout the body. Overproduction of NOX-mediated oxidative stress is heavily monitored via phosphorylation. NADPH oxidase plays a crucial role as a catalyst in producing superoxide species through the transfer of electrons from NADPH to oxygen and its subsequent transfer into extracellular space. The presence of neurofibrillary tangles and Aβ senile plaques in neuronal and glial cell populations in AD exacerbates the pathogenicity of the NOX pathway. Aβ plaques are principally involved in NOX activation in microglia and astrocytes wherein the cytosolic p47 phox component migrates to the membrane. Several in vitro and in vivo models

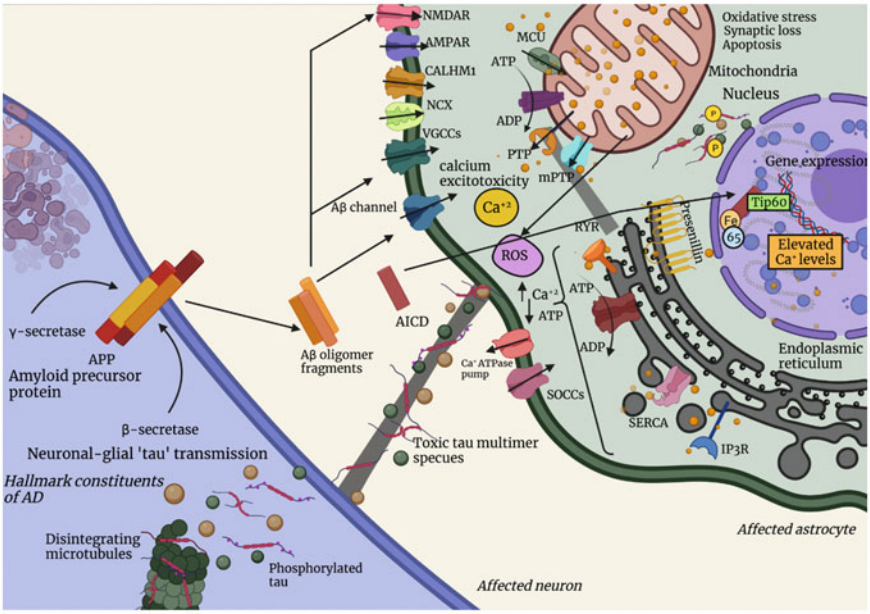


Fig. 3 The above illustration shows the neuronal–glial oxidative stress interactions in the presence of AD's hallmark constituents, amyloid-beta, and interneuronal neurofibrillary tangles. Due to the existence of chronic amyloid-beta and tau fibril deposits, numerous receptors situated on the surface membrane of neurons, astrocytes, and astrocytic mitochondria, endoplasmic reticulum, and nucleus, stimulate an uncontrolled influx of calcium ions and the depletion of ATP and NAD molecules leading to neuronal dysregulation. Toxic oligomeric AD species traverse across the neuro-glial spaces, facilitating the cascade initiation of cellular apoptosis, oxidative stress, and synaptic loss, ultimately culminating in neurodegeneration

demonstrate the implications of NOX in AD development (Qin et al. 2006). Human astrocyte cell lineages have been evidenced to produce ROS species through NADPH oxidase activities, although no ROS source has been reported in astrocytes derived from human brain progenitor cells. Responses to calcium ionophores and PK-C activation also contribute to astrocyte ROS generation. Primary astrocytes expressing IL-1b ± IFN-c exacerbate the production of ROS species. Even though NOX2 and NOX4 are prevalently expressed in astrocytes, NOX2 is regarded as the predominant NOX isoform present in human astrocytes (Abramov et al. 2005). NOX-mediated ROS is found in reactive astrocytes implicating their role in astrocytic oxidative damage (*the implications of oxidative stress on astrocytes are depicted in* Fig. 3). The metabolic configuration of astrocytes vastly relies on the process of glycolysis, allowing them to contribute glycolytically mediated lactate to neurons to ensure a high energy-efficient system and sustain complex neural functions.

Astrocytes have been studied less extensively in contrast to neurons for mitochondrial dynamics. As a safeguarding cell type in the neuronal environment via cushioning of excess glutamate, astrocytes discard surplus levels of glutamate

released from synaptic clefts by processing them to glutamine via tricarboxylic acid cycle and glutamine synthetase and ultimately generating the mitochondrial ATP. Aβ peptides promote the reduction in mitochondrial potentials of astrocytes and increase intracellular calcium concentrations whilst not affecting the neurons. Even though less concentrated Aβ aggregates motive enhanced GSH release from the astrocytes through Cx43 hemichannels and ABCC1 transports and provides protection against oxidative stress (Ye et al. 2015), its chronic exposure displays a concomitant decline in astrocytic GSH levels describing Aβ's dual nature (Abramov et al. 2004). Reduced GSH and oxidated proteins associated with energy metabolism are primarily linked with alterations in transcription factor regulation, including OXPHOS genes, reduced TFAM, NRF2, and PGC-1α in hippocampal regions of the AD brain (Sheng et al. 2012). Aβ exposure to astrocytes can elicit mitochondrial fragmentation and depolarization, contributing to heightened ROS production and metabolic dysfunctions. Thus, it affects the mitochondrial membrane potential of astrocytes, suggesting the susceptibility of astrocytic mitochondria in AD. The finding demonstrates that toxic fatty acids released due to Aβ from overstimulated neurons might induce cytotoxicity, specifically if they are not metabolized due to astrocyte mitochondrial impairment (Ioannou et al. 2019).

6 Neuroinflammation: An Inevitable Phenomenon in Reactive Astrocytes

In current times, scientists have proposed a novel classification of reactive astrocytes, i.e. A1 and A2 reactive astrocytes, respectively. A1 is primarily induced as a result of neuroinflammation and secrete neurotoxins leading to rapid cellular death of oligodendrocytes and neurons, whilst A2 is involved in conservative neuroprotective features, promoting neuronal cell survival and tissue repair. The conception of A1 sub-population of astrocytes is confirmed by the presence of biomarker genes including C3, MX1S, and CFB (Li et al. 2019), which otherwise are not present in A2 subtypes. These details suggest that even if astrocyte activation can be neuroprotective at times, it can also stimulate the secretion of pro-inflammatory signal mediators, particularly when afflicted with Aβ accumulation in AD (Henstridge et al. 2019). The physiological responses of glial cells, i.e. microglia and astrocytes, are chiefly involved in phagocytizing cellular fragments and aggregated proteins (Heneka et al. 2015). The assemblage of aberrant tau fibrils and senile plaques supports the pathogenic framework of AD, which augments the release of pro-inflammatory cytokine mediators such as IL-1, IL-6, TNF-α, and IL-1β. Astrocytes express a diverse set of receptors that recognize and specifically bind to Aβ such as lipoprotein receptor-related protein (LRPs), scavenger receptor (SR), receptor for advanced glycation end products (RAGE), and membrane-associated proteoglycans. RAGE binding to S100/calgranulins and DNA-binding protein HMGB1/amphoterin stimulates the cascade activation of

several intracellular pathways including NF-kB, MAPK, ERK, JNK, and S100B pointing Aβ as a crucial neuroinflammation initiator in AD (Hofmann et al. 1999; Gonzalez-Reyes and Rubiano 2018; Hori et al. 1995). When co-cultured with glial cells, the presence of Aβ aggregates aggrandizes the reactive astrocyte populations whilst activating astroglial NF-kB factor and complement signalling, expediting impaired synaptic density, potentiates the production of pro-inflammatory mediators through astrocyte's response to LPS and SR ultimately culminating in neurodegeneration (Lian et al. 2015; Murgas et al. 2012). Microglia, the resident macrophage cell lines of the central nervous system (CNS), are activated and develop into disease-associated macrophages (DAMs) (Sarlus and Heneka 2017) in response to systemic inflammations and neurobiological adaptations in brain tissue. Likewise, activated microglia can turn resting astrocytes into neurotoxic reactive astrocytes by secreting inflammatory cytokines like TNF and IL-1 (Liddelow 2019). Astrocytes, like microglia, have pro-inflammatory and neuroprotective cellular subtypes. Inflammatory astrocytes mediate the existence of pro-inflammatory factors (IL-1, TNF-, NO), which are known to cause secondary inflammatory responses, detrimental to the survival of astrocytes in the long run. Conversely, neuroprotective reactive astrocytes upregulate a variety of neurotrophic factors such as IL-4, IL-10, and TGF-beta through anti-inflammatory cytokines. Fluctuations in Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CN), observed in glial cells, are pronouncedly affected due to Aβ-induced neural damage, further leading to hyperactivation of its substrate, NFAT, and ultimately resulting in cognitive decline and neuroinflammation (Furman and Norris 2014; Pleiss et al. 2016).

6.1 Nuclear Factor Kappa B (NF-κB) Pathway

The NF-κB signalling pathway is crucial during development and inflammatory processes. The NF-κB pathway is essential to initiate astrocytic differentiation and maintain their multipotential and proliferation properties, as its early inhibition induces NPC apoptosis and impedes their differentiation. According to recent studies, the NF-B pathway exerts a dual basic role during NPC transition into astrocytes: it enhances astrocyte specificity, but its sustained expression hinders differentiation. Specifically, in AD, it has been demonstrated that overexpression of inflammatory signals and mediators in the proximity of Aβ deposits and neurofibrillary tangles are associated with higher degrees of neurodegeneration. Prolonged glutamate excitotoxicity of astrocytes has been observed to drive cytotoxic, oxidative stress-induced upregulation of NF-kB through epidermal growth factor receptor (EGFR) signalling. Activation of the previously sequestered heterodimeric transcription factor, i.e. NF-kB to its inhibitor IkB-α, has been proposed to possess both neurotoxic and neuroprotective roles with the consequences expected to be dependent on duration, timing, and level of activity (Meffert et al. 2003). Studies on mice models with Cre-mediated deletion of inhibitor complex IkB-α wherein NF-kB was selectively activated to assess their effect in neuron-astrocyte co-cultures

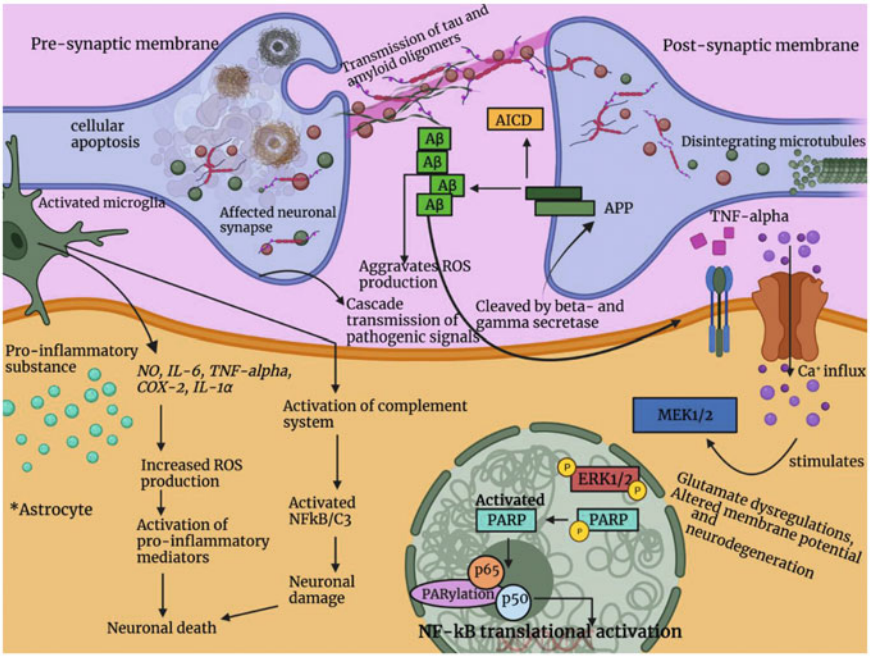


Fig. 4 The diagram portrays the neuroinflammatory interactions among neurons, astrocytes, and microglia. Amyloid-beta peptides and tau fibrils, for their neurodegenerative features, promote the uncontrolled influx of pro-inflammatory signal mediators and ROS production, activate the complement systems, elevate calcium influx, and stimulate the PARP-1/NF-kB neuroinflammation

demonstrated that complement factor C3 is an essential astroglial NF-kB target that engages in neuronal–glial interactions via neuronal C3aR and the deviant elevations in their signalling loop affect excitatory synaptic function and dendritic morphology through calcium dysregulation. NF-kB/C3 is induced in astrocytes as a result of their interactions with Aβ, mainly focused on human AD brain and APP/TG mice (Fatoba et al. 2021).

6.2 *Poly (ADP-ribose) Polymerase-1 (PARP) Pathway*

PARP, an 18-membered family of nuclear proteins, critically regulates cellular processes such as inflammation, genomic stabilities, DNA repair, and cellular apoptosis through their putative interactions with innumerable transcription factors (Herceg and Wang 2001). ssDNA or dsDNA breaks due to oxidative stress are known to activate DNA repair protein as PARP-1. PARP-1 utilizes NAD^+ to produce poly(ADP-ribose) polymers (PAR). This utilization catalyses the sequential cleavage of NAD^+ to nicotinamide and ADP-ribose. Their uncontrolled activities result in energy depletion, promoting cellular degradations (Bürkle 2005; Abeti et al. 2011). Intra-nuclear expressions of PARP and PAR have been ascertained at much

higher proportions in AD than in healthy brains (Love et al. 1999). The excessive activation of PARP-1 under pathological conditions may lead to an accumulation of PAR, a novel signalling molecule that induces programmed cell death, or consumes a large amount of NAD^+, resulting in an energy crisis and necrotic cell death (Herceg and Wang 2001). PAR/PARP-1 co-localization with MAP-2, tau protein, and Aβ leads to PAR-induced apoptosis-inducing factor release, increased depositions of senile plaques and tau fibrils, and pathogenesis of AD brains (Martire et al. 2015). PARylation of the ReIA (p65) sub-component of NF-kB by PARP-1 aggravates the activation of NF-kB, promoting chronic neuroinflammation. PARylation accelerates the synthesis of NF-kB transcription complex through the dissociation of NF-kB from PARP-1, promoting the NF-kB binding to DNA sites (Szabó and Ohshima 1997). As a result, morphological alterations occur as well as activation of microglial cells and release of NO and cytokines (Martínez-Zamudio and Ha 2014) (Fig. 4 *portrays the PARP-1/amyloid/NF-kB interactions*). Aβ40-42 susceptibilities to mixed co-cultures of hippocampal neurons and glial cells from Sprague Dawley rats reflected increased expression of PAR in GFAP-positive astrocytes leading to NAD^+ depletion, loss of mitochondrial potential ($\Delta\psi m$), reduced oxygen uptake, and ultimately Aβ/PARP-1 induced neuronal death-4. TNFα-mediated PARP-1 activation necessitates sustained calcium influx, MEK1/ERK2 kinase phosphorylation, and PC/PLC activation promoting transcriptional activity of NF-kB and pro-inflammatory effects on primary cortical astrocyte and microglia co-cultures (Vuong et al. 2015).

7 Therapeutic Compounds and Clinical Trials

7.1 Fasudil

Initially known for its role as a potent vasodilator, Fasudil, an efficacious Rho-kinase inhibitor, since then has been utilized for its application in treating cerebral vasospasm induced due to subarachnoid haemorrhage, pulmonary hypertension, and cognitive impairments. Fasudil attenuates the ROCK (Rho/Rho-kinase) signalling, frequently involved in oxidative stress and inflammation. Administration of 25 mg/kg/day Fasudil in eight-month-old APP/PS1/TG mice models resulted in rescued cognitive deficits and restricted microglia activation and alters the pro-inflammatory settings to anti-inflammatory ones and subsequently transits reactive astrocytic phenotype from A1 to A2 through its inhibitory interactions on MyD88, NF-kB, and TLR4 expressions (Guo et al. 2020). Studies on combined doses of Fasudil with another group of ROCK inhibitors, Y-27632 in C57BL/6 mice astrocyte cultures showed increased ((3)H)-D-aspartate (D-Asp) uptake, elevated expressions of EAAT1, and EAAT2 along with the reduced abundance of F-actin (Lau et al. 2011). Fasudil treatment to neural C17.2 stem cells promoted neurite outgrowth and differentiation in neuronal cells and astrocytes along with elevated levels of MAP-2, GFAP, and DCX through modulation of NOTCH signalling (Chen et al. 2015).

7.2 *Selegiline (L-Deprenyl)*

Selegiline is primarily regarded as an irreversible, selective antagonist of monoamine oxidase B and promotes the synthesis of monoamine neurotransmitters in the brain, i.e. dopamine and serotonin. They suppress astrocytic GABA levels through inhibition of MAO-B, suggesting the restoration of impaired learning (Piccinin et al. 1990), memory deficits, spike probability, and synaptic plasticity in an A-β-independent manner in AD (Jo et al. 2014). The actions of selegiline are dose-specific wherein administration of larger doses (>20 mg/day) potentially capitulates its specificity to MAO-B. Long-term selegiline treatment regime often produces statistically ineffectual and disappointing effects at reversing learning and cognitive deficits in APP/PS1/dE9 mice models (Jo et al. 2014). Even though selegiline provides an antioxidant and neuroprotective contour and positive behavioural and cognitive scores in several neuropathological conditions, its study in AD remains elusive. **KDS2010**, a novel, reversible MAO-B inhibitor variant's treatment to APP/PS1/TG mice (4-week; 10 mg/kg/day), demonstrated prolonged attenuation of GABA production and maintained spike probability ameliorated cognitive impairments whilst suppressing abnormal tonic GABA currents in a concentration-dependent manner (Park et al. 2019).

7.3 *Ceftriaxone*

Ceftriaxone is chiefly reported as third-generation β-lactam cephalosporin antibiotics, intended for the treatment of diverse sets of bacterial, bone joint, urinary tract, and intra-abdominal infections in the body. The administration of ceftriaxone validates that they profoundly facilitate EAAT2 and GLT-1 (rodent homolog of EAAT2) expression in human astrocytes and rodent models. Ceftriaxone-induced EAAT2 promoter activation displays positive signalling with NF-kB binding site at position-272, indicating their potent glutamate transport-modulating property. In terms of astrocytes, ceftriaxone is highly regarded for its critical role in instigating EAAT2-specific expressions, restraining neuronal cell death due to glutamate toxicity, thus asserting neuroprotective functions in vitro and in vivo animal models (Rothstein et al. 2005).

7.4 *Riluzole*

Riluzole, possessing benzothiazole scaffold, was originally authorized by U.S. FDA for its disease progression attenuating aspect in patients with amyotrophic lateral sclerosis (ALS) in 1995 (Yoshizumi et al. 2012). They selectively block NMDA receptors, kainate receptors, and TTX-sensitive Na^+ ion channels, all of which

correspond to damaged neuronal cells. Although riluzole's efficiency on glutamate receptors is contestable, newer studies do suggest their interests as a glutamate uptake. In addition to enhancing glutamatergic synaptic activity, riluzole is suggested to enhance glutamate uptake via glial transporters and decrease glutamate leakage to extra-synaptic NMDA receptors. Striated astrocytes cell cultures, prepared from E15/E16 Swiss mouse embryos and enhanced with riluzole (100 μM) for three days, represented selective upregulation in GLT-1 protein levels and glutamate uptake whilst showing non-significant alterations in GLAST protein levels (Carbone et al. 2012). 5-month riluzole treatments in early-onset 5XFAD mice models suggested rescued effects at substantially attenuating Aβ species including APP mRNA, Aβ oligomers, Aβ40-42 levels, gene expressions of pathology associated astrocytes, microglia, and neurons and maintaining NMDA receptor expression in the hippocampus (Okamoto et al. 2019).

7.5 SNAP-5114

SNAP-5114, an effective γ-aminobutyric acid transporter 3/4 (GAT3/4) inhibitor, assists the notion of reversing altered GABA expressions in reaction astrocytes, observed in AD. (*S*)- SNAP-5114, an analogue obtained from SNAP-5114 parent compound. Hippocampal slices of the 5XFAD mouse model, when treated with SNAP-5114, substantially mitigate altered tonic GABA currents in dentate granule (DG) cells whilst reversing cognitive memory and LTP impairments (Böck et al. 2020; Wu et al. 2014).

7.6 LDN/OSU-0212320

LDN/OSU-0212320 is a notable pyridazine-derived activator of EAAT2 protein level leading to its increased expression whilst extenuating neuronal loss and neurodegeneration in time- and concentration-dependent manner in primary neurons and astrocytes cultures. Wild-type FVB/N TG male mice treated with LDN/OSU-0212320 demonstrated upregulation in total GLT-1 expression in the hippocampus, striatum, and cortex domains and a small but significant amplification in glutamate clearance rates, postulating that they facilitate the GLT-1 production in peri-synaptic processes rather than cellular proportions such as synaptosomes (Wilkie et al. 2021; Takahashi et al. 2015). In PA-EAAT2 rat astrocyte cell lines, they upregulated EAAT2 expression via translational activation. They are known to display enhanced synaptic integrity, improved learning and cognition profile, and restored EAAT2 protein levels and Aβ senile plaque burden in APP/Sw/Ind mice model (Takahashi et al. 2015). Treating rTg4510 mice models with *LDN/OSU-0215111*, an advanced pyridazine-derived compound, speculated normalization of pathological incidences observed in AD through constant, direct reduction in toxic phosphorylated tau (ptau)

levels by modifying their antagonistic action on tau kinase, glycogen synthase kinase-3 beta (GSK-3β), suppressing disease progression at the moderate phase of AD along with restoring cognitive scores and synaptic integrity (the therapeutic compounds and their chemical structures are described in Table 1) (Foster et al. 2019).

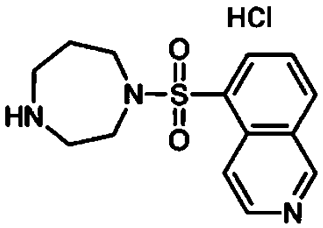
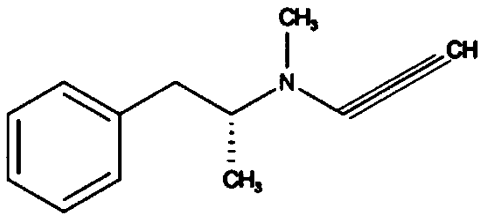
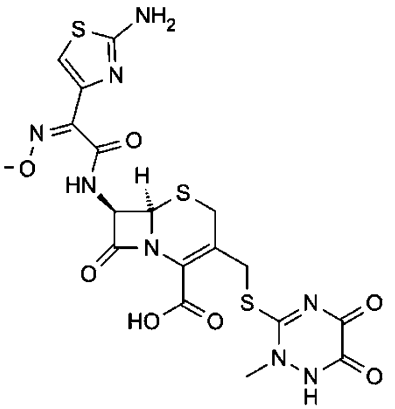
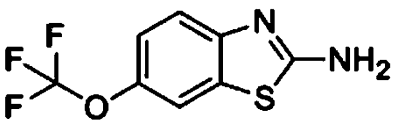
7.7 Alpha-Tocopherol (Vitamin E)

Many studies postulate the beneficial applications/supplementations of alpha-tocopherol in producing a disease-attenuating effect in patients and TG animal models with AD, PD, ALS, and CNS dysfunctions. Alpha-tocopherol transfer protein (TTP), specifically expressed in GFAP-positive cerebellar astrocytes, stimulates the influx of alpha-tocopherol to neighbouring neurons, curtailing incidences of oxidative stress (Ulatowski et al. 2022). Human glioblastoma cell lines treated with TRF and α-TCP elucidated the preservation of mitochondrial membrane potential (MMP) upon glutamate excitotoxicity and declined lipid peroxidation, oxidative stress, and glutamate-mediated cell death, along with promoting neuroprotection and neuronal survival in astrocytes (Selvaraju et al. 2014).

8 Future Works and Conclusion

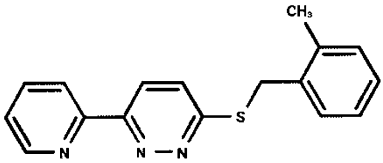
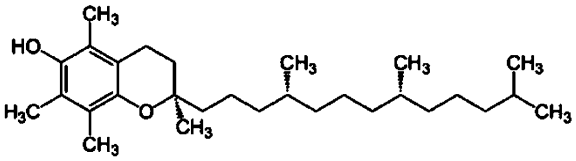
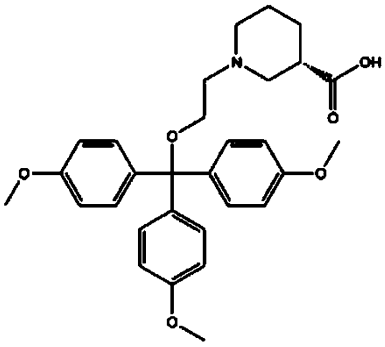
AD-associated Aβ plaques and tau fibrils have been shown to modulate the functional dynamics of healthy neuronal and glial cell residents in the CNS, reflecting deteriorating conditions in the neuronal–glial interrelated connections. Even though studies on astrocytes have not been widely ascribed as much as neurons, recent researches suggest their impact on AD's pathological progression. Treatment regimens put forth continuous approaches to developing concise disease-attenuating compounds to treat AD successfully. Glial cells, astrocytes in specific, respond to CNS insults with variations in their molecular, morphological, and functional prospects. Henceforth, astrocytes play crucial roles in the aetiology of several neurodegenerative diseases. Current understandings of the roles of reactive astrocytes are still in their nascent stage. The pathological notions of astrocytes due to neuroinflammation, Aβ senile plaques, NFTs, glutamate, and another neurotransmitter uptake, as well as oxidative stress in the CNS, often reflect therapeutic targets for producing pragmatic disease-attenuating effects. Of all the studies enumerating the neuroprotective and pathological motives of reactive astrocytes in vitro and in vivo study models, evaluating its outline at the biomolecular level and deciphering the region- and mechanism-specific progression appears noteworthy. The development of 'omics' techniques and futuristic breakthroughs delivers pioneering interventions to address the global burden of dementia-related disorders and encourages us to provide viable alternatives to conventional treatments and supports the

Table 1 Therapeutic compounds and their chemical structures

Compounds	Chemical structures
1. Fasudil	Molecular formula: $^{C}14^{H}17^{N}3^{O}2^{S}$  M. wt: 291.36 g/mol
2. Selegiline	Molecular formula: $^{C}13^{H}17^{N}$  M. wt: 187.2808 g/mol
3. Ceftriaxone	Molecular formula: $^{C}18^{H}18^{N}8^{O}7^{S}3$  M. wt: 554.57 g/mol
4. Riluzole	Molecular formula: $^{C}8^{H}5^{F}3^{N}2^{OS}$  M. wt: 234.199 g/mol
5. LDN/OSU-0212320	

(continued)

Table 1 (continued)

Compounds	Chemical structures
	Molecular formula: $^{C}17^{H}15^{N}3^{S}$  M. wt: 293.39 g/mol
6. *Alpha-tocopherol*	Molecular formula: $^{C}29^{H}50^{O}2$  M. wt: 430.71 g/mol
7. SNAP-5114	Molecular formula: $^{C}30^{H}35^{NO}6$  M. wt: 505.6 g/mol

The above table demonstrates the chemical structures of astrocyte associated. *AD* disease-modifying agents

evidence of potent AD targets. In line with the rising prevalence in individuals affected with neurodegenerative conditions, extensive and advanced research works dedicated to developing safer, more efficacious, and reliable molecules will diversify and facilitate a more detailed view of the underplayed prospects of AD. In this review, we have provided concise insights into the morphological and fundamental functions of astrocytes in both physiological and pathological settings, provided conceptions of the possible therapeutic interventions involved, and elucidated the embryonic and adult glial cell genealogy observed in normal and AD-related astrocytes. Along with it, we have also concentrated on presenting graphical representations of astrocytes.

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Consent to Participate: Yes.

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Targeting Mitochondrial Dynamics as a Restorative Approach in the Treatment of Alzheimer's Disease

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Abstract The progressive damage and deprivation of neuronal cholinergic neurons lead to critical loss of learning and memory-based brain function, characterized as Alzheimer's disease (AD). This disorder is associated with two main hall marker proteins in neurofibrillary tangles, i.e. amyloid-beta (Aβ) plaques and hyperphosphorylated tau along with α-synuclein, but none of them explains the complete aetiology of AD. Various attempts have been made to relate the complex interlinkage of tau and Aβ proteins with many other proteins to understand the pathophysiology of AD. Mitochondrial dynamics—a fusion and fission balance—has been linked to AD as its imbalance leads to elevated fragmentation of mitochondria and irregular mitochondrial distribution resulting in neuronal apoptosis and decline of cognitive function leading to AD. Aβ plaque formation in neuronal mitochondria imbalances the mitochondrial dynamic process by increasing the fission and reducing the fusion phenomenon. In this chapter, we tried to explore the role of different proteins such as Drp1, Fis1, Mff, MiD49, MiD51, and endophilin-B1 (mitochondrial fission proteins) and Mfn1/2, Opa1, Oma1, HDAC6, AIM2, and cAMP/PKA (mitochondrial fusion proteins) in the progression of AD, which would be targeted as potential targets for the treatment of AD in future.

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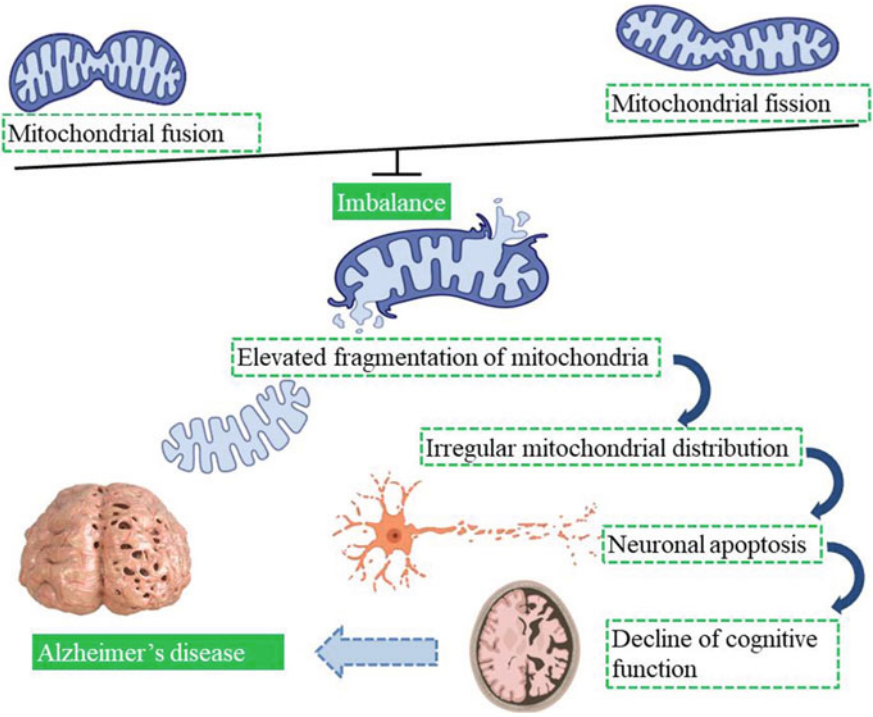
Graphical Abstract



Keywords Alzheimer's disease (AD) · Mitochondrial dynamics · Apoptosis · Mitochondrial fission proteins · Mitochondrial fusion proteins

Abbreviations

AD	Alzheimer's disease
AIM2	Absent in melanoma 2
APP	Amyloid precursor protein
Aß	Amyloid-beta
Ca^{2+}	Calcium
CL	Cardiolipin
Drp1	Dynamin-related protein 1
ER	Endoplasmic reticulum
ETC	Electron transport chain
Fis1	Mitochondrial fission protein 1
HDAC6	Histone deacetylase 6
IMM	Inner mitochondrial membrane

l-OPA1	Long-type optic atrophy protein 1
MAPT	Microtubule-associated protein tau
Mff	Mitochondrial fission factor
Mfn1/2	Mitofusins 1 and 2
MiD49 and MiD51	Mitochondrial dynamics proteins of 49 and 51 kDa
Oma1	Overlapping with the M-AAA protease homolog
OMM	Outer mitochondrial membrane
Opa1	Optic atrophy protein 1
PS1	Presenilin 1
PS2	Presenilin 2
s-OPA1	Short-type optic atrophy protein 1
SPs	Senile plaques
YME1L	Yeast mitochondrial AAA metalloprotease like 1 ATPase

1 Introduction

A neurodegenerative syndrome is a group of incurable and life-threatening brain disorders in which neurons lose their ability over time and eventually dies. Over the last 50 years, an increase in the lifespan of people's lives has resulted in a rise in the estimated incidence of neurodegenerative problems, correlated with ageing (Yang et al. 2021). Alzheimer's disease (AD) is one such neurodegenerative ailment that has a massive impact on millions of individuals (Coyle et al. 1983). As per the Global Burden of Disease Study, AD is among the fastest-growing death-causing disorders (Fitzmaurice et al. 2017). Presently, approx. 55 million or more individuals are suffering from dementia across the globe where 10 million new cases are detected every year (https://www.who.int/news-room/fact-sheets/detail/dementia). The main symptom associated with this disorder is impairment in cognitive and memory function due to the gradual and specific neuronal death in the forebrain along with other regions of the brain (Brookmeyer et al. 2007). The aetiology of AD has been attributed to a number of variables. These may be biological factors (like ageing, sex-specific, and body weight), environmental factors (e.g. way of living, toxicants, and cerebral injury), genetic factors (e.g. the mutation in amyloid precursor protein [APP], presenilin 1 [PS1], and presenilin 2 [PS2] genes in AD and susceptibility genetic variants in sporadic cases), and many others (Qiu et al. 2009).

Generally, loss of synapse and atrophy of neurons present in the hippocampus region and of the cerebral cortex results in the development of AD. Clusters of misfolded proteins named amyloid-beta (Aβ) plaques and neurofibrillary tau tangles (NFTs) cover the whole brain in severe AD (Sheppard and Coleman 2020). The major component of amyloid or senile plaques (SPs) is highly insoluble and is composed of polypeptide fibrils, which are proteolysis-resistant, generated by Aß cleavage. β-secretase (BACE1) and γ-secretase are two main enzymes that cause the sequential breakdown of APP and result in the production of Aβ38, Aβ40, and Aβ42

variants of Aβ peptides (Anand et al. 2017). Another tangle of tau proteins is a microtubule-related protein, which is expressed by the gene name microtubule-associated protein tau (MAPT) (Binder et al. 2005). This protein helps in the efficient transportation between neuronal systems by attaching to microtubules and also helps in the normal development and functioning of neuronal cells. Hyper-phosphorylation of tau proteins at an abnormal rate results in the emergence of insoluble tangles of tau protein in neurons and abrupt the functioning of the microtubule (Yiannopoulou and Papageorgiou 2013).

The severity of cognitive deterioration and histological anomalies is used to classify AD into four main steps, which are described as follows (Dickson and Weller 2011; Kumar and Tsao 2018; Calabrò et al. 2021):

- Preclinical: This is the initial stage with no significant symptoms; thus, this stage is frequently missed. Mild cognitive impairment is observed with modest memory loss, affecting the entorhinal cortex (first) and the hippocampus (later).
- Mild AD: Cognitive symptoms tend to emerge during this phase, which is associated with loss of memory, inability to memorize new things, and difficulty in problem-solving and judgement as pathological alterations enter the cerebral cortex region.
- Moderate AD: The severity of symptoms worsens in this phase as pathological damage expands in different regions of the cerebral cortex, which regulates language, thought processing, and sensory abilities along with behavioural issues and social disengagement tendency.
- Severe AD: At this level, the pathological damage is thought to have spread throughout the whole cortex and there is a full loss of individual independence for daily tasks. The affected person's cognitive abilities deteriorate with the emergence of additional systemic symptoms, such as dyspraxia, i.e. trouble in performing learned motor activities properly, olfactory disturbance, insomnia, with extrapyramidal signs such as dystonia, akathisia, and parkinsonian effect.

The human nervous system consumes a significant amount of energy as several neural functions are energy-intensive in nature, and this demand is fulfilled by mitochondria as it is the primary site for the origin of energy, supplying ATP via oxidative phosphorylation (Wang et al. 2020). To regulate the ionic gradients with the resting membrane potential along with the packaging of neurotransmitters into synaptic vesicles to be released at the synaptic cleft demands a significant amount of ATP concentration (Saxton and Hollenbeck 2012). Many researches have reported that mitochondria present in neurons and peripheral cells of AD patient and in AD cell models are fragmented (Zhu et al. 2013). Therefore, excessive mitochondrial fragmentation can affect ATP formation, which results in mitochondrial dysfunction and that can be lethal for the sustainability of neurons (Yang et al. 2021).

Repeated coordinated rounds of fission and fusion maintain the architecture of a cellular mitochondrial network, which is termed "mitochondrial dynamics". When single mitochondrion separates into double daughter mitochondria, it is known as mitochondrial fission, which is a multiphase process. This process is controlled by the binding of GTPase dynamin-related protein 1 (Drp1) at the mitochondrial

constriction site mediated by endoplasmic reticulum that oligomerizes Drp1 and results in the allocation of dynamin 2, which terminate the splitting of the membrane. On the contrary, mitochondrial fusion is a double-step process where optic atrophy 1 facilitates the joining of the inner mitochondrial membrane (IMM), while mitofusins 1 and 2 mediate the attachment of the outer mitochondrial membrane (OMM) along with other post-translational modifications, which coordinate this processes (Tilokani et al. 2018). Elongated and interconnected mitochondria are produced when there is an elevation in the rate of mitochondrial fusion process, while the increase in the mitochondrial fission phenomenon results in mitochondrial fragmentation (DuBoff et al. 2013). The activity of mitochondrial fission proteins has been linked to the deposition of α-synuclein along with plaques of Aβ and phosphorylated tau proteins, within the brain of Alzheimer-diseased patient (Yang et al. 2021).

2 Mitochondrial Dynamics: Synchronized Cycle of Fission and Fusion

To fulfil the different energy demands of highly polarized axons and dendrites of nerve cells, the mitochondrial dynamic process is regulated under which an extended mitochondrial network is formed in the somatodendritic region, while axons have fragmented mitochondria (Overly et al. 1996; Popov et al. 2005). There are clusters of dynamin-related GTPase proteins such as Drp1, mitofusins 1 and 2 (Mfn), and optic dominant atrophy 1 (Opa1), which regulate the shape and network of mitochondria and drive these mitochondrial fission and fusion processes to fulfil the demand.

2.1 Mitochondrial Fusion

The process of joining two adjacent mitochondria by fusing their outer membranes (OMM) followed by combining inner mitochondrial membranes (IMMs) and matrix is known as mitochondrial fusion, and the process is mediated by majorly three GTPase-Mfn1, Mfn2, and optic atrophy 1 (OPA1) (Nunnari et al. 1997; Meeusen et al. 2004). The Mfn configuration has two domains, i.e. HR1 and HR2 domains, which face towards the cytosol. The trans-facing HR2 or GTPase domain present in mitofusins promotes the interaction of two adjacent mitochondrial outer membranes. The tethering induces dimerization and conformational changes on Mfns and causes mitochondrial docking and elevates the number of contact sites present on the mitochondrial membrane. Finally, OMM fusion is accomplished via GTP-dependent oligomerization, which is accompanied by IMM fusion driven by OPA1 and CL (cardiolipin), respectively. On both sides of the mitochondrial

membrane, the association between OPA1 and CL fuses the two IMM, which is mediated by OPA1-dependent GTP hydrolysis (Tilokani et al. 2018). Therefore, mitofusin turnover regulates the mitochondrial fusion followed by ubiquitination and then removed by proteasomal degradation (Tanaka et al. 2010; Leboucher et al. 2012). mRNA interweaving of inner mitochondrial peptidases, for example, OMA1 and QW, produces long type (L-OPA1) and short type (S-OPA1), respectively (Anand et al. 2014). A study reported that conditional deletion of Mfn2 gene in dopaminergic neurons leads to defective mitochondrial transportation and progression along with decreased mitochondrial volume (Pham et al. 2012).

2.2 Mitochondrial Fission

The mitochondrial fission process primarily involves GTPase protein, i.e. dynamin-related protein 1, also termed as DLP1 or DNM1 (Palmer et al. 2011a, b). To initiate the process of fission, the multiplication of mtDNA firstly indicates the location for the recruitment of ER in the matrix. Simultaneously, Drp1 oligomers reside at equilibrium in between the cytoplasm and mitochondria, and also before this maturation and oligomerization steps, Ca^{2+}-mediated constriction takes place at the joining site of ER–mitochondria in IMM. Later on, at the ER site where the membrane pre-constriction has started, oligomerized Drp1 gets collected along with other proteins like ER-bounded inverted formin-2 (INF2), which triggers actin nucleation, and Spire1C, which polymerizes at the contacted site of mitochondria–ER. The pre-constriction at mitochondria is mediated by the mechanical force provided by the actin cable contraction through myosin IIa. Also, mitochondrial fission factor (Mff), FIS1, and mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51) drive Drp1 towards the constricted site and induce oligomerization to create a circular structure. Pre-existing site of constriction at the mitochondrial site is enhanced by GTP hydrolysis, and ultimately, the Drp1 protein-mediated constriction site terminates through DNM2 recruitment and results in the production of two distinct mitochondria (Smirnova et al. 2001; Fröhlich et al. 2013).

3 Connection of Mitochondrial Dynamics in AD

AD is a neuronal degenerative condition characterized mostly by the depletion of cholinergic neurons in regions of the brain, which are necessary in regulating and maintaining cognitive, speaking, and memory function, resulting in early memory impairment that can develop into broad cognitive impairment. Phosphorylated tangles of tau protein and Aβ plaques are two prominent biomarkers of AD (Bird 2018).

Overexpression of APP impairs mitochondrial dynamics, i.e. fission/fusion, resulting in excessive fragmentation of mitochondria and aberrant dispersion across

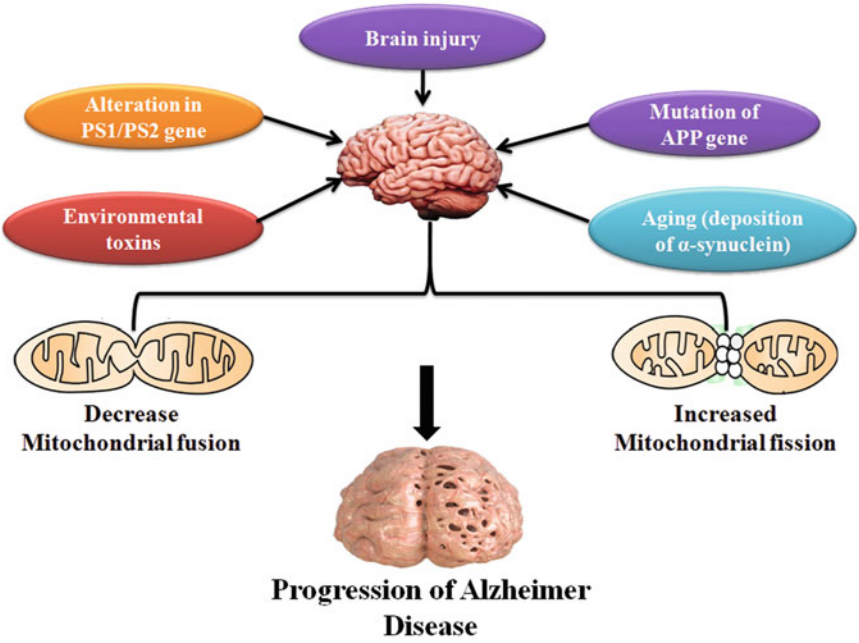


Fig. 1 Risk factor of Alzheimer's disease

the perinuclear zone (Wang et al. 2008). Also, as per a study, caspase-split tau alleviates TRAK2-mitochondrial coupling and reduces the number of ATP generated for mitochondrial motility, creating a negative outcome on mitochondrial transport (Quintanilla et al. 2020). In mouse models of AD, altered mitochondrial dynamics, transportation, and its distribution, everything gets modified in Alzheimer's condition (Fig. 1) (Pigino et al. 2009; Du et al. 2010; Wang et al. 2010) and also reported that APP-mutated mice, which have a large amount of accumulated Aβ, have been linked to reduced ATP production by affecting complex III and complex IV of ETC (electron transport chain) and decreased oxygen consumption (Caspersen et al. 2005).

Many studies, by using immunofluorescence techniques, have discovered a favourable relationship between Drp1 (a mitochondrial fission protein) and tau protein (phosphorylated), which promotes an association between Drp1 and Aβ and ultimately leads to increased mitochondrial fragmentation. As per other studies, it has been reported that at an initial level of AD progression, Drp1 expression was higher than Marf expression (it is homologous to human MFN2 protein). However, the extent of Mfn2 and Mfn1 within the brain of Alzheimer's subject is dramatically downregulated as the disease progresses (Manczak et al. 2011). As a result, a decrease in the production of fusion elements (genes) is anticipated to occur in AD

later and is linked to the progression of disease. However, it has been identified that there is a modification in the rate of protein expression whose reasons are uncertain. Also, the alteration in GTPase causing mitochondrial fragmentation is related to inducing further progression in AD situation, which is needed to be explored more.

4 Proteins Implicated in Mitochondrial Fusion Process and Their Relation/Role in AD

4.1 Optic Atrophy Protein 1 (Opa1)

Optic atrophy-1 is a kind of GTPase that is essential for the fusion of the inner membrane of mitochondria. Inside the inmost mitochondrial membrane, OPA1 modulates mitochondrial fusion and structure of cristae (Santarelli et al. 2015). OPA1 has eight different mRNA split variants that are regulated for tissue-specific expression (Ishihara et al. 2006). They are divided into two types as follows: long isoforms (L-OPA1) bind to IMM, while short isoforms (S-OPA1) are found within intermembrane space (IMS) near the OMM (Fülöp et al. 2013). OPA1 contributes to cristae remodelling by oligomerizing two subunits of L-OPA1, while one subunit of S-OPA1 later reacts with some other complexes of protein to modify the structure of cristae and causes mitochondrial fusion in collaboration with Mfn1 and Mfn2. The level of OPA1 was reduced in AD, which supports mitochondrial fragmentation (Fig. 2, Table 1).

