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REVIEW ARTICLE

Current and novel therapeutic molecules and targets in Alzheimer's disease



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Alzheimer's disease (AD) is a neurodegenerative disorder in which the death of brain cells causes memory loss and cognitive decline, i.e., dementia. The disease starts with mild symptoms and gradually becomes severe. AD is one of the leading causes of mortality worldwide. Several different hallmarks of the disease have been reported such as deposits of β -amyloid around neurons, hyperphosphorylated tau protein, oxidative stress, dyshomeostasis of biometals, low levels of acetylcholine, etc. AD is not simple to diagnose since there is no single diagnostic test for it. Pharmacotherapy for AD currently provides only symptomatic relief and mostly targets cognitive revival. Computational biology approaches have proved to be reliable tools for the selection of novel targets and therapeutic ligands. Molecular docking is a key tool in computer-assisted drug design and development. Docking has been utilized to perform virtual screening on large libraries of compounds, and propose structural hypotheses of how the ligands bind with the target with lead optimization. Another potential application of docking is optimization stages of the drug-discovery cycle. This review summarizes the known drug targets of AD, *in vivo* active agents against AD, state-of-the-art docking studies done in AD, and future prospects of the docking with particular emphasis on AD.

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Introduction

Alzheimer's disease (AD) is one of the most common causes of dementia in the society. AD is generally classified into two types: (1) early onset/familial AD (FAD); and (2) sporadic AD (SAD).¹ The malfunctioning and gradual death of neurons in the disease results in loss of memory and cognitive functions. The disease is characterized by accelerated accumulation of amyloid β ($A\beta$) plaque around neurons and hyperphosphorylated microtubule associated tau protein in the form of neurofibrillary tangles within the cells.^{2,3} The degradation of hyperphosphorylated tau by the proteasome system is also inhibited by the actions of $A\beta$. Amyloidogenic pathway results from a mutation and replaces the normal pathway in which α -secretase acts on the amyloid precursor protein (APP), a membrane protein, followed by γ -secretase forming a harmless peptide but the amyloidogenic pathway involves the breakdown of APP by β -secretase followed by γ -secretase, and results in the formation of $A\beta$ plaque, whose major constituent is the 42 residue long $A\beta_{42}$.^{4,5} AD is a progressive neurodegenerative disorder characterized by progressive loss of memory, declining cognitive function, decreased physical function, and ultimately the patient's death due to the death of the brain cells (Fig. 1).⁶ The progression of AD can be broken into three basic stages: (1) preclinical (no signs or symptoms); (2) mild cognitive impairment; and (3) dementia.⁷

Recent reports suggest that > 4.7 million people of ≥ 65 years of age are living with AD in the USA.⁸ AD is predicted to affect one in 85 people globally by 2050.^{9,10}

$A\beta$ oligomers and plaques are potent synaptotoxins, block proteasome function, inhibit mitochondrial activity, alter intracellular Ca^{2+} levels,^{11,12} and stimulate inflammatory processes.^{13,14} The above processes contribute to neuronal dysfunction. Hyperphosphorylation of tau protein leads to the accumulation of neurofibrillary tangles within the neurons.³ As a result the biochemical and synaptic communication between neurons is disrupted which results in the gradual death of the cells.¹⁵ The majority of the cases of AD are SAD.¹⁶ FAD is caused by autosomal dominant mutations in either APP or the presenilin-1 or -2 gene/protein.^{17,18} A gene known as the *Apo-e4* is one of the factors associated with higher chances of sporadic AD.¹⁹ The risk factors for SAD include aging leading to a gradual deterioration of function, presence of the apolipoprotein E4 (APOE4) allele, and vascular diseases such as stroke and cardiac disease.^{20,21}

Computer-aided drug design or computational drug discovery has been one of the major tools applied in drug discovery programs used to reduce the cost and process time. The major parts of computer-aided drug design are structure based drug design, ligand based drug design, and sequence based approaches. The most widely used chain for drug discovery and designing seems to be target