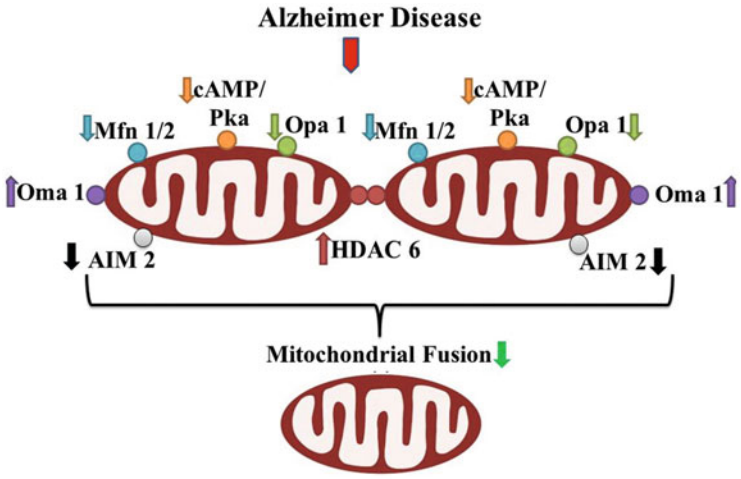


Fig. 2 Mitochondrial fusion in Alzheimer's disease

Table 1 Mitochondrial fusion proteins involved in AD

Sr. No.	Protein	Mechanism	References
1.	OPA1	OPA1 contributes to cristae remodelling by oligomerizing two subunits of L-OPA1, while one subunit of S-OPA1 later reacts with some other complexes of protein to modify the structure of cristae and causes mitochondrial fusion in collaboration with Mfn1 and Mfn2. The level of OPA1 was reduced in Alzheimer's disease	Fülöp et al. (2013)
2.	CAMP/PKa	Overexpress Drp1 protein while no change in the production rate of proteins implicated in mitochondrial fusion. CAMP/PKa downregulated in Alzheimer's disease	Chen et al. (2012)
3.	Oma1	OMA1 is a protease enzyme present in mitochondria. Its inner membrane is metalloendopeptidase that has a proteolytic effect inside the intermembrane region. Alzheimer's brain autopsy has shown lowered OPA1 protein concentration, denoting increased OMA1 level in these tissues	Wang et al. (2007), Noh et al. (2020)
4.	Mfn1/Mfn2	Both mitofusins 1 and 2 are transmembrane proteins, which assemble in homodimer and heterodimer to fuse the OMMs. Enhanced Mfn2 expression inhibits AßO deposition depicting its protective role in AD	Wang et al. (2009)
5.	AIM 2	AIM2 inhibition enhances MFN2 overexpression, which improves mitochondrial fusion, according to molecular docking	Sita et al. (2020)
6.	HDAC 6	AD models have reported that HDAC6 expression elevates dramatically in the hippocampus and other key areas of the brain	Guedes-Dias et al. (2015)

4.2 Overlapping with the M-AAA Protease Homolog (OMA1)

OMA1 is a protease enzyme present in mitochondria. Its inner membrane is metalloendopeptidase that has a proteolytic effect inside the intermembrane region (Käser et al. 2003). OMA1 is inactive under typical conditions, but it responds quickly to mitochondrial stress, such as depletion of membrane potential or an overabundance of reactive oxygen species (Noh et al. 2020). Under such condition, yeast mitochondrial AAA metalloprotease like 1 ATPase (YME1L) and overlapping with the M-AAA protease homolog (OMA1) combinedly digest OPA1 and result in s-OPA1 production (Anand et al. 2014). On the contrary, one study also reported that also L-OPA1 is released in the space present in between mitochondrial intermembrane under the influence of OMA1, which induces fragmentation of mitochondria, cytochrome c release, and apoptosis (Olichon et al. 2003; Duvezin-Caubet et al. 2006; Pérez et al. 2018). However, analysis of two different Alzheimer's brain autopsies has shown lowered OPA1 protein concentration, denoting increased OMA1 level in these tissues (Fig. 2, Table 1) (Wang et al. 2007; Manczak et al. 2011).

4.3 Histone Deacetylase 6 (HDAC6)

Histone deacetylases (HDACs) are an enzyme that deacetylates the residues of lysine present in histones and other non-histone proteins found in the nucleus, cytoplasm, and mitochondria. HDACs have a deacetylase activity that is opposite of enzyme histone acetyltransferases (HATs) as various studies have shown that HDAC/HAT catalytic balance is important in maintaining brain homeostasis (Saha and Pahan 2006). Histone deacetylases (HDACs), especially HDAC6, have been involved in the aetiopathogenesis of AD (D'Mello 2009; Fischer et al. 2010; Li et al. 2011). Different AD patients and AD models have reported that HDAC6 expression elevates dramatically in the hippocampus and other key areas of the brain. Overexpression of HDAC6 reduces α-tubulin acetylation, resulting in decreased intracellular transport, and its inhibition using tubastatin A (TBA) enhances mitochondrial fusion (Fig. 2, Table 1) (Guedes-Dias et al. 2015).

4.4 Absent in Melanoma 2 (AIM2)

It is a protein inducible by interferon or also called as absent in melanoma 2. The structurally similar proteins, Aim2/AIM2, are expressed by AIM2-like receptor (ALR) family genes, which belong to the T1 IFN-inducible PYHIN protein family (ALR genes) (Brunette et al. 2012; Choubey 2012; Connolly and Bowie 2014). The Aim2 activation of inflammasomes and pyroptosis have a role in inflammation related to AD including neurotoxicity ultimately leading to neurotoxicity as Aim2 deletion reduces the Aβ accumulation in the region of the hippocampus and cerebral cortex (Choubey 2019). In NSCLC cells, AIM2 localizes to mitochondria, and AIM2 suppression results in increased mitochondrial fusion and reduced cell growth (Fig. 2, Table 1). AIM2 inhibition enhances MFN2 overexpression, which improves mitochondrial fusion, according to molecular docking (Qi et al. 2020).

4.5 cAMP/PKA

cAMP, i.e. cyclic adenosine monophosphate, is connected to the modulation of mitochondrial dynamics and OXPHOS in mammals. A study reported that elevated level of intracellular cAMP overexpress Drp1 protein, while no change in the production rate of proteins implicated in mitochondrial fusion (like OPA1, Mfn1, Mfn2) was observed in ONH astrocytes (Ju et al. 2019). While another study reported that a higher level of BACE1 (β-site APP-cleaving enzyme 1, which degrades APP) lowers phosphorylation of the cAMP response element binding protein (CREB), response of protein kinase A (PKA), and cAMP levels, knockdown of BACE1 gives reverse outcome, therefore denoting that AD is regulated by BACE1 via cAMP/PKA/CREB pathway (Fig. 2) (Chen et al. 2012).

4.6 Mitofusins 1 and 2 (Mfns 1 and 2)

Both mitofusins 1 and 2 are transmembrane proteins, which assemble in homodimer and heterodimer to fuse the OMMs (outer mitochondrial membrane) together with their GTPase activity (Sita et al. 2020). Many studies have reported that the hippocampus region of AD brain has less number of proteins involved in mitochondrial fusion (Wang et al. 2009) as the Mfn2 gene was detected upon the short arm of chromosome 1 (1p36), which is implicated in AD (Hiltunen et al. 2001) in numerous studies. Therefore, enhanced Mfn2 expression inhibits AβO deposition depicting its protective role in AD (Fig. 2, Table 1) (Sita et al. 2020).

5 Proteins Implicated in Mitochondrial Fission Process and Their Relation/Role in AD

5.1 Dynamin-Related Protein 1 (Drp1)

The gene named DNM1L encodes for dynamin-related protein 1, belongs to the family of dynamin proteins, and has major participation in mitochondrial fission (Kandimalla et al. 2021). A study reported that in AD, Drp1 raised mitochondrial fragmentation and diminished fusion and synaptic activity (Fig. 3, Table 2). In AD neurons, these aberrant connections lead to the growth of defective mitochondria and concluded that decreased Drp1 is advantageous in symptomatic-transgenic tau (P301L) mice (Gandre-Babbe and van der Bliek 2008).

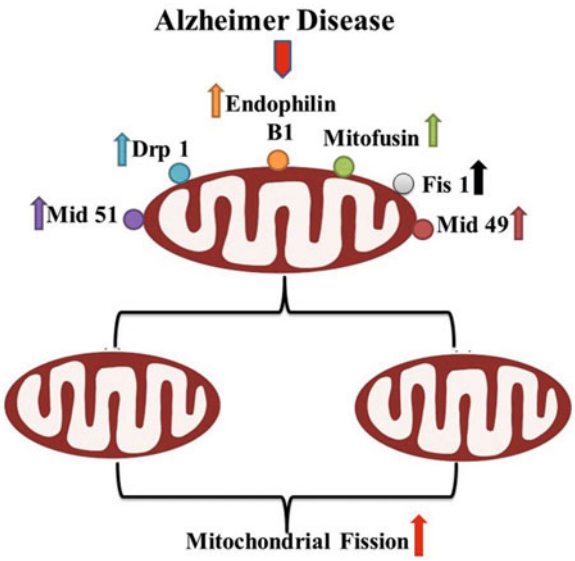


Fig. 3 Mitochondrial fission in Alzheimer's disease

Table 2 Mitochondrial fission proteins involved in AD

Sr. No.	Protein	Mechanism	References
1.	Drp1	In Alzheimer's disease, Drp1 raised mitochondrial fragmentation and diminished fusion and synaptic activity	Kandimalla et al. (2021)
2.	Fis1	Fis1 overexpression in cultured neurons would phenocopy AD-like mitochondrial distribution abnormalities	Palmer et al. (2013)
3.	Mitofusin (Mff)	Mitochondrial fission factor (MFF) is an outer membrane protein of mitochondria that regulates mitochondria fragmentation	Otera et al. (2010)
4.	Endophilin-B1	Endophilin-B1 deficiency increased the build-up of amyloid, implying that endophilin-B1 deficiency has a dual function in AD	Wang et al. (2015)
5.	MiD49 and MiD51	MiD49 and MiD51 are two adaptor proteins, which target Drp1 without the support of Mff or Fis1. High endogenous MiD forms targeted Drp1 multimers that facilitate OMM scission; however, amplification of heterologous MiD promotes fusion	Atkins et al. (2016)

5.2 *Mitochondrial Fission Factor (MFF)*

Mitochondrial fission factor (MFF) is an outer membrane protein of mitochondria that regulates mitochondria fragmentation (Otera et al. 2010). During fission, MFF and the proteins Fis1, MiD49, and MiD51 bring the cytosolic protein Drp1 to the mitochondrial surface (Fig. 3, Table 2) (Palmer et al. 2011a, b; Zhang et al. 2020). One of the studies has revealed that there is an elevation in the level of MFF protein present in matured oligodendroglial cells subjected to oligomeric $A\beta_{1\text{-}42}$ (toxic Aβ peptide); however, no variations in the mitochondrial dynamics process were observed (Zhang et al. 2020).

5.3 *Mitochondrial Fission Protein 1 (Fis1)*

Fis1 is a kind of outer membrane protein of mitochondria whose C-terminal domain is exposed towards cytoplasm (James et al. 2003; Lees et al. 2012) This protein is required for mitochondrial-dynamin-mediated fission; however its precise role is to assemble different fission machinery and also to select an unknown scission site at mitochondria (Wang et al. 2009). In one study, AD neurons exhibited increased level of Fis1 and lesser neurite development, with fewer mitochondria than normal controls. Fis1 overexpression in cultured neurons would phenocopy AD-like mitochondrial distribution abnormalities, but Fis1 eradication failed to improve optimum neurite development, despite the better connection of mitochondria (Fig. 3, Table 2) (Palmer et al. 2013).

5.4 *Mitochondrial Dynamics Proteins of 49 and 51 kDa (MiD49 and MiD51)*

MiD49 and MiD51 are two adaptor proteins, which target Drp1 without the support of Mff or Fis1 (Atkins et al. 2016). High endogenous MiD forms targeted Drp1 multimers that facilitate OMM scission; however, amplification of heterologous MiD promotes fusion by deactivating Drp1. MiD51, on the other hand, has a one-of-a-kind ability to bind ADP in its nucleotidyltransferase domain. MiD51 suppresses Drp1 in the absence of ADP, but ADP increases MiD51-mediated fission, implying a connection between metabolism and fission (Fig. 3, Table 2) (Cuddeback et al. 2001).

5.5 *Endophilin-B1*

Endophilin-B1, also recognized as Bif-1 or SH3GLB1, is a polyfunctional protein that imparts its role in autophagy and apoptosis, along with maintenance of mitochondrial morphology and its function (Fig. 3, Table 2) (Pierrat et al. 2001). Endophilin-B1b and endophilin-B1c (collectively known as endophilin-B1b/c) are neuroprotective isoforms that are selectively decreased by amyloid in mice with mutant APP and PSEN1 (abbreviated APP/PSEN1). Endophilin-B1 deficiency increased the build-up of amyloid, implying that endophilin-B1 deficiency has a dual function in the aetiology of AD, altering both amyloid degradation and neuronal viability (Wang et al. 2015).

6 Conclusion

According to the findings of this study, mitochondrial dynamics have a key role in mitochondrial fission and fusion protein expression, both of which govern AD. It regulates the onset and development of AD via modulating mitochondrial signalling pathways. We investigated the many signalling systems that are targets in the progression of AD. As a result, mitochondrial fusion proteins play an important role in AD treatment. Along with this, mitochondrial fission proteins accelerate the development of AD. These findings might help researchers develop new-generation medications that utilize mitochondrial dynamics signalling systems, which could be highly effective in the treatment of AD.

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Epigenetic Therapy for Alzheimer's Disease

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Abstract Alzheimer's disease (AD) is a progressive neurodegenerative disorder with no effective treatments. Increasing evidence suggests that the epigenetic machinery in AD is perturbed and plays a significant role in the development and progression of the disease. Current research focuses on the mechanisms by which DNA methylation, histone modifications, and noncoding RNAs influence AD pathology. Implementing new drug targets is imperative because the existing therapeutic strategies for AD are neither cost-effective nor beneficial in decelerating disease progression. Here, we summarize the present understanding of the role of epigenetics in AD and provide insight into the prospective therapeutic interventions based on reversing these epigenetic modifications. There is also a discussion of the difficulties in translating these therapeutic options from the bench to the bedside.

Keywords Alzheimer's disease · Epigenetics · Epigenetic therapy · DNA methylation · Histone modifications · miRNA therapy

1 Introduction to Alzheimer's Disease

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder that is clinically characterized by a progressive deterioration of memory and cognitive functions. It is the leading cause of dementia, with over 55 million people affected worldwide (WHO 2021). Further, the incidence of AD is projected to double every 20 years, at least until 2040. Diffuse and neuritic extracellular amyloid plaque development brought on by excessive beta-amyloid (A) buildup and neurofibrillary tangles (NFT) composed of hyperphosphorylated microtubule-associated tau (p-tau) protein are the main neuropathological hallmarks of AD (Mayeux and Stern 2012). Besides these typical manifestations, synaptic and neuronal losses are seen during AD. Although the etiological mechanisms that drive the pathological alterations are

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not entirely understood, AD is considered a polygenic and complex disease with environmental and epi (genetic) factors known to play a role (Reitz et al. 2011).

Over 95% of AD cases are sporadic, and age is one of the most significant nonmodifiable risk factors associated with it (Li et al. 2019). The less incident familial form of the disease (fAD) is caused by single inherited genetic mutations, including single-nucleotide polymorphisms (SNPs) and mitochondrial DNA mutations, and typically has an early disease onset between 30 and 60 years of age. The three most studied genes in which mutant variants bring about fAD are Aβ-precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*). All the known disease-causing variants influence Aβ production, although the precise mechanism by which these genetic variations result in fAD is unknown. Mutation in *APP* is directly responsible for the aggregation of Aβ in senile plaque. The *PSEN1* and *PSEN2* genes, which encode for presenilin 1 and presenilin 2, respectively, play a crucial role in regulating the activity of the β-secretase that catalyzes the proteolytic cleavage of the APP (Bieschke 2013). Apart from these deterministic genes, the apolipoprotein E ε4 (*APOE*ε4) variant is also known to significantly raise an individual's risk of developing AD (Cacabelos et al. 2005, 2012; Strittmatter et al. 1993). It has been suggested that APOE-ε4 may impact AD pathology by interacting with APP metabolism and Aβ accumulation and enhance tau hyperphosphorylation. Furthermore, according to genome-wide analyses, the human genome contains roughly 600 genes that may be involved in the genesis of AD (Cacabelos and Teijido 2018). Other antecedent risk factors associated with the disease include cardiovascular disease, type II diabetes, hypertension, obesity, traumatic brain injury, and smoking (Mayeux and Stern 2012).

There are only a few FDA-approved medications to treat AD symptoms like memory loss, learning difficulties, confusion, agitation, and other symptoms, despite the incidence of AD rising due to the growing aging population. The dysfunction of the cholinergic system in the symptomatology of AD has long been established (Davies and Maloney 1976; Kása et al. 1997), and thus, the first strategy for AD treatment involved the use of cholinesterase inhibitors such as donepezil and rivastigmine. Additionally, N-methyl-D-aspartate (NMDA) receptor antagonist, memantine, has also been considered for its ability to reduce neuronal excitotoxicity. However, these drugs offer only partial and transient symptomatic relief and have severe side effects (Du et al. 2018). Aducanumab is an immunotherapy-based intervention that works by clearing abnormal Aβ deposition. However, it has adverse effects, including local brain swelling and microhemorrhages (Perlmutter 2021).

Therefore, there is an urgent need for a more potent AD medication that eliminates the symptoms and slows the disease's progression. Furthermore, only 1% of AD cases exhibit genetic polymorphism, which indicates the involvement of other factors that could be beneficial in the effective diagnosis and therapy of the disease. In order to address the shortcomings of the current gene and medication options, epigenetics in AD machinery has attracted much attention in recent years.

2 Epigenetics and Its Role in AD

The term "epigenetics" refers to changes in gene expression that are not caused by genetic changes; instead, it refers to meiotically and mitotically inherited changes in gene expression that merely affect the phenotype without altering the genotype (Xu et al. 2012). These biochemical and physiological changes include DNA methylation, posttranslational histone modifications, or changes in noncoding RNA expression. Environmental exposures have a critical role in epigenetic alterations and therefore provide a mechanistic link between the genome and environment in determining phenotype. By affecting chromosome compaction and DNA accessibility, epigenetic changes can dynamically control gene expression.

Aging has a major role in causing aberrations in epigenetic machinery, thus increasing the risk for neurodegenerative disorders like AD. These age-related accumulations of aberrations lead to defects in neuronal cell development and transport and impair the glial cells' metabolism, which culminates in cell death (Delgado-Morales et al. 2017). Further, it has been speculated that epigenetic alterations have a key role in processes like learning and memory (Mikaelsson and Miller 2011; Van den Hove et al. 2014). DNA methylation and histone modifications, in particular, are vital for memory formation. Understanding the epigenetics associated with disorders like AD can thus help us to determine the key players involved apart from genetic factors.

To date, more than 20 epigenetic mechanisms that promote Aβ formation, reactive oxygen species (ROS) generation, immunological disorders, and neuronal cell death in AD have been reported (Bufill et al. 2020). In addition, accelerated epigenetic age is a hallmark of the AD brain, particularly within the prefrontal cortex (Levine et al. 2015). Epigenetic age, a biomarker of aging, is measured from DNA methylation levels along specific cytosine-guanine dinucleotide (CpG) sites that correlate with chronological age. Since epigenetic age is directly linked to cognitive abilities, people with an accelerated epigenetic age would experience AD symptoms much earlier (Marioni et al. 2015). The major epigenetic modifications associated with AD, which could be potential targets for therapy, are discussed below (Fig. 1).

2.1 DNA Methylation

DNA methylation refers to the addition of methyl group to the fifth position of cytosine in the CpG dinucleotide context within a CpG island by DNA methyltransferases (DNMTs). Aberrant DNA methylation activates and represses genes based on their occurrence within the gene. For example, promoter hypermethylation and hypomethylation correlate with gene expression suppression and activation. Besides, gene body methylation correlates with active gene expression. The extent of methylation at the promoter region decreases the gene expression either by blocking the transcription factor binding site or promoting the binding of

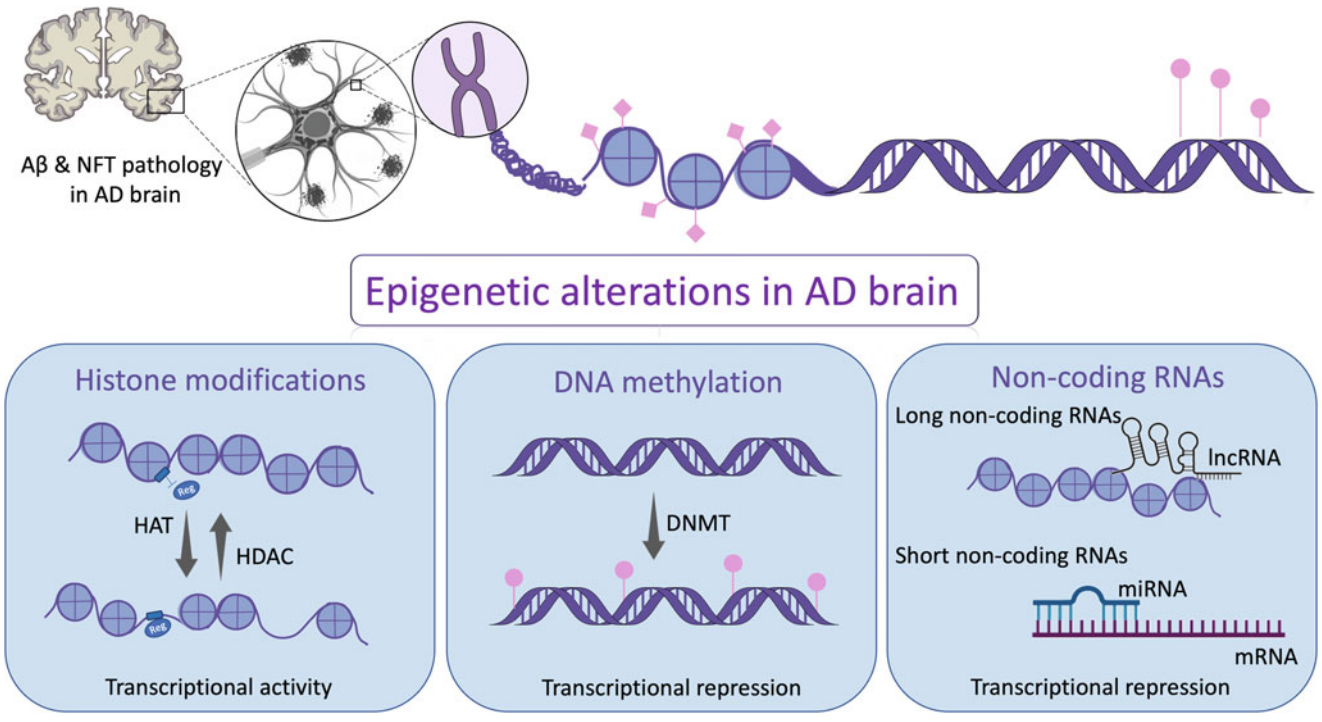

Fig. 1 Epigenetic alterations in AD brain: The three important epigenetic modifications in AD are histone modifications, DNA methylation, and aberrant noncoding RNA expression. Increased levels of histone deacetylases, DNA methyltransferases, and abnormal expression of long noncoding RNAs and microRNAs are observed in AD. These epigenetic modifications impact the transcription level, thereby altering the gene expression. (*DNMT:* DNA methyltransferases, *HAT:* histone acetyltransferases, *HDAC:* histone deacetylases, *lncRNA:* long noncoding RNA, *miRNA:* microRNA, *NFT:* neurofibrillary tangles)

repressors (Moore et al. 2013). The four DNMTs identified in humans are DNMT1 (Bestor et al. 1988), DNMT2 (Yoder and Bestor 1998), and DNMT3a/3b (Okano et al. 1999).

Aberrant DNA methylation frequently occurs in AD. Aberration in the methylation of specific proteins like APP causes excess secretion of β-secretase that correlates directly with deficiency of vitamin B6, B12, and folate (Zhang et al. 2018). Further, the microtubule-associated protein tau (MAPT) methylation suppresses the gene and can impact the tau protein level (Bufill et al. 2020). Both the events above are considered one of the early biomarkers for pathogenic genes involved in AD progression. The hypermethylated promoter region of specific genes, for example, brain-derived neurotrophic factor (*BDNF*), spectrin beta 4 (*SPTBN4*), *COASY* that encodes of coenzyme A synthase, thromboxane A2 receptor (*TBXA2R*), SPRINT, and sorbin and SH3 domain containing 3 (*SORBS3*) gene, indicates a transition from mild cognitive impairment (MCI) to AD (Di Francesco et al. 2015; Kobayashi et al. 2016; Xie et al. 2017; Yokoyama et al. 2017). Neprilysin (*NEP*), sortilin-related receptor 1 (*SORL1*), and ATP-binding cassette transporter A7 (*ABCA7*) are implicated in the Aβ process, and their methylation state in AD could thus be directly linked to increased Aβ levels (Yu et al. 2015). Additional gene loci associated with Aβ load and tau tangles in AD include HLA class II histocompatibility antigen DRB5 beta chain (*HLADRB5*), bridging integrator 1 (*BIN1*), and solute carrier family 24 members 4 (*SLC24A4*) (Yu et al. 2015).

Additionally, a global hypomethylation resulting from reduced DNMT levels and perturbed vitamin B complex metabolism is often considered an epigenetic hallmark of AD. In some *in vitro* and mouse models with vitamin B complex and folate deficiency, global hypomethylation of certain genes like β-secretase 1 (*BACE1*), breast cancer gene 1 (*BRCA1*), *APOE, PSEN1*, and triggering receptors expressed on myeloid cells 2 (*TREM2*) was seen to be associated with β-amyloid deposition (Fuso et al. 2008, 2011; Marques et al. 2012; Ozaki et al. 2017; Wang et al. 2008). Upregulation of specific neuroinflammatory and cell death-associated genes like CD33 molecule (*CD33*), complement receptor 1 (*CR1*), and tumor necrosis factor-α (*TNF-α*) is also reported (Cacabelos and Torrellas 2014; Chouliaras et al. 2012; Mastroeni et al. 2011; Teijido and Cacabelos 2018).

2.2 Histone Modifications/Chromatin Remodeling

Chromatin remodeling is an important mechanism that enables chromatin rearrangement to make it transcriptionally accessible, i.e., to facilitate transcription factors to come in contact with DNA and control gene expression. It plays a significant role in maintaining genome integrity.

Histones, the primary protein component of chromatin, are also subject to epigenetic alterations that contribute to altered gene expression. The addition of an acetyl group by histone acetyltransferases (HATs), a process called histone

acetylation, is linked to the activation of transcription (Gujral et al. 2020). Conversely, chromatin becomes more compressed, and transcription is suppressed due to histone deacetylation by HDACs (Wawruszak et al. 2019). Studies have shown an increase in the levels of HDACs during mild cognitive impairment (MCI) to AD progression (Mahady et al. 2018). Further, the HAT/HDAC equilibrium that regulates transcription is perturbed during aging and AD progression (Saha and Pahan 2006). Overexpression of HDAC2 in the hippocampus and prefrontal complex has shown a decrease in dendritic spine density and synaptic plasticity and is further associated with the downregulation of genes involved in learning and memory (Gräff et al. 2012; Guan et al. 2009; Levenson et al. 2004). A direct correlation between increased phosphorylation of tau protein with elevated HDAC6 level is reported in a mouse model of AD. Thus, it can be an important marker for improving cognitive impairment (Ding et al. 2008). There is a significant reduction in the acetylation levels of histone H4, causing downregulation of Egr1, c-Fos, and BDNF in the frontal cortex and hippocampus of transgenic mice recapitulating AD pathology causing memory impairment (Gao et al. 2022; Hendrickx et al. 2014). In addition, the class III HDACs, called sirtuins (SIRT 1-7), are found to affect dementia positively; they reduce tau aggregation by preventing the acetylation of their lysine 28 residue (Cohen et al. 2011). It is significant to note that the expression of these HDACs is found to be reduced in mouse models of aging and certain neurological disorders (Julien et al. 2009; Kanfi et al. 2012; Quintas et al. 2012; Sasaki et al. 2006; Sommer et al. 2006).

Phosphorylation and ubiquitination are other epigenetic modifications implicated in chromatin remodeling and governing gene expression. Chromosome condensation occurs during cell division, transcriptional control, and DNA damage repair, and histone phosphorylation is a crucial intermediate step in these processes. The phosphorylation of histone H3 at serines 10 and 28 and histone H2A at T120 controls chromatin compaction and chromatin structure and function (Smith 1998). Histone H2AX phosphorylation in astrocytes is linked with AD. For example, DNA damage results in rapid phosphorylation of H2AX at the S139 region in the astrocytes of AD (Myung et al. 2008). Further, evidence of phosphorylation of H4 in neuroblastoma and H3 in the CA1 region of the hippocampus region suggests its importance in cognitive dysfunction (Myung et al. 2008). In addition, ubiquitination is also associated with Aβ production and disrupts APP's function through factor E4B (Gireud-Goss et al. 2020). While H2A ubiquitination is found to be reduced in AD, the *Bmi1* gene, which forms a protein complex and helps in H2A ubiquitination, is found silenced in AD (Flamier et al. 2018).

2.3 Noncoding RNAs

Noncoding RNAs (ncRNAs), such as circular (circRNAs), long ncRNAs (lncRNAs), and micro RNAs (miRNAs), contribute to the pathophysiology of AD by affecting transcription levels of genes belonging to specific metabolic pathways.

Several lncRNAs, which are non-protein coding transcripts that exceed 200 nucleotides, are found to be abnormally expressed in AD. For instance, BACE1-antisense (BACE1-AS), 51A, 17A, SOX2OT, GDNFOS, and NDM29 are associated with AD pathogenesis (Wu et al. 2013). BACE1-AS is seen to be overexpressed in AD patients and transgenic mice models, enhancing the mRNA levels of *BACE1* and thus Aβ production (Faghihi et al. 2008; Hébert and De Strooper 2009; Wu et al. 2013). AD patient's superior and precentral frontal gyri displayed an age-dependent rise in lncRNA expression (Zhou et al. 2019).

Recently, much attention has been drawn to miRNAs in the pathophysiology of AD. miRNAs constitute a class of 19–24 nucleotide long ncRNAs that act as negative regulators or gene silencing factors by binding to complementary regions on specific mRNA targets and are shown to affect the expression of *APP*, *BACE1*, and *PSEN,* which influence Aβ metabolism and tau phosphorylation (Wang et al. 2013). Further, they play a significant role in neurogenesis, insulin resistance, and innate immunity, thus being used as presymptomatic markers and for early diagnosis of the disease (Wu et al. 2013). Molecular studies involving in vitro AD models have found consistently dysregulated miRNAs like miR-298 and miR-328 that were linked to greater levels of the protein BACE1 (amyloid precursor protein converting enzyme) leading to overproduction of Aβ (Boissonneault et al. 2009).

Recent evidence from functional analyses to identify differentially expressed miRNAs and their target genes suggest that pro-inflammatory miRNA-7, miRNA-9-1, miRNA-23a/miRNA-27a, miRNA-34a, miRNA-125b-1, miRNA-146a, and miRNA-155 are upregulated in the superior temporal lobe of AD patients (Pogue 2018). miRNA-485, miRNA-4723, and miRNA-149 are differentially expressed in AD, and administering these miRNAs in an in vivo mouse model had an impact on regulating synaptic genes and significantly reduced Aβ binding to synapses (Zolochevska and Taglialatela 2020). miRNA-mRNA pairs implicated in protein folding were abundant in the parietal lobe, while the frontal lobe showed increased miRNA-mRNA pairs involved in synaptic transmission, protein degradation, and apoptosis (Li et al. 2021). Additionally, several miRNA subtypes have been linked to AD vulnerability and may serve as blood-based biomarkers of AD (Sabry et al. 2020; Wu et al. 2020; Yılmaz et al. 2016).

Epigenetic modifications such as the ones described above play a crucial role in the neurobiological processes that lead to AD pathology. Evidently, targeting these epigenetic alterations is a novel avenue for treating AD. It may further allow for a better understanding of the fundamental mechanisms of the disease and enable the detection of new drug-resistance-related changes.

3 Epigenetic Therapy

As discussed earlier, AD is a complex and multifaceted disorder that involves multiple factors and symptoms, including cognitive, behavioral, and neuropsychiatric comorbidities (Dubois et al. 2016). Thus, identifying effective targets for therapy

has always been a great challenge. Implementing new drug targets is imperative because the existing therapeutic strategies for AD are neither cost-effective nor beneficial in decelerating disease progression. Increasing evidence suggests that abnormal epigenetic changes play a key role in the etiology of AD. Incidentally, identifying epigenetic modifications linked to AD could be beneficial in developing innovative therapeutic approaches.

The use of activators and inhibitors of DNA methylation, histone modifications, and miRNA-based therapeutics are a few potential therapeutic strategies to target AD (Fig. 2).

3.1 DNA Demethylation Modulators

There are numerous approaches to targeting DNA methylation for therapeutic purposes in AD. The global hypomethylation observed in AD could potentially be targeted for AD management. Hypomethylation of AD-related genes like APP, PS1, and PS2 has been implicated in learning and memory deficits (Liu et al. 2014). Further, overexpression of DNMT3a2 within the hippocampus has been reported to increase global methylation levels and enhance memory in aged mice (Oliveira et al. 2012).

In this regard, patients with AD may benefit from dietary supplementation of folic acid, vitamin B12, betaine, and other methionine sources that act as cofactors in DNA methylation. Adequate dietary supplementation has been shown to increase the levels of methyl donor S-adenosylmethionine (SAM) for DNA methylation processes (Chan and Shea 2008; Kok et al. 2015; Li et al. 2015; Wang et al. 2013). Further, increasing the level of SAM has decreased APP, PSEN1, and BACE1 expression levels, thereby reducing Aβ levels (Fuso et al. 2005). However, in AD patients, no cognitive improvement was found while testing betaine, a methyl donor traditionally used to treat homocystinuria. While the small sample size and absence of a placebo-treated control group in this study could have masked the actual efficacy of betaine, more recent animal model studies show promising results (Chai et al. 2013; Ibi et al. 2021; Leiteritz et al. 2018; Miwa et al. 2011).

Apart from this, the scope of DNA demethylating agents, or DNMT inhibitors, in alleviating AD symptoms is also being explored by researchers. The use of DNMT inhibitors, in addition to traditional therapy, may prove to be effective, considering that several genes like *NEP, LINE 1, and SORB3* are hypermethylated in AD. The FDA has already approved DNMT inhibitors such as 5-azacytidine (azacytidine) and 5-aza-2'-deoxycytidine (decitabine) for the treatment of diseases like leukemia (Christman 2002), Friedreich's ataxia, and fragile X syndrome (Sherzai et al. 2020). The use of natural products as DNMT inhibitors, such as curcumin derivatives, psammaplins, and catechins, is also being considered for AD therapy (Cuadrado-Tejedor et al. 2013; Nebbioso et al. 2012). Among them, a polyphenol in green tea, epigallocatechin-3-gallate (EGCG), presents a promising treatment agent for neurodegenerative disorders (Bieschke 2013; Dragicevic et al. 2011).

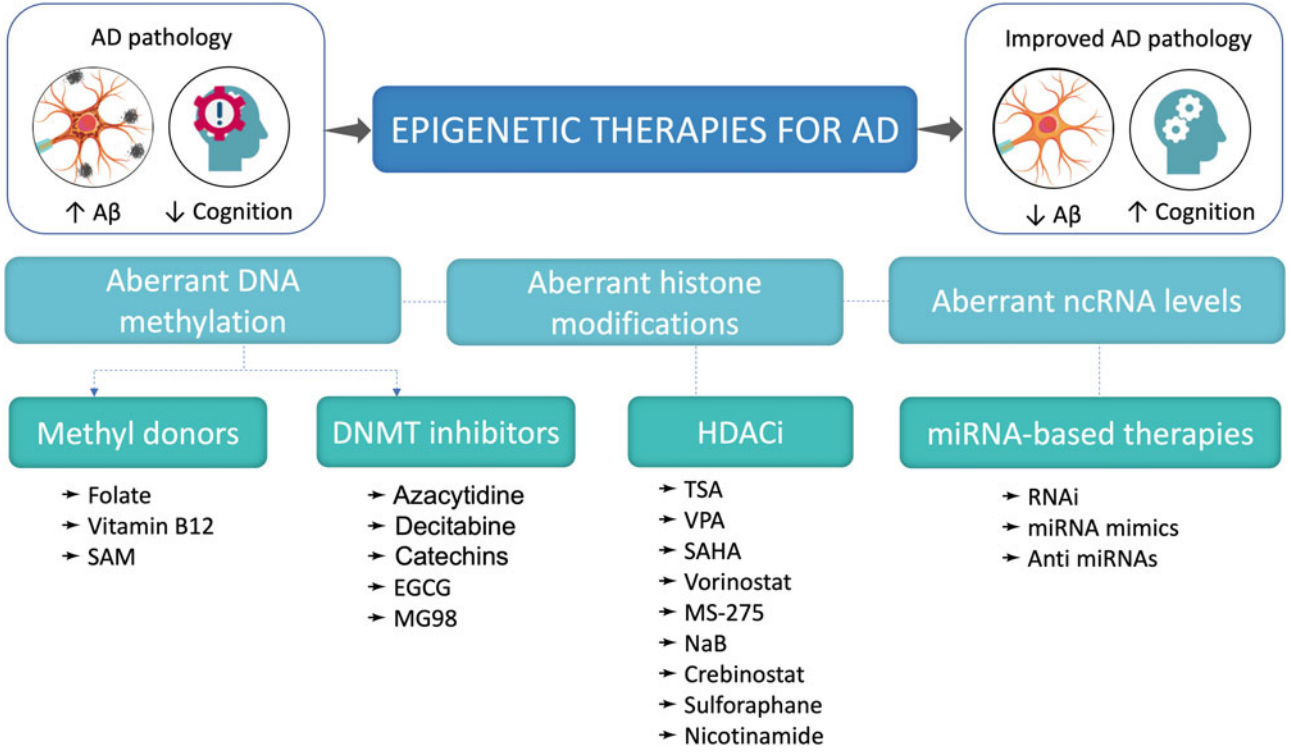

Fig. 2 Potential epigenetic therapies for AD: Therapies targeting the aberrant epigenetic machinery include methyl donors and DNMT inhibitors or demethylating agents for aberrant DNA methylation, histone deacetylase inhibitors for histone modifications, and miRNA-based therapies for abnormal noncoding RNA levels. Studies suggest that implementing these therapies aids in alleviating AD pathology and improves cognition. (*EGCG:* epigallocatechin gallate, *HDACi:* histone deacetylase inhibitors, *NaB:* sodium butyrate, *RNAi:* RNA interference, *SAHA:* suberoylanilide hydroxamic acid, *SAM:* S-Adenosyl methionine, *TSA:* trichostatin A, *VPA:* valproic acid)

Future treatment strategies to reduce DNA methylation may favor the use of DNA demethylating agents targeting specific sequences like oligonucleotide antisense inhibitor MG98 (Plummer et al. 2009; Stewart et al. 2003; Winquist et al. 2006). Another precise and effective way to control site-specific DNA methylation is the use of interspaced short palindromic repeats (CRISPR)—deactivated Cas9 (dCas9) editing systems (Urbano et al. 2019).

3.2 Histone Deacetylase Inhibitors

Among the most promising drugs made for the treatment of AD are histone deacetylase inhibitors (HDACi) that are found to have a therapeutic role in repressing the functional targets of HDAC involved in critical regulatory functions like memory formation, neurotrophic, and neuroprotective properties that regulate imbalances in protein acetylation level and transcription (Yang et al. 2017). HDAC2 and HDAC3 have been shown to repress memory formation, whereas HDAC5 supports memory formation. Thus, drugs such as CI-994 (HDAC2 antagonist) and drugs that increase HDAC5 may be helpful for AD. Certain HDACi drugs like trichostatin A (TSA), 4-phenyl butyric acid, valproic acid (VPA), suberoylanilide hydroxamic acid (SAHA), vorinostat, MS-275, sodium butyrate (NaB), and crebinostat showed enhancement in cognitive function after administration (Göttlicher et al. 2001). In addition, certain HDACi like sulforaphane accompanied due to an increase in the expression of the promoter region of BDNF led by acetylation of H3 and H4 exhibits promising results (Kim et al. 2017). Features like longer half-life, the ability to penetrate the blood–brain barrier, and moderating expression of genes involved in Aβ production are some specialized features developed in recent HDACiW2 therapy (Fischer et al. 2007). These potential benefits of HDAC, including tau protein phosphorylation, promoting dendritic spine density, and learning memory, make HDACi an excellent option for treating AD. Valproic acid sodium salt (HDACI inhibitor) is approved for the treatment of epilepsy and bipolar disorders already (Yao et al. 2014). Another drug, nicotinamide (Vit-B2, a class III HDAC inhibitor), also showed promising effects in delaying cognitive impairment (Green et al. 2008). Certain HDACi drugs under study, which are under phase II clinical trials, are RDR-929, etc. Finally, in recent studies, certain nonspecific epigenetic therapies like blood plasma therapy have been found to impact cognitive function significantly. Apart from that, cognitive stimulation, physical exercises, administration of circulating blood factors like glycosylphosphatidylinositol (GPI)-specific phospholipase D1 (Gpld1), and liver-derived GPI-degrading enzyme have been found to have a promising role in establishing novel therapies (Horowitz et al. 2020).

3.3 miRNA-Based Therapy for AD

miRNA therapy has drawn its interest mainly because of its nature to act as a molecular target for disease therapy or as a possible disease biomarker for gene expression studies (Friedman et al. 2009). Particularly, miRNAs are broadly distributed throughout the neurological system, where they play an essential regulatory role in processes like synaptic plasticity, neuronal differentiation, neurite outgrowth, and dendritic spine shape (Cao et al. 2016).

Currently, the Aβ peptides and tubulin-associated unit (tau) proteins are the only biomarkers for AD identified so far. Moreover, miRNAs are stable enough to directly target the genes at the molecular level causing AD, such as presenilins, BACE-1, APP, TOMM40, and BDNF (Liu et al. 2014). In addition, miRNA amplification through PCR is highly sensitive, and analysis is a comparatively easy process. Also, it is more cost-effective than recognized biomarkers like structural MRI and positron emission tomography (PET) for molecular neuroimaging (Kumar et al. 2017).

AD biomarkers may be more effectively accomplished by using serum as biological fluid instead of CSF and performing cluster analysis of a miRNA family instead of looking at a single miRNA (Denk et al. 2018). Considering the available evidence, a mimic or an antagonist of miRNAs could be investigated in AD treatment. The function of miRNA can be inhibited through the action of single-stranded antisense oligonucleotide or by administering certain compounds that can prevent AD by modulating the miRNA expression either by promoting its synthesis or by using a synthetic double-stranded oligonucleotide miRNA that can mimic the functions of endogenous miRNA (miRNA mimic). Automated gene analysis will likely promote the development of miRNA treatment medicines for AD in the next few years (Shadfar et al. 2015).

4 Current Challenges for Epigenetic Therapy in AD

While epigenetic therapy may seem like a prospective intervention for AD, certain limitations must first be addressed. For instance, it must first be reviewed whether all epigenetic modifications can be restored with such therapy. Since epigenetic changes are sometimes quite complex, the potential side effects of these drugs should not be overlooked. Determining precise targets for an epigenetic-based treatment is further complicated by the extensive and diverse list of epigenetic abnormalities linked to various illnesses. In order to provide more targeted treatment, this method necessitates a systematic characterization of the most important pathogenic epigenetic markers. This goal could be accomplished by identifying the epigenetic markers observed in the early stages of the disease and comprehending the epigenetic defects

that are seen with normal aging to distinguish the differences between normal and pathological aging.

Other considerations while targeting epigenetic modifications for AD therapy are pharmacogenomics related, including toxicity, brain penetration, and genomic regulation (Cacabelos et al. 2016). One major problem is identifying molecules or drugs that can easily cross the blood–brain barrier (BBB). Although DNMT and HDAC inhibitors can surpass this limitation, other factors, including their efficiency and delivery, require further validation (Hockly et al. 2003; Kwa et al. 2011). The genotoxicity and stability issues of DNMT inhibitors and side effects of HDAC inhibitors, including reactive oxygen species-mediated DNA damage, have been well documented (Cuadrado-Tejedor et al. 2013; Petruccelli et al. 2011). Thus, more studies are warranted to improve these drugs' efficacy and safety.

5 Conclusion and Future Prospects

In conclusion, an increasing body of evidence indicates that epigenetic changes are crucial to the pathogenesis and development of AD. Although extensive research has been done on identifying and developing a therapy for alleviating AD-related impairments, the complex pathophysiology and lack of thorough understanding of all the molecular underpinnings pose a challenge to effective drug discovery against AD. In this regard, treatments targeted at reversing the aberrant epigenetic alterations associated with AD could pave the way to an effective therapeutic intervention that not only curbs the symptoms but attenuates AD-related pathology. DNA methylation modulators, HDAC inhibitors, and miRNA-based therapies are some of the prospective targets. However, future approaches should focus on more reliable biological outcomes by thoroughly characterizing the molecule with minimal side effects. Additionally, findings suggest that some epigenetic markers may manifest earlier during the disease progression, suggesting its role as an effective diagnostic tool for early detection.

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Declarations **Ethics Approval and Consent to Participate**

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

Not applicable.

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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Exploring the Diverse Roles of GSK-3β Kinase in Alzheimer's Disease

Gadde Shareena, Dileep Kumar, and Nanasaheb Thorat

Abstract Alzheimer's disease, a progressive neurodegenerative disorder, is a global health concern whose consequence affects an individual's cognitive scores, behavioral patterns, mood swings, and psychology. Globally, the condition is prevalent among elderly population groups. The inclusion of fibrillary lesions (NFTs) and senile plaques (Aβ) constitutes two contentious diagnostic factors contributing to AD-related deterioration. A compendium of continual mistranslated dialogs among several protein kinases, phosphatases, and transcription factors leads to misinterpreted signaling, thus forming the early manifestations of the condition. Neurodegenerative disorders, such as Alzheimer's disease, have been linked to abnormalities in biochemical signals. Glycogen synthase kinase-3 (GSK-3) is a widely acknowledged regulator engaging in the formation and subsequent advancement of AD pathophysiology along with controlling several critical targets involving neuronal deterioration. Consequently, they are regarded as a critical region of interest in the disease-modifying diagnosis of Alzheimer's disease (AD), where anomalous expression of this enzyme has indeed been implicated with overexpression of Aβ peptide, further initiating a cascade sequence leading to hyperphosphorylation of tau proteins. Hence, the focus on elucidating potential GSK-3 inhibitors aids in halting the disease progress and helps us observe the interlinking among several mechanisms of cellular functions involving GSK-3. This review ascertains the in-depth knowledge regarding GSK-3 and summarizes the surrounding elements responsible for alleviating its negative impact in pathological conditions. Along with it, we highlight the emergence of several clinical

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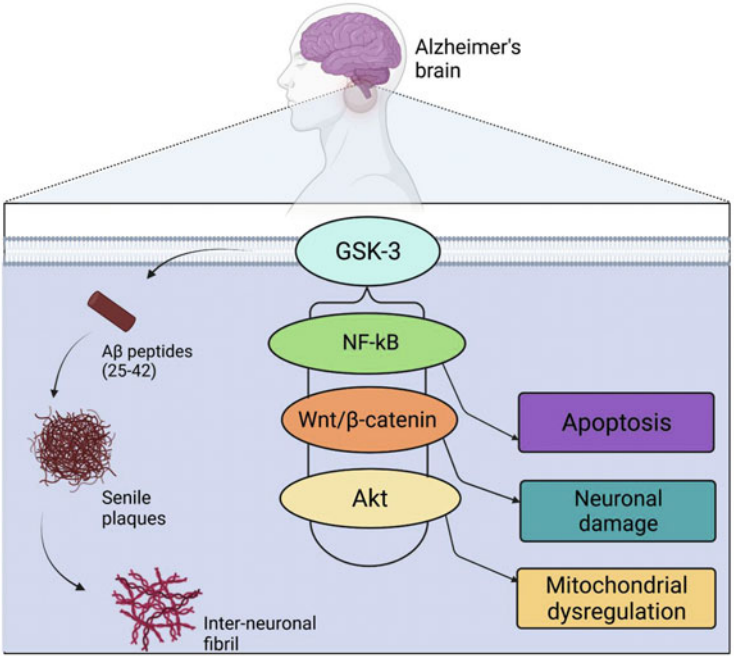
therapeutics and their studies to observe the potentials of GSK-3 inhibitors and assist our commands in understanding their effects on AD targets.