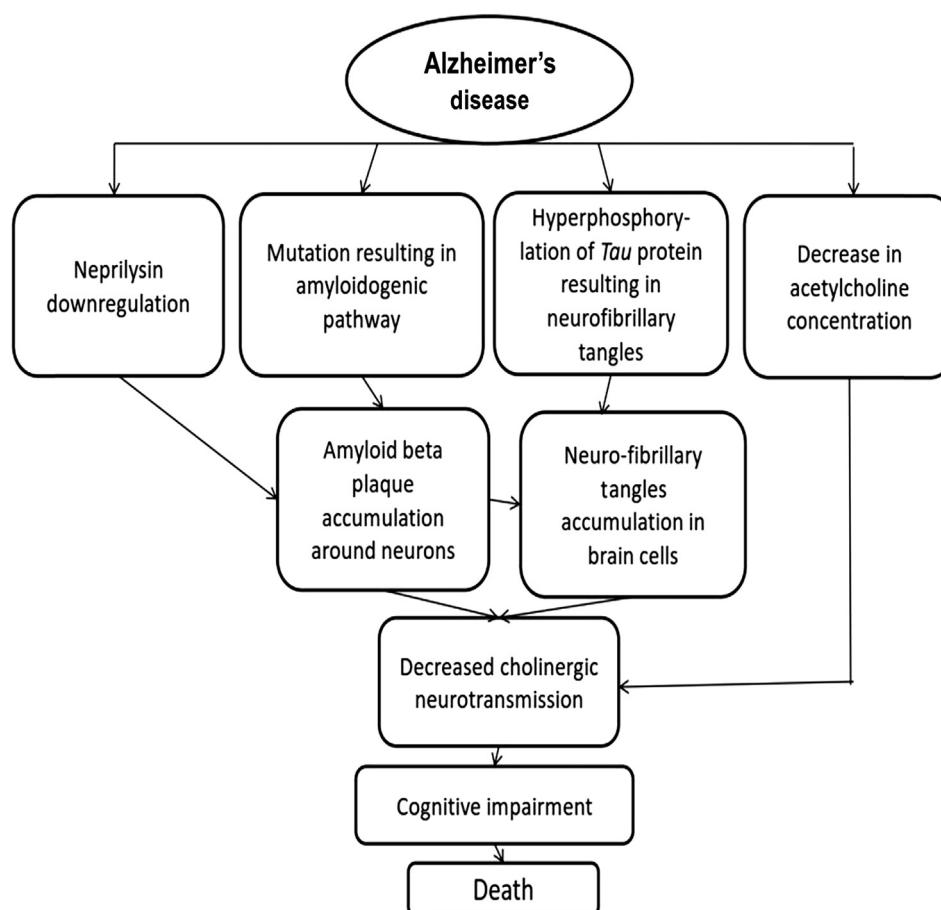


Figure 1 Alzheimer's from disease to death.

identification—molecular docking—quantitative structure-activity relationship—lead optimization. Docking is a computational approach that predicts the favored orientation of the binding of one molecule (ligand) to the second molecule (receptor) to form a stable or firm complex. Docking is a software based program used to envisage the affinity and activity of binding of small molecules to their targets by using scoring functions. Molecular docking software has two core components: (1) a search algorithm (used to find the best conformations of the ligand and receptor); and (2) score function (a measure of how strongly a given ligand will interact with a particular receptor).^{22,23} This review is strongly focused on targets, ligands, and docking advances which have been used to search the best inhibitors or stimulators for AD therapy (Fig. 2).

Conventional drug target networks in AD

Acetylcholinesterase

Damage to the acetylcholine (ACh) producing cholinergic neurotransmission has been shown to be possibly associated with the memory deficits in the brain of patients with AD.²⁴ Some forms of learning and plasticity in the brain cortex are dependent on the presence of ACh.^{25,26} The neurotransmitter ACh is released from nerve fibers during cholinergic transmission, which binds to the designated receptors on other cholinergic nerve fibers and conveys the message to generate a response. The cholinesterase enzymes [mainly acetylcholinesterase (AChE) present in the synaptic cleft of cholinergic neurons] decrease the concentration of ACh by hydrolyzing the molecule.²⁷ Cholinesterase inhibitors bind to these enzymes resulting in increased concentration of

ACh in the synapses.²⁸ The resulting accumulation of ACh causes continuous stimulation of the muscles and glands that potentiate the parasympathetic activities like vasodilatation, constriction of pupils of the eyes, increased production of sweat, saliva, and tears, slow heart rate, mucus secretion in the respiratory tract, and constriction of bronchioles.²⁹ The development of AChE inhibitors is based on the finding that disruption of the cholinergic pathways in the cerebral cortex and basal forebrain contributes to the cognitive impairment of AD patients.^{30,31} There are currently four drugs that act as an AChE inhibitor and are approved for symptomatic relief; namely donepezil, galantamine, rivastigmine, and tacrine.³²