Graphical Abstract



The graphical abstract depicts the critical role of GSK-3 in Alzheimer's disease.

Keywords Alzheimer's disease · GSK-3 · Aβ peptide · Tau protein · NFTs · GSK-3 inhibitors

Abbreviations

AD	Alzheimer's disease
AICD	Amyloid precursor protein intracellular domain
AKT/PKB	Protein kinase B
APC	Adenomatous polyposis coli
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
Aβ peptide	Amyloid-beta peptide
BACE1	Beta-secretase 1
CBP	CREB-binding protein
CD marker	Cluster of differentiation marker

CDK-5	Cyclin-dependent kinases 5
CK1	Casein kinase 1
CREB	cAMP response element-binding protein
CSF	Cerebrospinal fluid
FTD	Frontotemporal dementia
GPCR	G-protein-coupled receptors
GSK-3	Glycogen synthase kinase 3
IL	Interleukins
IMAP	Inositol monophosphate phosphatase
IR	Insulin receptor
JNK	c-Jun N-terminal kinase
LRP	Low-density lipoprotein
MAP	Microtubule-associated proteins
MAP3K	Mitogen-activated protein kinase
MAPT	Microtubule-associated protein tau
MLK3	Mixed lineage kinase-3
MTBR	Microtubule-binding region
mTORC1	Mammalian target of rapamycin
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NFTs	Neurofibrillary tangles
NO	Nitric oxide
PHFs	Paired helical filaments
PI3Ks	Phosphoinositide 3-kinases
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
PS1	Presenilin 1
PSP	Progressive supranuclear palsy
ROR2/RTKs	Receptor tyrosine kinases
TG mice	Transgenic mice
TNF-α	Tumor necrosis factor alpha
Wnt	Wingless/integrated
β-TrCP	β-transducin repeat-containing protein

1 Introduction

Scientific and technological advancements have enhanced quality of life and prolonged longevity. The number of patients suffering from neurodegenerative illnesses is steadily growing. Dementia-related neurodegeneration is the seventh major cause of death, impacting about 55.2 million people globally (Arvanitakis et al. 2019). Alzheimer's disease is the most prevalent neurodegenerative disorder, associated with dementia, accounting for nearly 70% of the cases. Presently, AD dementia affects approximately 6.5 million Americans aged 65 and over today. This figure might rise to 13.8 million by 2060 if no medical breakthroughs are achieved to

prevent, slow, or cure AD (Breijyeh and Karaman 2020). AD is clinically diagnosed by progressive cognitive decline and memory loss. Early signs include troubled recalling of people and conversation. Patients experience reduced communication, behavioral changes, disorientation, trouble in recalling events, and depression, as the illness progresses. Histopathologically, AD is defined by the presence of Aβ neuritic plaques and NFTs formed by MAP tau (Meraz-Ríos et al. 2013).

Even though AD is extensively researched, the underlying molecular mechanisms are unknown, with no curable therapy present. Many signaling pathways related to AD pathology are being investigated to discover potential therapy targets, such as GSK. GSK-3 is considered to be a critical element in AD pathogenesis since it plays an important role in cell metabolism and signaling in both healthy and diseased states. This kinase's deregulation impairs all of the major hallmarks of the illness, namely memory, neurogenesis, tau phosphorylation, amyloid production, and synaptic function.

Glycogen synthase kinase 3 (GSK-3) was first identified as a protein kinase that phosphorylates and suppresses glycogen synthase. Initially discovered as a glycogen synthesis regulator in insulin response (Hughes et al. 1993), GSK-3 is a signal transduction protein serine/threonine kinase that is ubiquitously expressed and abundant in cellular processes throughout the body. Although it mainly exists as a cytosolic protein, a higher degree of GSK-3 activity is found in cortical neurons' nucleus and mitochondrial compartment along with the hippocampus (Takahashi et al. 2000), amygdala (Leroy and Brion 1999), and cerebellar regions (Yao et al. 2002) in the brain.

To date, two isoforms of the protein have been identified, *GSK-3α and GSK-3β* (Woodgett 1990; Doble and Woodgett 2003). These enzymes have almost identical (~95%) catalytic domains, with the significant difference in an additional glycine-rich N-terminus sequence found in the *α*-isoform (Mukai et al. 2002) *(the contrast between the two isoenzymes is observed in Fig. 1)*. Each isoenzyme is encoded by a homologous gene wherein GSK-3β's role is predominant in the neurodegenerative studies observed in animal models (Duda et al. 2018; Jope and Roh 2006; Kitagishi et al. 2012).

GSK, a crucial protein kinase, is among the most active kinases in the human body. It mediates the addition of phosphate molecules at N-terminus onto serine and

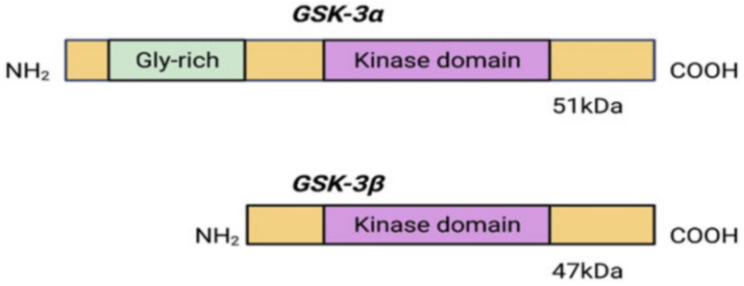


Fig. 1 Illustration portrays the GSK-3 isoenzymes, i.e., GSK-3*α* and GSK-3β

threonine residues, with a relatively higher affinity for serine (Stamos et al. 2014). This mediation to add phospho-groups is observed in over 40 distinct substrates (Sutherland 2011; Jope and Johnson 2004). The substrate phosphorylation by GSK-3 enzymes suppresses its activity. GSK-3 enzymes regulate a number of cellular signaling pathways, including metabolism, energy use, motility, transport, growth, and apoptosis (Jope and Johnson 2004; Woodgett 2001). There are multiple diverse inhibitors (>30) of GSK-3β being tested to determine their potential therapeutic properties for Alzheimer's disease and other maladies such as bipolar disorder (Li et al. 2010; Sproule 2002), chronic inflammation, type II diabetes (Nikoulina et al. 2000), Parkinson's disease (Kozikowski et al. 2006), and several cancers (Jope et al. 2007; McCubrey et al. 2016). Accumulating evidence suggests that the GSK-3β expresses multifocal interests in AD pathology for its direct participation in promoting Aβ pathology (Ma et al. 2006) and accelerates tau hyperphosphorylation (Lucas et al. 2001; Spittaels et al. 2000) in the brain tissues. Its presence is universal in several pro-inflammatory and pro-apoptotic pathways such as Wnt/β-catenin (He et al. 1995; Pierce and Kimelman 1995), NF-kB (Rinnab et al. 2008), AKT/PI3K (Jope and Roh 2006), and intrinsic apoptosis signaling (Hoeflich et al. 2000). Hence, it is suggested that the study of GSK-3β activation is highly associated with brain aging and cascade of deteriorating AD events. This paper aims to provide a thorough knowledge of GSK-3β and its inhibitors in order to gain insight into its molecular structure, function, and regulation. Several aberrant mechanisms are also outlined which are driven to produce pathogenic, inflammatory responses.

2 GSK-3β Affects Tau, from Normal Functioning MAPs to Aberrant NFT Deposits

The GSK-3β activity is associated with its domains observed in secondary structures. The protein kinase comprises of α-helical domain present at the C-terminal and β-strand domain where they lie between the amino acids, 139–343 and 25–138, respectively (Ter Haar et al. 2001). The ATP-binding site, which is responsible for the catalytic activities of the kinase, is present at the interface between the glycine-rich loop and activation loop. The aligning of the domains promotes active conformation and effective binding to the ideal substrates. The ATP-binding sites enforce the enzymatic activity that hydrolyzes ATP to ADP, releasing an inorganic phosphate molecule and energy. The phosphorylation is majorly observed in two different ways, i.e., direct phosphorylation and priming followed by phosphorylation, wherein the latter is 100–1000 times more effective (Thomas et al. 1999).

Degenerative events in Tau physiology result from unreliabilities in protein kinases and phosphatases. Though several kinases jointly cause the putative rise of the disease, GSK-3β acts as a gateway to the upcoming pathological events. GSK-3β activity is monitored and controlled directly by phosphorylation at two distinct

phospho-sites, Ser21/9 of GSK-3*β/α* and Tyr279/216 of GSK-3*β/α*. The binding at these sites either inhibits or increases their activity, respectively (Verhees et al. 2013; Dajani et al. 2001). Several tau posttranslational modifications undermine tau–microtubule association and thus facilitate its misfolding and mislocalization. The cytoplasmic tubule network, which supports axon function in mature neurons, is quite noteworthy. Tau proteins assist and regulate microtubule formation and stability, rendering them the most studied MAPs (Drewes et al. 1998). The disparities and interaction of traditional tau with several kinases and A*β* peptides lead to its hyperphosphorylation, prompting tau's disengagement from microtubules and subsequent aggregation to form insoluble NFTs, evidencing its diminishing solubility and eloquent reactivity to phospho-specific tau antibodies (Sternberger et al. 1985). Nonetheless, many studies have concluded that the presence of tau hyperphosphorylation and NFTs pave the way for neuronal death in AD.

Tau results as an alternately spliced product at 2,3,10 exons of the MAPT gene on chromosome 19 (Goedert et al. 1991), demonstrating that six distinct isotypes occur in adult human brains, ranging from 352 to 441 amino acids. The tau variants contrast from each other by the occurrence of either three or four repetitive regions in the carboxy-terminus (positive site; C-terminal) and the absence or presence of one or two inserts in the amino-terminus (negative site; N-terminal) region (Guo et al. 2017). The comparison between adult tau and fetal tau varies for their differences in microtubule binding, wherein 4R (R1–R4) found in adults is more impactful than the fetal isoforms, at eliciting microtubule assembly with 3R (R1, R3, R4) (Stamos et al. 2014) for the exclusive presence of KVQIINKK at R1-R2 inter-regions in 4R isoforms which enhances binding affinity to several folds and promotes microtubule polymerization (Goode and Feinstein 1994; Panda et al. 1995).

The translation of tau and its composition varies with the progression of disease and tauopathy involved, such as progressive supranuclear palsy (PSP), Alzheimer's disease (AD), and frontotemporal dementia (FTD). The expression of GSK-3β is evidently observed in both normal tau and aberrant tau deposits in the AD brain (Sun et al. 2002; Ferrer and Puig 2002; Yamaguchi et al. 1996). Even though the functional basis of this phenomenon is unknown, a linking role between them is significantly evident. The *in vitro* studies demonstrate that both GSK-3*α* and GSK-3β hyperphosphorylated tau at phosphorylation sites are related to AD. Consistent with several findings, the activation of GSK-3β facilitates neuronal synapse loss (Gomez-Isla et al. 1996) and neurodegeneration (Gong and Iqbal 2008). In *In vivo* and cultured neuronal systems, overexpression of GSK-3β elicits multisite phosphorylation in PHF-like epitopes, presumably in conjunction with other factors including tubulin and other kinases, heparin, a polyanion compound (Fiol et al. 1990; Chun et al. 2004). Splicing factor complexes, primarily from the serine/arginine-rich (SR) family, catalyze pre-mRNA splicing. The RT-PCR analysis reveals the role of GSK-3β, sometimes applicable with the presence of A*β*, to modulate the translation by phosphorylating SC35 protein, a promoter of which regulates the splicing of exon 10 in tau in cell-cultured cortical neurons (Greenberg and Davies 1990; Takashima et al. 1998a, b). The splicing also observes a reduced expression of 4R tau isoforms whose MTB affinity is more remarkable than that to

3R isoforms. This approach leads to GSK-3β-mediated reduced microtubule binding. Additional concerning outcomes of GSK-3β overexpression are observed for their role in hippocampal neurodegeneration (Avila et al. 2006), impaired cognition (Gómez de Barreda et al. 2010), learning deficits and increased LTD, and decreased LTP via altered NMDA receptors (Peineau et al. 2007; Salcedo-Tello et al. 2011). While GSK-3β phosphorylates nearly 29 AD-relevant phospho-sites on tau protein, many GSK-3β substrates necessitate a pre-phosphorylation via a "*stimulatory,*" priming kinase at a region proximal to the targeted GSK-3β residue. The pre-phosphorylation on unphosphorylated substrates is mediated by PKA, CK1, CK2 and MAP kinases, including CDK-5 (Kockeritz et al. 2006; Zhang et al. 2003; Polakis 2002). This phosphorylated residue binds at a serine/threonine, four residues from the C-terminal to the site on GSK-3β (Frame and Cohen 2001), e.g., CDK-5, whereas few of them can prime at five or six residues from the GSK-3β target site, e.g., CRMP2, and considerably enhance affinity and selectivity for specific substrates (Cole et al. 2006; Leroy et al. 2010). As the capacity of GSK-3β's propensity to phosphorylate a substrate is dynamically reliant on the functional activity and physiology of the priming kinase, which is regulated via a multitude of elements such as cell type and cellular context, this diverse distinctive feature adds several layers of influence. The priming phenomenon is relatively reversible as the prime substrates for GSK-3β either undergo positive regulation or negative regulation for nearly 100 proteins, suggesting the fundamental regulatory role of this enzyme in several cellular processes. Some mutation, such as arginine to alanine by phospho-site 96, preferentially suppresses human GSK-3β-induced primed substrates phosphorylation (Frame et al. 2001; Twomey and McCarthy 2006).

3 GSK-3β Affects the Integrity of Aβ Senile Plaques and Influences its Pathophysiology

A clear-cut diagnosis for Alzheimer's disease is established on the presence of its hallmark constituents, including senile plaques and NFTs. The upregulation in constituents drives AD pathogenicity. While both NFTs and Aβ play a crucial role in AD, tau has a less proactive role in the absence of senile plaques describing their interdependency. GSK-3β indulges itself in several intermediate pathways of Aβ production, ultimately promoting neurodegeneration (Kim et al. 2006).

Familial AD displays its prevalence in nearly 1–2% of all AD cases. So far, nearly three genes have been discovered, studied, and linked to early-onset dementia, i.e., APP, PSEN1, and PSEN2. Mutations in one of these genes are responsible for causing familial AD. The presence of apoE 4 is considered an understudy for its role in AD. The gene's polymorphic alleles (mainly ε4 allele) are the most prominent genetic risk determinants of late-onset AD (Corder et al. 1993). The APP processing mutations are observed in familial AD. The amyloid cascade hypothesis concludes

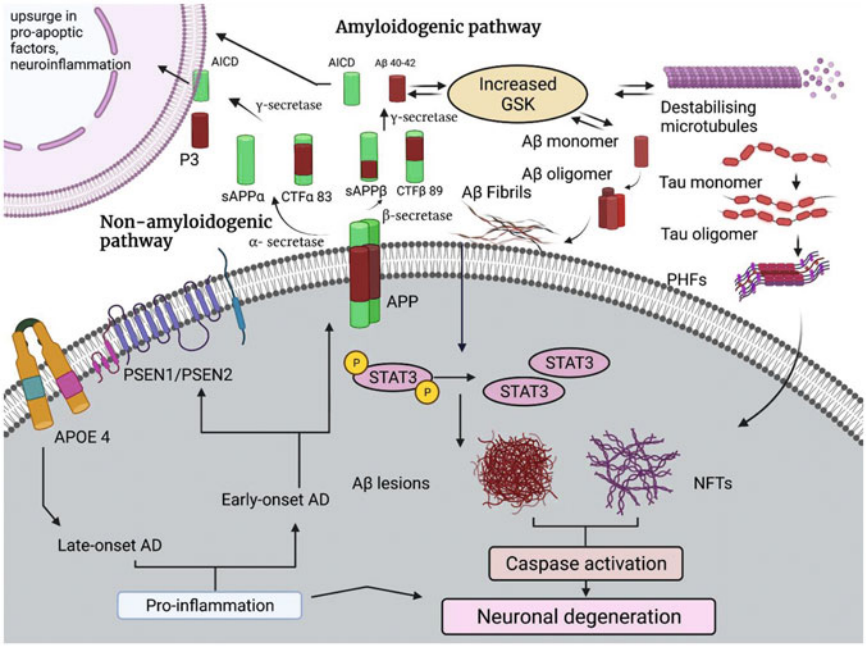


Fig. 2 Above illustration demonstrates the overexpression of GSK-3β evident in the pathology of β-amyloid and NFTs formation, as observed in AD

that the initial phases of aberrant Aβ peptide production led to the subsequent neurodegeneration observed in AD (Verdile et al. 2005). The *β*-amyloid plaques consist of *β-amyloid* peptides. The peptides are the cleavage product of a precursor protein, APP, expressed ubiquitously in the brain and spinal cord of the CNS. The proteolytic degradation of APP by a series of secretases, proteases, results in forming several A*β* species. GSK-3β indulges itself in several intermediate pathways of A*β* production, ultimately promoting neurodegeneration. In the amyloidogenic pathway, the Aβ species are synthesized due to the rise of mutations in the APP gene. APP is endo-proteolytically degraded, sequentially by BACE-1 enzymes (Vassar et al. 1999), at Asp+1 and Glu+11. The approach produces a C-terminal fragment, β-CTF, and a large N-terminal soluble fragment, sAPP-β. The γ-secretase complex, generally composing four subunits, i.e., Aph-1, presenilin (PS), Pen-2, and nicastrin (Nct), (Kimberly et al. 2003), further degrades β-CTF eventually releasing AICD and Aβ40-42. They tend to form aggregates, giving rise to neuritic plaques and other intractable oligomeric peptides forms, found as deposits in AD (Soriano et al. 1999) *(the emphasis of the Aβ and tau pathology is described in Fig. 2).* In the non-amyloidogenic pathway, APP is proteolytically cleaved by α- and γ-secretases, respectively. The disintegrated APP produces a C-terminal fragment, α-CTF, and a soluble N-terminal APP fragment, sAPP-α. The process follows cleavage by γ-secretases, ultimately giving rise to P3 fragments. While Aβ-related

studies conclude that P3 fragments are not engaged in the amyloid plaque core activity, they play critical roles in neuronal death and pro-inflammatory responses. Studies on human-derived cell lines, SH-SY5Y neuroblastoma, show that the P3 fragments induce cellular apoptosis via JNK phosphorylation (Norton et al. 2002). Thus, they do not portray significance as observed in classical Aβ pathways. In AD, the activity of α secretases is restricted due to the overexpression of GSK-3 isoforms through the inhibition of PK-C and ADAM enzymes (family of α-proteases), thus producing insoluble deposits in the brain.

Besides stimulating Aβ production through both pathways, evident studies correlate the steep decline in sAPP-α, a soluble neuroprotectant present in the CSF fluids with the GSK-3β activation, observed in AD. GSK-3β regulates Aβ production by modulating the functions and expression of presenilin. The observed AD-related mutations in PS1 augment its binding affinity to GSK-3β and, as an outcome, boost its tau-directed kinase activity. Hence, the enhanced interactions between PS1 and GSK-3β lead to increased tau phosphorylation (Takashima et al. 1998a, b). GSK-3β-induced BACE-1 expression heightens key pro-inflammatory epigenetic markers via NF-kB signaling, an overexpressed phenomenon in AD (Chen et al. 2012). Recent studies discovered that Aβ inhibits GSK-3β and that GSK-3β negatively affects Wnt/catenin transcription by remapping TCF4 from the BACE1 gene, thus suppressing the Wnt pathway. Several *in vitro* studies have suggested that Ab species upregulates GSK-3 signaling by suppressing PI3 kinase/Akt signaling (Abbott et al. 2008), thus preventing inhibitory phosphorylation of GSK-3 isoforms, i.e., GSK-3α and GSK-3β. This signaling drives APP processing intensity and establishes a cascade loop for ab production. Consistent with the role of GSK in the Wnt pathway, amyloid-ß promotes the GSK-3β regulation for its binding action to frizzled (Fz) receptor (Magdesian et al. 2008) along with exhibiting antagonistic actions on the IR receptors (IR) (Busciglio et al. 1995; Townsend et al. 2007).

4 Signaling Pathways and Their Implications with GSK-3β: A Central Key Mediator

4.1 Wnt Signaling Pathway

The Wnt signaling pathway involves the association of protein to the cell surface receptors often aided with coreceptors such as LRP and ROR2, wherein specific, the Wnt protein attaches itself to the extracellular, N-terminal, cysteine-rich regions of *frizzled (Fz) receptor* (a subgroup of GPCR family) which leads to the membrane recruitment and activation of scaffold protein and disheveled (MacDonald et al. 2009). The two forms of pathways involving Wnt signaling, e.g., canonical and non-canonical pathways, are based on its approaching response concerning β-catenin, wherein GSK-3β involves itself in the canonical signaling pathway. The

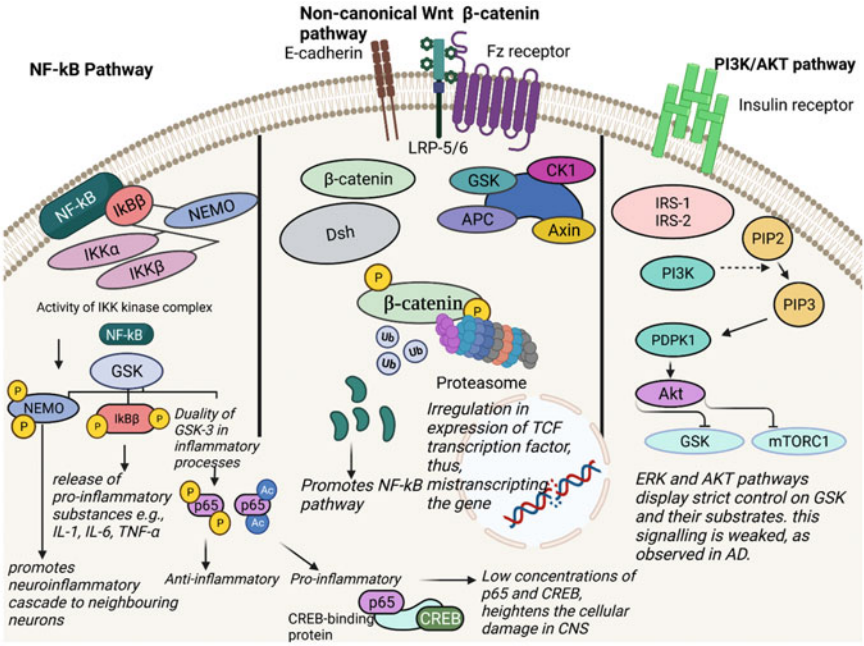


Fig. 3 Function of GSK-3 in numerous cellular pathways related to Alzheimer's disease. GSK-3 is implicated in three principal pathways, where its significance in the synthesis and progression of several pro-inflammatory and pro-apoptotic signals is considerable

canonical pathway associated with β-catenin involves its accumulation and translocation from the cytoplasm to the nucleus, where it serves as a prominent co-transporter for several transcription factors from the TCF/LEF family. β-catenin plays a significant role in regulating and coordinating the expression of genes by regulating cell–cell adhesion., rendering it a dual-role protein. As observed in some studies, GSK-3β inhibits the Wnt β-catenin pathway as it participates in the destruction complex, along with APC, Axin, and CK1 (Minde et al. 2011, 2013). Hence, GSK-3 functions as a negative regulator of β-catenin protein.

The GSK-3β-mediated *β*-catenin phosphorylation results in its proteasomal degradation by E3 ubiquitin ligase (β-TrCP) (MacDonald et al. 2009). This approach leads to increased expression of the NF-κB, thus mediating the NF-κB pathway. Hence, an ordered inversely proportionate link is observed in the Wnt pathway and NF-κB pathway, both of which show a clearly defined participation of GSK-3β protein kinase, as observed in cortical neurons associated with CNS *(Fig. 3 portrays the neuronal damage induced due to overexpressed GSK-3β in Wnt signaling)*. APC protein, a tumor suppressor gene whose mutations cause colorectal cancer (Fodde et al. 2001), is a subunit of the destruction complex that provides a well-established function in MT polymerization, axonal development, and polarity. The development of neuronal growth and polarity necessitates a target signaling between APC and PAR3 polarity kinase at the terminals of prospective axons (Rubinfeld et al. 1996;

Hart et al. 1998). The presence of GSK-3β protein kinase disrupts its mechanistic approach. The binding of GSK-3β to APC protein promotes APC phosphorylation leading to reduced MT stability (Zumbrunn et al. 2001) and irregular neuronal polarities at emerging axonal tip (Shi et al. 2004) and, therefore, neurodegeneration. Though research on APC protein suggests a relation with Alzheimer's disease, more substantial and specific characteristics of their linkage are yet to be known.

GSK-3β protein kinase plays a massive part in supporting the pro-inflammatory pathways, which leads to neurodegeneration in the brain. The degree of inflammation through GSK-3β is based on the activation of microglia and astrocytes, which aid in releasing several harmful neurotoxins, thus causing neuronal toxicity (Spangenberg and Green 2017; Green and Nolan 2012). Microglia, whose activation is inevitable for the role of GSK-3β, participates in neurodegeneration as a chronic source of neurotoxic indicators, interleukin-1β, cytokines, IL-1, IL-6, TNF-α, NO, and reactive oxygen species (ROS). Studies on transgenic mouse models suggest that the activation of the microglia is a prompt response to the upregulation of β-amyloid protein sediment. A stimulus for the chronic activation of the microglia is pertinent in neurodegenerative processes, e.g., LPS, where the LPS binds to TLR-4 receptors on the microglia, thus upregulates the MHC class II, CD40, CD80, and CD86 activation markers, thus involving the JNK pathway, mediated by MLK3 (Lehnardt et al. 2002). The GSK-3β plays a crucial role in the regular functions and dimerization of MLK3 and promotes the pro-inflammatory pathways to multiple folds (Hanisch and Kettenmann 2007; Vidyadaran et al. 2012). It results in neuroinflammation on neighboring neurons and promotes neuronal death, as seen in several neurodegenerative disorders such as AD and Parkinson's disease (PD). Research investigations suggest that GSK-3β contributes to inflammation via the production of pro-inflammatory cytokines and thus increases cell activation and migration. Thus, overexpression of GSK-3β causes NF-κB-mediated transcription. Furthermore, Aβ may interfere with normal Wnt pathway function, preventing Wnt from suppressing GSK-3β, and directly activate GSK-3β elevating tau phosphorylation. Taken together, many studies support the concept that GSK-3β represents the biochemical connection between both AD pathophysiologies (Aβ and tau) (Hernández et al. 2010).

4.2 NF-κB-Mediated Transcription

NF-κB, existing as homodimer and heterodimer proteins, is expressed abundantly in the CNS. They demonstrate their significance in maintaining cellular behaviors such as inflammation and cellular apoptosis. The pathogenic role of NF-kB in Alzheimer's has been linked to its interaction with amyloid-β and GSK-3β functionality. Understanding the pathway would help us elucidate the relevant substrates and inhibitors involved to restrict the transit of this cascade. It is an inducible transcription factor family that comprises NF-B1 (p50), NF-B2 (p52), RelA (p65), RelB, and c-Rel. Under general circumstances, NF-κB is bound to the IKK complex,

rendering its inactivation in the cytoplasm (Tak and Firestein 2001). So, we can assume that the phosphorylation of IκB can initiate the cascade responses of this pro-inflammatory pathway. The NF-κB component is triggered by a plethora of intracellular and extracellular stressors, notably T-cell and B-cell activation, PMA, oxidative stress, bacterial LPS, and certain viral infections (Rothwarf et al. 1998; Yamaoka et al. 1998). The IκB *kinase* (*IKK*) complex comprises two significant kinases that form the catalytic domains, IKKα and IKKβ, highlighting epitope specificity at s32 and s36 in the N-terminus of IκBα, predisposing it to its proteolytic destruction and the regulatory domain, NEMO/IKK γ (Karin and Ben-Neriah 2000). Activated NF-κB enhances the activity of IκB *kinase (IKK)* complex, which downregulates IκB activity via its phosphorylation, followed by ubiquitin system degradation by the ATP-dependent 26S proteasome (Gilmore 2006; Basseres and Baldwin 2006; Hayden and Ghosh 2004). The interaction between IκB and IκB *kinase (IKK)* is a critical regulatory step in maintaining the homeostasis of this pathway, which can be counterproductive at times. GSK-3β engages in the toll-like receptor (TLR) pathway by phosphorylating the IκB *kinase (IKK)* complex at ser8, ser17, ser31, and ser43 in the N-terminal regions. This interaction induces the activation of NF-kB by phosphorylation of its inhibitor complex. In certain instances, GSK-3β promotes anti-inflammatory and pro-inflammatory action by directly phosphorylating the p65 subunit at serine 468 and acetylating the p65 unit at lysine 310, respectively (Holger et al. 2004). Along with it, GSK-3 promotes the binding of both p65 and CREB transcription factor to their coactivator, CREB-binding protein (CBP), wherein the consequences outcome reduced CREB expression, thus fuelling the cascade of inflammatory responses via promotion of chemokines, cytokines, e.g., IL-6, IL-10, TNFα, and MCP-1. GSK-3β-induced reduction IL-10 expression depicts an inverse proportionality to other cytokines in aid in the subsequent promotion of degenerative processes observed in AD (Holger et al. 2004) *(the depiction of GSK-3 on NF-kB is portrayed in Fig. 3).*

4.3 Insulin/IR/PI3K/AKT Pathway

Insulin receptors are transmembrane receptors consisting of three primary components: two α units and one β-unit linked to each other. They bind to the IR receptors, frequently expressed in neurons, microglia, and astrocytes, constituting an α and β-unit. The β-unit's two critical components, tyrosine residues and tyrosine kinase enzyme, are activated through interactions with insulin receptors, where the kinase enzyme phosphorylates the residues and subsequently activates the entire IR receptor complex (Johnstone et al. 2003). Upon conformational modifications, activated IR promotes PI3 kinase. The attachment of PI3 kinase to IR causes its activation and the conversion of PIP2 to PIP3 in the cell membrane, facilitating AKT/PKB protein kinase activation *(Fig. 3 demonstrated the mechanistic role of GSK-3β in PI3K pathway).* Its role is involved in several pathways such as inhibiting GSK-3β, promoting glycogenesis through activation of glycogen synthase, upregulated

expression of GLUT-4 glucose co-transport, reducing the expression of hyperphosphorylated tau, and deregulation of mTORC1, which increases lipids and protein synthesis in the cell (Johnstone et al. 2003; Vannucci et al. 1998), thus controlling the cognitive profile of the brain. The presence of AD leads to a less significant or weakened signaling in this pathway, causing elevated GSK-3β concentrations, thus resulting in neuronal death and accelerated axonal degeneration.

4.4 Apoptosis and Neurodegeneration

Apoptosis, a highly regulated and functioned activity, is considered programmed cellular destruction of the cells based on various factors. Cell death is triggered by caspases that cleave specific proteins in the cytoplasm and nucleus to trigger apoptosis in the endoplasmic reticulum (ER) (Song et al. 2002), mitochondria, and lysosomes (Leber et al. 2010). While apoptosis aids in demolishing the inconsiderate cells out of various systems in our body, its mistranslation can be proven to be detrimental, as observed in Alzheimer's disease. The implications that lead a cell to its apoptotic stages are complicated pathways. Once initiated, the process follows cascade events. The stimuli associated with apoptosis can be provided for the action of caspases in the system through cleaving specific proteins in the cytoplasm and nucleus. Caspases are considered a significant culprit in the creation of apoptotic activities. The *in vitro* and *in vivo* cell cultures suggest a relationship between apoptosis and AD. These caspases are the same guys seen in ab plaque formation. GSK-3β plays a dual opposing role in the pathways associated with apoptosis; i.e., it upregulates the apoptosis in the intrinsic pathway and downregulates the same in the extrinsic pathway. Several pro-apoptotic kinases are implicated in the etiology of Alzheimer's disease (Youle and Strasser 2008). Though a concise overview is challenging, CSF concentration estimates could be appropriate forecasting tools for monitoring the cognitive decline associated with it. Dysregulation of apoptotic signals occurs in several conditions, including cancer, caspase-8 deficiency, and autoimmune illnesses, in which excessive cell death is essential in various neurodegenerative diseases. As a result, it is critical to dissect the architecture of the apoptosis pathways.

An intrinsic apoptotic pathway is mainly an event led in the mitochondrial compartments of the cell. External stimuli such as ER stress or oxidative stress enforce the dissipation of the membrane potential of mitochondria. Bak and Bax, belonging to the Bcl-2 family of proteins, are vital mediators for transmitting the pro-apoptotic signal to the mitochondria (Youle and Strasser 2008). The duality of Bax is observed where it sequestered anti-apoptotic and oligomerized in the membrane, thus disrupting the electric potential of the mitochondria. The cytochrome c released with the aid of Bax and Bak leads to its ATP-based binding to the Apaf-1 and later to pro-caspase-9, forming a protein complex, apoptosome (Shakeri et al. 2017). The complex acts on the precursor pro-caspase-9, resulting in its cleavage, thus releasing caspase-3. GSK-3β promotes the pathway through its multivariate

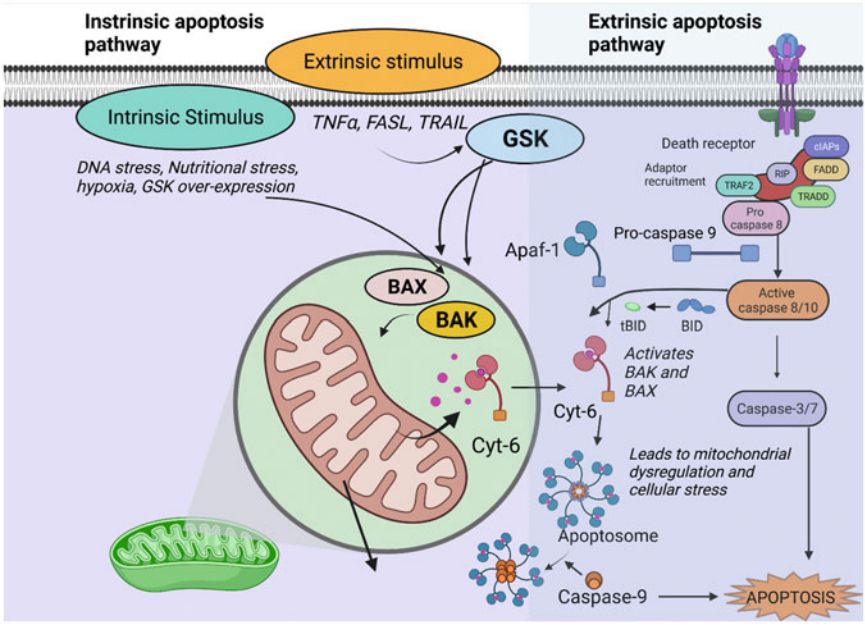


Fig. 4 Schematic diagram indicates the mitochondrial dysregulations associated with GSK-3 expression. The elevated expression of GSK-3 leads to the imbalanced electric potential of mitochondria, hence its dysregulation and apoptosis

regulation of gene transcription factors *(Fig. 4 displays the pathogenic mechanisms played by GSK-3β).* The GSK-3β implicates its role through its isoforms. While the role played by nuclear GSK-3β is significant, there is also a dynamic function exhibited by cellular GSK-3β. GSK-3β phosphorylates Bax at s163 epitope and enhances the degradation of the anti-apoptotic Bcl-2 family member (Wang et al. 2010).

5 GSK-3 Inhibitors

5.1 Lithium

Since the late nineteenth century, lithium has been found to exhibit a wide range of medical properties (Cade 1949). The drug was used to prevent psychotic episodes and manic symptoms associated with bipolar disorder (Machado-Vieira et al. 2009). In recent years, the discovery has defined its role as a viable treatment for Alzheimer's disease. The actions on GSK-3β are attributed to the cationic activity of lithium and its existence as a cationic salt. It has been shown to downregulate the activity of several enzymes such as inositol monophosphate phosphatase IMPA,

BPNT, GSK-3α, and GSK-3β (Freland and Beaulieu 2012). The competitive inhibition of Mg+2, a cofactor of GSK-3β, downregulates the protein kinase activity (Ryves and Harwood 2001). Another approach follows the direct phosphorylation at N-terminal serine residues of GSK-3. The activation of the AKT pathway by lithium corresponds to reduced GSK-3 levels via disruption of AKT/βArr2/PP2A signaling (Beaulieu et al. 2008), typically stimulated by D2R by DA (Beaulieu et al. 2005). This leads to GSK-3β inhibition at Ser9 (Jope 2003) and prevents the hyperphosphorylation of tau protein. Cell culture and transgenic animal models display a declining trend in tau neurotoxicity and impaired cognition, following reduced Aβ pathology (Nocjar et al. 2007; Forlenza et al. 2012). Postmortem studies of AD brains depict an abnormal rise in IMAP, a key Myo-inositol regulator (Shimohama et al. 1998). Lithium mediates the deregulation of Myo-IMAP and mTORC, thus halting the pathways associated with neurodegeneration (Sarkar et al. 2005; Sarkar and Rubinsztein 2006). Assessments on the PS1xAPP mice model showed that chronic oral dose-dependent lithium interventions reduced neuronal loss and axonal dystrophies, wherein it is modulated by compacting Aβ plaques and lowered their toxic oligomers along with upregulation of HSP (Trujillo-Estrada et al. 2013).

5.2 Tideglusib

Tideglusib compounds can be considered the most advantageous GSK-3β inhibitors due to their prodigious efficacy observed in Alzheimer's disease and other neurological disorders. Tideglusib, belonging to the class thiazolidinedione (TDZD), is an irreversible, ATP-independent molecule that phosphorylates GSK-3β protein kinase at the cys199 motif. The double APP/TG mouse model studies show the marked effect of tideglusib in repressing and preventing tau phosphorylation and hence reduced NFTs and amyloid load in patients with AD (Serenó et al. 2009). Animal model studies displayed that they exert anti-inflammatory, neuroprotective effects, and neurogenic features (Morales-Garcia et al. 2012). Tideglusib acts as a PPAR agonist as it features regulation and repression of Aβ physiology and molecules associated with pro-inflammatory processes and enhancement of mitochondrial activity, thereby preventing aggressive apoptosis (Markus and Michael 2008).

5.3 SAR502250

It is a selective GSK-3β inhibitor whose roles are yet to be discovered for AD. The compound shows efficacy in treating several neurodegenerative and neuropsychiatric disorders. It is a complicated inhibitor whose complex pharmacological roles are yet to be defined and classified. The molecule exerts its effect mainly on the isoforms of Aβ peptides and tau phosphorylation and improves the cognitive profile of patients with mild-to-moderate AD.

In vivo statistical analysis conducted on SAR502250 on hyperphosphorylation of tau (S396) in P301, TG mice displayed its reduced expression in the cortex and spinal cord in a dose-dependent manner (Griebel et al. 2019). Clinical studies conducted on E18 Wistar model rats employed hippocampal neuronal cells at embryonic stages. The effects of Aβ25-35-induced cell death studies showed that SAR502250 attenuated the toxicity at concentrations of 100 nM and 1 μM, respectively (Griebel et al. 2019), generated as a result of intraventricular injection of the peptide whose toxicity is similar to that of Aβ1-40, reducing the oxidative stress, neuroinflammation, and rapid glial activation produced as a result of it (Zussy et al. 2011). Many studies have demonstrated that the compound improves the cognitive function of AD brains in numerous animal models (Tsaltas et al. 2007; Verhees et al. 2013). They aid in promoting hippocampal neurogenesis in adults and alter the synaptic plasticity of neurons aiding in long-term memory improvements (King et al. 2014).

5.4 CHIRs

The CHIRs compounds are purine analogs initially developed and created by Chiron Pharmaceuticals. They are classified as aminopyrimidine compounds and significantly attenuate many protein kinase-associated pathogenic roles in AD. Many CHIR analogs, notably laduviglusib (CHIR99021), CHIR98014, and CHIR98023 (Ring et al. 2003), demonstrate potential substrate-specific inhibition of GSK-3-mediated tau phosphorylation and prevent cellular apoptosis in rat neural cells (MAIN 27). Laduviglusib facilitates autophagy and is a Wnt/β-catenin signaling pathway stimulant. The compounds exhibit selectivity for both isoforms, namely GSK-3α and GSK-3β. While a limited number of studies have been conducted on CHIRs, their potentiating activities suggest a novel response toward GSK-3 inhibition with little effect on other kinases (Ring et al. 2003).

5.5 Indirubins

The bis-indole indirubin isolated from Danggui-Shaoyao-San (DSS) is a traditional Chinese medicine used in the treatment of a wide range of diseases, for their antiproliferative and anti-depressive properties. It was initially identified as a CDK inhibitor before being revealed to be a strong GSK-3β inhibitor. The analog 6-bromoindirubin shows more substrate selectivity for GSK-3β than CDK-5, while 6-bromoindirubin-3′-oxime (6BIO) exhibits nearly 16-fold heightened selectivity for GSK-3β. TG mice research indicates tau phosphorylation in cultured cortical neurons (Martin et al. 2009). Further, they mimic the Wnt signaling pathway (Sato et al. 2004) and display their expression in mouse ES cells to enhance self-renewal

and pluripotency. Another substance, Indirubin-3′-oxime, which prevents the pro-inflammation responses reduces tau hyperphosphorylation, Aβ deposition, and spatial cognition deficits in APP/PSEN-1-TG models (Ding et al. 2010).

5.6 Sb-216763

SB-216763 is an ATP-competitive, aryl-indole maleimide-derived intervention developed by SmithKline Beecham. The physiological role of SB-216763 is implicated in a wide range of complex diseases such as Alzheimer's disease and ischemia, wherein they act as neuroprotectants and prevent PI3K signal-facilitated neuronal death. The same assessments are observed for SB- 415286, considered as other maleimide derivatives sets. It is a potent, selective GSK-3β inhibitor whose multivariate role is implicated in several pathophysiologies. In the postnatal AD rat model, SB-216763 reduces Aβ-induced neuronal toxicity while also lowering the extent of tau phosphorylation in the hippocampus (Selenica et al. 2007), caspase-3 activation, and JNK kinase activity (Hu et al. 2009). This synergistic action is associated with several therapeutic outcomes, e.g., lowered expression of pro-apoptotic conditions and cell death. HEK293 cell culture study observes that the medication affects the expression of phospho-tau by binding at Ser202, hence reducing its levels in AD (Cross et al. 2001). Along with it, they induce inhibitory phosphorylation at Ser9. This outcome is possible due to the mediation of p90rsk, which phosphorylates tau at GSK-3β specific phospho-epitope, i.e., Ser396. They induce the promotion of canonical Wnt β-catenin pathway, neuroprotective features, and glycogen synthase stimulation in the liver cells for the acumen of GSK-3 inhibition. The clinical tests conducted on cultured MEFs display that promoting β-catenin by the SB-216763 expressed the presence of pluripotent specific genes, Oct4, Sox2, and Nanog. The compound is believed to exhibit self-renewal properties of pluripotent, making it the first GSK-3β inhibitor with pluripotent properties (Kirby et al. 2012).

5.7 Tolfenamic Acid (TF)

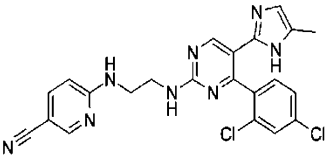
The compound is known for its NSAID activity, wherein they inhibit major protein kinases as such SP1-mediated CDK-5 and GSK-5 activity. Mice models suggest their activity in reducing the tau hyperphosphorylation induced by GSK-3β and diminishing the effects of phosphorylated PP2A and enhancing its activity (Zhang et al. 2020). The transgenic hTau mice models depict that TF alleviates cognitive and learning impairments by decreasing the expression APP and BACE1 (Subaiea et al. 2013; Adwan et al. 2015). The western blot analysis showed a lower significance of hyperphosphorylation tau in AD when treated with TF (Chang et al. 2018).

5.8 Paullone Compound

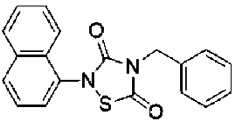
Paullones, a benzazepinone class, are potent inhibitors of two protein kinase families: cyclin-dependent kinases and glycogen synthase kinase. Paullones have shown to be a limited yet effective class of molecules that serve a significant role in treating certain malignancies and Alzheimer's disease. Kenpaullone, in particular, has been shown to be a competitive inhibitor of CDK-5 via its binding to an ATP-binding site. Many experimental transgenic organism studies describe the effectiveness of paullone compounds, primarily kenpaullone and alsterpaullone, for their role as potent GSK-3 inhibitors (Castelo-Branco et al. 2004). While kenpaullone reduces the overexpression of APP and deregulates the Aβ peptide synthesis, it also enhances the maturation of precursor cells into dopamine neurons. Alsterpaullone and Kenpaullone are ATP-competitive compounds shown to bind preferably with GSK-3β. Alsterpaullone suppresses tau phosphorylation *in vivo* at sites frequently phosphorylated by GSK-3β in cultured neurons of AD (Leost et al. 2000) and inhibits NMDA-induced LTD in the hippocampus region (Peineau et al. 2008) *(Fig. 5 represents the chemical structures of various GSK-3β inhibitors)*.

6 Conclusion and Future Prospect

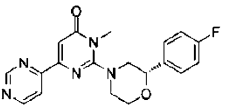
GSK-3 has been identified as a crucial target in the etiology of AD. GSK-3 overexpression is prevalent in the CNS of AD individuals, and its significance is widely studied for its relation with tau and Aβ pathogenesis. GSK-3 has a conserved role in the regulation of several signaling pathways for its involvement in engaging with different substrates and molecules. An upsurge in GSK-3 levels is observed in association with Alzheimer's disease and several other neuropathological disease conditions. This increment leads to the progression of NFTs, Aβ, along with causing neuronal toxicity and neuroinflammation. While many of the inhibitors can produce promising therapeutic effects in the systems, many fail at producing the threshold required. Even though studies on underlying molecular mechanisms of target kinases have not been widely ascribed, recent researches suggest their impact on AD's pathological progression. As a result, extensive research is needed to explain the roles of tau and GSK3 in physiopathology and structural abnormalities, as well as to investigate the effectiveness of novel AD therapeutics. Although nearly 30 GSK-3 inhibitors have been discovered and studied so far, most of their clinical trials are still in the preliminary phases and have shown several instances of drawbacks. GSK-3 has a multifactorial effect in AD, wherein GSK-3 in association with other protein kinases and transcription factors produces a cumulative detrimental impact in AD patients. In this review, GSK-3's mechanism of action, correlation with several signaling pathways, pathogenic cascade, and its association with Aβ and tau phosphorylation, has been described. In addition, various GSK-3 inhibitors, their actions, and substrate effect have also been highlighted.



Ladvuglusib CHIR-99021



Tideglusib



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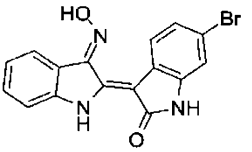
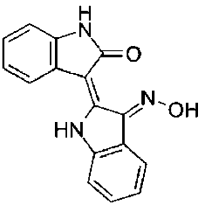
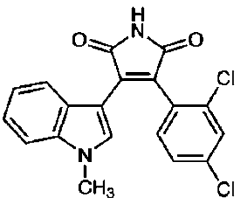


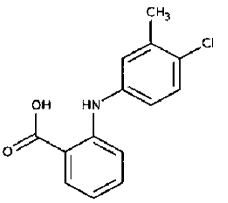
6-bromoindirubin-3′-oxime
(6-BIO)



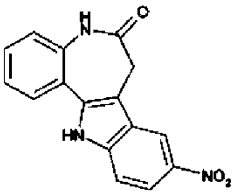
Indirubin-3′-oxime



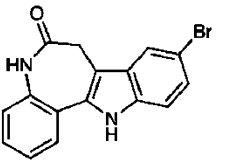
SB-216763



Tolfenamic acid



Alsterpaullone



Kenpaullone

Fig. 5 Above diagrams demonstrate the chemical structures of various GSK-3 inhibitors

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Role of Target Fishing in Discovery of Novel Anti-Alzheimer's Agents: In Silico Applications

Anjali Murmu, Balaji Wamanrao Matore, Purusottam Banjare, Nilesh Kumar Pandey, Nikita Chhabra, Lomash Banjare, Sourav Basak, Jagadish Singh, and Partha Pratim Roy

Abstract Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by progressive degeneration in the brain and shrinking (atrophy) of the brain size with the association of multiple factors/targets. The traditional "one medicine, one target" approach tends to be insufficient due to the complexity of AD. Till now, no established strategy for the treatment of AD is available. Therefore, it is of prime importance to catch the promising targets from the reported several targets. Target fishing here plays a pivotal role in a drug discovery without prior knowledge about their role in AD. Computational approaches are now considered an integral component of drug discovery with exceptional theoretical assumptions over the decades. Ligand and structure-based in silico approaches are able to provide a rational solution to the answer of target fishing. With this background, the objective of the book chapter was mainly to describe the powerful in silico tools for the optimization of the target based on literature reports and in silico tools available for target prioritization.

Keywords Alzheimer's disease · In silico · Docking · Pharmacophore · QSAR · Target fishing · Target prioritization

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Abbreviations

ACAT	Acyl cholesterol acyltransferase
ACHE	Acetylcholinesterase
AD	Alzheimer's disease
APP	β-amyloid precursor protein
Aβ	Amyloid β
BCHE	Butyrylcholinesterase
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CLU	Clusterin
COX-2	Cyclooxygenase-2
CR1	Complement receptor 1
GABA	γ-aminobutyric acid
GPCRs	G-protein-coupled receptors
GRN	Progranulin
HIF-1α	Hypoxia-inducible factor 1α subunits
HMG	3-hydroxy-3-methylglutaryl l
LRRK2	Leucine-rich repeat kinase 2
MAO-B	7 mono amino oxidase-B
MAPT	Microtubule-associated protein tau
NMDA	Glutamate N-methyl D-aspartate
PICALM	Phosphatidylinositol-binding clathrin assembly protein α-synuclein
PSEN1	Presenilin 1
PSEN2	Presenilin 2

1 Introduction

Every organ in the body is impacted by aging, which is fundamental to neurodegeneration and dementia. One of the most pervasive diseases, dementia, affects the lives of millions of individuals worldwide (World Health Organization 2019; Sivakumar et al. 2020; Khan et al. 2020). Alzheimer's disease (AD) is a severe, protracted neurological condition that is irreversible; it damages the brain, which causes memory, cognitive, and behavior problems, mostly in the elderly. The death of brain neuronal cells is the cause of these symptoms (Mehrazar et al. 2020). Although the etiology of AD is unclear and complicated, a variety of theories have been put up that the multifactorial pathology of AD is driven by the absence of acetylcholine (ACH), which influences the progressive deterioration of brain tissue (Murphy and Levine 2010). The neurodegenerative processes seen in AD are caused by the buildup of extracellular β-amyloid in senile plaques, loss of cholinergic activity in specific brain regions, intracellular neurofibrillary tangles, including the hyperphosphorylated tau protein, and neuroinflammation (Rajmohan and Reddy 2017) (Fig. 1).

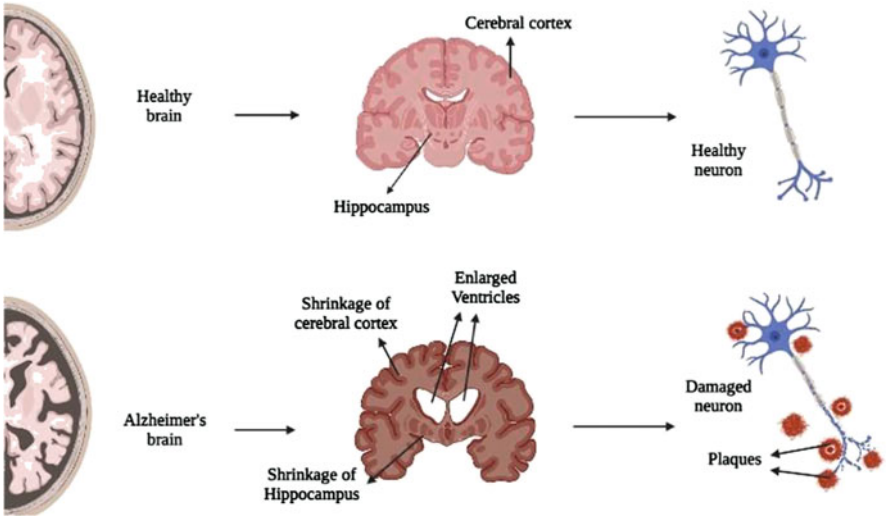

Fig. 1 Comparison of healthy brain and brain of Alzheimer's patient

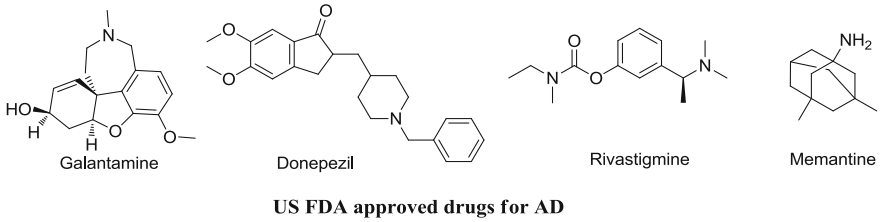

Fig. 2 Chemical structures of US FDA-approved drugs for AD treatment

Inhibiting cholinesterase enzymes (CHEs) are one of the main methods for raising the level of ACH, which is crucial for attention, learning, memory, and motivation. Acetylcholinesterase (ACHE) and butyrylcholinesterase (BCHE) are two widely dispersed cholinesterases that belong to the hydrolase enzyme class. There is an imbalance between ACHE and BCHE in AD patients (Acar Cevik et al. 2019; Rullo et al. 2019; Zaib et al. 2021). Available AD medications like three acetylcholine inhibitors (donepezil, rivastigmine, and galantamine) and one N-methyl-D-aspartate (NMDA) receptor antagonist (memantine) are currently used clinically (Marucci et al. 2021). The chemical structures of US FDA-approved drugs for AD treatment are shown in Fig. 2.

These medications, however, were only able to enhance the cognition and level of dementia in AD patients; they could not prevent the disease's progression. Rather, they can cause a number of adverse effects, including gastrointestinal problems, hepatotoxicity, and peripheral side effects. However, despite the fact that several anti-AD bioactive substances were created based on factors such as the deposition of

β-amyloid (Aβ) plaques, the majority of them were unsuccessful (Czarnecka et al. 2019).

AD has a complex etiology. The idea of "magic bullets," or medications that have incredibly high target specificity, is an ancient and venerable one in medicinal chemistry and drug development (Galati et al. 2021). The traditional "one medicine, one target" approach is insufficient given the complexity of AD. Many neurodegenerative diseases categories, including AD, have experienced a dramatic paradigm change in drug discovery as a result of recent research into ground-breaking network pharmacology (Pérez-Nueno 2015; Shaikh et al. 2021; Yao et al. 2021). For the design and development of drugs, it is essential to identify potential targets for a known bioactive molecule. Due to serious clinical side effects and cross-reactivity discovered during latter-stage clinical trials, many chemical compounds have failed to receive approval and reach the market over the previous few decades (Mohs and Greig 2017). There are various targets responsible for causing AD such as ACHE, BCHE, COX-2, MAO-B, NMDA, GABA-A, and GABA-B (Fang et al. 2017). Prioritization of potential targets plays a very important role in the treatment of AD. Conventional methods for identifying potential targets with good accuracy include protein affinity isolation and subsequent mass spectrometric analysis, as well as approaches based on mRNA expression (Ziegler et al. 2013). However, experimental approaches are expensive in terms of resources and time. Because of these limitations, in silico target fishing is considered a promising alternative for target identification. In contrast to virtual screening, which is used to search large libraries of compounds for molecules that are most likely to bind a specific target, the aim of target fishing, also known as in silico reverse screening, is to identify the most likely targets of a query molecule (Jenkins et al. 2006). Owing to the complexity of the treatment of AD, polypharmacology was adopted to combat the neurodegeneration. The idea of creating single molecules that can simultaneously control a number of targets involved in the intricate neurodegenerative cascade was created by the field of polypharmacology (Makhoba et al. 2020). In order to treat AD, multi-target-directed ligands (MTDLs), which simultaneously modulate many pathogenic factors, have attracted the interest of scientists and have been produced globally (Blaikie et al. 2019; Rossi et al. 2021). This chapter gives a detailed emphasis on AD, its cause, types, and different stages, and advances of target fishing and target prioritization are summarized in a later section.

2 Causes and Risk Factors for AD

Aging, genetics, head injuries, vascular illnesses, infections, and environmental variables are among the risk factors for AD (Fig. 3), which has been thought of as a complex disease (heavy metals, trace metals, and others). It is currently unclear what causes the pathological alterations in AD (Aβ, NFTs, and synaptic loss). The fundamental causes of AD are thought to be two of the many hypotheses that have been put forth: Some contend that cholinergic dysfunction is a significant risk factor for AD, while others contend that changes in the generation and processing of

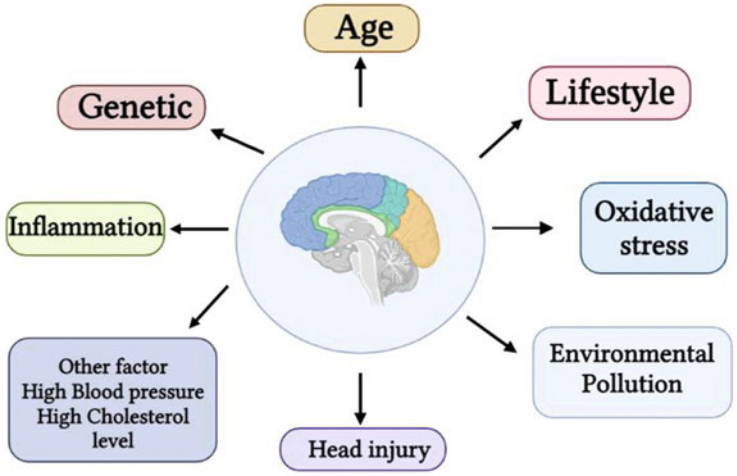

Fig. 3 Different factors responsible for the cause of AD

β-amyloid protein are the primary beginning factors (Anand and Singh 2013; Armstrong 2019).

3 Types of AD

3.1 Sporadic Form of AD (SAD)

SAD is also known as late-onset form of AD (LOAD), which reveals itself after 65 years of age. This kind of AD is basically seen in 95% of the older generation. Symptoms of the disease begin insidiously and worsen over time. At present, only a provisional diagnosis can be made and usually takes place during the final phase of the illness (Spina et al. 2021). Definite diagnosis requires postmortem evaluation. All cases of sporadic Alzheimer's disease are accompanied by a characteristic pathological process, but this process is much longer than the clinically recognizable phase of the disease (Duara et al. 1993; Braak and Del Tredici 2012). The gene associated with late-onset AD is shown in Fig. 4.

3.2 Hereditary Familial Form of AD (FAD)

FAD is categorized as early-onset of disease (EOAD) correlated with the mutation of gene. AD has a strong genetic correlation with three genes: β-amyloid precursor protein (APP) and the genes for the presenilin 1 (PSEN1) and presenilin 2 (PSEN2) proteins (Mendez 2017) (Fig. 4). Alterations within these genes are directly correlated with plaques formation. Literature data demonstrated that subjects inheriting

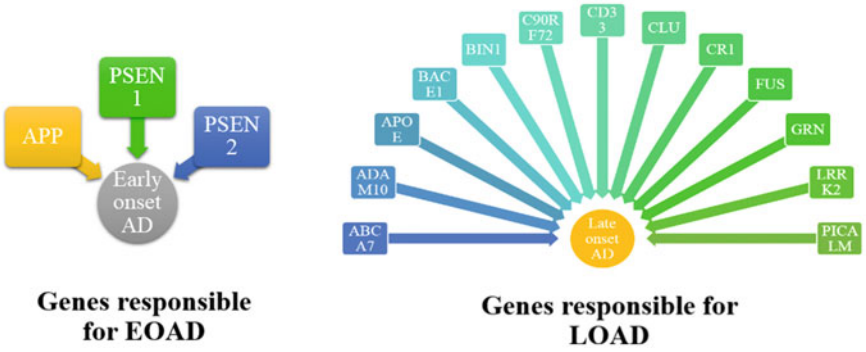

Fig. 4 Different genes responsible for causing AD for both EOAD and LOAD. (Note: *APP* β-amyloid precursor protein, *PSEN1* presenilin 1, *PSEN2* presenilin 2, *CD33* CD33 molecule (sialic acid-binding Ig-like lectin 3), *CLU* clusterin, *CR1* complement receptor 1, *FUS* FUS RNA-binding protein, *GRN* progranulin, *LRRK2* leucine-rich repeat kinase 2, *PICALM* phosphatidylinositol-binding clathrin assembly protein α-synuclein)

mutations within APP or PSEN1 genes are guaranteed to develop AD, while those inheriting mutations within PSEN2 gene have a 95% chance of developing the disease. The cases of AD caused by alterations within the three genes are labeled as autosomal dominant familial AD, because of its inheritance model (Campion et al. 1999; Qin et al. 2020). It generally develops within 60 years of age, sometimes as early as 30 years of age. For this reason, it is also often indicated as early onset AD (EOAD). This form presents a clear molecular background, and it is easily recognized since it runs in families. Up to 5% of all AD cases are of this type (Wu et al. 2012).

However, both forms of AD share similar pathological processes leading to specific molecular manifestations, such as extracellular β-amyloid (Aβ) deposition, hyperphosphorylation tau protein and its intracellular accumulation oxidative stress, neuroinflammation, mitochondrial impairment, and ion metabolism disorders (Duyckaerts et al. 2009).

4 Stages of AD

The severity of cognitive impairment and the histopathological changes are the key criteria used to classify AD clinically. Typically, there are four steps listed:

4.1 Preclinical Stage of AD

Due to the absence of any serious symptoms, this phase is frequently disregarded. Typically, it is categorized as mild cognitive impairment. The earliest pathogenic

alterations start to manifest in this stage, first affecting the entorhinal cortex and the hippocampal region later. Subjects at this stage have minor memory loss from a symptomatologic perspective, with relatively few long-term memories affected (Oboudiyat et al. 2013; Parnetti et al. 2019).

4.2 Mild Stage of AD

Cognitive symptoms started to appear in this stage. During this period, the cerebral cortex is affected by pathological changes. From a symptomatologic perspective, coupled with memory loss, there is an inability to recall new knowledge, forgetting things and appointments, followed by impairment in decision-making, executive functioning, and problem-solving. Additionally, the participants display personality changes, mood fluctuations, and a lack of spontaneity. Additionally, uncertainty and disorientation are frequently observed conditions (Kirova et al. 2015; Wattmo et al. 2016).

4.3 Moderate Stage of AD

The severity of the symptoms continues to worsen in this stage. The areas involved in language, thought, and sensory processing are also affected by pathological impairment (cerebral cortex). Behavioral issues and a propensity for social withdrawal start to show up in addition to an aggravation of symptoms from earlier phases. Language dysfunction and visuospatial skill deficits follow (Reisberg et al. 1996; Apostolova 2016).

4.4 Severe Stage of AD

Subjects fully lose their ability to function independently at this phase. All of the cortical areas are thought to be affected at this point by the pathological damage. The affected person's cognitive functioning reaches its lowest point, and additional systemic symptoms such as dyspraxia (difficulty performing learned motor tasks), olfactory dysfunction, sleep disturbances, and extrapyramidal motor signs like dystonia, akathisia, and parkinsonian symptoms start to manifest (Reisberg et al. 1996; Herrmann 2007).

5 Targets Associated with AD

5.1 *Acetylcholinesterase*

ACHE is an enzyme, which is found in the postsynaptic region of neurons that catalyzes the breakdown of acetylcholine neurotransmitter to choline and acetic acid. Inhibition of ACHE can be a promising treatment against AD. Since the level of acetylcholine tends to decrease, the use of ACHE inhibitors can be a better therapeutic strategy to control this multifactorial neurodegenerative disorder (Li et al. 2022).

5.2 *Glutamate Receptor Ionotropic AMPA1/AMPA-2 Receptor*

AMPA receptors are activated upon the binding of β-amyloid. AMPA allows the influx of Ca^{2+} ions, which leads to increase in intracellular concentration of Ca^{2+} in cytosol and mitochondrial membrane. When the mitochondrial membrane is flooded with Ca^{2+}, it leads to dysregulation of mitochondria and causes programmed cell death by caspase-dependent and caspase-independent pathways. Studies have reported that the AMPA antagonists also reduce the dysregulation of neurons (Gasparini et al. 2013).

5.3 *β-Amyloid A4 Precursor Binding Protein (APP)*

The APP is sequentially hydrolyzed by β and γ secretases, which leads to the generation of β-amyloid. The excessive deposition of β-amyloid leads to the formation of amyloid plaques and causes AD (Hoozemans et al. 2008).

5.4 *Beta-Secretase (BACE1)*

It is commonly known as β-site amyloid precursor protein cleaving enzyme 1. It promotes the production of β-amyloid with the help of γ-secretase, which leads to initialization and breakdown of the extracellular domain of APP. Several promising BACE1 inhibitors are emerging as potential drugs for the prevention and treatment of AD (Chowdhury and Kumar 2020).

5.5 Butyrylcholinesterase

ACHE and BCHE, both neurotransmitters, are present in the postsynaptic region of neurons in every healthy brain. The contribution of BCHE in maintaining the concentration of ACH is comparatively less than ACHE. The major difference between ACHE and BCHE is that in case of AD the level of ACHE remains constant or decreases, but the level of BCHE increases. Some studies showed that BCHE also contributes to the decomposition of β-amyloid plaques, which occurs in a very early stage (Ejaz et al. 2022).

5.6 Cyclin-Dependent Kinase-5 (CDK5)

It is one of the unique members of cyclin-dependent kinases, which contribute to the initiation of AD. It plays a vital role in the development of the central nervous system along with neuronal migration, differentiation, synaptic functions, and memory consolidation. The pathological conditions of AD hyperactivate the CDK5 receptors, which triggers hyperphosphorylation of APP and tau proteins. This hyperphosphorylation leads to the formation of β-amyloid plaques and causes AD (Xie et al. 2017).

5.7 Mono Amino Oxidase-B (MAO-B)

MAO-B is associated with the synthesis of γ-aminobutyric acid (GABA). The GABA is produced by reactive astrocytes, and its release inhibits the synaptic transport and reduces the spike probability, which leads to loss of memory function and causes AD. The irreversible MAO-B inhibitors are used to cure AD, which completely restores learning and memory function (Rullo et al. 2019).

5.8 Glutamate N-Methyl D-Aspartate (NMDA)

It is one of the most abundant excitatory neurotransmitters present in the central nervous system of humans. The balanced quantity and signaling of NMDA are essential for healthy neuronal function. Excessive stimulation of glutamatergic signaling leads to excitotoxicity or nerve cell death. Several studies also suggest that excitotoxicity occurs due to excess accumulation of Ca^{2+} in the intracellular portion of neurons, which ultimately leads to the initiation of AD (Hansen et al. 2018).

5.9 Phosphodiesterase Type 4A/Type 4B/Type 9A (PDE4A/PDE4B/PDE9A)

The phosphodiesterase breakdowns the 3′-phosphodiester bonds in cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) and forms 5′-cyclic nucleotides. It regulates the concentration of cAMP and cGMP to perform various pharmacological activities for proper cell functioning. Several studies suggested that abnormal cAMP signaling can cause AD (García-Osta et al. 2012).

5.10 Tumor Necrosis Factor Alpha (TNF-α)

TNF-α is widely known for its antitumor along with anti-inflammatory activity. The presence of TNF-α around the β-amyloid in postmortem of human AD brains confirms that the binding of TNF-α with TNFR1 is required for the apoptosis of neurons. The concentration of TNFR1 proteins is comparatively higher in AD than in non-demented brains (Chang et al. 2017).

5.11 5-Hydroxytryptamine 4 (5HT4)

5HT4 is a G-protein-coupled receptor, when activated it modulates neurotransmission along with enhancement in the release of acetylcholine, which aids in memory formation. In several cellular and animal models when administrated with 5HT4 agonists, the release of soluble α-amyloid increases several folds, whereas the decrease in β-amyloid secretion was noticed. In some studies, 5HT4 agonists are also mentioned for inducing neurogenesis in the hippocampus along with the enteric system by activation of cyclic AMP (Lalut et al. 2017).

5.12 Myeloperoxidase (MPO)

MPO is the most abundant protein present in neutrophils. It is a major mediator of neutrophil oxidative outbursts. It catalyzes the formation of numerous oxidative substances. Some studies reported higher concentration of MPO in the plasma along with immunoreactive cells in the brain of the patient suffered from AD (Volkman et al. 2019).

5.13 Gamma-Secretase

It is a type of protein-digesting enzyme that breaks down the transmembrane domain of APP and aids in the formation of β-amyloid protein, which is the main protein component that gets deposited in the brain and causes AD. It is considered as one of the main targets in developing anti-Alzheimer therapeutics (Hur 2022).

5.14 Sigma 1 Receptor

Sigma 1 receptor is one of the emerging and potential targets for the development of anti-Alzheimer therapeutics. It is a type of opioid receptor and is present abundantly in the central nervous system. The sigma 1 receptor can affect the TCA cycle and hence protect the neurons from oxidative stress. Studies have confirmed the decreased concentration of sigma 1 receptors in AD patients, and also, an agonist for the same receptor provides some positive results in AD patients (Kargbo 2021).

5.15 Pyruvate Dehydrogenase Kinase

The transcription of pyruvate dehydrogenase kinase is induced by the hypoxia-inducible factor 1α subunits (HIF-1α). Its main motive is to decrease mitochondrial oxygen consumption, which is achieved by phosphorylating and inhibiting pyruvate dehydrogenase. The same blocks the conversion of pyruvate to acetyl Co-A as a result of which the supply of pyruvate for the TCA cycle is stopped. Hence, the mitochondrial consumption of oxygen decreases, which decreases oxidative stress, one of the potential causes of AD. Some recent studies showed comparatively less concentration of pyruvate dehydrogenase kinase in postmortem of cortical tissue from AD patient to control patient brain sample (Newington et al. 2012).

5.16 Cyclophilin D

Cyclophilin D is among one of the well-studied cyclophilins and is an integral portion of the mitochondrial permeability transition pore. The opening of cyclophilin D leads to cell death. Studies have shown that the cortical mitochondria, which lack cyclophilin D, are resistant to β-amyloid protein. The unavailability of cyclophilin D also confirms the protection of the neuron from β-amyloid deposition and also from oxidative stress, which leads to cell death (Du et al. 2008).

5.17 PPAR-γ

It is a type of ligand-activated nuclear receptor that coordinates the metabolism of lipid and glucose. The concentration of PPAR-γ is comparatively high in the AD brain. Such types of receptors can improve inflammatory responses. Some recent studies on animal models confirmed the decrease in β-amyloid plaque deposition on the administration of PPAR-γ agonist (Jiang et al. 2008).

5.18 Cholesterol Acyltransferase

It is also known as acyl cholesterol acyltransferase (ACAT) or sterol O-acyltransferase. It plays a vital role in cellular cholesterol homeostasis and forms cholesteryl esters from free cholesterols by transferring the fatty acyl group of fatty acyl-CoA to the 3β-hydroxy moiety of cholesterol. Some recent studies showed that blocking ACAT leads to significant positive results on AD (Shibuya et al. 2015).

5.19 HMG–CoA Reductase

It is chemically 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase. It is one of the universal pathways present in almost all living entities, which is responsible for the synthesis of lipophilic molecules such as cholesterol. The increased level of intracellular cholesterol leads to the deposition of abnormal β-amyloid, which initiates the primary symptoms of AD (Zipp et al. 2007).

5.20 12/15-Lipoxygenase

The increased oxidative stress may be one of the potential causes of AD. 12/15 LOX is considered as major source of causing oxidative stress. 12/15 LOX is supposed to play a key role in oxidizing polyunsaturated fatty acids, which leads to the formation of hydroperoxyacids, which behave as pro-oxidant mediators. The concentration of 12/15 LOX along with its metabolites was significantly high in AD-affected brains in comparison with normal brains (Praticò et al. 2004).

5.21 α7-Nicotinic Acetylcholine Receptors (α7-nACHR)

Some studies suggested that the activation of nicotinic acetylcholine receptors provides neuroprotection. This neuroprotection is necessary for the maintenance of the integrity of cholinergic neurons. Also, increased β-amyloid concentration inhibits the nACHR function, which amplifies the β-amyloid toxicity. Some recent studies conclude that the interaction of nACHR and β-amyloid performs maintenance of normal physiology and induces molecular etiology of AD (Caterina and Kelly 2012).

5.22 Glycogen Synthase Kinase 3 Beta (GSK-3β)

GSK-3β is a kinase whose main function is the phosphorylation of serine and threonine sites of different substrates. It can be given a name of master key among all AD targets as a disturbed concentration of GSK-3β can influence all other major pathophysiological parameters of AD such as phosphorylation of tau protein, production of β-amyloid, neurogenesis, memory, and synaptic functions (Balaraman et al. 2006).

5.23 Gamma-Aminobutyric Acid A/B Receptor (GABA-A/GABA-B)

The GABA-A receptors are involved in the neuroprotection against β-amyloid-induced toxicity and excitotoxicity. Considering this as a base the activation of GABA-A receptors could be developed as a potential therapeutic approach in not only AD but also other neurodegenerative disorders in which excitotoxicity plays a crucial role (Louzada et al. 2004).

5.24 Catechol O-Methyltransferase (COMT)

The main function of COMT is to transfer a methyl group from S-adenosylmethionine to the benzene ring of some catecholamine neurotransmitters like dopamine, epinephrine, and norepinephrine, which results in the complete degradation of catecholamines. Besides this mechanism, COMT also serves a major role in the degradation of dopamine in the brain. Some studies showed the level of dopamine in AD patient got impaired. Catechol estrogen is also a substrate of COMT. Some allelic variant of COMT modulates estrogen level in human

samples, which dysregulates the estrogen metabolites, which are majorly implicated in AD (Perkovic et al. 2018).

5.25 Cyclooxygenase-2 (COX-2)

COX is considered as rate-limiting enzyme needed for the production of prostaglandins. COX-2 is considered as inducible form of COX, which is generally responsible for inflammatory responses, but some studies show that it is also responsible for some neuronal functions. The concentration of hippocampal COX-2-immunopositive neurons was comparatively low in the brain of AD patient. Also, the non-selective COX inhibitors give better result in influencing β-amyloid in comparison with COX-2 (Xiang et al. 2002).

5.26 Histamine Receptor H3 (H3R)

H3R antagonist has been used in the treatment of various diseases related to the central nervous system including AD. The central histaminergic fibers have their origin from the tuberomammillary nucleus present in the posterior hypothalamus and have their branches in different portions of the brain including cerebral cortex, amygdale, and hippocampus. Hence, histamine is majorly related to a large number of physiological functions such as cognition, memory, and learning (Vohora and Bhowmik 2012).

5.27 Heat Shock Proteins

Some in vitro studies showed that HSP60 prevents β-amyloid accumulation by closing the pathway at the molecular level, which causes fibrillogenesis of peptides. So, the production of more HSP60 may be work as a potential drug. Even the role of the HSP is quite controversial (Campanella et al. 2018).

5.28 Inducible Nitric Oxide Synthase (iNOS)

The increased concentration of iNOS leads to an increase in the level of NO, and an increased level of NO induces the pathophysiology of many complex multifactorial disorders including AD. The inhibition of iNOS is considered as one of the potential therapeutic solutions for AD (Minhas et al. 2020).

5.29 Nuclear Factor Kappa-B Kinase Alpha/Beta (IKK α/β)

The nuclear factor kappa-B kinase plays a crucial role in the induction of AD. Any damage to this leads to undesirable phenotypic changes, which include neuroinflammation, activation of microglia and oxidative stress, and programmed cell death. Studies have shown that NF-kappa-B signaling pathway has its involvement in brain functioning, especially synaptic plasticity and balancing between learning and memory. Any impairment to this pathway may alter the neuronal dynamic, which can induce many neurodegenerative disorders including AD (Jha et al. 2019).

5.30 Mitogen-Activated Protein Kinase 14 (MAPK p38α)

Some researchers have reported that the concentration of MAPK p38α is low in healthy human brain when compared to demented brain. This low concentration confirms the degradation of BACE1 along with autophagic flux. In AD, the impairment of autophagic lysosomal degradation is seen. The increased level of MAPK p38α causes a further decrease in autophagic flux and an increased level of BACE1 and β-amyloid plaque formation, which induce AD (Alam and Scheper 2016).

5.31 Adenosine Receptor A2A

A2A is a member of purinergic receptor family, which plays a vital role in the pathophysiology of various types of neurodegenerative disorders including AD. Among the family of adenosine receptors, the most promising target is the A2A subtype (Merighi et al. 2022b). In the brain, it is located at pre-synaptic and post-synaptic levels in cortex, striatum, and hippocampus, and effects of glutamate release, microglia, and activation of astrocytes lead to various neurodegenerative disorders including AD. Some in vivo and in vitro studies show that A2A adenosine receptor antagonist can be used as a solution for AD (Merighi et al. 2022a).

5.32 Microtubule-Associated Protein Tau (MAPT)

MAPT is a type of tau protein, which is found in the healthy brain and is associated with axonal transmission, assembly, and stabilization of microtubules. In some pathological situations, MAPT becomes hyperphosphorylated. The formation of

aggregates in neurofibrillary tangles can be considered as one of the main causes of AD (Jayapalan and Natarajan 2013).

5.33 Muscarinic M1/M2 Receptor

Muscarinic receptors are the G-protein-coupled receptors (GPCRs) that mediate acetylcholine-induced neurotransmitter. There are five subtypes of muscarinic receptor (M1-M5). Among these, M1 and M2 were postulated to be the most promising therapeutic targets for AD. M1 receptor signals through Gq/11 G protein and M2 signals through Gi/o G protein. M1 microglia works at the injured site at the end stage of AD, when M2 microglia fails to repair the same. M1 and M2 receptor agonists could be more efficacious in the treatment of AD (Lebois et al. 2018; Avery et al. 1997).

5.34 Metabotropic Glutamate Receptor 2/Receptor 3 (mGLU2/mGLU3)

Metabotropic glutamate receptors may have a vital contribution to the pathogenesis of AD as the various groups of mGLU regulate neuronal cell death and survival. Some studies suggested that activation of mGLU receptors protects neurons from excitotoxic degeneration by inhibition of glutamate release. Some researchers reported that the potent and selective mGLU2 agonists provide protection against excitotoxicity in vitro. The involvement of mGLUR2 in the pathogenesis of neuronal cell death and survival indicates that it could play a role in the pathogenesis of AD (Lee et al. 2004).

5.35 Insulin-Degrading Enzyme (IDE)

IDE plays a vital role of cleaning the soluble β-amyloid both extracellularly and intracellularly and also effluxes the extracellular β-amyloid into the blood through blood–brain barrier. The kinetics of β-amyloid formation dominantly depends on the concentration of IDE and hence the decrease in the concentration of IDE, either due to age or other pathological conditions can induce AD. The level of somatostatin can also be considered as one of the potential factors for affecting the kinetics of β-amyloid formation and triggering AD as it stimulates the IDE expression and its secretion in microglia (Kurochkin et al. 2018).

5.36 Mitogen-Activated Protein Kinase 8/Kinase 9/Kinase 10 (JNK1/2/3)

The MAPK pathway includes the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 pathways. Researchers have reported that all the mentioned pathways are found active in vulnerable neurons in people suffering from AD, which marks the involvement of MAPK in the pathology and pathogenesis of AD. Some studies show that the MAPK pathways get activated by many extracellular and intracellular stimuli, which include peptide growth factors, cytokines, hormones, and various cellular stressors including oxidative stress. A variety of cellular activities are under regulation of MAPK signaling pathway including proliferation, differentiation, survival, and death so any deviation from the pathway may trigger various neurodegenerative disorders including AD (Kim and Choi 2010).

6 In Silico Studies on Different AD Targets

AD etiology is still unknown and complex, and various types of targets are associated, which are responsible to cause AD, as mentioned in the previous section. Table 1 comprises published data regarding in silico studies of one decade (2010–2022) associated with AD of various targets collected from PubMed database (PubMed, National Library of Medicine 2023).

7 Target Fishing

A new method being utilized in drug development is called "*in silico* target fishing," which aims to find potential protein targets for a query molecule. Using this approach, it is possible to clarify the biological processes and mechanism of action of substances whose goal is not yet known (Jenkins et al. 2006). Target fishing can also be used to identify drug candidate off-targets, allowing for the recognition and prevention of any potential side effects. Because of these factors, target fishing has grown to be a crucial strategy for polypharmacology, drug repurposing, and the discovery of new therapeutic targets (Patel et al. 2015). An in silico approach can be quicker, less expensive, more effective for particular protein structures, and thus simpler to use. Experimental target fishing, on the other hand, can be time-consuming and challenging to implement because of the multitude of interactions that may occur for a single small molecule with different protein targets (Ciriaco et al. 2022). Target fishing is a more enticing approach to drug development due to

Table 1 Different targets associated with AD and their in silico studies

Sl. No.	Target	Location	Total in silico studies	Total hit compounds
1.	Acetylcholinesterase (ACHE)	Muscles and neurons	256	478
2.	Glutamate receptor ionotropic AMPA1 receptor (AMPA-1)	CNS neuronal brain	–	–
3.	Glutamate receptor ionotropic AMPA2 receptor (AMPA-2)	CNS, hippocampus outer layer of cortex	–	–
4.	Amyloid beta A4 precursor-binding protein (APP)	Neurons	59	44
5.	Beta-secretase (BACE1)	Pancreas, title in glial cell in brain	107	129
6.	Butyrylcholinesterase (BCHE)	A-glycoprotein synthesized in the liver	155	142
7.	Cyclin-dependent kinase-5 (CDK5)	Post-mitotic central nervous system (CNS)	4	2
8.	Catechol O-methyltransferase (COMT)	Liver, kidney, and nerve cell in brain	1	1
9.	Cyclooxygenase-2 (COX-2)	Stomach and parenchymal cells	11	13
10.	Gamma-aminobutyric acid A receptor (GABA-A)	Your brain and spinal cord, mammalian CNS	–	–
11.	Gamma-aminobutyric acid B receptor (GABA-B)	Brain, hippocampus, thalamus, basal ganglia, hypothalamus	–	–
12.	Glycogen synthase kinase 3 beta (GSK-3β)	Skeletal muscle	13	32
13.	Histamine receptor H3 (H3R)	Pre- and post-sympathetic nerve and blood vessels and heart	2	3
14.	Heat shock protein 90 (HSP 90)	Chromosome 14	1	1
15.	Inducible nitric oxide synthase (iNOS)	Primarily expressed in the brain and neuronal tissue	4	5
16.	Nuclear factor kappa-B kinase alpha (IKK α)	Epithelial cells squamous cell	1	1
17.	Nuclear factor kappa-B kinase beta (IKK β)	Epithelial cells squamous cell	2	16
18.	Mitogen-activated protein kinase 8 (JNK1)	MAPK8 gene, chromosome 10	–	–
19.	Mitogen-activated protein kinase 9 (JNK2)	Chromosome 11	–	–

(continued)

Table 1 (continued)

Sl. No.	Target	Location	Total in silico studies	Total hit compounds
20.	Mitogen-activated protein kinase 10 (JNK3)	Gene/chromosome −5	2	2
21.	Monoamine oxidase-B (MAO-B)	Serotoninergic neurons and on the outer mitochondrial membrane of astrocytes	27	44
22.	Mitogen-activated protein kinase 14 (MAPK p38α)	Many cell types, brain in lungs	1	1
23.	Microtubule-associated protein tau (MAPT)	Neurons and dendrites and exons	43	54
24.	Metabotropic glutamate receptor 2 (mGLU2)	Brain	–	–
25.	Metabotropic glutamate receptor 3 (mGLU3)	Both pre- and post-synaptic elements in neurons	–	–
26.	Glutamate (NMDA) receptor (NMDA)	Neurons system synaptic trans. In brain spinal cord	15	45
27.	Phosphodiesterase type 4A (PDE4A)	Lungs, smooth muscles, epithelial cell	1	1
28.	Phosphodiesterase type 4B (PDE4B)	Immune cells, epithelial cells, and brain cells	1	1
29.	Phosphodiesterase type 9A (PDE9A)	Lower urinary tract	1	2
30.	Tumor necrosis factor alpha (TNF α)	Macrophages and limited quantities by B cells	18	16
31.	5-hydroxytryptamine 1A (5HT1A)	Brain, presynaptic autoreceptors	–	–
32.	5-hydroxytryptamine 2A (5HT2A)	Dorsal horn of the spinal cord	–	–
33.	5-hydroxytryptamine 3A (5HT3A)	Both PNS and CNS	–	–
34.	5-hydroxytryptamine 4 (5HT4)	Gut	6	3
35.	5-hydroxytryptamine 6 (5HT6)	Hippocampus nucleus	1	1
36.	Myeloperoxidase	Immune cells, neutrophilic, leukocytes and lymphocytes[1,2], monocytes	6	6
37.	Insulin-degrading enzyme	Endosomes and plasma membrane; it can also occur in the cytosol(1)	3	4
38.	Gamma-secretase	Subcellular compartments, such as the ER, ER-Golgi, TGN, endosomes, and plasma membrane	29	64
39.	Adenosine receptor A2A	Brain	1	1

(continued)

Table 1 (continued)

Sl. No.	Target	Location	Total in silico studies	Total hit compounds
40.	Sigma 1 receptor	Deeper laminae of cortex, Purkinje cells in brain, endoplasmic reticulum	–	–
41.	Muscarinic M1 receptor	Cerebral cortex, gastric, and salivary glands.	–	–
42.	α4-nACHR	Peripheral and CNS	–	–
43.	α7-nACHR	Cholinergic nerve system in the brain	3	15
44.	Muscarinic M2 receptor	Smooth muscles and cardiac tissue	–	–
45.	PPIase pin 1	Neurons	–	–
46.	Pyruvate dehydrogenase kinase	Mitochondria	–	–
47.	Cyclophilin D	Mitochondria, ER	1	31
48.	PPAR-gamma	Heart, muscle, colon, kidney, pancreas, and spleen	1	52
49.	Cholesterol acyltransferase	Lipoprotein	–	–
50.	HMG–CoA reductase	ER, long-terminal domain in the cytosol	1	1
51.	12-lipoxygenase	Brain, hypoglossal nerve	–	–

the potential to combine it with docking and virtual screening studies and the growing number of Web-based tools that have recently been developed (Cereto-Massagué et al. 2015). This method enables the evaluation of potential polypharmacology and drug repurposing applications, the prediction of side effects, and the determination of the mechanism of action of the bioactive molecule in addition to the prospective drug–target interaction and its mechanism of action (Wang et al. 2013; Shahid et al. 2021). Depending on the type of information used, the computational approaches used in target fishing can be divided into two groups: ligand-based and receptor-based methods (Fig. 5).

In large-scale virtual screening, ligand-based methods are preferable to structure-based methods due to their lower processing requirements, more flexibility, and increased potential for machine learning. When a molecule has a considerable degree of resemblance to compounds that are already known, ligand-based techniques are unquestionably preferred. Contrarily, the advantage of receptor-based approaches, like docking-based methods, is that they can be used to anticipate compounds that represent newly undiscovered chemical space. In addition, the ligand-based technique enables its use without the need for structural understanding of the target receptors, in contrast to receptor-based approaches that demand structural knowledge of prospective receptor targets (Sánchez-Cruz and Medina-Franco 2021).

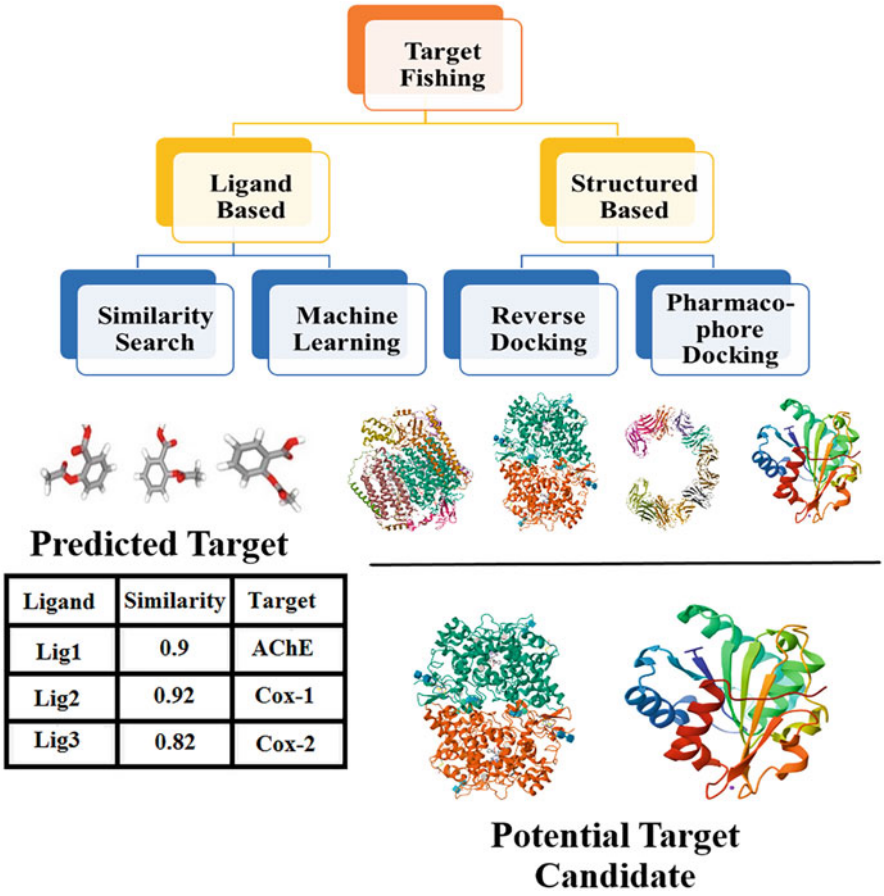

Fig. 5 Different in silico approaches used for target fishing

7.1 Ligand-Based Target Fishing

Due to their independence from the availability of protein structures and the ease of their implementation, ligand-based techniques are frequently utilized in conventional target fishing strategies. As a result, even novice users may apply these techniques, which typically produce accurate findings for SAR analysis and activity forecasts (Rognan 2010; Maggiora et al. 2014). The rationale for this tactic is founded on the similarity principle, which holds that two molecules with similar structural patterns might interact with similar targets by sharing similar bioactivities (Wale and Karypis 2009). For this reason, in order to carry out a ligand-based reverse screening, ligand databases with activity annotation on known targets are required. Target–compound interactions reported on publicly accessible databases like ChEMBL (Papadatos et al. 2015) and PubChem (Wang et al. 2017) and

Table 2 Different Web tools currently used for ligand-based target fishing

Web tool	Description	References
SwissTargetPrediction	Similarity in both 2D and 3D to known ligands	Daina et al. (2017)
CSNAP3D	A network algorithm for measuring 3D chemical similarity	Lo et al. (2015)
MolTarPred	Search for 2D similarities using ECFP4 fingerprints	Peón et al. (2019)
TargetHunter	2D similarity search based on ECFP6 fingerprints	Wang et al. (2013)
TarPred	Searching for molecules with a KNN-based fusion score	Liu et al. (2015)

information about FDA-approved drugs or drug candidates like those in the freely available Drug bank (Wishart et al. 2018) and Therapeutic Target Database (TTD) (Li et al. 2018; Lomelino et al. 2018) have become easier to find in recent years due to the increased availability of chemical data. Different Web tools available currently for ligand-based target fishing are available in Table 2.

7.2 Receptor-Based Target Fishing

In receptor-based methods, ligand–target pairs are predicted using knowledge of the protein structure. These approaches forecast not only the potential off-targets of small molecules but also their potential binding patterns inside the receptors, which are essential for comprehending their mode of action and logically developing selective compounds (Xie et al. 2011). Reverse docking and target fishing using pharmacophores are the two main techniques used. Three-dimensional (3D) protein structures are necessary for receptor-based methods. While docking-based target prediction only requires the 3D structure of the target and the active site location, which can be determined by a co-crystallized ligand or through pocket identification algorithms, receptor-based pharmacophore searches require at least one reference co-crystallized complex as input (Rognan 2010; Schomburg et al. 2014). Table 3 lists the many receptor-based target fishing Web tools.

8 Target Prioritization

Target prioritization is a most essential step in any drug discovery, especially in the field of complex disorders like cancer and neurodegenerative diseases (Matore et al. 2022a; Jellinger 1998). The pathophysiology of AD is still not clear that is why researchers are continuously finding new drug targets to get promising drug

Table 3 Different Web tools currently used for receptor-based target fishing

Web tool	Description	References
idTarget	Reverse docking approach based on divide-and-conquer method and using all protein structures in the PDB	Wang et al. (2012)
TarFisDock	Reverse docking using the potential drug target database (PDTD)	Li et al. (2006)
DPDR-CPI	Reverse docking using proteins from PDB and PDBBind, combined with ML models for target predictions	Luo et al. (2016)
ACID	Reverse docking with an automated consensus inverse docking protocol	Wang et al. (2019)
Drug ReposER	Pharmacophore approACH based on sub-structural similarity to the binding interfaces of known drug binding sites	Ab Ghani et al. (2019)
LigAdvisor	2D ligand–receptor interactions based on similarity estimations	Pinzi et al. (2021)
PLIP	Binding site alignment or similarity among molecular structures and residue sequences	Salentin et al. (2015)
PharmMapper	Target identification based on pharmacophore mapping procedure	Liu et al. (2010)

candidate (Jayadev 2022; Scheltens et al. 2016). To date, more than fifty AD-associated targets were identified, but since two decades not a single drug molecule was approved. The one probable reason behind this failure is an inappropriate target selection. Due to this, it is a very essential and difficult task to prioritize a promising target on the principle of "one disease"–"one target"–"one drug" (Velmurugan et al. 2020). It is harder to prioritize and select one single target for disease like AD. Most of the scientists are focused on one of the targets of ACHE, BCHE, and BACE1, to avoid this kind of failure. Based on the literature data, we prioritized some promising targets that could be useful for future AD drug design. The prioritized targets are shown in Fig. 6.