Computational biology approaches, primarily drug designing and molecular docking, have opened a new way for designing and developing more potent targets against disease. Besides the above marketed AChE inhibitors, many new modified synthetic and natural compounds have been shown to have potential cholinesterase activity. The presence of peripheral anionic site (PAS), besides the catalytic site on AChE, has been implied in promoting amyloid fibril formation and its colocalization. Novel flavonoid derivatives have been designed which can bind to both the mentioned sites of AChE and inhibited it better as compared with the conventional rivastigmine and donepezil.³³ In another study, derivatives of four flavonoids namely quercetin, rutin, kaempferol, and macluraxanthone were tested chemically and computationally. Macluraxanthone and quercetin derivatives were found to have very good inhibitory activity against the cholinesterase.³⁴ Several modified novel carbamates have been synthesized and have been tested *in silico* and *in vitro* and have been found to have very good AChE inhibitory activity.³⁵ Some novel compounds such as pyridopyrimidine, synthesized *in vitro*,

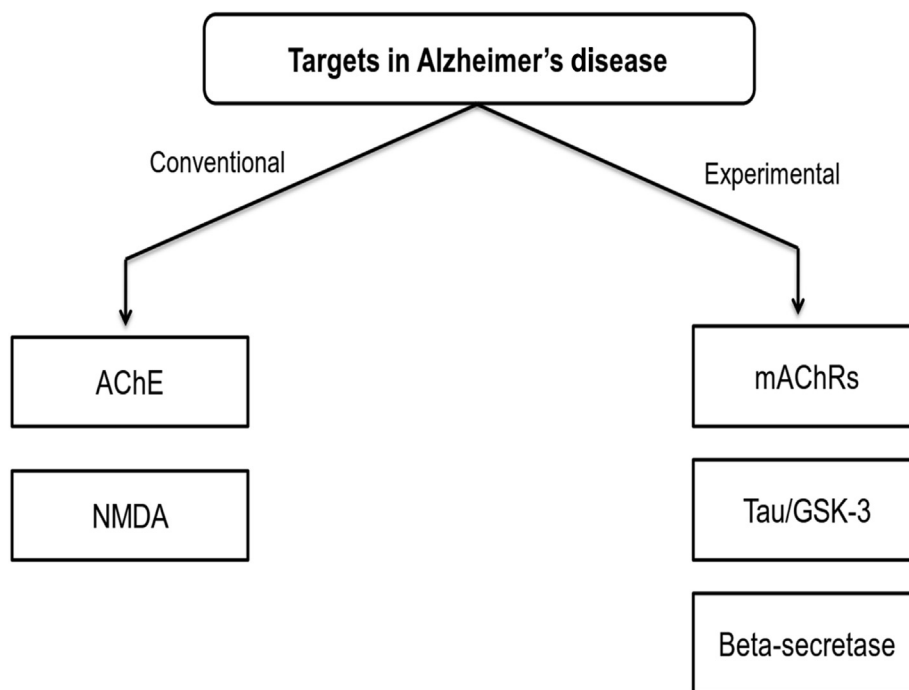


Figure 2 Major targets in Alzheimer's disease therapy. AChE = acetylcholinesterase; GSK-3 = glycogen synthase kinase-3; mAChR = muscarinic acetylcholine receptors; NMDA = N-methyl-D-aspartate.

have shown to possess greater AChE inhibitory action than the marketed drug galantamine, as reported through the molecular docking as well as *in vitro* studies.³⁶ Recently pyridonepezil and 6-chloro-pyridonepezil (the hybrid of donepezil and aminopyridine) have been reported to be more potent cholinesterase inhibitors than the single donepezil molecule through various *in silico* and *in vitro* studies. These compounds are considered to be dual inhibitors as they bind to both the catalytic site and the PAS.^{37,38} Many piperazine derivatives were also reported by researchers to be AChE inhibitors and a few were dual-site inhibitors as well.³⁹