The discovery of novel drugs for AD is going in three ways:

- Discovery of single-targeted or selective inhibitors.
- Discovery of dual-targeted inhibitors.
- Discovery of multi-targeted inhibitors.

8.1 Prioritized Targets for Discovery of Single-Targeted or Selective Inhibitors

The research group in this category is working on NMDA, APP, β-amyloid, etc. Due to the availability of several promising targets, researchers are adopting in silico techniques to prioritize single targets (Matore et al. 2022b). Due to the complex pathology of AD, this approach is not appropriate to develop efficient drug.

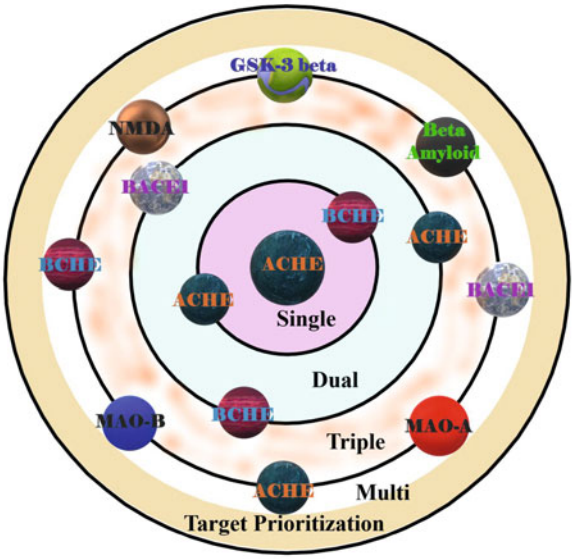

Fig. 6 Prioritized targets for single, dual, triple, and multi-targeted drug design for AD

8.2 Prioritized Targets for Discovery of Dual-Targeted Inhibitors

After the failure of a single-targeted approach, researchers were shifted toward the designing of dual inhibitors (Knez et al. 2017). The dual inhibitors aim to target AD pathogenesis pathway in two ways (Agatonovic-Kustrin et al. 2018; Carradori et al. 2018). These approaches also have difficulty in choosing targets. The literature suggested the use of cholinesterase (CHE); i.e., ACHE-BCHE combination is promising for designing dual inhibitors. There is one combination of CHE and BACE1 (ACHE-BACE1 or BCHE-BACE1). Another promising approach is the use of CHE and MAO (ACHE-MAO-A, ACHE-MAO-B or BCHE-MAO-A, BCHE-MAO-B). Apart from this use of ACHE-APP, ACHE-GSK-3β, ACHE-NMDA, BACE1-APP, BCHE-GSK-3β, BCHE-NMDA, and BACE1-NMDA are the promising targets. In multi-target drug design, there is the use of four or more targets that are also adopted. In such cases, choosing targets from the abovementioned dual or triple combinations will be beneficial. The use of two different dual-target or triple-target combination is promising in multi-targeted drug design.

8.3 Prioritized Targets for Discovery of Multi-Targeted Inhibitors

In the current scenario, designing multi-target inhibitors is a novel and promising approach to tackle complex disorder like cancer and AD, but the prioritization and selection of appropriate targets are still challenging (Hopkins 2008; Makhoba et al. 2020; Ma et al. 2010). In this case, the inverse docking and inverse pharmacophore are the most suitable and promising in silico approaches (Furlan et al. 2018; Schomburg et al. 2014). Based on the different literature studies and our understanding, we prioritized some target combinations that could be useful while designing multi-targeted drug for AD. The first combination is the use of three targets ACHE-BCHE-BACE1. Other triple target-based combinations are ACHE-BACE1-AAP, ACHE-BACE1-NMDA, ACHE-BACE1-GSK-3β, ACHE-BACE1-MAO-A, ACHE-BACE1-MAO-B, BCHE-BACE1-AAP, BCHE-BACE1-NMDA, BCHE-BACE1-GSK-3β, BCHE-BACE1-MAO-A, BCHE-BACE1-MAO-B, ACHE-BCHE-COLQ, etc.

8.4 In Silico Target Prioritization

Nowadays, the toughest task of target prioritization is made easy by different in silico tools and techniques (Baig et al. 2017). Along with the software packages, there are a number of freely available Web servers that can accurately predict the target affinity and prioritize accordingly. Molecular docking (inverse docking) and pharmacophore modeling (inverse pharmacophore) are still novel approaches (Furlan et al. 2018; Schomburg et al. 2014). There are some in silico studies listed below that show the prioritization of targets for AD.

Yu Jeong Shim et al. (2022) prioritized a single common gene target for a very rare disorder cerebral adrenoleukodystrophy and a very common neurodegenerative disorder, i.e., AD by using in silico approach. They used gene set enrichment analysis and weighted gene correlation network analysis for the identification of novel therapeutic target. They proposed four novel targets that are CD44 molecule, fibroblast growth factor 2, annexin A5, and beta-2-microglobulin. These targets are novel and could be useful in the further discovery of anti-AD drugs (Shim et al. 2022).

Parmi Patel et al. (2022) performed molecular docking of the antiviral drug (valacyclovir) against the different AD-associated targets, which are acetylcholinesterase (ACHE), amyloid beta, phosphorylated-tau, butyrylcholinesterase (BCHE), and beta-secretase 1 (BACE-1). Upon the analysis of docking scores and interaction, they found that ACHE is a most promising target for valacyclovir. The in silico results were validated by performing in vivo experiments, and both the results were correlated. This study suggests the use of ACHE target for the development and repurposing of antiviral drugs against AD (Patel et al. 2022).

Jessica Binder et al. (2022) developed a machine learning (ML)-based model to find out potential gene target for AD. Upon final screening and analysis of results, they prioritized five genes, which are highly associated with AD such as FRRS1, CTRAM, SCGB3A1, FAM92B/CIBAR2, and TMEFF2. These are the newly reported gene targets that can be useful in combinations with some existing targets ACHE or BCHE or BACE1 to get new combinations for multi-target drug design (MTDD) (Binder et al. 2022).

Muhammad Ayaz et al. (2022) performed a molecular docking study to prioritize the potential target for AD. In this study, they performed docking of different compound sets against the three targets of AD, which are BACE1, MAO-A, and MAO-B. The docking results suggested that the BACE1 is the most potential target for the given set of compounds (Ayaz et al. 2022).

Karim Raafat (2020) has prioritized four potential targets for AD by applying in silico network pharmacology approach. In this study, they utilized 6842 phytochemical compounds isolated from North African plant open database. From this study, they prioritized MAO-B, HMG-CoA, BACE1, and GCR target for AD. This target combination is novel and could be promising for MTDD (Raafat 2020).

Yan Hu et al. (2019) developed a predictive model to identify the potential drugs and targets for AD. The different machine learning algorithms were used to prioritize the drugs and targets for AD. Upon the ROC analysis and random forest prediction, they suggested the following order: ACHE, BCHE, MAO-B, BACE1, and Tau protein, respectively (Hu et al. 2019).

Chaoyun Li et al. (2019) performed an inverse docking study to prioritize the essential targets and potential compounds from the natural polyphenols for AD. They used different potential targets and compounds present within the KEGG AD pathway. Initially, they performed docking of selected compounds to seven protein targets. Upon the analysis of docking results, they prioritized APP and BACE, which are the promising targets. Finally, selected compounds were screened for in vivo potential and to validate the prioritized targets. The prioritized APP-BACE combination could be a promising dual target for design novel drug candidates against AD (Li et al. 2019).

Katarina Kores et al. (2019) performed an inverse docking for the resveratrol by using the CANDOCK algorithm. They collected approximately 38,000 protein structures from the RCSB PDB database and completed a docking study. They validated the docking method by performing the redocking of twenty-five cocrystal ligands in respective protein structures. Finally, they prioritized twenty human targets and five targets from other organisms for different diseases. Amongst the twenty targets, lysine-specific histone demethylase 1A and dynamin-1 are linked with AD and showed strong binding energy, i.e., –44.4614 and –42.0596 kcal/mol, respectively. This study suggested another novel dual-target combination for future drug design against AD (Kores et al. 2019).

Veronika Furlan and coworkers (2018) developed a novel inverse docking approach to prioritize the potential AD targets for curcumin by using the CANDOCK algorithm. In this study, they collected 13,553 protein structures from the RCSB PDB database. The molecular docking of curcumin was performed

against all the selected proteins. The inverse docking results suggested that the 3,5-cyclic phosphodiesterase 4D and 17-β-hydroxysteroid dehydrogenase type 10 will be an efficient dual target for AD treatment (Furlan et al. 2018).

8.5 Target Prioritization Based on the US FDA-Approved Drugs

The discovery of novel drugs for the effective treatment of AD is going on since the last one century, and till date, only four drugs were approved by US FDA (Barthold et al. 2020). The prioritization of appropriate targets is essential and challenging. For the target prioritization, we analyzed the most common targets for all drugs, which will be helpful in further drug discovery (Oboudiyat et al. 2013). The drug donepezil acts on almost 10 gene targets, galantamine is active against 20 gene targets, memantine has almost eight gene targets, and rivastigmine is having only two gene targets. The details of gene targets with respect to drugs are given in Table 4. These data clearly indicate that the ACHE and BCHE are the most effective targets for AD drug discovery. As we see most of the approved drugs act on more than one target, so it is necessary to target multiple receptor to get a better efficient drug.

8.6 Target Prioritization Based on the Literature Evidence

In the current scenario, some researchers are working on the new target to get findings; some are working on existing targets, which are validated, and some are working on combined targets. We prioritized three well-existing and two new targets for AD drug discovery (Blaikie et al. 2019). To prioritize these targets, we analyzed

Table 4 Different US FDA-approved and associated gene targets for AD

Sl. No.	US FDA-approved drugs for AD	Gene target	Reference
1	Donepezil	ABCB1, *ACHE*, BACE1, *BCHE*, HRH3, HTR2A, KCNH2, MAOB, SIGMAR1, and SLC6A4	Zhou et al. (2021), Clevleand Clinic (2023)
2	Galantamine	*ACHE*, *BCHE*, CHRFAM7A, CHRNA1, CHRNA10, CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA9, CHRNB1, CHRNB2, CHRNB3, CHRNB4, CHRND, CHRNE, CHRNG, and IDH1	
3	Memantine	CHRFAM7A, DRD2, GRIN1, GRIN2A, GRIN2B, GRIN3A, HTR3A, and SLC22A1	
4	Rivastigmine	*ACHE* and *BCHE*	

the last one decade in silico studies against different AD targets. During the literature search, we find out 256 in silico studies against the ACHE, 155 studies against BCHE, and 109 studies against the β-secretase and these studies yielded 478, 142, and 129 potential hits. These three targets were prioritized amazing % of getting promising hit. The details of the targets and associated number of studies and hit compounds are given in Table 1.

8.7 *Target Prioritization for Dual- or Multi-Target Inhibitors*

Designing dual or multi-targeted inhibitors for complex disorders is a novel and prominent approach, but the selection of an appropriate combination of targets is a still challenging task (Michalska et al. 2017). The researchers must have to choose proper combination of two or more targets. We screened multi-target-based in silico studies of AD and prioritized one dual and one triple target. The ACHE and BCHE are a most promising dual target and validated by using different in silico approaches like molecular docking, pharmacophore modeling, and QSAR (Jyothi and Yellamma 2016; Lee and Jun 2019; Ma et al. 2010). Most of the studies are molecular docking-based, and very few are pharmacophore- and QSAR-based. One triple target combination was also prioritized as ACHE, BCHE, and BACE1. The combination of these three targets is effective as it blocks the major pathway of AD pathogenesis. Among the multi-target studies, ACHE-BCHE-BACE1 is widely used and has promising results. All the studies of this combination were carried out by using molecular docking. The details of target combinations, PDB IDs, and in silico techniques are reported in Table 5. Many other dual or triple- or multi-target reports were also available, but most of the study contains one or two or all three targets of table X with MAO-A (Youdim and Bakhle 2006; Khan et al. 2017; Hagenow et al. 2020), MAO-B (Reis et al. 2018; Chowdhury and Kumar 2020), APP (Binder et al. 2022), FAAH (Leuci et al. 2022), NMDA (Wang and Reddy 2017; Gazova et al. 2017), GSK-3β (Oukoloff et al. 2019; Mazumder and Choudhury 2019; Fronza et al. 2019), β-amyloid (Gazova et al. 2017), TNF-α (Yang et al. 2020b), COX1 and COX2 (AlFadly et al. 2019), etc.

8.8 *Target Prioritization-Based Brain-Specific Neighborhood Networks*

We analyzed multi-omics brain-specific neighborhood networks for the most promising prioritized targets ACHEAcetylcholine esterase (AChE), BCHE, and BACE1, which is available at AlzGPS (https://alzgps.lerner.ccf.org/) (Clevleand Clinic 2023). The multi-omics network gives the interconnecting gene network and disease-associated genes targets, which could be helpful in speedup drug discovery and

Table 5 Different target combination, in silico techniques, and PDB IDs for AD

Sl. no.	In silico technique	Target combination	PDB ID for target	Reference
1	Molecular docking	ACHE and BCHE	4EY4, 4TPK, 4BDT, 4BDS, 4EY6, 4BDS, 4EY7, 4XII, 4EY7, 4BDS, 4EY7, 4TPK, 4EY7, 4TPK, 4EY7, 4BDS, 2CKM, 1P0I, 4EY7, 1P0I, 5FPQ, 2Y1K, 4EY7, 4BDS, 1B41, 1P0I, 1B41, 1P0I, 1EVE, 1P0I, 4EY7, 4TPK, 4EY7, 4TPK, 4EY7, 4TPK, 4M0F, 4TPK	Sang et al. (2020), Lima et al. (2020), Sobolova et al. (2020), Riazimontazer et al. (2019), Kohelová et al. (2019), Ghamari et al. (2020), Zhu et al. (2018), Hepnarova et al. (2018), Czarnecka et al. (2018), Horton et al. (2017), Jyothi and Yellamma (2016), Korabecny et al. (2015), González-Naranjo et al. (2014), Spilovska et al. (2013)), Yang et al. (2020a), Gao et al. (2021), Lamie et al. (2022), Kilic et al. (2023)
	Pharmacophore modeling			Yıldız et al. (2022), Dhamodharan and Mohan (2022), Wu et al. (2022), Adeowo et al. (2022), Lakra et al. (2022)
	QSAR modeling			Komatović et al. (2022), Ejaz et al. (2022), Işık et al. (2022)
2	Molecular docking	ACHE, BCHE, and BACE1	4EY6, 1P0M, 4D8C, 4EY7, 4TPK, 5I3V, 1EVE, 1P0I, 1TQF, 4M0E, 1P0M, 4ZSR, 1EVE, 1P0I, 4D8C, 4PQE, 1P0I, 2WJO, 1ACJ, 4BDS, 1 W51	Kumar et al. (2020), Haghighijoo et al. (2020), Panche et al. (2019), Yu et al. (2020), Panek et al. (2018), Lee et al. (2018), Fereidoonnezhad et al. (2018)

development (Baloni et al. 2022). Upon analyzing this network, we suggest that APPAmyloid precursor protein (APP) and COLQ target could be beneficial while prioritizing target for multi-target drug design. APPAmyloid precursor protein (APP) is showing an associated network with ACHE and BACE1, which are highly prioritized targets. This suggests that APPAmyloid precursor protein (APP) could be used as the third target along with ACHE and BACE1 for multi-target drug discovery. Another one gene is COLQ, which showed a commonly associated network with ACHE and BCHE, and it could be used as another target in combination with ACHE and BCHE to get promising multi-targeted drugs (Fig. 7).

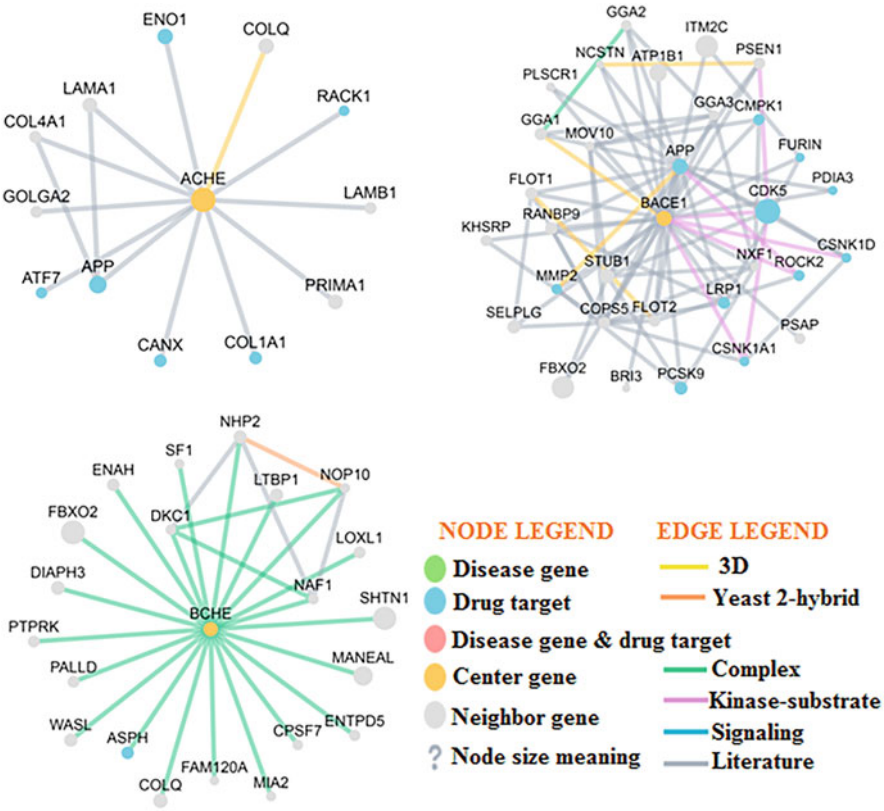

Fig. 7 Multi-omics brain-specific neighborhood networks of ACHE, BCHE, and BACE1

9 Conclusion

In this chapter, we attempted to highlight the importance of target fishing in the context of AD. The initial discussions were made on the causes and risk factors of AD, different targets associated with the pathogenesis of AD, and different types of AD. The next sections explained target fishing, target prioritization, in silico tools or approaches, application of in silico technique, and their importance in the discovery of anti-Alzheimer drug discovery. The exact pathophysiology of AD is still unknown, but till date more than fifty targets were identified. Due to this, target prioritization and selection of appropriate targets are very challenging. We tried to address various in silico tools or approaches that can be useful to overcome this kind of challenge. The structure-based inverse docking and inverse pharmacophore have numerous advantages and are promising approaches for target fishing. The ligand-based pharmacophore and QSAR approaches are less useful as they required

quantitative data. We also discussed the adopted different approaches to prioritize promising targets for AD. The first section described different targets associated with US FDA-approved drugs and identified two common targets, i.e., ACHE and BCHE. The second section illustrated the prioritization of ACHE, BCHE, and BACE1 by analyzing in silico studies on different AD targets and their % of hit identification. Finally, the prioritization of APP and COLQ gene targets by studying multi-omics brain-specific neighborhood networks for ACHE, BCHE, and BACE1 was discussed. With respect to different approaches used for AD drug discovery, we suggested different target combination (Fig. 6). ACHE, ACHE-BCHE, ACHE-BCHE-BACE1, and ACHE-BCHE-BACE1-NMDA are the first choice targets for designing of single-, dual-, triple-, and multi-targeted inhibitors. In a multi-target approach, it is necessary to incorporate two of the prioritized targets to get promising anti-Alzheimer hits.

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Multi-Target-Directed Ligand Approach in Anti-Alzheimer's Drug Discovery

Vaishali M. Patil, Neeraj Masand, Vertika Gautam, Shikha Kaushik, and Dee Wu

Abstract Alzheimer's disease is a multifactorial neurodegenerative syndrome and has raised concern related to global health and economy. Numerous targets have been analyzed toward discovery and development of potential therapeutics. Some of the single-target-based Food and Drug Administration (FDA) approved drugs include donepezil, galantamine, rivastigmine, and memantine which can improve the patient condition but fail to completely cure the disease. Single-target therapeutics have limitations to cure the disease due to complicated pathogenesis and complex network formed by the associated signaling pathways. Thus, the multi-target-directed ligand (MTDL) approach has gained importance as the potential anti-Alzheimer's drugs having the advantages of synergistic effect with improved cognition and regulating its progression. In the present chapter, multi-target-directed approaches are discussed with coverage of design strategies and promising compounds reported in recent years. Some of the well-explored targets like acetylcholine esterase (AChE), ß-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), glycogen synthase kinase 3ß (GSK-3ß), monoamine oxidases (MAOs), metal ions in the brain, N-methyl-D-aspartate (NMDA) receptor, and phosphodiesterases (PDE)

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are described focusing on their contribution toward cognitive neurodegeneration leading to Alzheimer's disease (AD).

Keywords Alzheimer's disease (AD) · Multi-target-directed ligand (MTDL) · Acetylcholine esterase · BACE-1 · Natural compounds

Abbreviations

5-HT	5-Hydroxytryptamine
AChE	Acetylcholine esterase
AChEI	Acetylcholine esterase inhibitor
AD	Alzheimer's disease
APOE	Apolipoprotein E
BACE!	ß-site amyloid precursor protein-cleaving enzyme 1
BBB	Blood–brain barrier
BuChE	Butyrylcholinesterase
CB1	Cannabinoid-based 1
ChAT	Acetyltransferase
FDA	Food and drug administration
GSK-3ß	Glycogen synthase kinase 3ß
JNK	c-Jun N-terminal kinase
MAO	Monoamine oxidases
MTDL	Multi-target-directed ligand
NFT	Neurofibrillary tangles
NMDAR	N-methyl-D-aspartate receptor,
NQO1	NAD(P)H quinone oxidoreductase
PAMPA	Parallel artificial membrane permeation assay
PDE	Phosphodiesterase
ROCK	Rho-associated protein kinase
ROS	Reactive oxygen species
α-M	α-Mangostin

1 Introduction

Alzheimer's disease (AD) is a progressive, multifaceted, and multifactorial neurodegenerative disease (Yilmaz 2015). It has phylogenic nature and possesses cross-talk among various signaling cascades. The complex pathophysiology of AD consists of aggregation of pathological proteins, impaired neurotransmission, increased oxidative stress, and/or microglia-mediated neuroinflammation. Various AD hypotheses supported by experimental data have been proposed, and they play an important role in its pathogenesis (Fig. 1) (Hardy and Higgins 1992; Selkoe and

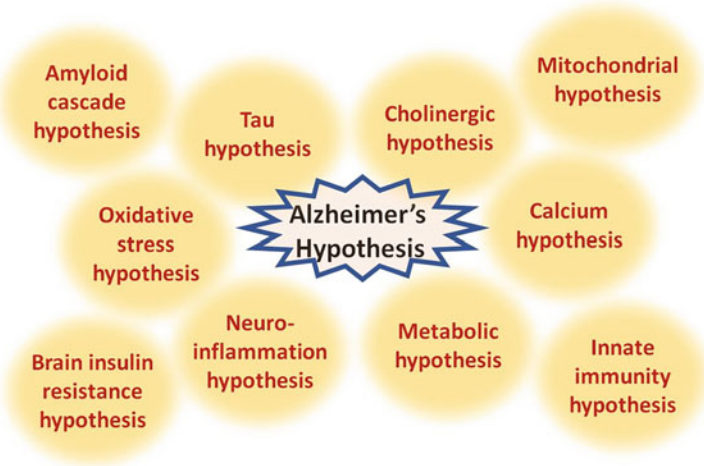


Fig. 1 Various proposed hypotheses for Alzheimer's disease

Hardy 2016; Iqbal and Grundke-Iqbal 1996; Iqbal et al. 2016; Perry et al. 1977, Bartus et al. 1982; Moreira et al. 2010; Swerdlow et al. 2010; Coyle and Puttfarcken 1993; Zhu et al. 2004; McGeer et al. 1994; de la Monte 2009; Deng et al. 2009; Hoyer 2000; Iqbal and Grundke-Iqbal 2005; Gong et al. 2016; Khachaturian 1994; Guillot-Sestier et al. 2015; Masand et al. 2017; Gupta and Patil 2020).

Several hallmarks of AD involved in pathological progression are oxidative stress, neuroinflammation, synaptic dysfunction, deprivation of cholinergic function, amyloid plaques, and neurofibrillary tangles (NFTs) (Canter et al. 2016; Busche et al. 2019). Thus, it can be defined as a disorder regulated by enzymes/receptors like acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), N-methyl-D-aspartic acid (NMDA), ß-secretase 1 (BACE1), and muscarinic and signaling pathways (c-Jun N-terminal kinase (JNK)) (Martin et al. 2013). In addition to this, based on the review of clinical studies, some of the important targets are amyloid, tau, apolipoprotein E (APOE)/lipids and lipoprotein receptors, neurotransmitter receptors, neurogenesis, inflammation, oxidative stress, cell death proteostasis/proteinopathies, metabolism and bioenergetics, vasculature, growth factors and hormones, synaptic plasticity/neuroprotection, gut–brain axis, circadian rhythm, and epigenetic regulators which were of interest for clinical studies in 2022 (Fig. 2) (Turgutalp et al. 2022; Cummings et al. 2022). Drugs acting as antagonists of these enzymes/pathways have limited success to control the symptoms and fail to stop or reverse the disease progression (Savelieff et al. 2019).

The prevalence of AD is increasing, and $>$ 50 million elderly are living with it (Li et al. 2022b). Research efforts have focused on the reported AD-related subpathogenesis without any success to put forward disease-modifying therapeutics. Unfortunately, no effective therapy for the prevention or treatment of AD is available. No disease-modifying drugs are available in the market, and very low clinical success is reported for this class of drugs. In addition to this, aspects related to the

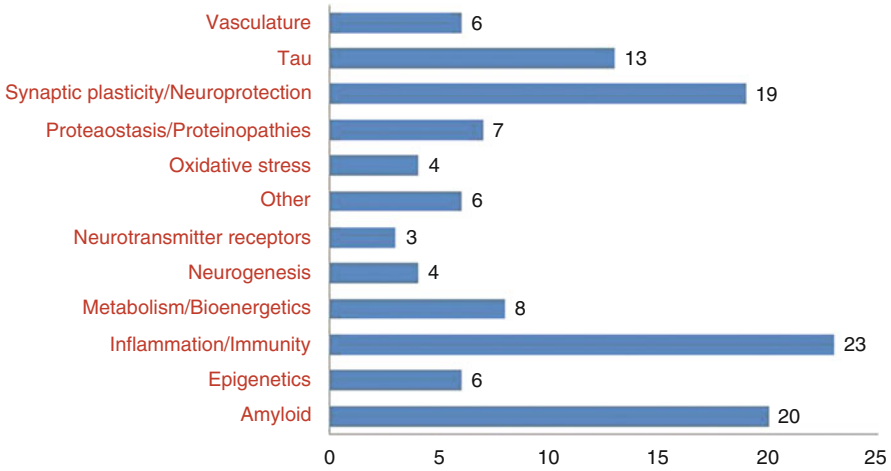


Fig. 2 Number of agents entered in the clinical phase of evaluation (during the year 2022) and their anti-AD mechanisms

onset and progression of this neurodegenerative disease are still unexplored. Researchers are focusing on newer therapeutic targets toward their efforts to identify definite and direct therapeutics. Despite the huge number of preclinical and clinical studies (> 4000), only a few drugs have been approved for clinical use and there is requirement for drugs to prevent, delay the onset of neurodegeneration, slow the disease progression, and improve the AD-associated symptoms (Cummings et al. 2022). Earlier FDA approved only five drugs for the treatment of AD, which include tacrine, donepezil, galantamine, rivastigmine, and memantine with recent addition of Leqembi (lecanemab-irmb) through accelerated approval pathway (https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-alzheimers-disease-treatment). Tacrine has been discontinued due to its hepatotoxicity (Watkins et al. 1994). Among the various reported approaches, multi-target-directed ligands (MTDLs) have several advantages when compared to single-target or combination therapeutics, but none of the reported agents have entered the clinical phase of development. In this chapter, an overview of the MTDL approach for the development of anti-AD therapeutics has been discussed along with its potential to address the associated limitations with various examples from the preclinical phase of development.

2 Single-Target, Multi-Target, and Combination Therapeutics

For AD drug development, the most promising strategies are combination therapeutics, MTDL therapeutics, and drug repurposing (Barthélemy et al. 2020). Among them, the drug repurposing approach consumes less time and requires less

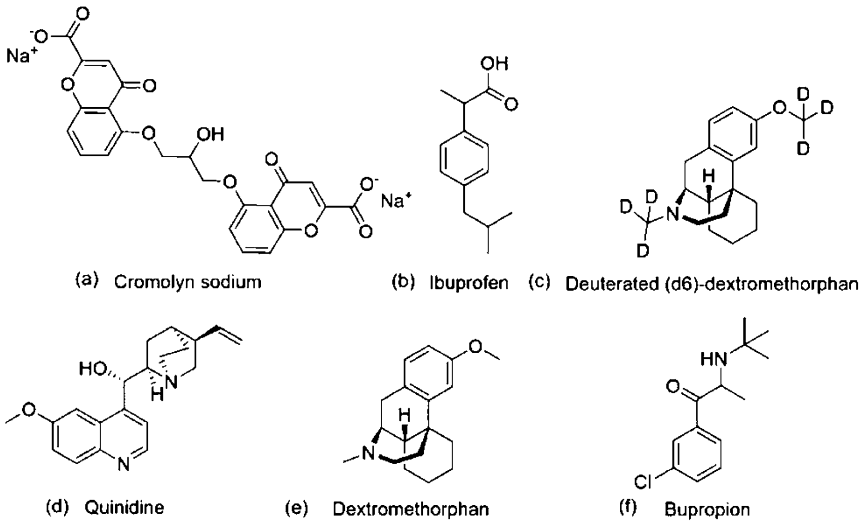


Fig. 3 Chemical structures of compounds used for anti-AD combination therapeutics (**a**, Cromolyn sodium; **b**, Ibuprofen; **c**, Deuterated (d6)-dextromethorphan; **d**, Quinidine; **e**, Dextromethorphan; **f**, Bupropion)

Table 1 Details of anti-AD combination therapeutics in phase III of clinical studies

Clinical trial ID	Details of combination	AD target	Ref.
NCT02547818	ALZT-OP1 (cromolyn and ibuprofen)	Amyloid and inflammation	Panza et al. (2016), Hori et al. (2015), Brazier et al. (2017), Pasqualetti et al. (2009), Weggen et al. (2001), Zhang et al. (2018)
NCT02442765, NCT02442778, NCT02446132	AVP-786 (deuterated (d6)-dextromethorphan and quinidine)	Agitation	Garay and Grossberg (2017), Wilkinson et al. (2019)
NCT03226522	AXS-05 (bupropion and dextromethorphan)	Agitation	O'Gorman et al. (2019), Ahmed et al. (2019), Stahl (2019)

investment, while a few combination therapeutics have been selected for phase III clinical studies (Fig. 3 and Table 1). In case of multi-target approach, drug combinations are used to prepare a single formula. There are some challenges associated with combination therapeutics such as drug–drug interactions due to the release or blockage of certain enzymes involved in metabolism. It alters drug concentrations leading to absence/reduced efficacy or toxic effects. In case of elderly patients taking several drugs, it increases the chances of drug–drug interactions.

In the last decade, multi-target or multifunctional drugs have gained importance as potential therapeutics for various diseases having complex and multifactor pathophysiology and drug resistance cases (Talevi et al. 2012; Koeberle and Werz 2014;

Talevi 2015). In case of complex disorders or diseases having resistance issues like AD, cancer, malaria, mycobacterium tuberculosis, and diabetes, simultaneous modulation of multiple targets can help to heal or reduce the disease condition (de Freitas et al. 2018; Makhoba et al. 2020; Benek et al. 2020). MTDLs are based on the use of one active ingredient (Zhou et al. 2019). In AD, MTDLs are helpful as they can focus on more than one subpathologies simultaneously and to establish a better approach. The current single-target anti-AD therapeutics have limitations like low efficacy and inability to control associated symptoms. Due to the diverse pathogenesis, the single-target anti-AD drugs have limitations and there is requirement of understanding the multifunctional or multi-target strategies for the development of potential drugs.

3 Multi-Target-Directed Strategies

The causative factor behind AD is characterized as a disease caused by the systemic breakdown of physiological networks of the brain (Hopkins 2008; Barabási et al. 2011). The complex pathophysiology observed with AD and lack of success with single-target strategies has emphasized the need for "one drug/ligand, multiple targets" as a better approach. Murphy et al. (2004) have emphasized on rational designing of multi-target directed ligands which can modulate multiple targets of interest related to the pathological condition. These are developed with the objectives to improve efficacy and/or safety and thus may provide wider application in clinical practice. Some of their merits compared to combination therapy include retention of all advantages of combination therapy with some additional benefits like absence of/less drug–drug interactions, reduced risk of adverse effects due to reduced polypharmacy, simplified dosage regimen causing better patient compliance, and requirement of less number of clinical trials (Proschak et al. 2019; Bolognesi 2013; Woodcock et al. 2011; Center for Drug Evaluation and Research 2013; Ibrahim and Gabr 2019). In addition to this, single-target agents demonstrate short and temporary effects and multiple target agents have chances of higher success rate (Rossi et al. 2021).

Among the well-reported anti-AD MTDLs, cholinesterase inhibitors present an interesting category. In addition to cholinesterase inhibition, the other biological properties targeting the factors involved in the intertwined pathogenesis are important to design and develop MTDLs. AChE inhibition along with antioxidant properties is an interesting strategy (Cruz et al. 2017).

In the past decade, studies focusing on the design and synthesis of multifunctional ligands targeting different AD pathways have been reported (Cabrera-Pardo et al. 2020; Bhatia et al. 2021). Hybridization of pharmacophores is another interesting approach where each of them is retained its nature for interaction with specific targets and thus can produce multiple pharmacological activities. It has the advantage to overcome the administration of multiple drugs and thus provides patient-friendly dosage regimen. Numerous hybrid analogs using bioactive pharmacophore moieties have been found useful (Uddin et al. 2021).

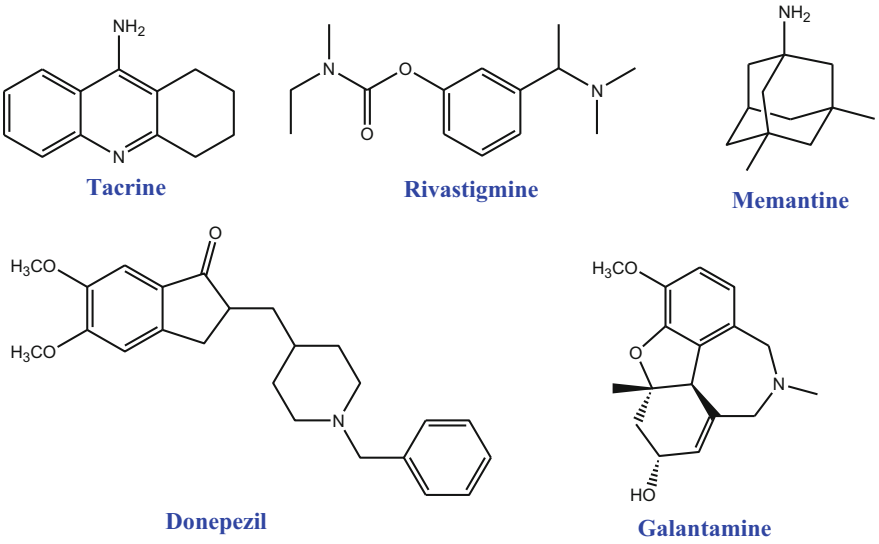


Fig. 4 Chemical structures of approved anti-AD AChEIs (Tacrine, Rivastigmine, Memantine, Donepezil, and Galantamine)

3.1 AChEI-Based MTDLs

Among Alzheimer patients, varying levels of acetylcholine, acetyltransferase (ChAT), and acetylcholinesterase (AChE) have been observed and has served as vital target in the development of first-generation anti-AD drugs. Few drugs acting through this mechanism have been approved (Fig. 5). Critical involvement of BChE in amyloid β aggregation makes it an important anti-AD drug target (Greig et al. 2002; Lane et al. 2006), but its inhibitors are associated with inevitable side effects. In a recent manuscript, Mishra et al. (2019) have reviewed various anticholinesterase hybrids of tacrine, donepezil, rivastigmine, resveratrol, galanthamine, huperzine, ferulic acid, indole, curcumin, lipoic acid, acridine, coumarin, ciproxifan, chalcone, etc., having anti-AD properties (Fig. 4). Some of the important categories studied under anti-AD MTDL category targeting AChE along with other related targets are described here.

3.1.1 Dual AChEIs Acting on ACh Hydrolysis Sites (Catalytic and Peripheral Anionic Sites)

AChE and BChE active sites are surrounded by numerous subsites which can be differentiated based on the residues. These structural features govern their potency and selectivity toward both enzymes (Dias and Viegas 2014). Studies have explained

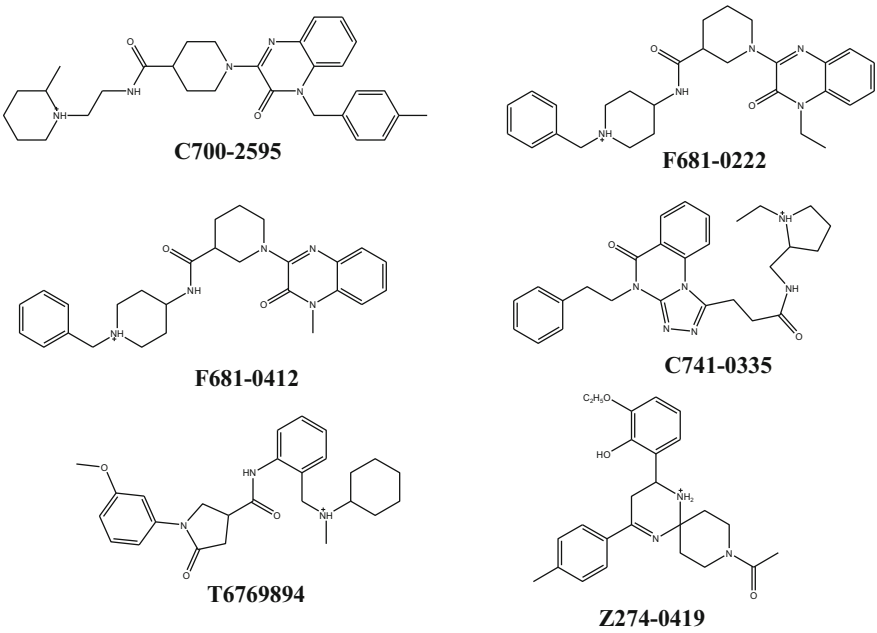


Fig. 5 Chemical structures of AChE and BACE1 inhibitory MTDLs

the role of peripheral catalytic site for reducing the cognitive deficit and lowering AChE-induced Aß aggregation. It leads to disease-modifying effect and thus helps to overcome cognitive deficit (Hosea et al. 1996; Mallender et al. 2000; Radic et al. 1991; De Ferrari et al. 2001; Inestrosa et al. 1996; Small et al. 1999; Bartolini et al. 2003; Hoyer et al. 2008).

3.1.2 AChE and BACE1 Inhibitors

For the last 2½ decades, amyloid hypothesis is the conventional approach for Alzheimer's research. The full-length amyloid precursor protein is split into fragments by ß-secretase 1 (BACE1) and initiates Aβ formation and deposition (Dias and Viegas 2014). Aβ deposition has been correlated with tau protein, inflammation, oxidative stress, etc., and thus specifies its role in anti-Alzheimer's MTDL discovery. In addition to rational approaches (discussed in the later part of this chapter), computational methods like ligand-based screening and scaffold hopping were performed to identify a few dual inhibitors (Fig. 5) from a dataset of approximately three million compounds and were further validated using in vitro (AChE $IC_{50} = 4–7$-μM and BACE-1 $IC_{50} = 50–65$ μM) and in vivo analysis (Stern et al. 2022).

3.1.3 AChEIs and Antioxidant

Oxidative stress is an important contributor for initiating Aß aggregation, tau protein hyperphosphorylation, acute inflammation, and neuronal apoptosis in Alzheimer's disease. Simultaneously inhibiting ROS formation has a conducive role (Dias and Viegas 2014; Nesi et al. 2017; Pohanka 2018). The extract of *Carpolobia lutea* has shown concentration-dependent dual activity and has promising potential as MTDLs for the management of neurodegenerative disorders (Nwidu et al. 2017). The well-reported AChEIs, i.e., donepezil and rivastigmine, have shown reduced antioxidant properties when evaluated for fluoride-induced oxidative stress models (Ferreira-Vieira et al. 2016; Goschorska et al. 2018). Thus, MTDLs from this category can be considered as potential Alzheimer's therapy.

3.1.4 AChEIs and Voltage-Dependent Ca^{2+} Channel Blockers

In Alzheimer's pathogenesis, Ca^{2+} influx stimulates γ-secretase pathway through lowered Aß production and tau hyperphosphorylation. Raised Ca^{2+} levels cause mitochondrial disruption and activate apoptotic cascade followed by cell death (Tan et al. 2012; Dias and Viegas 2014). Blockage of Ca^{2+} channels shows their mechanism by protecting against Aß oligomer (Wareski et al. 2009). Specifically, the L subtype of Ca^{2+} channel blockers are verapamil, diltiazem, isradipine, and nimodipine found to have contributing effect, and hence, it is a good strategy to prevent neuronal cell death (Mattson and Chan 2001; Qin et al. 2009).

3.1.5 AChEIs and Glutaminergic Receptor Inhibitors

Altered cerebral glucose and glutamate concentrations cause Aß plaque deposition, and $>$ 40% of neuronal synapses are glutaminergic. Earlier studies have described the effect of impaired glucose metabolism on glutamate receptor-mediated signaling pathways which cause impaired cognition among Alzheimer's patients (Hoyer 2004). A broad range of inhibitors of glutaminergic receptors (NMDA, AMPA, mGluR5, mGluR2/3, EAAT2) have shown promising preclinical and/or clinical results in improving cognitive functions (Bukke et al. 2020). AChE inhibitor bis (7)-tacrine has shown NMDA inhibitory role (Li et al. 2005). It can slow AD pathogenesis and improve associated cognition. Thus it is a promising approach for the development of anti-AD MTDLs.

3.1.6 AChEIs and CB1 Receptor Antagonists

Certain cannabinoids have exhibited neuroprotection against Aß leading to memory improvement. Additionally, the role in tau hyperphosphorylation, oxidative stress, and inflammation has been reported (Aso and Ferrer 2014). CB1 receptor

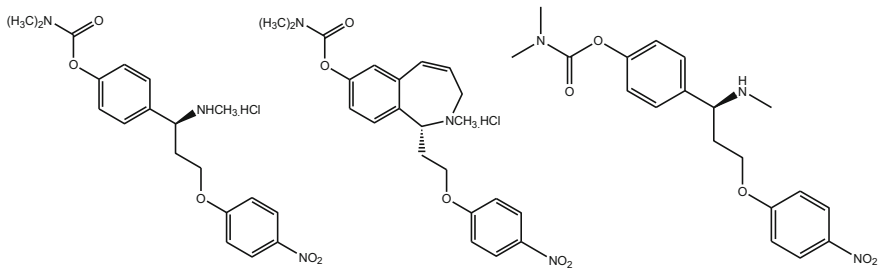
antagonists (increase ACh level in cortical and hippocampal neurons) have also been reported (Huang et al. 2011; Lange et al. 2010). Cannabinoid-based anti-Alzheimer's agents offer advantages like broader coverage of properties by selecting suitable combinations, and during the early phase of dementia, it has the potential to control the progression of neurodegeneration (Aso and Ferrer 2014).

3.1.7 AChEIs and NMDA Receptor Inhibitors

AChEIs have the potential to recover cognition and can act as disease-modifying agents, while NMDAR antagonists can contrast neurodegeneration (Rosini et al. 2015) (Glynn-Servedio and Ranola 2017). A combination formulation of donepezil and memantine has been put forward for clinical evaluation (http://www.adamaspharma.com). Herein, we focused on some multi-targeted ligands having symptomatic relief by blocking AChE and neuroprotection by NMDAR antagonism. During design, AChE and/or NMDAR inhibitory fragments were integrated into a single molecule. Some of the compounds considered for designing MTDLs under this category are carvedilol and tacrine, galantamine, and memantine for Carbacrine and Memagal, respectively (Rosini et al. 2015).

3.1.8 AChEIs and Serotonin Transporter (SERT) Inhibitors

Limitations associated with AD therapeutics can be addressed by the use of multi-targeted approach involving AChE and SERT, where the later can resolve the dose-dependent side effects of AChEIs. Literature reports highlight the preclinical evaluation of dual inhibitors against these potential targets as anti-AD therapeutics (Kogen et al. 2002; Toda et al. 2003, 2010; Rodriguez-Lavado et al. 2020). Few drugs have exhibited potent activity such as (*S*)-*A*, (*R*)-*B* (Lyketsos et al. 2000; Kogen et al. 2002), and RS-1259 (Fig. 6) (Abe et al. 2003, Toda et al. 2010). RS-1259 has shown simultaneous inhibition of AChE and SERT in the brain using in vivo models after oral administration and using in vitro models.



(*S*)-A **(*R*)-B** **RS-1259**

Fig. 6 Chemical structures of AChE and SERT inhibitory MTDLs

3.2 Phosphodiesterase (PDE) Inhibition-Based MTDLs

AChE inhibitors have demonstrated limited efficacy and development of tolerance after prolonged use, and it encouraged to use them in combination with other drugs. When compared with other reported targets, phosphodiesterases (PDEs) are emerging as promising targets for developing inhibitors to contrast neurodegeneration (Ribaudo et al. 2020; Sheng et al. 2022). In particular, selective small molecules targeting PDE4, PDE5, and PDE9 isoforms are being studied to explore alternative strategies against AD in light of their brain localization and of their role, to different extents, in cognitive processes (Zuccarello et al. 2020). Recently, many strategies have been tried to design and synthesize dual inhibitors of PDE subtypes and AChE to combat the multifactorial aspect of AD.

3.2.1 Tadalafil Analogs

In an attempt to synthesize first-generation dual-target inhibitors of AChE and PDE5, using drug repositioning and redeveloping strategy, Ni et al. synthesized a series of tadalafil derivatives (19 compounds). Inhibition of these tadalafil derivatives against AChE and BuChE was determined by the modified Ellman's method. The compounds exhibited good AChE activity ($IC_{50} < 1$ μM) and moderate BuChE activity. Following an IMAP-FP (immobilized metal ion affinity-based fluorescence polarization) assay, the most potent AChE inhibitors were found to show good or moderate PDE5 inhibitory activity (IC_{50} values of 0.050–3.231 μM). One of the essential features of a successful anti-AD drug is good BBB permeation. A parallel artificial membrane permeation assay (PAMPA) was employed, and the compounds exhibited good BBB permeability. In vivo studies on the mouse model showed an effect comparable to that of donepezil. These compounds proved to be potential selective dual-target AChE/PDE5 inhibitors and will be an excellent lead compound for further research. Figure 7 shows the structure of the active compound (Ni et al. 2018).

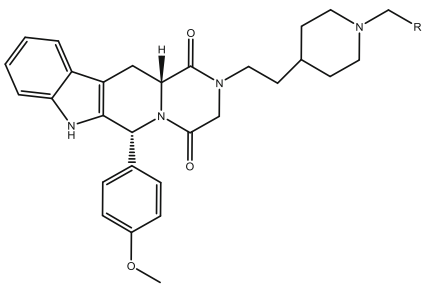


Fig. 7 Representative structure of tadalafil derivatives

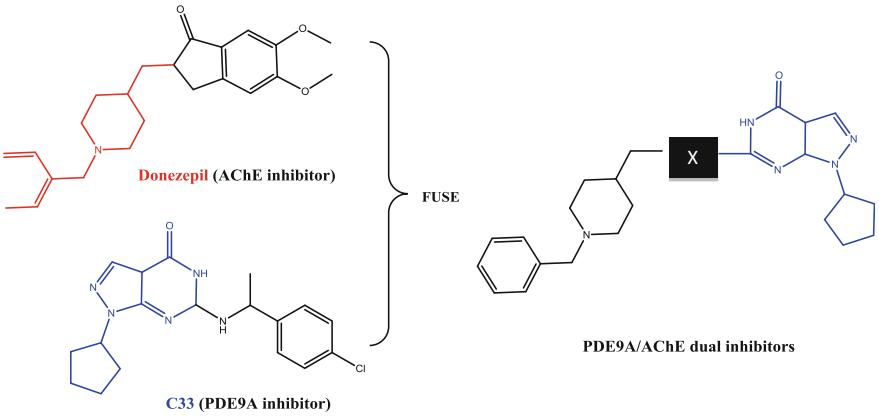


Fig. 8 Rational design of PDE9A and AChE dual inhibitors

3.2.2 Donepezil and Pyrazolo[3,4-d]Pyrimidinone (Pharmacophore of PDE)

In another work performed by Hu et al., dual inhibition of PDE and AChE was achieved by combining the pharmacophore of donepezil and pyrazolo[3,4-d] pyrimidinone (pharmacophore of PDE) using different linkages. A series of dual-target AChE/PDE9A inhibitor compounds Fig. 8 were designed, synthesized, and evaluated as anti-Alzheimer's disease (AD) agents. Among these targets, two compounds exhibited excellent and balanced dual-target AChE/PDE9A inhibitory activities (AChE: IC_{50} = 0.048 μM; PDE9A: IC_{50} = 0.530 μM and AChE: IC_{50} = 0.223 μM; PDE9A: IC_{50} = 0.285 μM). Moreover, these two compounds also possess good BBB penetrability and low neurotoxicity. It was found that they could ameliorate learning deficits induced by scopolamine and improve cognitive and spatial memory in Aβ25–35-induced cognitive deficit mice in the Morris water-maze test. This work produced promising candidates that possess potential inhibition of PDE/AChE (Hu et al. 2019).

3.3 Monoamine Oxidase-Based MTDLs

The oxidative damage is promoted by the increased monoamine oxidase B (MAO-B) level which generates free radicals (Riederer et al. 2004; Tripathi et al. 2013). Selective MAO inhibitors have been demonstrated for metal chelation or AChE inhibition, and therefore, targeting MAO is an important approach to improve cognition- and control-associated symptoms.

Recently Oh et al. (2021) have reported AChE and MAO-B dual inhibitory potential of some ellagic acid analogs which were derived from *Castanopsis*

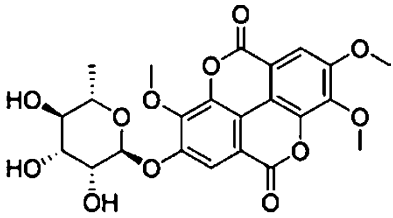


Fig. 9 Chemical structure of 4′-*O*-(α-L-rhamnopyranosyl)-3,3′,4-tri-*O*-methylellagic acid. (AChE: $IC_{50} = 10.1$ μM; binding energy = −8.5 kcal/mol; MAO-B: $IC_{50} = 7.27$ μM)

cuspidata var. sieboldii. In vitro and docking studies have identified 4′-*O*-(α-L-rhamnopyranosyl)-3,3′,4-tri-*O*-methylellagic acid as a potential analog supported with lesser toxicity (Fig. 9).

3.4 Metal Chelation-Based MTDLs

The metal hypothesis of Alzheimer's defines the role of metal ions (Fe^{2+}, Zn^{2+}, Cu^{2+}, Al^{2+}) in cognitive loss and neurodegeneration. It is required to maintain their homeostasis for normal neuronal functioning (Salvador et al. 2010; Bush 2013; Sastre et al. 2015). Higher concentrations of certain bivalent metal ions like Zn^{2+}, Fe^{2+}, and Cu^{2+} are associated with amyloid plaque formation, and Al^{2+} leads to the degradation of some neurotransmitters (ex. MAO) and generation of reactive oxygen species (ROS) (Lovell et al. 1998; Zatta et al. 1999, 2009; Dias and Viegas 2014). Thus, studies are warranted focusing on chelating agents as novel AD therapeutics by incorporating functional moieties which can target other AD pathways (Sharma et al. 2018). Thus metal chelators with AChE/MAO/BACE-1 inhibition and/or antioxidant properties are an important MTDL approach. This category of MTDLs can be subclassified as Aß-aggregation-based, AChE-based, MAO-based, and BACE-1-based metal chelating agents. Studies supporting the beneficiary effect of AChE and metal chelators in the treatment of Alzheimer's disease have been well-reported. Compounds interacting with Aß and capable to chelate Zn^{2+} and Cu^{2+} ions have shown bifunctional properties in Alzheimer's models (Figure) (Choi et al. 2014; Braymer et al. 2010; Jones et al. 2012). Some of the well-documented MTDLs based on inhibition of Aß aggregation are *N*-(pyridin-2-ylmethyl)aniline, *N*1,*N*1-dimethyl-*N*4-(pyridin-2-ylmethyl)benzene-1,4-diamine, pyridine-triazole derivatives, and quinoline–triazole.

A synthetic rhodamine-B-based molecule (Rh-BT, Fig. 10) has exhibited Aß aggregates by capturing redox metal ions and confirms multifunctional nature (Pradhan et al. 2020). It has shown stability in serum along with BBB permeability suggesting its potential for Alzheimer's treatment. Synthetic hybrid derivatives of tacrine with 8-hydroxyquinoline (*MC-AChE1*) and ferulic acid (*MC-AChE 2*) exhibited Cu^{2+} chelation and Aß reduction (Fernández-Bachiller et al. 2010; Xie et al. 2013; Fu et al. 2016). *MC-AChE1* produces AChE and BuChE inhibition (at nano- and subnanomolar concentrations) and *MC-AChE 2* inhibits AChE by

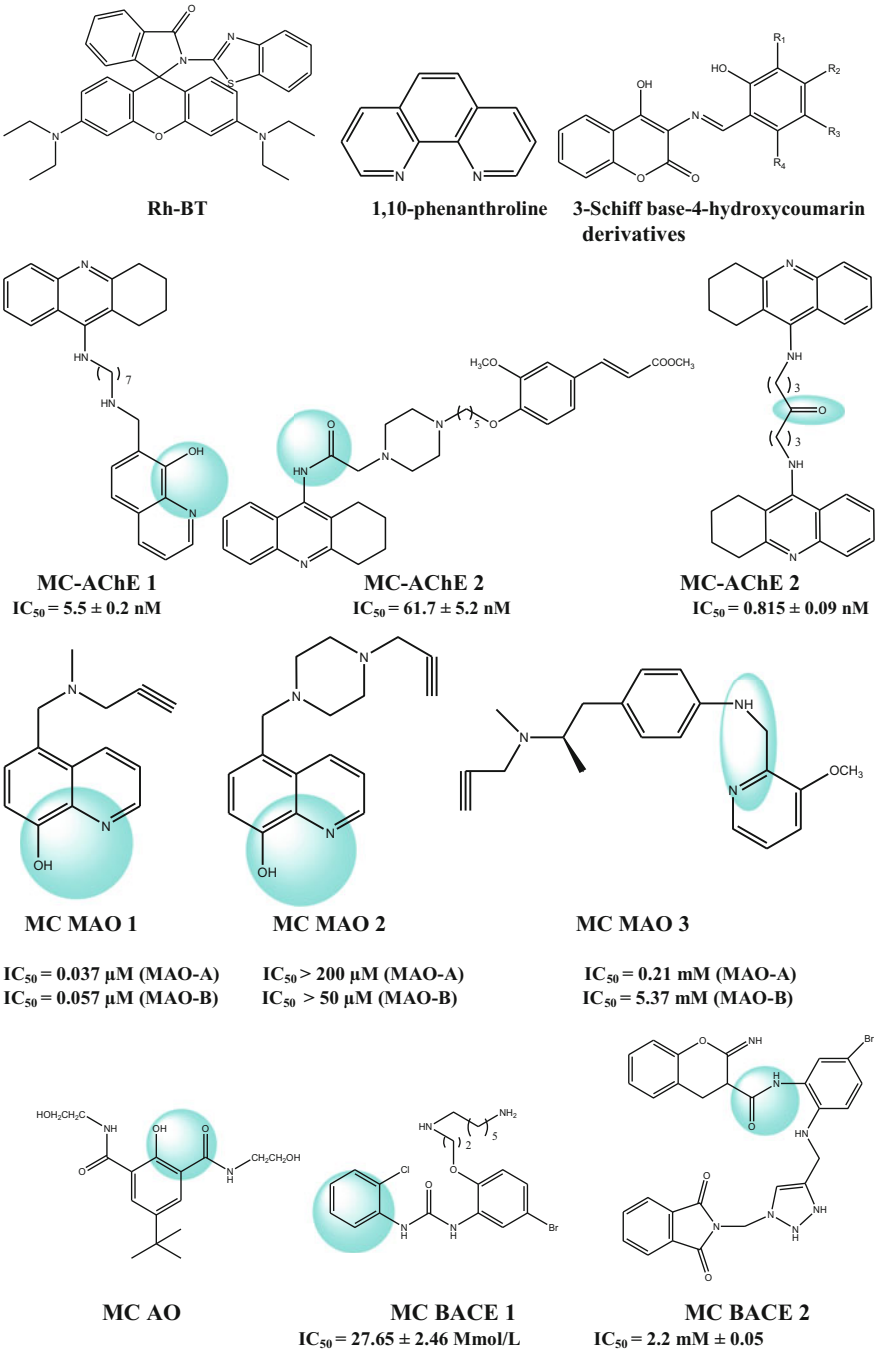


Fig. 10 Metal chelator-based anti-AD MTDLs (blue color indicates binding sites for metal chelation)

binding at the mod-gorge site. The *bis*(7)-tacrine (*MC-AChE 3*) interacts with anionic and catalytic sites to regulate AChE-induced Aß aggregation and shows Cu^{2+} chelation (Bolognesi et al. 2007). A novel chelator of Fe^{2+}, Cu^{2+}, and Zn^{2+} was designed using pharmacophoric features of rivastigmine and donepezil, and *MC MAO 2* has exhibited APP regulation and lowered oxidative stress (Zhang et al. 2013). Indanone metal (Fe^{2+}, Cu^{2+}, and Zn^{2+}) chelating derivative having piperidine moiety through ethylene linkage has blocked AChE at micromolar concentration and 14 times more potency as compared to donepezil (Meng et al. 2012). Similarly, metal chelating 1,10-phenanthroline has been evaluated for its inhibitory effect against AChE, i.e., aryl acylamidase and esterase activity (Chitra et al. 2013). Molecular docking and dynamics simulation have confirmed hydrogen and hydrophobic interactions with Phe295 and residues of the peripheral binding site, respectively, which supports experimental results with inhibition at micromolar concentration.

Among the category of MAO-based metal chelating agents, 8-hydroxyquinoline and propargyl nuclei hybrids (*MC MAO 1, MC MAO 2,* and *MC MAO 3*) were derived based on MAO inhibitory anti-Parkinson drugs (rasagiline and selegiline) (Fig. 10). Among them, the latter is most potent with MAO-B IC_{50} of 0.21 μM supported by docking interactions and can produce antioxidant and chelation properties for Fe^{2+}, Cu^{2+}, and Zn^{2+} (Youdim et al. 2005; Xie et al. 2022). During the evaluation of metal chelation properties of MAO-B inhibitors, 3,5-diaryl-4,5-dihydroisoxazoles have failed to bind with Fe^{2+} and Fe^{3+} (Meleddu et al. 2017).

3-Schiff base-4-hydroxycoumarin derivatives have reported significant potential as anti-AD agents during in vitro studies (Wang et al. 2015). The most potent compound is suggested to act by MAO (A and B) and self- and copper-induced Aß aggregation inhibition, antioxidant, and biometal chelation effects. The anti-AD properties at the micromolar level suggest it as a promising lead molecule.

A prochelator has been proposed showing Cu^{2+} chelating properties on interaction with BACE-1 (Folk and Franz 2010). It has the ability to inhibit Aß aggregation by sequestering the metal ion from Aß. Among this category, some reported BACE-1 inhibitors have been modified to derive metal (Fe^{3+} and Cu^{2+}) chelating agents like 1,3-diphenylurea analogs (*MC BACE1*) (Huang and Mucke 2012) and iminochromene carboxamides with aminomethylene triazole analogs (*MC BACE2*) (Iraji et al. 2017) supported by docking interactions at the active site of the enzyme.

3.5 BACE1 Inhibitor-Based MTDLs

In AD, BACE-1 and GSK-3ß are the important targets involved in the formation of senile plaques and NFTs. Studies suggest that dual inhibition of these targets will show conducive action (Prati et al. 2018). Few series of BACE-1 and GSK-3ß dual inhibitors have exhibited potent inhibition along with neuroprotective and good pharmacokinetic properties in relation to oral bioavailability and BBB penetration (Di Martino et al. 2016; Rampa et al. 2017). The fragment-based approach has been

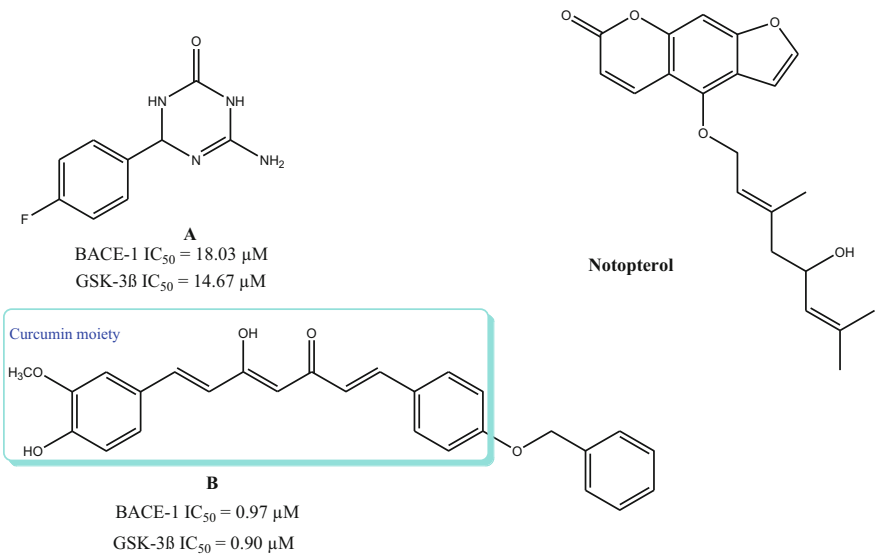


Fig. 11 Details of some BACE-1 and GSK-3ß dual inhibitors

implemented to design and derive dual inhibitors having cyclic amide and guanidine moieties. The most promising compound (Fig. 11a) has shown potent dual inhibition without neurotoxic properties (Rampa et al. 2017). Similarly, curcumin-based inhibitors (Fig. 11b) have exhibited balanced dual inhibition along with neuroprotective properties induced through NAD(P)H quinone oxidoreductase 1 (NQO1) (Di Martino et al. 2016). It has the potential for further evaluation due to BBB permeation.

A natural dual inhibitor notopterol (Fig. 11) has shown a potential to ameliorate AD-associated cognitive deficit in animal models by dual inhibition of BACE-1 and GSK-3ß at micromolar concentration, i.e., IC_{50} of 26.01 µM and 1.0 µM, respectively. The binding profile was further established using docking and dynamics studies showing protein stability in the binding complex (Jiang et al. 2020). Recently, Bajad et al. (2022) utilized both structure and ligand-based approaches such as virtual screening, homology modeling, docking and dynamics studies, drug-likeness screening, and assessment of pharmacokinetic and toxicity properties. It proposed two potential dual inhibitors, i.e., ZINC22551247 and ZINC668197980 (Bajad et al. 2022).

Based on the requirement of agents having multiple effects to comply with the complex pathogenicity of AD, MTDLs targeting toxicity of ROS, serotonergic receptors (5-HT_4 and 5-HT_6 receptors), neuroinflammation, etc., are under evaluation (Lanthier et al. 2019; Benek et al. 2020). Simultaneous targeting of several subpathologies will contribute to derive a better approach to obtain effective anti-AD agents.

4 Rational Design of MTDLs

For the design of MTDLs, it is important to have information about pharmacophores having affinity for diverse disease targets. Such pharmacophores are linked/fused/merged to get a single molecule. In case of linked MTDLs, the linker group may be cleavable or non-cleavable and their large size causes reduced bioavailability. The pharmacophores are partially overlapping each other in fused MTDLs, while the merged ligands have higher overlapping resulting in simple molecules with low molecular weight (Zhou et al. 2019). Some examples of anti-AD MTDLs like memoquin, xanthone-flavonoid derivatives, etc., are discussed here.

AChE has been reported to trigger Aß aggregation, and its peripheral anionic site facilitates fibril formation by interaction with Aß. Thus, for effective inhibition of Aß aggregation, blockage of AChE peripheral anionic site can be considered as an effective strategy (Li et al. 2018).

The first rationally designed compound from this category is memoquin. It is a 1,4-benzoquinone–polyamine hybrid of AChE and muscarinic inhibitor polyamineamide caproctamine with potent antioxidant and neuroprotective 1,4-benzoquinone (Cavalli et al. 2007; Prati et al. 2014). Memoquin is formed by replacing the inner polymethylene chain with benzoquinone nucleus. The structural details are presented in Fig. 12 (Bolognesi et al. 2009). The hydrophobic and planar

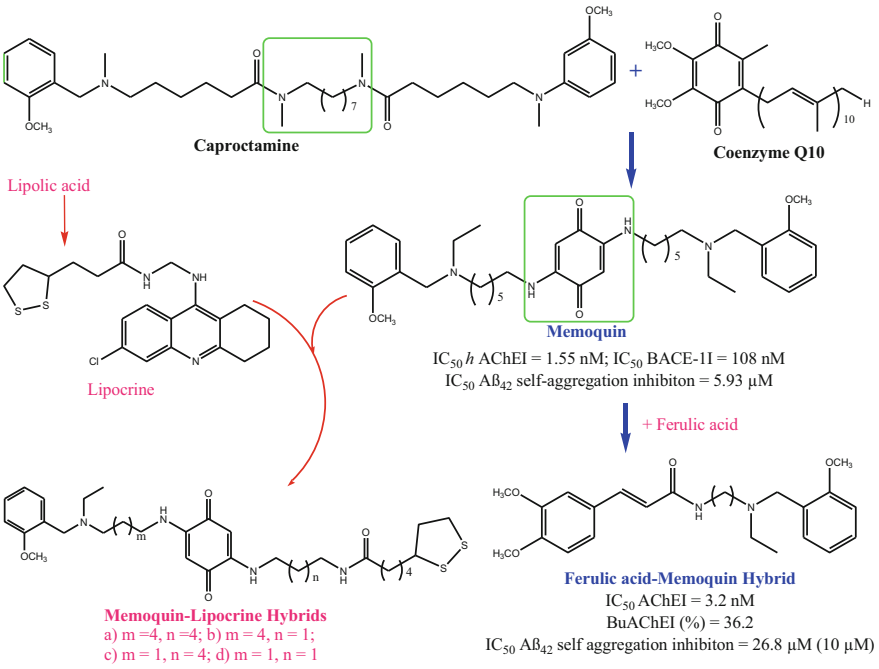


Fig. 12 Design of memoquin and its hybrids with ferulic acid and lipocrine (obtained from lipoic acid)

pi-system contributes toward protein–protein interactions in the Aß fibrillogenesis. It has demonstrated the potential to explore AChE and self-induced Aß aggregation based on the potential observed for ferulic acid–memoquin hybrids (Fig. 12) (Bolognesi et al. 2009; Pan et al. 2016; Ortiz et al. 2019). Multi-targeted profile for memoquin is established through in vitro studies which include inhibition of AChE and Aß aggregation induced by it, BACE-1, and free radical generation. Studies also support its oral bioavailability, BBB permeability, and safety profile. It confirms tolerance on prolog administration and its ability to restore cholinergic deficit, reduced expression, and accumulation of Aß and decreased tau protein phosphorylation (Cavalli et al. 2007; Bolognesi et al. 2009). As a next step to these findings, the mitochondria targeting antioxidant, alpha-lipoic acid, has been used to prepare memoquin hybrid analogs with an aim to prolong the onset/prevent/cure Alzheimer's disease (Bolognesi et al. 2009). Lipocrine, i.e., prepared by conjugating 9-amino-6-Cl-1,2,3,4-tetrahydroacridine with alpha-lipoic acid, has exhibited anti-AChE and antioxidant properties. The newly reported memoquin–lipocrine MTDLs (Fig. 12) has superior potential to prevent and cure AD through multiple antioxidant mechanisms. It involves reduced ROS production and mechanisms mediated through NQO1. These analogs have shown lesser inhibitory properties against AChE and BChE. Studies also confirm the requirement of lipoyl fragment at position 2 of the benzoquinone for receptor interactions.

The next important MTDLs from the AChE inhibitory category (reversible and non-competitive inhibitor) are based on tacrine and donepezil. Such analogs have demonstrated the ability to lessen the neurodegeneration associated with cholinergic damage and participate in other AD-related mechanisms (Fig. 13). The dual binding at AChE peripheral and catalytic sites has been implemented for the tacrine, and donepezil-based AChEIs have additional anti-AD properties against Aß aggregation, MAO, and oxidative stress. MTDLs based on this important scaffold are presented in Figs. 13 and 14 (Zagorska and Jaromin 2020).

In the development of AChE and BACE-1 MTDLs, pharmacophores of inhibitors from both categories are linked to design novel analogs. In this direction, the evaluation of hybrid analogs of donepezil (AChEI) with isophthalamide (Zhu et al. 2009) and 2,4-disubstituted pyrimidine (Mohamed et al. 2011, 2012) led to the identification of dual inhibitors with blockage of $A\beta_{1-40}$ production. In other studies, AChEI tacrine scaffold was fused with BACE-1 inhibitory flavonoid fragment of 4-oxo-4*H*-chromene (Fernández-Bachiller et al. 2012), huperzine A (Camps et al. 2000; Pérez-Areales et al. 2019), and benzofuran (Zha et al. 2016) to get some potent dual inhibitors of AChE and BACE-1, while few of them have exhibited interesting antioxidant activity. Tacrine and donepezil scaffolds are subjected to various structural modifications for AChEIs along with other AD targets to develop potential therapeutics (Ismaili et al. 2017). The potent compounds from such series are presented in Fig. 15.

The potential role of Rho-associated protein kinase (ROCK) for Alzheimer's treatment has been reported (Aguilar et al. 2017), and it helped to design PT109 (Fig. 16), i.e., based on lipoic acid and fasudil. Fasudil scaffold possess well-reported anti-AD contributing properties like blockage of ROCK and kinases,

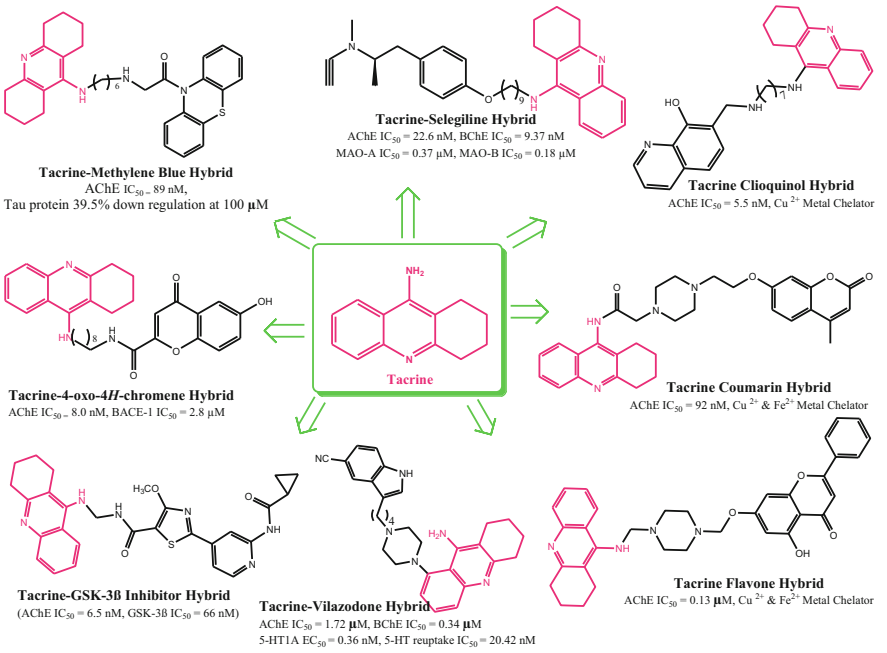


Fig. 13 Structure and activity details of tacrine-based hybrids as MTDLs

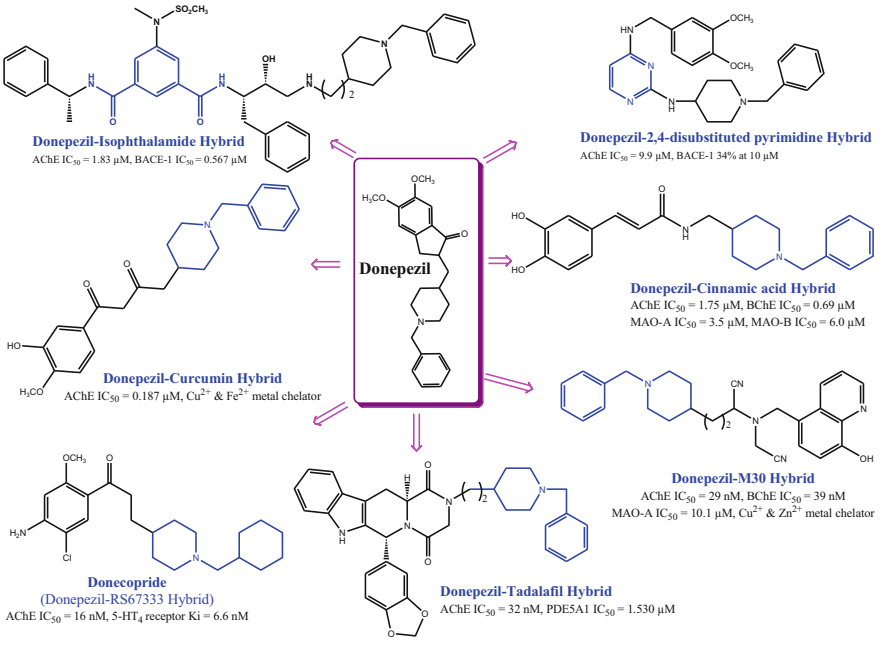


Fig. 14 Structure and activity details of donepezil-based hybrids as MTDLs

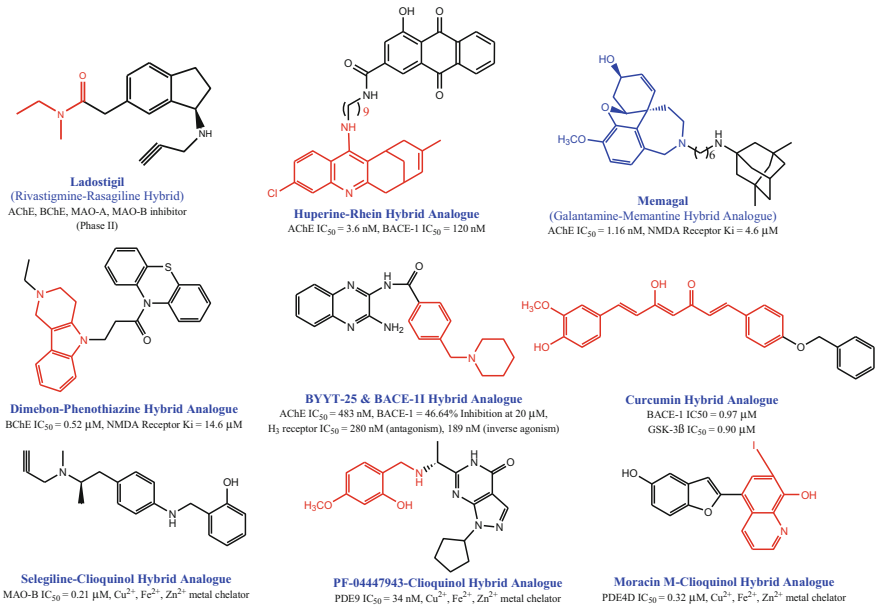
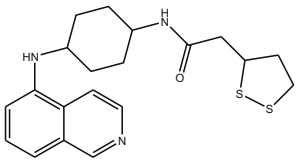


Fig. 15 Structure and activity details of some reported anti-AD MTDLs

Fig. 16 Chemical structure of *PT109*



and anti-inflammatory, antioxidant, and morphological alterations in neural stem cell line (Chen et al. 2017, 2020). It is a multifunctional ligand with broad-spectrum anti-Alzheimer's properties like reduced levels of *p*-Tau, *p*-JNK, etc., which have been demonstrated by in vivo and in vitro methods (Fig. 16).

5 Natural or Nature-Inspired Compounds

Studies have reported a large number of anti-AD natural substances such as huperzine (Serrano et al. 2016), rhein (Li et al. 2019), chelerythrine (Marasco et al. 2021), chalcone (Thapa et al. 2021), curcumin (Mukherjee et al. 2021), berberine (Akbar et al. 2021), resveratrol derivatives (Akbar et al. 2021), and coumarin (Li et al. 2022a). The vast pool of diverse phytoconstituents and their anti-AD potential warrants further studies (Noori et al. 2021). Recently, reported anti-AD drugs include sodium oligomannate; i.e., GV-971 is an oligosaccharide

from marine sources (Wang 2017; Xiao et al. 2021). It highlights a novel strategy for AD management through gut dysbiosis-promoted neuroinflammation (Wang et al. 2019).

Apart from the synthetic strategy used to design the dual-target inhibitors for AD, there are several instances where natural compounds that are inspired from nature are tested for their cholinesterase and PDE inhibition and many of them have shown promising results. Table 2 summarizes the studies on multi-target natural synthetic derivatives, and some reported phytoconstituents are presented in Fig. 17.

Five natural fungal secondary metabolites and one plant metabolite (compounds a to f, Fig. 18) have been selected for anti-AD evaluation based on their structural characteristics like heterocyclic nucleus, molecular weight, and presence of hydroxyl groups. They are evaluated for AChE, BChE, Aß peptide aggregation, and antioxidant properties (Piemontese et al. 2018). The study also aims to provide a possible solution for limitations observed with AChEIs. Additionally, heavy metal (copper (II) and zinc (II)) sequestering properties contribute toward the prevention of ROS production and prevent the formation of amyloid plaques. Aß peptide aggregation properties (at 100 μM) display the role of heterocyclic condensed ring system for disruption of ß-sheet conformation as per documented literature (Hiremathad et al. 2016). The study identified the following MTDLs as potential scaffolds for the development of new anti-AD drugs:

- Antioxidant and interaction with copper (II): tenuazonic acid (A), mycophenolic acid (C).
- AChE and $A\beta_{1-40}$ aggregation inhibitor: *epi*-Radicinol (B).
- AChE and BChE inhibitor: fungerin (F).

Alpha (α)-terpinyl acetate (Fig. 18g), an active phytoconstituent, has shown Alzheimer's disease-modifying activity (Chowdhury and Kumar 2020). It is obtained from *Elettaria cardamomum* L. Maton and exhibited significant interaction with multiple targets. It has demonstrated anticholinesterase, anti-aggregation, and neuroprotective properties and can mitigate symptoms along with disease-modifying activity.

Recently, a review on several anti-AD mechanisms of α-mangostin (α-M, Fig. 18h) which is purified from mangosteen supports its use as a promising molecule for multifactor treatment of Alzheimer's disease (Yang et al. 2021). The safety profile for over 100 years and diverse activity profile (against AChE, BuChE, Aß aggregation, inflammation, metal chelation, and ROS scavenger) has been well-documented through experimental results and clinical trials. MTDL derived from an inexpensive food waste, i.e., cashew nutshell liquid, has been combined with AChE/BChE tacrine nucleus, and it has been achieved by applying sustainable strategies (Fig. 19). The overall sustainability was maintained by adopting principles of green chemistry during synthesis of compounds. The screening was performed based on AChE/BChE selectivity and toxicity observed for hepatic, neuronal, and microglial cells (Rossi et al. 2021). Compound *5* has demonstrated high potency. Its crystal structure in complex with human BChE has been analyzed showing multiple interactions of the two aromatic nuclei at the active site gorge of human BChE.

Table 2 Multi-target natural and nature-inspired compounds

Category	Source/phyto-constituents	MOA	Effects	Ref.
Xanthines	*Coffea arabica* and *C. canephora*/caffeine	Non-competitive AChE inhibitor ($Ki = 175$ μM)	Neuroprotective and anti-inflammatory effects	Pohanka (2015)
		Interfere with intracellular cAMP and cGMP levels by acting as a weak, nonspecific reversible PDE inhibitor ($IC_{50} = 500$–1000 μM)		
Synthetic derivatives of xanthines	Propentofylline	Inhibits AChE ($IC_{50} = 6.40$ μM)	Improve cognition and dementia severity in mild-to-moderate AD	Mohamed et al. (2013)
	Pyrazolopyrimidinones	Inhibits PDE9 in the nM range (IC_{50} values <200 nM)		Singh et al. (2017)
	3-Isobutyl-1-methyl-xanthine (IBMX)	Nonselective inhibitor targeting PDE9 and other isoforms in the μM range ($IC_{50} = 230$ μM for PDE9)		Singh et al. (2017)
	Tacrine–pyrazolopyridine hybrid derivatives	The compounds demonstrated inhibitory activity on cholinesterases ($IC_{50} = 0.125$–0.412 μM for AChE and $IC_{50} = 0.245$–1.283 μM for BuChE) and even better activity on the PDE ($IC_{50} = 0.041$–1.307 μM)	Target AChE, BuChE, another enzyme involved in sustaining cholinergic tone, and PDE4D	Pan et al. (2019)
	Camel artemisia (*Peganum nigellastrum*) Indoline-2,3-dione and quinazoline derivatives	Inhibited AChE with IC_{50} values between 44 and 298 nM, and PDE5 with IC_{50} values between 17 and 746 nM		Zhou et al. (2017)

(continued)

Table 2 (continued)

Category	Source/phyto-constituents	MOA	Effects	Ref.
Flavonoids and coumarins	Rutin and its aglycone quercetin	Efficiently reduce AChE activity in rat tissues (25 and 50 mg/kg) also inhibit PDE5		Adefegha et al. (2018)
Polyphenolic acids	Nigerian plantain (*Musa sapientum*) extracts	Unripe peel aqueous extract showed inhibition of AChE (IC_{50} = 6.30 µg/mL) and PDE5 (IC_{50} = 3.10 µg/mL)		Oboh et al. (2017)
	Alligator pepper (*Aframomum melegueta*)	Inhibit AChE more efficiently (IC_{50} = 5.42 µg/mL)		Adefegha et al. (2017)
	Bastered melegueta (*Aframomum danielli*)	Show inhibition of PDE5 (IC_{50} = 7.24 µg/mL)		
	African walnut (*Tetracarpidium conophorum)*	Efficient in inhibiting AChE (IC_{50} = 0.87 µg/mL) and PDE5 (IC_{50} µg/mL)		Ademiluyi et al. (2019)
	Aqueous extracts of pulverized almond (*Terminalia catappa*) leaf and stem bark	Modulate the activity of AChE and PDE5 in the cardiac tissue of rats (100–200 mg/kg)		Dada et al. (2021)
	Leaves extract (aq.) of *Ocimum gratissimum*	Inhibit AChE (IC_{50} = 43.19–44.67 µg/mL) and PDE5 (IC_{50} = 44.23–53.99 µg/mL)		Ojo et al. (2019)

In silico approaches have been applied to search multi-target AD ligands based on reported information about chemical structures and their biological properties against crucial Alzheimer's targets, namely cyclin-dependent kinase 5, ß-secretase, MAO-B, glycogen synthase kinase 3ß, and acetylcholinesterase (Ambure et al. 2019). Linear discriminant analysis has been applied to derive five classification models and checked for their applicability domain using confidence estimation approach. Further, MTDLs were identified by screening natural database (InterBioScreen) using the derived validated in silico models. Drug-like properties

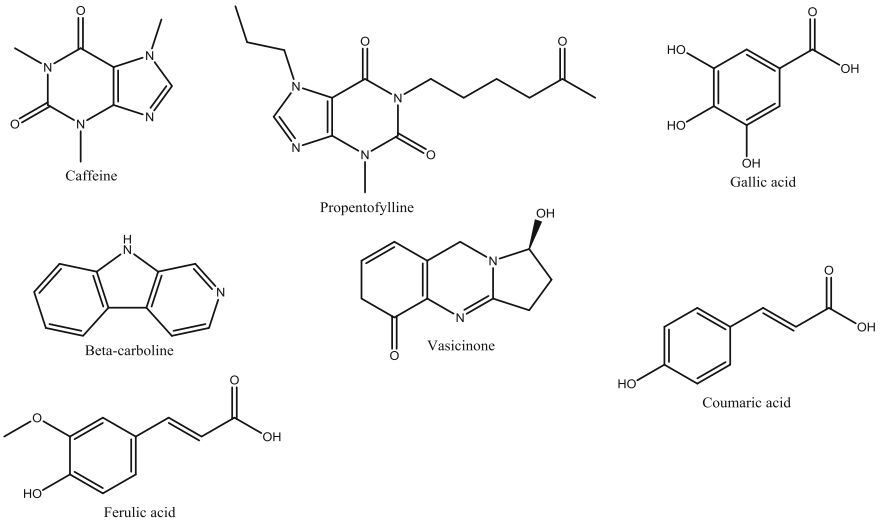


Fig. 17 Chemical structures of phytoconstituents reported in the investigations as MTDLs

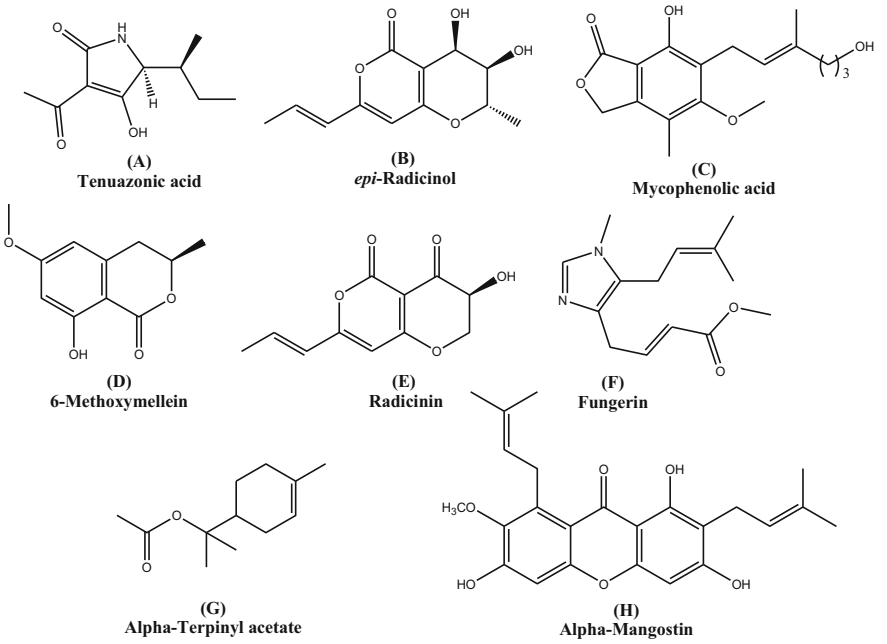


Fig. 18 Chemical structures of anti-AD MTDLs derived from natural origin

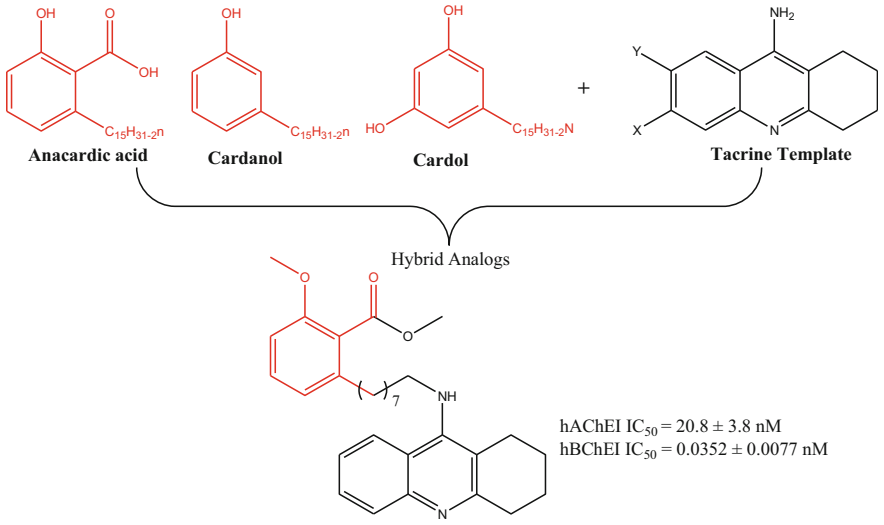


Fig. 19 Structural details of hybrid analogs derived from cashew nutshell liquid combined with tacrine template and the potent analog

Table 3 Details of natural MTDLs and their targets identified by screening InterBioScreen database

	AD targets	Compound
3 targets	CDK5, GSK-3ß, AChE	STOCK1N-31,193
	CDK5, BACE1, AChE	STOCK1N-68,100
	CDK5, BACE1, GSK-3ß	STOCK1N-83,050
2 targets	BACE1, AChE	STOCK1N-68,215, STOCK1N-69,729
	BACE1, GSK-3ß	STOCK1N-03648, STOCK1N-04548
	CDK5, AChE	STOCK1N-71,927, STOCK1N-76,042, STOCK1N-76,267
	CDK5, BACE1	STOCK1N-55,801, STOCK1N-67,973, STOCK1N-68,845
	CDK5, GSK-3ß	STOCK1N-36,270, STOCK1N-36,506, STOCK1N-38,926
	CDK5, MAOB	STOCK1N-50,225
	MAOB, GSK-3ß	STOCK1N-38,066, STOCK1N-38,837, STOCK1N-39,155

and molecular dynamic studies were performed for identified active ligands to analyze their potential as MTDLs against AD. The study outcomes are detailed in Table 3.

6 Conclusions

Alzheimer's disease (AD) is a multifactorial neurodegenerative disease, and a drug that targets a single protein would not provide a cure for this disease. Currently available drugs for AD are all palliative rather than curative. The lack of therapeutic effectiveness of the single-target drugs and multifactorial etiology of AD has led to the design of multi-target directed ligands for AD.

Malfunctioning of cholinergic transmission and glycation, formation of amyloid deposits, and oxidative stress have been proposed to be involved in pathogenesis and progression of the disease (Lane et al. 2018). In this connection, drugs sustaining the cholinergic tone have been developed to contrast the progressive cognitive decline that characterizes AD. In particular, AChE inhibitors such as donepezil are used for the symptomatic treatment of dementia, even if only moderate efficacy is observed in AD patients. Owing to their limited efficacy and problems of the onset of tolerance after long-term use of AChE inhibitors, they are encouraged to be used in combination with other drugs. Various natural and synthetic compounds have been evaluated for their multi-targeted mechanism for AD. The complex pathogenesis of AD is not addressed by available single-target therapeutics and fails to provide complete cure. Recent evidence-based research has focused on the development of MTDLs as anti-AD therapeutics to address the limitations and side effects associated with available therapeutics. MTDLs based on targeting different potential targets involved in Alzheimer's pathogenesis from synthetic or natural sources have exhibited promising results. During clinical use, some of the major limitations like drug-induced hepatotoxicity can be addressed by structural modifications or synthesis of hybrid analogs using hepatoprotective agents. Studies confirm the potential of MTDLs as an ideal pharmacological tool for tackling diseases having complex pathology. Among the various reported pathologies, AChE inhibitor-based multi-targeting has received considerable attention along with other important targets like BACE-1, GSK-3ß, NMDA, and PDE. There is a need for further extension for preclinical and clinical evaluation. Some well-reported anti-Alzheimer's agents like tacrine/donepezil/memoquin have been modified to develop MTDLs to reduce toxicity and side effects and to improve cognition. The success of MTDLs has been evidenced by their neuroprotective, anti-inflammatory, antioxidant, and inhibition of different AD pathogenesis in in vivo and in vitro models.

The Successful implication of MTDLs can eliminate the need to simultaneously administer multiple drugs with potentially different degrees of bioavailability, pharmacokinetics, and metabolism. It will also provide patients a simplified therapeutic regimen. Limitations associated with multi-targeted approach are complex activity profile, unpredictable pharmacokinetics, lack of BBB permeability, adverse effects, etc. In view of the demand for safe and potent AD therapeutics, more efforts are required. In the near future, active immunotherapy against both amyloid pathology and tau pathology in a single bivalent AD vaccine is worth investigating.

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Exploring the Role of Tau Proteins in Alzheimer's Disease from Typical Functioning MAPs to Aberrant Fibrillary Deposits in the Brain

Gadde Shareena and Dileep Kumar

Abstract Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to memory loss and cognitive function deficits in affected individuals, eventually affecting their motor functions. Among many other neurodegenerative conditions, AD is the leading cause of disability and dependency in the elderly population. The two principal essential markers of AD pathology include abnormal deposition neurofibrillary tangles (tau proteins) and amyloid plaques (Aβ peptides). Thus, tau proteins are of critical interest as the prominent indicator of disease mechanisms. Understanding the normal biological functions of tau and the role of PTMs (posttranslational modifications) is essential. In AD, the alteration of physiological tau proteins into aberrant misfolded proteins, such as oligomers, PHFs, and NFTs, due to the PTMs, leads to its interneuronal propagation along with Aβ plaques. It results in synapse loss, neurotoxicity, and neurodegeneration. Since extensive research has shown that Aβ-targeting medicines are toxic and less effective at attenuating AD pathology, tau-directed therapeutics have gained significant remedial focus in recent decades. Although current tau-related therapies provide transient symptomatic comfort, they do not treat the illness overall. Hence, modern research has focused on analyzing tau protein's mechanisms and complexities to produce effective disease-modifying drugs. This review presents a thorough understanding of tau proteins in AD pathogenesis, their origin, and their essential roles in physiological conditions. Additionally, various effective therapeutics targeting tau-associated PTMs such as hyperphosphorylation, acetylation, and methylation are described. Moreover, novel therapeutic strategies such as immunotherapy and oligonucleotide therapy have been mentioned. The clinical trials surrounding tau-related medications have also been highlighted.

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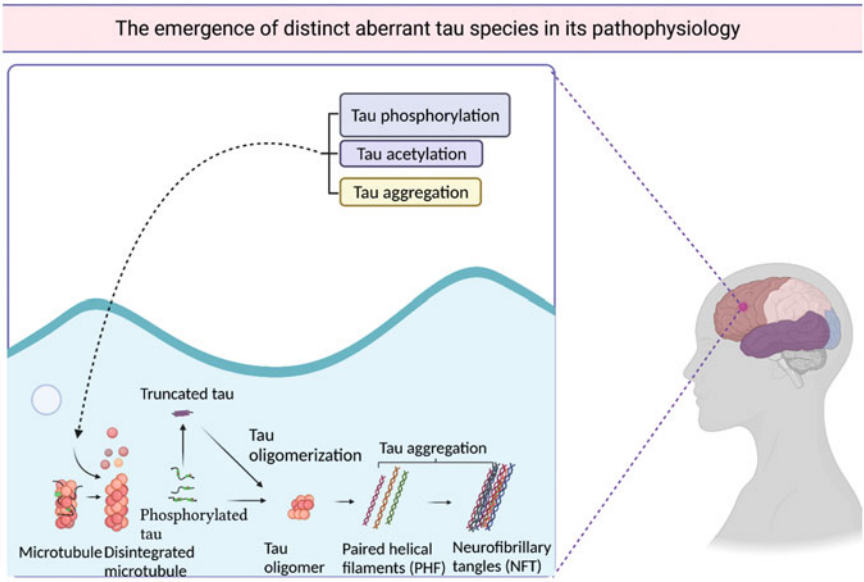
Graphical Abstract



The graphical abstract illustrates the pathological transformation of tau proteins to interneuronal fibrillary tangles (NFTs), due to posttranslational modifications, seen in AD.

Keywords Tau inhibitors · Biomarker · Tau protein · Immunotherapy · Aberrant tau · Posttranslational modifications

Abbreviations

AD	Alzheimer's disease
AIEC	Anterolateral entorhinal cortex
APP	Amyloid precursor protein
Aβ peptide	Amyloid-beta peptide
CDK-5	Cyclin-dependent kinases-5
CK1	Casein kinase 1
COX	Cyclooxygenase
CSF	Cerebrospinal fluid
GSK-3	Glycogen synthase kinase 3
HMTM	Hydromethylthionine mesylate
JNK	c-Jun N-terminal kinase
MAP	Microtubule-associated proteins
MAP3K	Mitogen-activated protein kinase
MAPT	Microtubule-associated protein tau

MT	Microtubules
MTBR	Microtubule-binding region
NFT	Neurofibrillary tangles
PHFs	Paired helical filaments
PI3Ks	Phosphoinositide 3-kinases
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
PMEC	Posteromedial subregion
PP2A	Protein phosphatase 2A
PTM	Posttranslational modifications
SMAP	Synthetic tricyclic sulfonamide PP2A activators
TG mice	Transgenic mice

1 Introduction

Neurological disorders have been seen throughout antiquity to substantially impact individuals' psychomotor and cognitive index, affecting their capacities. AD affects over 44 million individuals worldwide (Tahami Monfared et al. 2022), accounting for 60–80% of dementia-related disorders (Qiu et al. 2009). It indicates an incidence rate of 10.7% among affected individuals aged 65 and older. In the United States, nearly 6.2 million people aged 65 and older suffer from AD, with around 200,000 younger people suffering from early-onset AD. Women have a higher prevalence of AD as compared with men, reported at approximately 65% (Cunningham et al. 2015). According to the WHO, AD is the sixth major cause of death worldwide and the fifth major cause of mortality in Americans aged 65 and older. According to the 2019 statistics, official death records evidenced 121499 mortalities due to AD (Arvanitakis et al. 2019). Patients suffering from AD live between 3 and 11 years after the initial diagnosis, wherein a few survive 20 years and more. The severity of AD-related impairments at the initial diagnosis can affect life expectancy.

Along with age, untreated risk factors such as stroke, hypertension, and heart disease can affect an AD individual's life expectancy (Cechetto et al. 2008). AD is a pivotal contributor to impairment and dependency among the elderly, showcasing physical, psychological, social, and economic consequences. Memory loss is minimal in the initial phases of AD, but affected individuals lose their aptitude to deliver messages and respond to their environment with time (Jahn 2013).

The essential risk factors of AD include undiagnosed clinical depression, increasing age, head-related trauma, smoking, hypertension, behavioral alterations, physical inactivity, and cardiovascular risk factors. Aside from these factors, genetic predisposition contributes a crucial role in the pathophysiology of early-onset AD, typically observed in familial AD cases. Even though mutant genes of AD, such as the APOE-e4 gene, increase the probability of developing the disease, they sometimes do not outcome in an AD diagnosis (Reitz and Mayeux 2014). Tau lesions and

β-amyloid, two biomarkers that form aberrant brain deposits, are strongly associated with AD (Ittner and Götz 2011). Neuropathological autopsy assessments of neural tissue for neuritic plaques and interneuronal fibril tangles in the hippocampal and entorhinal regions are required for a definitive diagnosis. As shown in the autopsy studies, the potent development and spreadability of the plaques are observed in a foreseen pattern. The development of tau disease is a complex multivariate phenomenon. Tau homeostasis is controlled by various PTMs, subcellular localization, molecular chaperones, and mRNA splicing, which regulates tau turnover and microtubule binding (Hall et al. 2012). Tau's homeostatic balance is disrupted in AD, gradually losing its affinity for microtubules and accumulating as proteotoxic aggregates in the brain tissues. These disease-associated modifications of tau proteins result in repetitive neuronal dysfunctions, neurotoxicity, and neuronal apoptosis (Niikura et al. 2006). Along with it, the emergence of tau lesions among neuronal synapses leads to an uneven distribution of neurotransmitters (Francis 2005), cytoskeletal alterations, synapse loss (Jackson et al. 2019), and neuroinflammation (Bloom 2014). Currently, existing clinical approaches, although focusing on the aberrant tau species, primarily target and inhibit the enzyme acetylcholinesterase and, in some cases, reduce glutamatergic transmission (Breijyeh and Karaman 2020). This provides temporary symptomatic relief in the affected patients but does not eliminate the condition (Yiannopoulou and Papageorgiou 2013).

Furthermore, since the cognitive impairment associated with NFTs is substantially greater than that involved with Aβ lesions, recent studies have focused more on analyzing tau pathology (Näslund et al. 2000). Most researchers conclude that they exhibit complicated pathogenic functions such that they block the neural framework of the brain and interfere with the several mechanisms essential for cell survivability, respectively. The deterioration and neural tissue death are the conclusive reasons for their functioning in memory loss, personality changes, and difficulties carrying out daily tasks (Bekris et al. 2010).

This paper aims to provide a comprehensive understanding of tau proteins, their origin, and abnormal alterations in AD. The available tau inhibitors for particular PTMs have been discussed, as well as other novel therapeutic strategies such as immunotherapy and oligonucleotide therapy. Clinical trials involving potent inhibitors have also been described in the context of AD and other neurodegenerative diseases.

2 The Origin, Structural, and Functional Morphology of Tau-A Crucial Regulatory Protein

Tau proteins, which stabilize microtubules, were identified in 1975. They are typically found in the distal parts of axons. They are widely distributed throughout neuronal cells and tissues of the central nervous system (CNS), with negligible amounts in the somatodendritic regions and the nucleus (Tashiro et al. 1997).

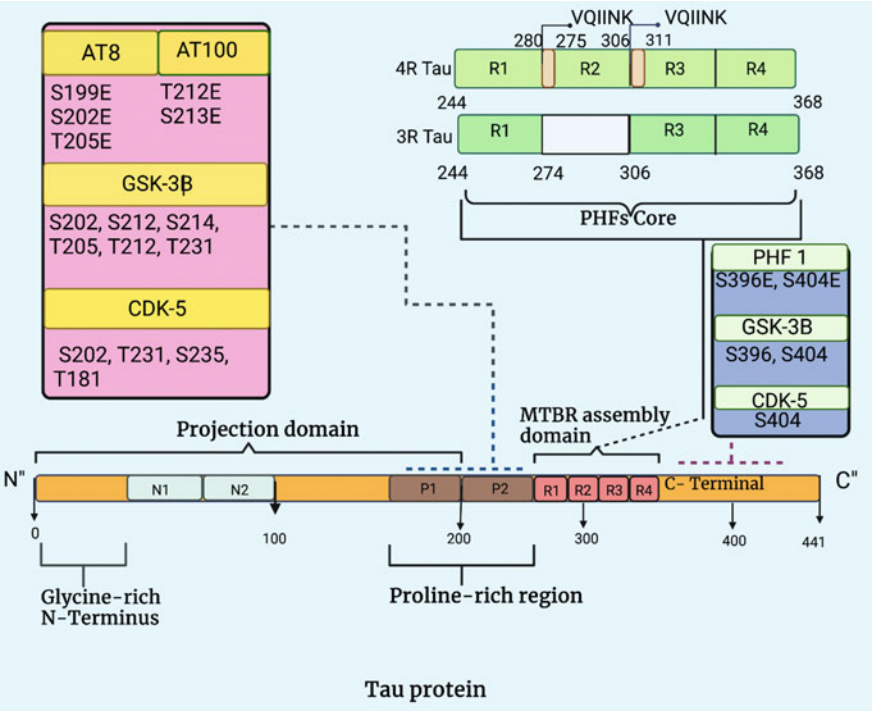


Fig. 1 The diagram represents the structural characteristic of tau protein, showcasing several domains, N-terminal and C-terminal while also, briefly describes the phospho-epitope, present for protein kinase bindings that determines its activity or inactivity

Microtubule structures and alterations in their features are implicated in the development of dementia-related neurological disorders since they play a vital role in controlling axonal stability and cell morphology. The tau protein and tubulin maintain a dynamic environment that allows tubulin assembly into microtubules (Cleveland et al. 1977). They are frequently diagnosed using PET nuclear imaging of neuronal tissues and CSF laboratory testing to detect tau-based biomarkers.

Alternate mRNA splicing of exons 2, 3, and 10 of the MAPT gene (microtubule-associated protein tau) on chromosome 17 results in a class of six highly soluble tau isoforms, each of which contains nearly 441 amino acid residues in matured human brain tissues (Goedert et al. 1991). It is distinguished by the presence/absence of one-to-many N-terminal inserts (0N, 1N, 2N), as well as the occurrence of three (3R) or four (4R) microtubule-binding repeats in the C-terminal region of tau (Fig. 1 portrays the structural characteristics of tau protein) (Ramkumar et al. 2018). Tau isoform interpretations are closely related to brain growth, with only the short isoform, 0N3R, indicated during neurogenesis. In contrast, the developed adult brain displays all six predominant isoforms with approximately equivalent levels of 3R and 4R isoforms (Goedert et al. 1989; Trabzuni et al. 2012).

Tau proteins and several other destabilizing MAPs like stathmin have been termed "intrinsically disordered proteins." They share a specific characteristic of being resistant to heat and acidic exposure while also having a minimum secondary structural composition (von Bergen et al. 2000). As a result of the numerous underlying dynamics, tau proteins are complex and challenging to establish. Tau plays an essential role in MT dynamics since it controls structural assembly, spatial arrangement, and dynamic characteristics. Tau binds to microtubules and either overtly or implicitly stabilizes them by functioning as a circumnavigation which improves their connectivity to a range of other cytoskeletal elements, like actin and neurofilaments. To obtain optimum system dynamics, support axonal development, and regulate vesicles and organelles trafficking through MTs, Tau and other MAPs bound to MTs are closely influenced by many variables, i.e., posttranslational modifications (PTMs) (Mandelkow et al. 1995; Ramkumar et al. 2018). Tau has been identified to be associated with regulatory proteins on a continual basis and regulate microtubule-mediated axonal transport and play a pivotal role in axonal divergence, ontogenesis, protrusion, trafficking, and neuroplasticity (Kempf et al. 1996; Takei et al. 2000).

Tau protein is widespread in neurons due to its intrinsic microtubule integration. It has risen to prominence due to the likelihood of its fibrillar deposition in neuronal cells and tissues in various neuropathological diseases, most notably AD. Neurofibrillary tangles (NFTs) are formed when tau biomolecules aggregate in brain tissues. Tangles develop when the microtubule protein tau is hyperphosphorylated, enabling it to dissociate from microtubules, generate aggregates, and enhance tau translocation and trafficking to the somatodendritic region, which leads to the dissemination of pathological tau. The higher levels of aberrant tau lead to intracellular tau–tau interactions, culminating in tau aggregation. Tau lesions are made up of diverse polymers whose composition varies depending on the disease and its stages. Tau emerges as straight filaments, monomers, oligomers, and paired helical filaments (PHFs) in AD (Meraz-Rios et al. 2010). The formation of phosphorylated pre-tangles and neuropil threads (Dehmelt and Halpain 2004), seen with the Gallyas silver stain, is detected decades before the complications occur in various tauopathies, revealing a remarkably consistent pattern.

In the brains of AD patients, the degree of phosphorylation reflects changes in the activity of abnormally activated protein kinases and phosphatases, which can be related to kinase overexpression or breakdown of the regulation (Imahori and Uchida 1997). The propagation of tau pathology in the brain in Tauopathies, according to experimental evidence, incorporates the propagation of abnormal neurotoxic tau species across neuroanatomically related brain areas. Increased cytosolic tau promotes tau–tau interactions in neurons, resulting in insoluble interneuronal neurofibrillary/gliofibrillary tangles (Alonso et al. 2008). The formed NFTs exhibit a detrimental effect on neuron signal transmission, contribute to blocking synapses, and lead to swollen axon terminals (Götz et al. 2006). However, on the other hand, PHFs and soluble lower mass tau aggregates are not responsible for their neurotoxic characteristics. The propagation is similar to a "prion-like" mechanism, wherein the aberrant tau is transmitted (Nussbaum et al. 2013). The underlying rationale for the

sequential appearance of tau pathology in the brains of tauopathy patients is that it emanates from the distinct vulnerable and susceptible regions of the brain to AD's pathophysiology, which is then demonstrated in the aging brain by the NFT lesions all across the brain. Lesions originate in the trans-entorhinal and anterolateral entorhinal cortex (alEC) and subsequently propagate to the posteromedial subregion (pmEC). These two subregions process different memory processes and interconnect other brain regions that handle object-related and spatial memory (Kerr et al. 2007). As opposed to the latter region, which may not be significantly affected until later in the disease process (Berron et al. 2017) and appear to show resilient to early tau pathology (Reagh et al. 2018), the former region, aIEC, is more susceptible to the effects of aging and preclinical AD neuropathology due to its early sensitivity to tau pathology. The former aIEC region is more affected than the later pmEC regions, which are not extensively impaired until the latter stages of the disease and appear to be resistant to early tau pathology. The aIEC regions possess early sensitivity to preclinical AD neuropathology and increasing age (Reagh et al. 2018; Braak and Braak 1985).

3 Posttranslational Modifications and Their Respective Therapeutic Inhibitors

3.1 Hyperphosphorylation

The phosphorylation pattern in AD varies with the advancement in its pathology. While during extensive research, a multitude of phosphorylation sites has been identified to modulate tau MT-binding and MT assembly activities, as well as to be involved in the development, morphogenesis, and axon maintenance in the neuronal framework. The abundance and frequency of interneuronal neurofibrillary tangles (NFT) in the neocortical regions correlate with dementia and surge with age, making it a plausible therapeutic target and a nascent topic for scientific interest. Acknowledging the functioning of several protein kinases, phosphatases, tau phosphorylation sites, and other numerous posttranslational modifications that regulate and control the phosphorylation of the protein at multiple domains is necessary to develop coherent tau-based therapeutic drugs (Fig. 2 represents several classes of protein kinases and their potential inhibitors). In the AD-associated brain, tau is abnormally hyperphosphorylated at several places, apparently due to multiple paired combinations of several protein kinases, promoting the conversion of normal tau to phosphorylated tau (Morishima-Kawashima et al. 1995), and hinders its microtubule assembly enabling potency. Understanding the regulatory modalities of tau phosphorylation is thus of crucial concern in establishing the rationale of tau aggregation formation and devising a protective approach to deal with these lesions in AD. Since hyperphosphorylation is considered the most distinguished signaling mechanism associated with NFT development, it has been postulated that the active suppression

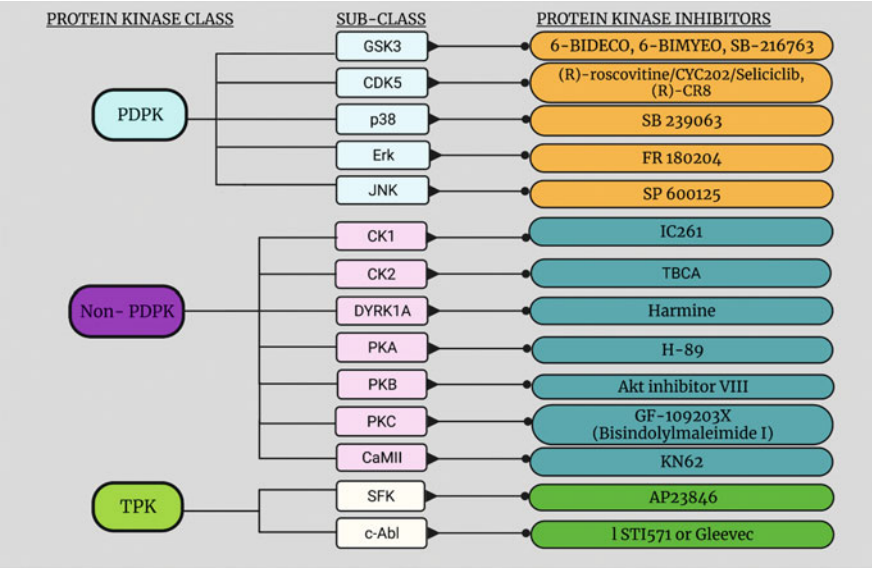


Fig. 2 Above tree diagram describes the sub-classes of protein kinases and their protein kinase-specific inhibitors, typically observed as a commonplace of therapeutic interventions in dementia-related conditions

of protein kinases associated with tau hyperphosphorylation due to (CDK5 and GSK3 of the AGC family (Šimić et al. 2016) can potentially phosphorylate tau at the majority of the identified AD locations.

Glycogen synthase kinase 3 (GSK-3) is a protein kinase that enables the transfer of phosphate molecules to threonine and serine amino acid residues. GSK-3 exists in two isoforms, GSK-3α and GSK-3β, encoded by a distinct gene. Compared to the β1 isoform, the β2 isoform displays reduced activity on tau phosphorylation (Mukai et al. 2002).

Phosphorylation by GSK-3 occurs at nearly 42 sites. Twenty-nine out of them are phosphorylated in AD neural tissues (Sergeant et al. 2008). GSK-3 increased expression in transgenic animals corresponds to progressive cognitive irregularities along with tau hyperphosphorylation and, as a result, neurodegeneration (Lucas et al. 2001) and neurotoxicity, ultimately causing cell apoptosis (Bhat et al. 2002; Hetman et al. 2000). Furthermore, GSK-3 is also engaged in cell proliferation, neural functions, oncogenesis, embryogenesis, programmed cell death, and immunogenic response pathways (Hooper et al. 2008; Rayasam et al. 2009). As demonstrated via in vitro assays using mammalian GSK-3β, Interestingly, Aβ plaques activate GSK-3β. The interactions promote tau hyperphosphorylation and their integral localization with interneuronal fibrils (Pei et al. 1997). Activated GSK-3β enhances its concentration in the frontal lobe region of the brain, further promoting neural tissue damage in the hippocampal region, cognitive deficits, and erythrogenic responses, enhances amyloid concentrations, causes a noticeable decline in

cholinergic levels in AD (Hoshi et al. 1996), and also accelerates the production of toxic tau multimers corresponding to neurotoxicity. Additional evidence supported by Cho JH and Johnson GV suggests the production of detergent-incongruous tau and thioflavin S-positive clumps in cultured cells from co-transfection of quantized tau at D421 and GSK-3 (Cho and Johnson 2004) or triple expression of wild-type tau, GSK3, and JNK (Sato et al. 2002). The clinical phase trials on GSK-3-mediated tau phosphorylation are performed on several pharmaceutical medications such as lithium chloride, AR-A014418, 6-BIO, 6-BIBIO, and SB 216763 understanding their respective functionalities and conclude their beneficial and detrimental natures, respectively.

3.1.1 Lithium Chloride

Lithium, a divalent metal ion, possesses stronger tendency to cross the blood-brain barrier, increasing its bioavailability in the bloodstream. It depicts effective pharmacological silencing on GSK protein kinase. In cell cultures, lithium prevents senile plaque-induced neurotoxicity and phosphorylation (Caccamo et al. 2007). Specific studies conducted on transgenic animals revealed that lithium elicited declining effects on phospho-tau levels and amyloid-β plaques, reduced NFT formation (Forlenza et al. 2014), and rejuvenated neuron loss.

3.1.2 SB 216763

SB-216763 is a synthetic ATP-based compound manufactured initially by GlaxoSmithKline. It competitively binds and inhibits GSK-3α and GSK-3β at equipotent ratios in microvascular endothelial cell lines and colorectal cancer cells. Hence, it acts as a potential downregulation mediator for GSK protein kinase and demonstrates minimal inhibitory potencies on 24 other protein kinases. Aside from avoiding neuronal cell death induced by the PI3-kinase pathway, it features powerful neuroprotective properties and intensifies glycogen production in liver cells. SB 216763 has been reported to keep mouse embryonic stem cells undifferentiated (Kirby et al. 2012).

3.1.3 Tideglusib

It is an irreversible ATP-independent GSK3 inhibitor chemical. Tau phosphorylation, cellular apoptosis, astrogliosis, senile plaque depositions, and cognitive dysfunctions are all reduced in AD model species (Serenó et al. 2009). In sub-population investigations for patients with mild AD, clinical assessments establish its type, which is linked to improved cognitive functions and lower CSF levels of secretase (Domínguez et al. 2012).

Cyclin-dependent kinase 5 (CDK5), regarded as a compendium of kinases comprising 17 amino acid residue sites in tau, was found as the target for CDK-5, depicting antagonistic activities on GSK-5. They are the most frequently occurring protein kinases after GSK-3, and current therapeutic approaches target them as potent pharmacological medications. They reportedly act as GSK-5 inhibitors, suggesting that CDK5 inhibition is highly likely to enhance tau phosphorylation. The physiological activity of CDK5, which contributes to neurodevelopmental and synaptic activity, is exclusively dependent on the binding of its regulating components, p35 or p39. Calpain, a calcium-dependent protease, cleaves p35 and p39 to produce p25 or p29 and then binds to CDK5. This process culminates in increased pathogenic expression. CDK5 phosphorylates tau at 9–13 domains by activating GSK3β (Liu et al. 2016).

3.1.4 Roscovitine (Seliciclib)

Roscovitine, an oral medication primarily associated with cancer studies, was first developed as an experimental neurodegenerative intervention by Tocris Bioscience. It is noted for its competitive binding properties to CDK5. These interactions prevent tau from being phosphorylated while lowering the concentrations of phosphorylated tau and inducing cellular death, ultimately eliminating undesirable aberrant tau (Khalil et al. 2015).

3.2 Microtubule Instabilities

Acetylated tau is a diagnostic biomarker for stable microtubules. It is decreased in tauopathies associated with AD. As anticipated, tau gradually disengages from axonal MTs due to hyperphosphorylation, which is known to diminish the protein's affinity for the MT by changing the dynamic of the tubulin polymer (Hempen and Brion 1996). It is known to generate a slew of adverse side effects, such as leukopenia and distal polyneuropathy, leading to axonal edema (Freedman et al. 2011). Due to dose-limiting effects, long-term therapy of tauopathy patients with this class of medicines may be problematic. They primarily stabilize microtubules, prevent cell division, and improve cellular density. Despite these significant challenges, several lines of research have found therapeutically valuable drugs for treating cellular diseases.

3.2.1 Epothilones (BMS-241027)

They are a distinctive group of natural cytotoxic substances that affect microtubule activity and were firstly identified from the myxobacterium S. cellulosum. They are among the only three MT-stabilizing medicines that progressed through clinical testing. BMS-241027 reversed a spatial memory loss in rTg4510 mice expressing

mutant human tau. It also decreased hippocampus neuronal loss and several forms of histological neuropathology. BMS-241027 restored behavioral and cognitive impairments, eliminated tau pathology, and reduced neuron loss (Zhang et al. 2012) which is relatively safe as it penetrates the blood-brain barrier. Epothilone D has been proven efficacious at low concentrations (Brunden et al. 2010).

3.3 Phosphatase Activity

Protein phosphatase 2A (PP2A) is a heterotrimeric phosphatase essentially found in healthy brains. It comprises three structural subunits: a structural A subunit, a regulatory B subunit, and a catalytic C subunit that regulates, monitors, and expresses different physiological activities of the phosphatase (Jones et al. 1993). PP2A is an essential neuronal protein whose modifications could lead to dysregulations in cytoskeleton integrity and promote cellular apoptosis. In the brains of AD patients, neuronal loss is detected alongside tau hyperphosphorylation, amyloid synthesis, and memory impairments. Compared with age-matched controls, the expression of PP2A and its promoters is significantly decreased in the brains of patients with AD, but PP2A inhibitors are upregulated. Remarkably, PP2A serves to regulate GSK3β, JNK, and CDK5 protein kinases, providing an alternative approach to modulating tau phosphorylation (Liu et al. 2005). It is a significant contributor to the hyperphosphorylation of tau at numerous sites where the decrease in the protein phosphatase(s) concentrations involving tau dephosphorylation is noticed. Ultimately, the activity of PP2A is reduced in the disease owing to reduced levels, increased inhibition, and alterations in its specificity and subcellular localization (Sontag and Sontag 2014; Sontag et al. 2007).

3.3.1 Synthetic Tricyclic Sulfonamide PP2A Activators (SMAPs)

SMAPs (DBK-1154 and DBK-1160) bind to the PP2A A scaffold subunit and induce PP2A conformational changes in the holoenzyme complex, leading to more efficient pharmacological actions as powerful PP2A agonists. Treatments with DBK-1154 and DBK-1160 reduce tau phosphorylation in N2a/APP cell lines in a dose-dependent manner. Although SMAP treatment reduces the expression of pT668APP and PS1, it does not affect the rat models' motor ability and anxiety. As shown in HHcy rat brains, DBK-1160 reduces APP-BACE1 and APP-Thr668 interactions and APP cleavage (Sontag et al. 2007). Another drug group, SCR-1693, a novel tacrine-dihydropyridine hybrid, did not demonstrate similar capability when utilized as positive controls. They drastically improve PP2A activity and contribute to tau phosphorylation and Aβ40-42 level reductions in the brain (Wang et al. 2015). SMAP treatment attenuated HHcy-mediated Aβ overproduction and tau hyperphosphorylation in HHcy-AD rat models, alleviated cognitive deficits and hippocampus neuronal synapse dysfunction, and avoided neuronal spine loss. SMAPs reduced overall tau phosphorylation via PP2A activation, prevented

HHcy-mediated hyperphosphorylation, improved memory, repressed Aβ generation via APP phosphorylation and secretase activity, and prevented HHcy-mediated synaptic dysfunction and spine loss in HEK-293/tau cells (Wei et al. 2020). Metformin, a PP2A agonist, affects PP2Ac-MID1-4 protein complexes associated with microtubules. The PP2Ac-MID1-44 regulates PP2Ac degradation and enhances PP2A activity by enabling PP2Ac to be accessible for holoenzyme activity.

3.4 Acetylation

Acetylation is undoubtedly one of the most significant variables in tau pathophysiology. It is a broadly acknowledged PTM in neuronal pathophysiology. Acetylation modulation plays a pivotal part in AD behavioral traits. It has been identified as a possible TBI blood biomarker that may demonstrate pathological overlapping between traumatic brain injury (TBI) and AD. Tau is associated with acetylation within the lysine-rich MTBRs (microtubule-binding region) (Cohen et al. 2011; Min et al. 2010). In vitro and cell-based experiments display activation and inactivation of Sirtuin1 deacetylase and p300/CBP by S-nitrosylated-GAPDH simultaneously, resulting in enhanced neuronal activity, thus acetylating tau and promoting its mislocalization (Morris et al. 2015). Acetylation of tau enhances its pathology by promoting tau mislocalization (Shin et al. 2021). Tau breakage, blocking ubiquitin binding, and delaying tau protein's turnover (Wang and Mandelkow 2016) ultimately resulted in neurodegeneration and neurobehavioral dysfunction. As a result, the cytosolic accumulation of tau in the cell becomes susceptible to aggregation and much more difficult to eliminate. The mass spectrometric investigations imply that the acetylation sites cluster within the microtubule-binding region (MTBR). The mass spectrometry studies observed four major acetylation sites (K163, K280, K281, and K369) in phospho-purified tau from cell cultures, corresponding to limited tau-associated MT assembly functions (Trzeciakiewicz et al. 2017) and affirmed the presence of acetylated tau sites cluster within the MTBRs.

3.4.1 Salsalate

Salsalate is an oral NSAID prodrug that belongs to the salicylates class of medicines. It has been marketed for decades in treating rheumatoid arthritis and osteoarthritis, substantially reversing tau-related dysfunction in tau-related animal models. It functions pharmacologically by inhibiting p300 HAT tau acetylation at Lys174 (Min et al. 2015), resulting in reduced expression of tau pathology, preserved volume in the hippocampal region, and facilitated cognitive profile in transgenic animal models (Congdon and Sigurdsson 2018). Furthermore, it modulates the expression of cyclooxygenase (COX) enzymes, lowering the production of prostaglandins, which pertains to its anti-inflammatory, analgesic, antipyretic, and antiplatelet activities.

3.5 Aggregation

The center of the Alzheimer's neurofibrillary tangle's constituent filaments comprises a truncated tau repeat domain fragment. This truncated segment acts as an initializing cascade factor for the transneuronal propagation of proteolytically stable oligomer species between neurons and previously healthy neighboring neurons. The potential upstream role of misfolded tau is considered highly responsible for tau protein aggregation. The 441-residue tau protein, primarily self-assembled in the microtubules, is mainly found to be unfolded. The mutations at VQIVYK (PHF6) and VQIINK(PHF6*), located in the second and third repeat units of MTB regions, respectively, form stable parallel β-sheet twisted filaments that are responsible for their tau aggregation-inducing activity in the brain (Takei et al. 2000; von Bergen et al. 2001) As demonstrated in the *in vitro* and silico studies, the β-hairpin configuration of the functional tau monomer is extensively disrupted during the p301 mutation, thus destabilizing the structure and enhancing neurodegeneration.

3.5.1 Curcumin (Diferuloylmethane)

The role of curcumin has been meticulously studied in the history of the Indian system of medicine and modern medicine for its effectiveness in a wide range of medical conditions such as cancer, chronic arthritis, respiratory tract infections, cardiovascular disorder, digestive ailments, allergy responses, liver conditions, and clinical mood swings. Curcumin is a fat-soluble, polyphenolic compound obtained as a bright yellow-orange pigment from the rhizomes of Curcuma longa species belonging to the Zingiberaceae family (Dong et al. 2012). They depict many pharmacological activities such as antioxidative, anti-inflammatory, anticarcinogenic, antidiabetic, hepatoprotective, nephroprotective, antirheumatic, and antineoplastic activities. Curcumin has been proven beneficial for its potential effects on neurogenesis and improved cognitive functions in aged transgenic mice modalities (Cox et al. 2015). It inhibits the amyloidogenic protein aggregation of tau lesions by reducing oligomeric tau (Rane et al. 2017) and interacting with the PHF6 domain (Bijari et al. 2018). The body's poor bioavailability and rapid degradation are correlated with the compound's fat-soluble nature, hindering its contribution to long-term usage in neurodegenerative therapies. However, the clinical studies related to curcumin were not proven efficacious, and hence, extensive studies led to the establishment of different related curcumin compounds to assist in AD.

3.5.2 Resveratrol

Resveratrol is a polyphenolic non-flavonoid containing phytochemicals and phytoalexin, which several plants produce as a response to an injury or when the plant is under attack by a wide range of pathogens, such as bacteria or fungi. It is found chiefly in the skins of red wine grapes (Xia et al. 2017). Numerous studies report its

high antioxidative, anti-inflammatory, anticarcinogenic, anti-cardioprotective, vasorelaxant, phytoestrogenic, and neuroprotective potencies (Baur and Sinclair 2006). Dietary wine consumption is directly proportionate to beneficial effects on AD. Its anti-tumor potential has been observed by many *in vitro* and *in vivo* studies that demonstrate that it can suppress various elements of carcinogenesis (Zykova et al. 2008; Varoni et al. 2006). Hence, it is favorably considered for AD-related potential therapies. The assessments performed on P301L tau transgenic models revealed the decline in the levels of tau phosphorylation at AT8 sites along with reduced Aβ fibrillary levels in several cell cultures (Feng et al. 2009) and APP/PS-1 transgenic mice (Porquet et al. 2014). It enhanced tau dephosphorylation via the PP2A stimulation pathway (Schweiger et al. 2017) along with modulating the expression of ERK1/2 and GSK3β protein kinase signaling pathways (Jhang et al. 2017).

3.5.3 Purpurin

Purpurin, a naturally occurring anthraquinone, is primarily a colorant found in the roots and tubers of Rubia species such as R. peregrine (wild madder) and Rubia tinctorum (common madder), belonging to the lipocalin family. The characteristic yellow-red colored dye is mainly employed in cotton printing natural hair dyeing and forms complexes with various metal ions (chelation compounds). The in vitro ThS fluorescence assay revealed that purpurin depicts an inhibitory effect on PHF6 peptide aggregation in a dose-dependent manner. They suppress tau fibril expansion by heparin via association with the PHF6 domain, thus downregulating the amyloid load. Purpurin supplementation improved eye neurodegeneration in the transgenic Drosophila fly model. It disassembled the accumulation of hTau besides crossing the blood–brain barrier (BBB), suggesting strong evidence in the treatment of tau-related dementia (Viswanathan et al. 2020).

3.5.4 Panax Ginseng (C.A. Mey. (Ginseng))

It is regarded as a prominent, valued perennial herb that has historically been used as a traditional medication in China, Korea, and several other regions of Southeast Asia. It is primarily obtained from the roots of plants in the genus Panax (Araliaceae). The pharmacological effects of the compound have been demonstrated on cancer, diabetes, and cardiovascular diseases and have been utilized for advancing immune function, central nervous system (CNS) functions, extenuating oxidative stress, antioxidant, and anti-inflammatory activities (Jung and Jin 1996). The presence of ginsenosides and gintonin characterizes ginseng. Ginsenosides, also called ginseng saponins, are the major pharmacologically bioactive components of ginseng.

The ginseng extracts provide symptomatic relief in patients with AD and constrain the disease's progression by curbing Aβ deposits and tau protein

hyperphosphorylation via the mediation of mitochondrial functioning, neuron transmission, cellular apoptosis, and oxygen free radicals (Rajabian et al. 2019). Based on the substantial *in vitro* and *in vivo* research studies on Korean red ginseng extracts, inhibition of tau aggregations was observed (Shin et al. 2020), wherein the inhibitory effect is suspected to be caused due to the surfactant action or antioxidative effects of ginseng as it contains saponin and flavonoid compounds. They have been shown to defend against various pathogenic cascades in AD, including β-amyloid formation, neuroinflammation, oxidative stress, mitochondrial dysregulations, and neurotoxicity.

3.5.5 Methylthioninium Chloride (Methylene Blue)

Although this therapeutic intervention was developed in the late 1800s, its acceptance and usage have been humongous over the past few years as clinical testing reveals its complete drug profile. It is successfully proposed in many neurodegenerative ailments from bipolar disorders (Alda et al. 2017) to Alzheimer's disease, besides their functioning as a histological dye and trace indicator and its hepatoprotectant and neuro-protectant features. These phenothiazine dyes have been known to show virucidal properties on infectious diseases such as AIDS-related Kaposi's sarcoma and to inactivate Staphylococcus aureus (Zolfaghari et al. 2009) and HIV-1 (Floyd et al. 2004). Moreover, it is currently being studied for bipolar disorder Alzheimer's and finds broad expression for its bactericidal, fungicidal, anti-inflammatory, and cardioprotective properties. Several findings were observed in the in vitro assay of methylene blue. It suppresses tau–tau associations and inhibits the clustering process. Its transmission to experimental mice models reduces the occurrence of hyperphosphorylated tau assemblies (Hosokawa et al. 2012) and prevents deterioration in the patient's cognitive profile (Stack et al. 2014). Methylene blue impedes tau assemblage through its pronounced action on modulating cysteine residues in tau protein. They prevent tau polymerization by confining tau monomers in an aggregation-incompetent frame, thus preventing the further spread of tau filaments and their toxic analogs, resulting in severe neuronal toxicity (Panza et al. 2016). Along with its proven bioavailability, as it crosses the blood–brain barrier, this compound was proven beneficial in significantly reducing tau pathology and improving cognitive traits in mice (May et al. 2004).

4 Hydromethylthionine Mesylate (HMTM) (Also K/as Leuco-Methylthioninium Bis(Hydromesylate))

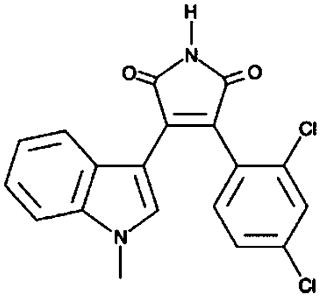
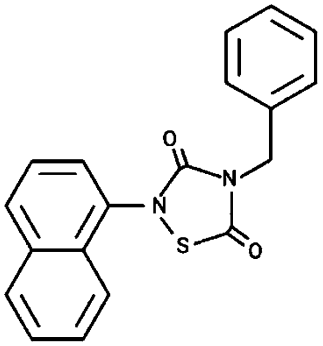
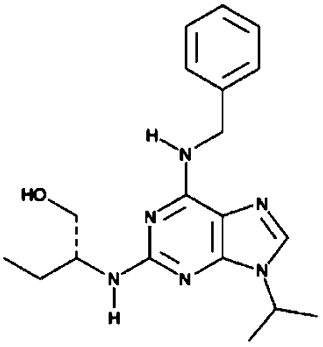
MTM is a second-generation tau–protein aggregation inhibitor with concentration-dependent pharmacological effects in the reduction of cognitive decline, tau aggregation, and brain atrophy. The medicine, developed by TauRx Pharmaceutical Ltd., has been shown to be a more effective derivative of methylthioninium chloride, and

it is the only tau targeting drug in phase 3 late-stage AD development. For cell absorption, methylthioninium chloride (MTC), which contains MT+ species, must be converted to leuco-methylthioninium (LMT). *In vitro*, methylthionine, the reduced form of LMT, was found to be the active species in preventing tau aggregation. The site of action in tau aggregates that are evident in both AD and FTD is within the proteolytically stable tau-unit core (Wischik et al. 2022). The methylthioninium (MT) moiety of HMTM has been suggested to augment pathological tau clearance and improve mitochondrial activity. The medication developed by TauRx Pharmaceutical Ltd. has been shown to be a more effective derivative, being the only tau targeting in phase 3, late-stage AD development. The effect of 8 mg of HMTM per day was effective at 7.5 points on the ADAS-Cog scale, which is three times higher than current AD treatment regimens. After 65 weeks of treatment, there was an 85% reduction in cognitive decline (Schelter et al. 2019). The MT moiety has been shown to have neuroprotective properties by suppressing microglial activation and increasing autophagy (10–20 nM range) (Wischik et al. 2022). In recent studies, HMTM was reported to prevent the formation of AD aggregates, increase hippocampal acetylcholine levels and complex IV in brain mitochondria, reverse glutamate release impairment in cerebral synaptosomes, and restore the activity of choline acetyltransferase in the basal forebrain (Riedel et al. 2019) (Table 1).

5 AD Immunotherapy—A Revolutionary Viewpoint to Effectual AD Medicines

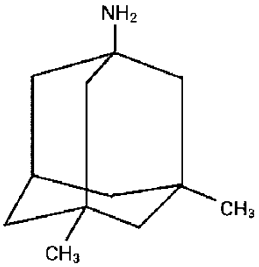
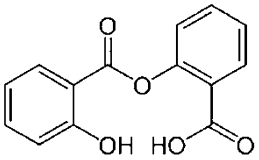
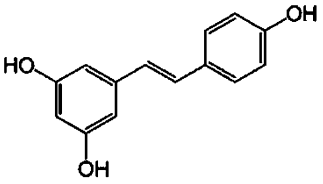
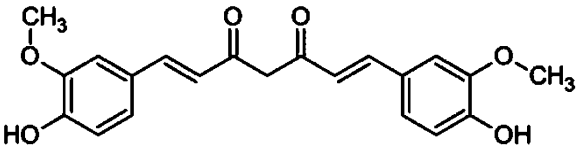
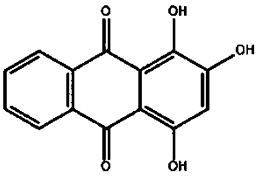
Immunotherapy entails comprehending the constant dialog mechanism between the immune system and multitudes of cancer cells. It has gained the interests of several researchers, physicians, and pharmaceutical corporations, for its potential as a disease-modifying approach owing to its multifaceted nature in treating a wide range of disorders such as cancer, neurological disorders, allergic disorders, and autoimmune disorders. Several years of research and testing have resulted in these approvals, which validate the efficacy of these medicines. Its clinical trials, which involve patient volunteers, are strictly supervised and monitored. Several possible immunotherapeutic techniques, including cell-based strategies like adoptive cell transfer, chimeric antigen receptor therapy, checkpoint inhibition, and agent-based approaches such as antibody therapy, are considered. Many immunotherapeutic agents are being tested in clinical trials to characterize several tau species linked to neurodegeneration and dementia. Antibodies may potentially curtail the disease advancements by halting the progression of abnormal tau. The establishment of therapeutic tau antibodies has emerged in antibody-derived imaging probes that are more specific than the dye-based tau epitope profile, which can eventually be used to determine the parameters of therapeutic antibodies for maximum efficacy and safety (Morgan 2011).

Table. 1 Table demonstrates the chemical structures of effective tau-related disease-attenuating therapeutic compounds

Compounds (1)	Chemical structure (2)
SB 216763	Molecular formula: $C_{19}H_{12}Cl_2N_2O_2$  M. wt: 371.22 g/Mol
Tideglusib	Molecular formula: $C_{19}H_{14}N_2O_2S$  M. wt: 334.4 g/Mol
Roscovitine	Molecular formula: $C_{19}H_{26}N_6O$  M. wt: 354.5 g/Mol

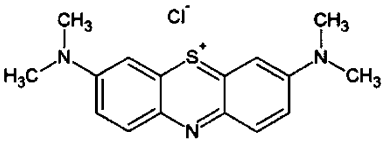
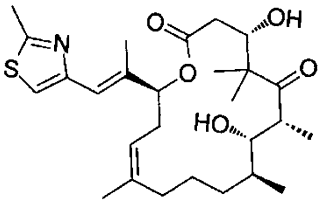
(continued)

Table. 1 (continued)

Compounds (1)	Chemical structure (2)
Memantine	Molecular formula: $C_{12}H_{21}N$  M. wt: 179.30 g/Mol
Salsalate	Molecular formula: $C_{14}H_{10}O_5$  M. wt: 258.229 g/Mol
Resveratrol	Molecular formula: $C_{14}H_{12}O_3$  M. wt: 228.25 g/Mol
Curcumin	Molecular formula: $C_{21}H_{20}O_6$  M. wt: 368.38 g/Mol
Purpurin	Molecular formula: $C_{14}H_8O_5$  M. wt: 256.21 g/Mol

(continued)

Table. 1 (continued)

Compounds (1)	Chemical structure (2)
Methylene blue	Molecular formula: $C_{16}H_{18}ClN_3S$  M. wt: 319.85 g/Mol
Epothilones-D (BMS-241027)	Molecular formula: $C_{27}H_{41}NO_5S$  M. wt: 491.68 g/Mol

Note: The chemical structures of numerous tau-directed inhibitors are shown in Table 1 column (1) which represents the powerful tau inhibitors, and the chemical structures of the substances are represented in column (2)

Active immunity is considered an attractive therapeutic approach as it aids in inducing sustained immune response with a small dose concentration. Fragmented tau or phosphorylated tau peptides are frequently used as the targeted approaches for active immunity. It has been observed to strengthen the tau physiology by approaching single or multiple phospho-epitopes at the amino terminus of normal or mutant tau (Fig. 3 illustrates the pathological alterations in AD and their counterpart immunotherapies in preventing such deterioration).

- C57BL6J mice (congenic stained) are widely approved testing subjects as these transgenic mice express different pathological alterations of AD, including showcase encephalomyelitis, axonal impairment, and aggravation properties.

Passive tau vaccination, as opposed to active tau immunization, provides a feasible and potent alternative to the potential hazards highlighted by active strategies, in which the individuals with AD do not develop antibodies and the adverse effects of immunization likely stay in the transient phase, lowering the occurrences of undesirable immunogenic responses. It also results in increased epitope-specific activity for the targeted epitope (Bard et al. 2000).

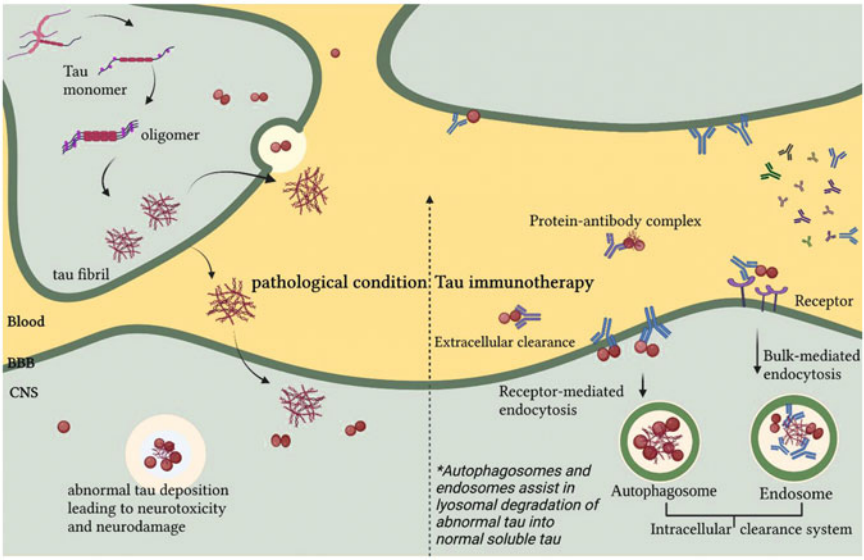


Fig. 3 Above schematic diagram portrays a comparative picturesque between typical pathological conditions observed in AD and the immunotherapeutic regimens, indicating immunotherapy, as a plausible area of interests in developing safer, and effectual drug compounds in preventing the pathological deterioration of AD

5.1 AADvac-1

Target Type: Aberrant Tau Species

The vaccine AADvac-1, considered an early intervention in the immunotherapy domain, has proven efficacious in slowing the progression of the tangle-associated behavioral expressions and reducing the concentration of tau aggregates in the brain. AADvac-1 is an artificial synthetic peptide vaccine that comprises 294–305 tau amino acid residue (Novak et al. 2017). A biotech company, Axon Neuroscience, initially manufactured it. The clinical trials were assessed in patients with mild AD for 24 months. The phase 1 pilot clinical trials for two doses of AADvac-1 were conducted (N=33) with primary progressive aphasia (PPA), a speech-impacting neurological condition in 18- to 85-year-old age groups, and phase 2 trials, to ensure the optimal safety profile and effectiveness of AADvac-1 in patients with moderate AD, were examined, with the phase 3 trials yet to be confirmed. The vaccine was found to be reducing blood NFTs levels alone. Despite having an excellent safety profile, the significant decrease in pTau in CSF and cognitive scores in participants was not definitive. While the immunization did not significantly enhance cognitive scores across the range of scientific analysis of all ages, a pre-arranged age sub-population analysis revealed a slower rate of cognitive decline in the younger participant pool. Even though the immunization therapy lowered the levels of

phosphorylated tau in the spinal fluids of the brain and spinal cord and cerebrospinal fluid (CSF), their CSF adjustments were not clinically conclusive (Novak et al. 2019).

5.2 ACI-35

The vaccine was initially developed by AC immune (Switzerland) and licensed by Janssen Pharmaceuticals.

It is a liposomal vaccine whose constituents comprise phosphorylated serine residues (S396 and S404) and tau fragments (393–408). Due to the vaccine's ineffective immunological response, ACI-35 was modulated, and ACI-35.030 was produced and displayed a solid immune response compared to the previous vaccine (Hickman et al. 2011).

5.3 AN-1792

Target Type: Amyloid-Related

It consists of a full-length AB peptide with qs21 adjuvant. The purpose of AN-1792 is to evoke an immune response on Ab plaques in the brain. Furthermore, its nature is attributed to preventing/reversing AD's pathological hallmarks, such as synaptic loss, gliosis, neuritic dysfunctions, and impaired performance in behavioral bioassay (Gilman et al. 2005).

A multinational phase 2a trial in 372 patient with mild-to-moderate AD revealed brain inflammation in four treated patients later proven to be meningoencephalitis. This adverse event affected 6% of all subjects. The development of AN-1792 was halted in 2002, but follow-up assessments of treated individuals continue. Postmortem pathology examinations of individuals who had received AN-1792 in phase 1 or 2a revealed that the vaccination had significantly removed plaque from the brain. It did not remove neurofibrillary tangles. It also revealed T-cell infiltration and inflammation surrounding leptomeningeal blood vessels, particularly around vascular amyloid. Only a tiny patient population treated with AN-1792 had a strong antibody response, especially antibodies directed against the amyloid's N-terminus. CSF studies revealed a tendency toward lower CSF phospho-tau concentrations in responders, and postmortem brain tissue phospho-tau analyses revealed a decrease in accumulated tau in neuronal processes. MRI scans at baseline and after therapy revealed a transitory increase in brain shrinkage in responders, which was deemed counterintuitive at the time (Vellas et al. 2009).

5.4 RG7345

As developed by Roche Pharma, RG7345 is a humanized monoclonal antibody that binds to the S422 domain of the tau's C-terminal phospho-epitope. They were discovered to be reducing the abundance of intractable, pathogenic tau in pS422, improve short-term mental functioning, and reduce the abnormal tau accrual in Alzheimer's disease (Collin et al. 2014).

5.5 RO7105705(Semorinemab)

It is an anti-tau IgG4 antibody, a strong indicator of a misdirected inflammatory response. It targets extracellular fibril lesions and implies reduced effector function to limit the cascade of microglial activations by binding to the N-terminus region. The antibody's pharmacological impact has been found to diminish aberrant tau forms and constrain their transmission from one cell to another, along with upregulation of plasma tau levels, as observed in P301L transgenic mice (Lee et al. 2016).

6 Conclusion

In this review, we have observed the roles of various potent inhibitors, which we might find helpful to briefly illustrate how these cascade factors gradually affect a person's brain tissue physiology. Several neurodegenerative diseases are yet to be recognized and analyzed. Thus, scientific investigations are critical in the field of neurodegenerative disorders. The wide range of viable therapies is likely to offer therapeutic benefits for effectively treating tau protein dysregulation. Though several scientific studies cannot fully comprehend the aberrant protein's origin role, futuristic research findings will enable our understanding of precise configurations of its origin, mechanisms, and effective therapies that will help eradicate this globally alarming neurodegenerative disorder. Although numerous vaccines and medications have been shown to have therapeutic properties, only a limited fraction of such medications depict positive effects on AD patients, with many of them still in the late stages of clinical trials. While the clinical trials prove these compounds' efficacy and safety, many are considered symptom-based intervention drugs rather than a permanent cure for the pathogenicity of the abnormal tau. Thus, it is necessary to develop new medicines to target and modulate the degenerative effects associated with Alzheimer's disease. Several critical pieces of evidence suggest the study of various tau inhibitors and the biological basis for tau protein. Knowing several mechanistic roles of pathogenic tau helps us identify novel interventions that focus on increasing the clearance of aberrant tau forms.

Nonetheless, the underlying causes of neurodegeneration and the molecular mechanism governing tau-mediated neurotoxicity must be understood. Despite the failure of some therapeutic medications to repair tau pathology, participant studies have demonstrated that they provide symptomatic vascular relief, allowing more time to concentrate on addressing neurodegenerative disorders. This review is of paramount significance as it helps us see and understand the more extensive biomolecular insight of what the general population merely perceives as a brain-affecting disorder. The comparative assessment of tau-related inhibitors and their pathology helps us portray an outline that highlights its subtypes, isoforms, and inhibition pathways associated with tau-related AD. Hence, it helps us focus on investigating the molecular and cellular pathways and processes involved in AD pathogenesis to support the development of effective disease-modifying agents. Upon looking into the futuristic prospects of AD, we realize that current studies focusing solely on a single crucial facet of its therapy are wholly inadequate to slow or eliminate this globally widespread disease. Integrating therapeutic interventions and identifying several other diagnostic markers in AD are essential. Since phosphorylated tau species are well-regarded for their critical roles in AD, exploring and developing possible drug target candidates and inventive research approaches would empower the acquisition of forthcoming tau inhibitors.

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The Overview of Drugs Used in Alzheimer's Disease and Their Molecular Targets

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Abstract Alzheimer's disease (AD) is a progressive condition in which degeneration of neuronal cells is observed in the brain. There are many drugs approved by the

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FDA for the treatment of AD and it includes galantamine, donepezil, rivastigmine, memantine, and aducanumab. Among the available drugs, galantamine, donepezil, and rivastigmine are acetylcholinesterase inhibitor and they enhance cholinergic neurons in the brain. Another drug memantine is N-methyl-D-aspartate (NMDA)-type glutamate receptor antagonist. It prevents excitotoxicity and neuronal cell apoptosis. Similarly, aducanumab is a recently approved drug which attenuates the accumulation of amyloid beta protein. There are many synthetic drugs and herbal bioactives that have been reported against AD. Some of the synthetic drugs and bioactives possess low bioavailability and BBB permeability. Due to this, they show less therapeutic action at the target site for the treatment of AD. Such therapeutic moieties can be incorporated into advanced drug delivery system to enhance their bioavailability and blood–brain barrier (BBB) permeability. The chapter describes an overview of the various treatment strategies as well as the nanomedicines that have been developed so for to treat AD.

Keywords Alzheimer's disease · Drugs · Treatment strategies · Herbal therapies

Abbreviations

ABC	Aducanumab
AChE	Acetylcholinesterase
AD	Alzheimer's disease
APP	Amyloid precursor protein
Aβ	Amyloid beta
BBB	Blood–brain barrier
BDNF	Brain-derived neurotrophic factor
CAT	Catalase
CNS	Central nervous system
CUS	Chronic unexpected stress
GPX	Glutathione peroxidase
H_2O_2	Hydrogen peroxide
IgG1	Immunoglobulin gamma 1
IL	Interleukin
MDA	Malondialdehyde
NFκB	Nuclear factor kappa B
NGF	Nerve growth factor
NLC	Nanostructured lipid carrier
NMDA	N-methyl-D-aspartate
NSC	Neural stem cells
Qu	Quercetin
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SD	Sprague Dawley

SLN	Solid lipid nanoparticles
SNEDDS	Self-nanoemulsifying drug delivery systems
SOD	Superoxide dismutase
TBI	Traumatic brain injury
TNF	Tumour necrosis factor
VEGF	Vascular endothelial growth factor

1 Introduction

Alzheimer's disease (AD) is a chronic condition in which deterioration of cholinergic neurons is observed in various parts of the brain, such as the cerebral cortex and hippocampus. Globally, more than 44 million people are suffering from AD till date (Vishwas et al. 2020). The symptoms associated with AD are categorized by cognitive dysfunction, psychotic abnormalities, and problems in speaking, walking, and performing daily activities. There are many pathogenic factors that are responsible for the disease progression, such as accumulation of amyloid beta (Aβ), formation of neurofibrillary tangle, enhanced oxidative stress, neuroinflammation, mitochondrial dysfunction, endoplasmic reticulum stress, degeneration of cholinergic neurons, and excitotoxicity (Vishwas et al. 2022a, b, c). There are various drugs available in the market for the treatment of AD, such as galantamine, donepezil, rivastigmine, memantine, and aducanumab (Argueta et al. 2022). Furthermore, there are many other drugs, bioactives, and advanced drug delivery system-based nanoparticles, which also produce significant effects against AD (Breijyeh and Karaman 2020). In this chapter, various treatment strategies and their molecular targets have been discussed.

2 Global Prevalence and Economic Burden

Globally, more than 44 million people have been reported with dementia in the year 2022 ("Alzheimer's and Dementia in India", 2022). Among them, about 70% of cases are due to AD. In Australia, approximately 0.487 million people have been diagnosed with dementia till 2022 ("Dementia statistics", 2022). In the USA, about 5.8 million people above 65 years of age have been affected by AD. The overall cost of health care, long-term care, and hospice services for people aged 65 and older with dementia was about $305 billion in 2022 (Alzheimer Association 2022). Further, it is expected that the number may increase up to 13.8 million till 2050 (Alzheimer Association 2022). About 4.72 million Australians have been diagnosed with dementia till the end of 2022, and this number is predicted to rise to 5.9 million by 2028 and 10.76 million by 2058 ("Dementia Statistics", 2022). In Japan, about 3.5 million people suffered from AD till 2016, and it is expected to reach up to 4.9

million till 2026 ("Japan will have the fastest growing prevalent cases of Alzheimer's", 2022). In China, the prevalence rate of dementia was 5.60% till 2019 (Jia et al. 2020). As per statistics, in India around 4 million people are suffering from AD ("Alzheimer's and Dementia in India", 2022).

3 Therapeutic Approaches

3.1 Cholinergic Hypothesis

Several studies have reported that degeneration of cholinergic neurons leads to cognitive dysfunction. Acetylcholine is a neurotransmitter, which is responsible for maintaining cognitive function. Degeneration of acetylcholine neurons leads to cognitive dysfunction and behavioural abnormalities. Acetylcholine is metabolized by the acetylcholinesterase enzyme, which is present in the synapse. It can degrade acetylcholine into acetic acid and choline. Acetylcholine metabolism reduces the level of acetylcholine in synapses and postsynaptic neurons (Chen et al. 2022b). Galantamine, donepezil, and rivastigmine are among the cholinomimetic drugs approved by the FDA for the treatment of AD (Vishwas et al. 2020). It inhibits acetylcholinesterase enzymes and enhances the levels of acetylcholine in postsynaptic neurons. Donepezil is used for all stages of AD, whereas galantamine and rivastigmine are used for mild and moderate AD. These drugs produce common side effects such as hepatotoxicity, nausea, abdominal pain, diarrhoea, loss of appetite, and vomiting (Ye et al. 2015). Recent studies suggest that acetylcholinesterase enzyme inhibitors attenuated the accumulation of Aβ proteins, which is one of the potential biomarkers of AD (Alzheimer's Facts and Figures Report 2022).

3.2 Glutamate Hypothesis

The development of AD is influenced by glutamate-mediated neurotoxicity. Since glutamate is the most prevalent excitatory neurotransmitter in the mammalian central nervous system (CNS), it has a critical role in most of the CNS functions (Gilles and Ertlé 2022). Glutamate binds with N-methyl-d-aspartate (NMDA) receptor. NMDA receptors are ligand-gated cationic channels. According to the glutamatergic hypothesis, excessive NMDA receptor activation can lead to an abnormal rise in intracellular calcium, which is responsible for neuronal cell death. Furthermore, degeneration of neuronal cells declines cognitive functions. Memantine is a NMDA receptor antagonist that has a quick on/off kinetics with a moderate affinity. It prevents excessive calcium influx brought by overstimulation of NMDA receptor. It reduces excitotoxicity and cell apoptosis and enhances cognitive functions (Wang and Reddy 2017).

3.3 *Aβ Hypothesis*

Aβ is a fragment of the protein called amyloid precursor protein (APP). The exact function of APP is not defined yet. The APP is cleaved by other proteins into portions that remain within and outside of cells. In some instances, Aβ is formed as one of the fragments when APP is cut into a variety of ways. Aβ is chemically "stickier" than other fragments generated by APP fragmentation. It accumulates in stages to form tiny amyloid plaques, which are characteristic of an Alzheimer's-affected brain. The fragments first form tiny clusters known as oligomers, then chains of clusters known as fibrils, and finally "mats" of fibrils known as beta-sheets.

These beta-sheets in later stages form clusters and accumulate in the brain. The amyloid hypothesis states that these phases of beta-amyloid accumulation impede cell-to-cell communication and activate immune cells. These immune cells (cytokines) initiate an inflammatory response, which leads to neuronal cell apoptosis.

Aducanumab (ABC) is the most recent drug approved by the FDA for the treatment of AD. It is available in transparent to opalescent and colourless to yellow solution form. It comes in single-dose vials and is administered via intravenous infusion. ABC is human immunoglobulin gamma 1 (IgG1) monoclonal antibody, which can bind and reduce both soluble and insoluble accumulated Aβ and plaque formation. ABC is used for the treatment of mild AD and dementia (Khanna et al. 2022). ABC binds to a linear epitope, which is released by Aβ amino acids 3 to 7. After binding with a linear epitope, it suppresses the aggregation of Aβ monomers, oligomers, and polymers. ABC also inhibits the phosphorylation of tau proteins and neurofibrillary tangles (Ali et al. 2022). ABC exhibits some common side effects like headache, diarrhoea, hypersensitivity, immunogenicity, confusion, delirium, altered mental status, and disorientation. Figure 1 depicts the mechanism of action of ABC and other anti-Alzheimer's drugs.

4 Oxidative Stress and Neuroinflammation

Oxidative stress and neuroinflammation play a key role in the progression of AD. Enhanced level of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to increased production of cytokinins such as tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, IL-1β, and nuclear factor kappa B (NFκB) (Agostinho et al. 2010; Mhatre et al. 2004). These inflammatory mediators also promote cellular apoptosis. In the Alzheimer's brain, several inflammatory indicators, such as higher levels of cytokines and their receptors, have been identified. These cytokines are responsible for various brain dysfunctions such as the production of neurotoxic free radicals, pro-inflammatory cytokines, and neurotoxic prostaglandins. Furthermore, pro-inflammatory cytokines may be involved in activating β-secretase and increasing Aβ formation, triggering a chain reaction of neurotoxic events in the brain (Mhatre et al. 2004). Several preclinical studies have been conducted where the results revealed that various neuroprotective drugs and

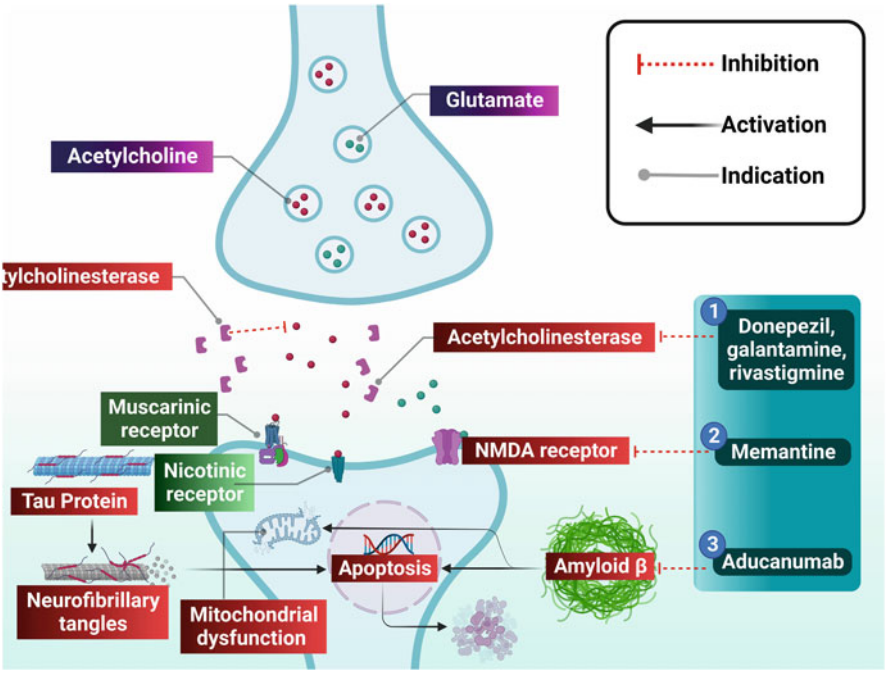

Fig. 1 Mechanism of action of galantamine, donepezil, rivastigmine, memantine, and aducanumab

bioactives attenuated oxidative stress and neuroinflammation. Some of the important studies are mentioned below.

In one of the studies, Yang and co-workers reported the neuroprotective effects of fisetin in mice. Lead is a heavy metal, and it can cause various neurotoxic effects in the brain such as cognitive dysfunction, enhanced oxidative stress, and neuroinflammation. The open maze test was conducted to check the behavioural studies. The study revealed that fisetin enhanced the latency (second) and reduced the number of errors in the open maze test. This further revealed the improvement of mice's cognitive function. Fisetin showed the antioxidant and anti-inflammatory effects by downregulating the levels of ROS and cytokines such as NF-κB p65, TNF-α and IL-6 (Yang et al. 2019). Another study was reported by *Zhang* et al., where fisetin has shown an antioxidant effect in traumatic brain injury (TBI) mice. TBI is a quite complex disorder that can cause primary and secondary brain injury. Brain injury causes calcium homeostasis, oxidative stress, and neuroinflammation. Fisetin has shown antioxidant and anti-inflammatory effects. It has shown neuroprotective effects by reducing primary and secondary brain injury (Zhang et al. 2018).

Another study showed antioxidant and anti-amyloid effects of quercetin in mice. In this study, oxidative stress was induced by hydrogen peroxide (H_2O_2). The H_2O_2 enhances the levels of ROS, neuronal toxicity, and mitochondrial dysfunctions. Quercetin (Qu) has attenuated the toxic effects of H_2O_2, which suppress oxidative

stress and mitochondrial dysfunction. It has also inhibited the accumulation of Aβ in hippocampus parts of the brain, which exhibit its anti-Alzheimer's effects (Godoy et al. 2017). Chen et al. have evaluated the antioxidant and anti-apoptotic effects of Qu in rat glioma C6 cells. Qu attenuated the levels of ROS and significantly inhibited oxidative stress in rat glioma C6 cells (Chen et al. 2006).

Lycopene is found in red-coloured fruits and vegetables including tomatoes, pomegranates, and watermelons. It is a carotenoid aliphatic hydrocarbon. Strong antioxidant, anti-inflammatory, anti-amyloidogenic, and anti-apoptotic characteristics have been linked to lycopene (Danysz et al. 2000). As a result, it has protective properties against neurodegenerative illnesses including AD and Parkinson's disease (Prema et al. 2015). According to Liu et al., lycopene can reduce the neuroinflammation brought on by beta-amyloid and suppress NF-B signalling in the brain's choroid plexus during the early stages of Alzheimer's disease. The production of Aβ neuroinflammation in rats was seen in this study. A water maze test was used to capture behavioural characteristics. The in vivo behavioural investigation against Aβ and lycopene showed neuroprotective effects. The anti-inflammatory and antioxidant properties of lycopene have been revealed in the biochemical research done so far (Liu et al. 2018).

Prakash et al. explored the role of lycopene in the restoration of BDNF and mitochondrial levels in AD, which is induced by β-amyloid. They discovered that animals exposed to β-A1–42 had changed brain-derived neurotrophic factor (BDNF) levels compared to the controls. In rats treated with β-A1–42, chronic treatment of lycopene improved memory retention, attenuated mitochondrial-oxidative damage, decreased neuroinflammation, and restored BDNF levels. These investigations showed that lycopene inhibits amyloidogenesis and protects against β-A1–42-induced cognitive impairment (Prakash and Kumar 2014).

Huang et al. reported that lycopene protected the neuron oxidative damage induced by butyl hydroperoxide. They have pre-treated the neural stem cells (NSCs) with lycopene and found that lycopene promoted the secretion of nerve growth factor (NGF), BDNF, and vascular endothelial growth factor (VEGF) from NSCs (Huang et al. 2018).

Qu is another phytoconstituent which has neuroprotective activity against AD. The neuroprotective action of Qu is primarily attributable to the suppression of polyglutamine aggregation, acetylcholinesterase (AChE), amyloid fibrillogenesis, 6-hydroxydopamine, 3-nitropropionic acid, and elevated ApoE level (Formica and Regelson 1995). Mehta et al. claim that Qu, at a dosage of 30 mg/kg, has been shown to lessen chronic unexpected stress (CUS), including anxiety and depression, and to enhance cognitive function. Along with the maze and open field, the behaviour of the mice was also investigated to examine CUS. Nitric oxide, pro-inflammatory cytokines (IL-6, TNF-a, interleukin-1 beta, and cyclooxygenase-2), and oxidative stress indicators (thiobarbituric acid reactive compounds and nitric oxide) are said to be increased by CUS. In the hippocampus, this further results in the apoptosis of neuronal cells. Qu substantially decreased the levels of all these inflammatory markers, which avoided neuronal cell injury (Mehta et al. 2017). Qu's effects on cadmium-induced neuronal damage in the frontal cortex of Sprague Dawley (SD) rats were documented by Unsal et al. (Mehta et al. 2017). Qu demonstrated

anti-inflammatory and antioxidant properties, which led to neuroprotective benefits. Several biochemical experiments, including catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX), and histopathological analyses, were carried out to evaluate these activities. The findings demonstrated that Qu has antioxidant properties by lowering the levels of SOD, SPX, and CAT in the rats' frontal brain and raising the levels of MDA. Qu also inhibited neuronal cell degeneration in the frontal cortex region of the brain, according to histopathological examinations (Mehta et al. 2017).

Similarly, curcumin is another important phytoconstituent, which has a significant effect against AD. With respect to Aβ in AD, Ferrari and colleagues addressed the chemical connections between curcuminoids and metal-chelating agents. They looked at substituted curcuminoids' metal-complexing potential as a novel possible AD therapeutics. Curcumin exhibited metal-chelating, anti-inflammatory, and antioxidant effects in addition to preventing the aggregation of mutant proteins such Aβ- and α-synuclein and huntingtin (Reddy et al. 2018a).

Traditional Chinese medicine uses the powerful antioxidant baicalein, which is derived from Scutellaria baicalensis Georgi. (Lamiaceae) (TMC). Additionally, baicalein has been shown to prevent Aβ-induced toxicity in PC12 cells, promote A-oligomerization and fibrillation, and cause performed A-amyloid fibrils to disaggregate (Lu et al. 2011). Baicalein has also been found to prevent Aβ-induced toxicity in PC12 cells, cause Aβ-oligomerization, fibrillation, and disaggregate preformed A-amyloid fibrils (Gu et al. 2016).

The myricetin polyphenolic molecule, also known as hydroxyquercetin, has neuroprotective properties against Aβ-induced neuronal cell damage. Myricetin's hydrophobic properties and low molecular weight facilitate BBB bridging, creating a good therapeutic environment. According to Shimmyo et al., myricetin has a dual function that excludes its impact on protein expression by reducing BACE1 activity (IC50 = 2.8 μM) and activating the α-secretase (ADAM10) in the cell-free enzyme activity. It has been discovered that myricetin has less of an effect on neuronal cells than was initially thought (Ramezani et al. 2016).

An isoquinoline alkaloid known as berberine was discovered in the plant Coptis chinensis Franch. (Ranunculaceae). Berberine has neuro-pharmacological effects that control APP processing and decrease Aβ protein levels. For 4 months, transgenic AD mice were given berberine orally at doses of 25 or 100 mg/kg per day, which shows a significant reduction in Aβ pathology and no effect on BACE1 protein levels (Asai et al. 2007).

Although the use of herbal medicines is expanding globally, their clinical applicability as medicines is constrained by their poor solubility, bioavailability, and pharmacological activity, as well as their physical and chemical instability and ease of degradation. Therefore, one potential technique for boosting their pharmacological action may be to create herbal medications with nanotechnology-based delivery systems. To assure their safety and efficacy in treating many types of illnesses, these nanotechnology-based delivery systems' development still has to be further examined, particularly with regard to their safety and toxicity profiles (Dewi et al. 2022). Some other herbal medicine and synthetic drugs and their anti-AD effects are listed in Tables 1 and 2.

Table 1 Herbal medicine and its bioactive component against AD

Name of herbal medicine and bioactives	Bioactive component	Animal models	Pharmacological effects	Reference
Lycium barbarum	• Polysaccharides	Homocysteine-induced toxicity in rats	• Reverse Aβ and homocysteine-induced apoptosis	Ho et al. (2010)
Moringa oleifera extract	• Qu 3-methyl ether	Homocysteine-induced AD in rats	• Improve cognitive function • Reduce oxidative stress	Mahaman et al. (2018)
Ginkgo biloba	• Qu • Kaempferol	Oxidative stress-induced cytotoxicity in SHSY5Y neuroblastoma cells	• Improve cognitive function • Improve motor functions	Blecharz-Klin et al. (2009)
Glycyrrhiza glabra	• Glycyrrhizin • Glycyrrhizic acid • Isoliquiritin	Diazepam-induced amnesia in rats	• Produce antioxidant • Anti-inflammatory effects • Improve memory function	Chakravarthi and Avadhani (2013)
Curcuma longa	• Curcumin • Turmerone • Germacrone • Ar-curcumene	Aβ-induced AD in Tg2576 mice brain	• Attenuate accumulation of Aβ • Inhibited Aβ	Reddy et al. (2018b)
Salvia officinalis extract	• Cineole • Borneol • Fumaric acid • Chlorogenic • Acid thujone • Tannic acid	Human patients	• Inhibition of acetylcholinesterase • Use for mild and moderate stages of AD	Akhondzadeh et al. (2003), Yang et al. (2005)
Rosmarinus officinalis	• Carnosic acid • Rosmarinic acid • Camphor • Caffeic acid • Ursolic acid • Betulinic acid	Lipoperoxidation-induced AD in rats	• Inhibitor of lipoperoxidation	Moss et al. (2003)

(continued)

Table 1 (continued)

Name of herbal medicine and bioactives	Bioactive component	Animal models	Pharmacological effects	Reference
Phyllanthus acidus L extract	• 4-hydroxybenzoic acid • Caffeic acid • Adenosine • Kaempferol • Hypogallic acid	Scopolamine-induced dementia and oxidative stress in rats	• Enhance cognitive and learning function • Produce antioxidant effects • Inhibit acetylcholinesterase enzymes	Uddin et al. (2016)
Lepidium meyenii (black maca)	• Macamides • Macaene	Ovariectomy induced by AD in mice	• Enhance cognitive functions • Inhibit neuroinflammation	Liu et al. (2011)
Siberian barberry	• Palmatine	Alzheimer's littermates mice	• Palmatine improves learning and cognitive function • Palmatine easily bypasses through BBB	Kiris et al. (2023)
Holothuria scabra extracts	–	Anti-amyloid effects in transgenic *C. elegans* model	• Inhibit Aβ • Reduce the paralysis and enhance the chemotaxis behaviour	Kleawyothatis et al. (2023)
Indian catechu methanolic extract	–	Aluminium chloride-induced AD in rats	• Inhibit acetylcholinesterase enzyme • Produce antioxidant effects	Elmorsy et al. (2021)
–	Rosmarinic acid	Inhibition of Aβ activities in endothelial cell	• Inhibition aggregation of Aβ	Hase et al. (2019)
–	Ursolic acid	$A\beta_{1-42}$-induced AD in mice	• Enhance hippocampal neurogenesis • Enhance cognitive functions • Attenuate $A\beta_{1-42}$	Mirza et al. (2021)
–	Hesperidin methylchalcone	$A\beta_{1-42}$-induced AD in Wister rats	• Attenuate $A\beta_{1-42}$ • Inhibit enzymes such as acetylcholinesterase and butyrylcholinesterase • Inhibit $A\beta_{1-42}$ neuroinflammation	Wang et al. (2023)

–	Fisetin	Galactose-induced oxidative stress in mice	• Inhibit oxidative stress • Inhibit ROS • Inhibit neuroinflammation • Enhance cognitive functions	Ahmad et al. (2021)
–	Lycopene	Lipopolysaccharide (LPS)-induced AD in rats	• Inhibit LPS level • Reduce neuroinflammation • Reduce degeneration	Temitope (2021)
–	Bromelain	Aluminium chloride-induced AD in rats	• Improve exploratory activity, cognitive function, anxiety, and depression in rats • Modulate thioredoxin binding protein	Eraky et al. (2023)
–	Lutein	Aβ-induced AD in rats	• Inhibit Aβ • Inhibit oxidative stress	Nazari et al. (2022)

Table 2 Several synthetic drugs against AD

Drugs	Animal models	Pharmacological effects	Reference
Thiazolidine-4-one	Streptozotocin-induced AD in rats	• Attenuate oxidative damages • Inhibit acetylcholinesterase enzyme • Inhibit tau protein	dos Santos et al. (2023)
Interferon-beta	AD in rats	• Attenuate oxidative damages • Inhibit amyloid formation • Inhibit tau proteins • Anti-inflammatory effects • Anti-apoptotic effects	Chavoshinezhad et al. (2023)
Sitagliptin	3xTg-AD triple transgenic AD	• Enhance learning and memory • Enhance regenerations of neuronal cells	Valverde et al. (2021)
Acetylsalicylic acid	Human	• Low dose of acetylsalicylic acid for long term treats all stages of dementia	Nguyen et al. (2022)
Fluoxetine	Aluminium chloride-induced AD in rats	• Activate Nrf2/HO-1 • Attenuate toll-like receptor 4/NLR family pyrin domain containing 3 (TLR4/NLRP3) inflammasome signalling pathway	Abu-Elfotuh et al. (2022)
Amoxapine	Aβ-induced AD in rats	• Attenuate accumulation Aβ multiple serotonin receptor 6 mediated targets, including β-arrestin2 and cyclin-dependent kinase 5 (CDK5)	Li et al. (2017)

5 Ongoing and Completed Clinical Trials of AD

At present, there are many clinical trials conducted on AD. Out of that, very few successfully completed all phases and were approved by FDA. Different ongoing (Aug 2018 to Jan 2023) and completed clinical trials on AD are listed in Tables 3 and 4.

6 Challenges Associated with Oral Delivery of Drugs and Bioactive

Various studies have shown that several drugs and bioactives have anti-Alzheimer's effects. Despite such activities, they are not able to produce neuroprotective effects in the brain. Therefore, attempts have been made to develop various drug delivery systems loaded with various drugs and bioactives (Fig. 2).

Table 3 Ongoing clinical trial for AD (Clinical Trials.gov 2023)

Intervention	Sample size	Phase	Design	Study start month	Estimated study completion date	NCT number
ABBV-916	195	2	R	Jan 2023	Dec 2024	NCT05291234
UPLIFT-AD	2000	NA	NR	Mar 2023	Nov 2024	NCT04520698
Wei Li Bai capsules	130	2	R	Jan 2023	Nov 2024	NCT05670912
MK-1942	408	2	R	Nov 2022	May 2026	NCT05602727
Active acupuncture, sham, and donepezil hydrochloride	160	NA	R	June 2022	Dec 2025	NCT05078944
Caffeine and placebo	248	3	R	Mar 2021	Nov 2024	NCT04570085
Probiotics	40	NA	R	Dec 2021	June 2023	NCT05145881
Sargramostim	42	2	R	Jun 2022	Jul 2024	NCT04902703
Memantine and placebo	88	4	R	Jan 2019	Jun 2024	NCT03703856
LX1001	15	1,2	NR	Aug 2018	Jan 2023	NCT03634007

R randomized, *NR* non-randomized, *NA* not applicable

7 Formulation Strategies for Improving the Bioavailability of the Drugs and Bioactives

In recent year, advanced drug delivery system-based micro- and nano-formulations are widely developed to increase the solubility, dissolution, bioavailability, and brain permeability of the drugs. Nanoemulsion, nanosuspensions, polymeric nanoparticles, polymeric micelles, carbon nanotubes, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLCs), and self-nanoemulsifying drug delivery systems (SNEDDS) are the examples of advance drug delivery systems.

Table 4 Completed clinical trial for AD (Clinical Trials.gov 2023)

Intervention	Sample size	Phase	Design	Study start month	Completion date	NCT number
Donepezil HCL	171	4	R	Mar 2014	Jun 2016	NCT02097056
Donepezil HCl	97	4	NA	Dec 2007	Sep 2018	NCT00571064
Donepezil	199	4	NR	Sep 2014	Jan 2022	NCT00381381
Carvedilol	29	4	R	May 2011	Feb 2018	NCT01354444
Galantamine	99	4	NA	Jan 2010	Dec 2012	NCT01054976
ENA713	222	4	NA	Sep 2013	Feb 2017	NCT01948791
Rivastigmine	208	4	NR	Nov 2007	Apr 2012	NCT00561392
Memantine	60	4	R	May 2007	Aug 2014	NCT00476008
Memantine	12	4	NA	Oct 2007	Jan 2014	NCT00551161
Memantine, placebo	265	4	R	May 2007	Dec 2009	NCT00469456
Memantine, placebo	277	4	R	Mar 2009	Sep 2012	NCT00862940
Rivastigmine transdermal patch	228	4	NA	Feb 2008	Jul 2011	NCT00622713
Donepezil hydrochloride	14	4	NA	May 2007	Nov 2021	NCT00477659
Rivastigmine patch	142	4	R	Oct 2007	May 2011	NCT00549601
Methylphenidate and placebo	60	4	R	Jul 2007	Nov 2015	NCT00495820
Cilostazol and placebo	46	4	R	Aug 2011	May 2014	NCT01409564
ENA713	121	4	NA	Apr 2012	Apr 2018	NCT01585272
Neuraceq (florbetaben 18F)	218	4	NA	Feb 2018	Nov 2018	NCT02681172
Simvastatin and placebo	49	4	R	Jun 2010	Jun 2017	NCT01142336
Florbetapir (18F)	96	4	NA	Apr 2014	Jul 2015	NCT02107599
Risperidone	180	4	R	Jan 2007	Apr 2013	NCT00417482
Ramipril	14	4	R	Sep 2009	Aug 2020	NCT00980785

R randomized, *NR* non-randomized, *NA* not applicable

8 Advanced Drug Delivery System-Based Drugs for the Treatment of AD

Several studies have been reported where advanced drug delivery system-based nano-formulations are used to reduce symptoms of AD. In one of the studies, Ahmad et al. found that Qu-loaded SNEDDS with the drug increased its bioavailability for the treatment of cerebral ischemia and degeneration of the neurons. The droplet size and zeta potential of Qu-loaded SNEDDS were found 94.63 ± 3.17 nm

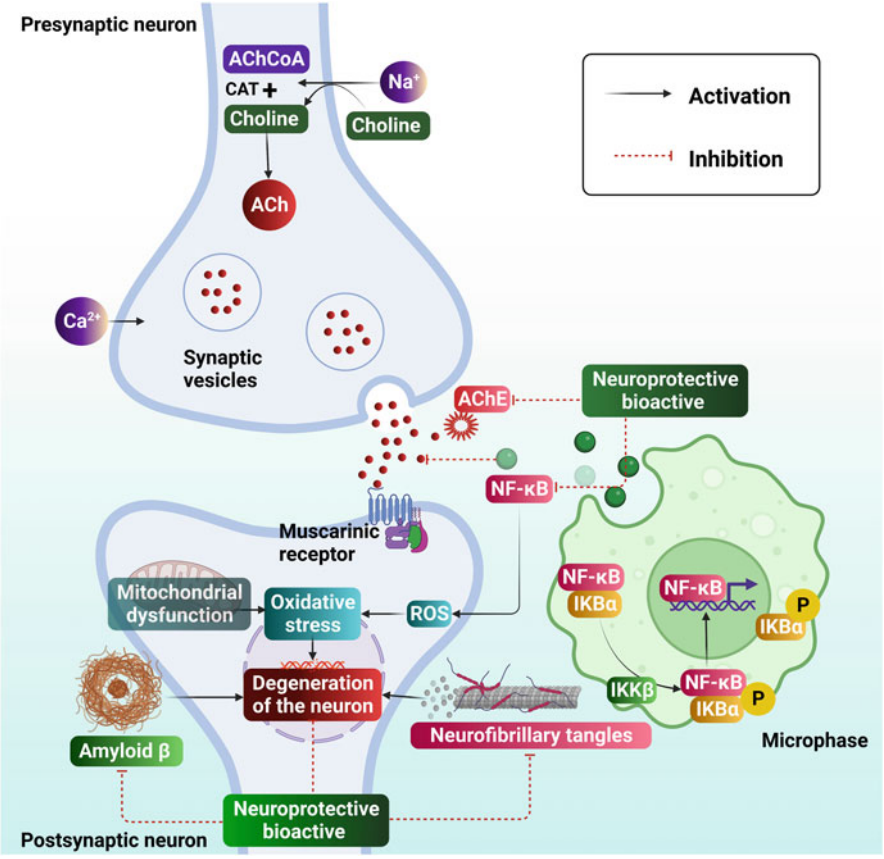

Fig. 2 Antioxidant and anti-inflammatory effects of neuroprotective bioactive substances

and − 17.911.0–17.91 ± 1.02 mV, respectively. The SNEDDS was developed for in situ formation of oil-in-water nanoemulsions (Ahmad et al. 2017). Qu-loaded gold–palladium (AuPd) nanoparticles were developed by Liu and co-workers against Aβ aggregation and mHtt genes for the treatment of AD and HD. Due to their lipidic composition and nano-size, Qu nanoparticles stimulated through autophagy and reduced Aβ aggregation in neuronal cells. These Qu nanoparticles also exhibited neuroprotective properties by attenuating the cytotoxicity generated by Aβ. The histopathological picture demonstrated that Qu-loaded AuPd nanoparticles exhibited no toxicity in the cortex, hippocampus, and thalamus regions of the mice brain as compared to the control group (Liu et al. 2019). Some of the important studies are mentioned in Table 5.

Table 5 Advanced drug delivery system-based various drugs and bioactive substance for the treatment of AD

Therapeutic moiety	NDDS	In vivo model	Method of preparation	Research highlights	References
Rivastigmine	Nanoparticles	Wistar rats	Spontaneous emulsification	• Enhance 1.96 folds of oral bioavailability compared with naïve rivastigmine • Reduce oxidative stress	Wilson et al. (2011)
Pomegranate	Nanoparticles	Aluminium chloride-induced Wistar albino rats	Spontaneous emulsification	• Increase 3.25 folds of oral bioavailability compared with naïve drug • Attenuate thiobarbituric acid level from cerebral cortex • Shown antioxidant effects	Almuhayawi et al. (2020)
Curcumin + selenium	Nanoparticles	5XFAD mice	Emulsion solvent evaporation process	• Inhibit Aβ aggregation	Huo et al. (2019)
Nanoparticle–chelator conjugates	Nanoparticles	AD transgenic animal	–	• Inhibit Aβ aggregate formation • Protect human cortical neurons from Aβ oxidative toxicity	Liu et al. (2009)
Aβ protein Antibody	Nanoparticles	AD transgenic mice (Tg2576)	Hyeon method	• Target cerebrovascular amyloid formation • Enhance passive immunization	Poduslo et al. (2011)
Qu	Nanoparticles	Scopolamine-induced amnesia	Spontaneous emulsification	• Enhance memory function • Reduce oxidative stress	Palle and Neerati (2017)

Phosphatidic acid or a modified apolipoprotein E-derived peptide (mApoE)-functionalized liposome	Liposomes	APP/PS1 Tg mice	Thin-film hydration	• Sequester A β 42 in human biological fluids	Conti et al. (2017)
mApoE–PA	Liposomes	APP/PS1 Tg male mice	Thin-film hydration	• Reduce brain Aβ aggregates • Reduce Aβ levels in mice brain	Balducci et al. (2014)
Qu	Lipid nanoparticles (SLN and NLC)	In vitro study in hCMEC/D3 cells	Hot homogenization technique	• Reduce fluorescence intensity of thioflavin T (ThT) in Aβ (1–42)	Pinheiro et al. (2020)
Thymoquinone	Nanoparticles	Mice	Single-emulsion solvent evaporation technique	• Reduce SOD and oxidative stress • Reduce aggregation of Aβ	Yusuf et al. (2021)
Selenium–chondroitin sulphate nanoparticles	Nanoparticles	SH-SY5Y cells	–	• Reduce aggregation of Aβ in SH-SY5Y cells • Reduce SPD, ROS, MDA, GSH-Px	Gao et al. (2020)
Erythropoietin	Solid lipid nanoparticle	Rats	Double-emulsion solvent evaporation method	• Reduce aggregation of Aβ • Enhance cognitive functions	Dara et al. (2019)
Chiral penicillamine-capped selenium nanoparticles	Nanoparticles	Mice	–	• Inhibit aggregation of Aβ • Enhance cognitive functions	Sun et al. (2017)
Zinc	Polymeric nanoparticles	APP23 mice	Double-emulsion solvent evaporation method	• Inhibit aggregation of Aβ • Attenuate pro-inflammation (IL-6, IL-8)	Vilella et al. (2018)

(continued)

Table 5 (continued)

Therapeutic moiety	NDDS	In vivo model	Method of preparation	Research highlights	References
Mesoporous silica	Gold nanoparticle	Mice	–	• Decrease aggregation of Aβ • Decrease ROS	Yang et al. (2016)
Curcumin	Magnetic nanoparticles	Mice	Antisolvent precipitation method	• Nanoparticles enhance penetration of the BBB	Cheng et al. (2015)
	Polylactide-co-glycolic acid nanoparticles	PC12 cells	–	• Inhibit aggregation of Aβ • Inhibit formation of Aβ	Barbara et al. (2017)
Sialic acid	Selenium nanoparticles	PC12 cells (neuroblastic cells and eosinophilic cells)	Homogeneous method	• Reduce aggregation of Aβ in • Anti-apoptosis effects	Yin et al. (2015)
Resveratrol and grape extract	Solid lipid nanoparticles	HEK293 cells	High-shear homogenization method	• Reduce aggregation of Aβ	Loureiro et al. (2017)
Chitosan	Polymeric nanoparticles	Rats	–	• Reduce oxidative stress • Enhance cognitive function	Manek et al. (2020)
Memantine	Nanoemulsion	Rats	Homogenization and ultrasonication methods	• Inhibit oxidative stress	Kaur et al. (2020)
Magneto-plasmonic nanoparticles	Metallic nanoparticles	Rats	–	• It works as a biosensor for the detection of tau protein	K.-L. Chen et al. (2022a)

Tanshinone IIA	Chitosan nanoparticles	–	–	• Increase Aβ • Increase UNC-51, BEC-1, ATG-7, LGG-1, and ATG-18 • Decrease oxidative stress	Zhang et al. (2022)
Doxorubicin	β-Cyclodextrin-poly (β-amino ester) nanoparticles	BME cells	–	• Increase permeability across the in vitro BBB models	Gil et al. (2012)
–	Gold nanoparticles	C57BL/6 inbred strain mice	–	• Increase permeation of BBB in both in vitro and in vivo models	Praça et al. (2018)
–	Gold nanoparticles	–	–	• Large AuNPs induce amorphous aggregates on the brain lipid bilayer • Decrease formation of Aβ	Kim et al. (2016)
–	Amine-modified gold nanoparticles, citrate-modified gold nanoparticles	–	–	• Increase positively charged gold nanoparticles attached to Aβ more tightly	Kim et al. (2016)
Qu	–	HBME cells, SK-N-MC cells, human astrocytes, without in vivo data	–	• Enhance paracellular drug delivery and protect neurotoxicity induced by Aβ	Kuo and Tsao (2017)

9 Conclusion

There are various pathological pathways associated with AD such as degeneration of cholinergic neurons, accumulation of Aβ, tau protein, oxidative stress, mitochondrial dysfunction, and neuroinflammation. The FDA has approved some of the drugs, which can reduce the symptoms of AD. These drugs include galantamine, donepezil, rivastigmine, memantine, and aducanumab. However, these drugs have certain limitations such as hepatotoxicity, nausea, abdominal pain, diarrhoea, loss of appetite, and vomiting. Looking at this, many plant-based bioactives have also been explored in their treatment as a nutraceutical therapy. Some of them include curcumin, Qu, lycopene, and fisetin. Even their formulations are available in the market. These nutraceuticals have shown good efficacy in minimizing the symptoms and complications of AD. However, these herbal drugs also suffer from some biopharmaceutical and physicochemical challenges such as poor solubility, drug loading in formulation, and gastrointestinal instability. The development of novel drug delivery systems for synthetic and herbal drugs has helped in overcoming the challenges associated with synthetic and herbal drugs and found very effective in treating the disease at much lower doses. Even there are many clinical trials that are ongoing on drug-loaded novel drug delivery systems, and some have even reached their second and third phases. However, it is still a farfetched dream to have a novel formulation that can completely cure the disease. Nevertheless, scientists are putting their relentless efforts in this direction, and hopefully, in the near future, one can expect a nanomedicine that can substantially treat AD.

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