N-methyl-D-aspartate receptor

Excessive activation of N-methyl-D-aspartate (NMDA) type glutamate receptors causes excessive and continuous Ca^{2+} influx through the receptor associated ion channel in AD patients.⁴⁰ Glutamate-mediated synaptic transmission is vital for the normal functioning of the nervous system with glutamate being the most important excitatory neurotransmitter in the brain.⁴¹ Hyperactivation of the NMDA receptor with glutamate leads to the production of free radicals and other enzymes that contribute to the death of neuronal cells. Glutamate is not eliminated properly and may even be inappropriately released, with the disruption of energy metabolism during acute and chronic neurodegenerative disorders. Furthermore, energetically compromised neurons become depolarized since in the absence of energy they cannot maintain ionic homeostasis. This depolarization relieves the normal Mg^{2+} block of NMDA receptor-coupled channels.⁴² Consequently, during ischemia and other neurodegenerative symptoms, excessive stimulation of glutamate receptors is supposed to occur. Therefore, NMDA receptor antagonists could be therapeutically beneficial in a number of neurological disorders like stroke, dementia, and neuropathic pain syndromes.⁴³ NMDA receptors are made up of different subunits like NR1 and NR2A-D (NR3A or B subunits in some cases also). The receptor is composed of a tetramer of these subunits. The subunit composition determines the pharmacology and other parameters of the receptor-ion channel complex. The alternative splicing of subunits, such as NR1, further contributes to the pharmacological properties of the receptor.⁴⁴ The drug memantine (the only marketed NMDA receptor antagonist) has a rapid blocking and unblocking activity with the receptor.⁴⁵ Since memantine is the single drug molecule available as the NMDA antagonist, a wide range of molecular docking studies are in the pipeline to select a number of novel and active ligands against this receptor in AD. In this process, some of the major novel ligands identified as having validated molecular docking results are 3-hydroxy-1H-quinazoline-2,4-dione derivatives,⁴⁶ 1-benzyl-1,2,3,4-tetrahydro- β -carboline,⁴⁷ 3-substituted-1H-indoles,⁴⁸ phenyl-amidine, and triazolyl-amidine derivatives,⁴⁹ etc. Since glycine has been identified as a coagonist of NMDA, there has been a wide search for finding novel antagonists that could block the glycine binding NR1 subunit of NMDA receptor.⁵⁰ Molecular docking studies have found ifenprodil and similar compounds as novel blockers of the NR2B unit of NMDA.^{51,52}

Novel targets in AD

Muscarinic and nicotinic ACh receptors

Muscarinic receptors (mAChR) are the ACh receptors found at various locations including the central nervous system (CNS) that form one of the G protein-receptor complexes in the cell membranes of certain neurons and other cells. It is evident that in the CNS, mAChRs are involved in memory, motor control, and learning process. These receptors are classified into five subtypes named M1–M5.⁵³ They play various roles, including acting as the main end-receptor stimulated by ACh released from postganglionic fibers in the parasympathetic nervous system. The M1-type mAChR, in the hippocampus and cerebral cortex, play a central role in cognitive processing, memory, and learning which are impaired in AD.⁵³ These cholinergic deficits which become a significant feature in AD can be restored via cholinergic activation. A few attempts have been done previously with muscarinic agonists which improved cognitive functions in patients but could not complete the trials as they were nonspecific and activated other subtypes too.⁵⁴ However, nicotinic receptors also respond physiologically to ACh and the $\alpha 7$ and $\alpha 4\beta 2$ subtype expressing neurons are particularly seen damaged in AD patients.⁵⁵ Over the years, certain M1 subtype selective agonists such as AF102B, AF150, AF267B, and AF292 of AF series drugs have been tried on patients with AD and the compound AF267B has been found to have excellent pharmacokinetics and can even penetrate the blood–brain barrier with oral administration whereas AF102B, AF150(S), and AF267B were found to have neurotrophic effects, elevated nonamyloidogenic APP, and decreased $A\beta$.⁵⁶ In AD amyloid formation decreases the ability of these receptors to transmit signals, thereby leading to decreased cholinergic activity. It has been reported that activation of M1 mAChRs can attenuate the Alzheimer's pathological features and restore cognitive functions, some mechanisms upregulate α -APP, and decrease hyperphosphorylated tau, since hypocholenergic effects also lead to formation of $A\beta$.⁵⁷ An M1 allosteric candidate from GlaxoSmithKline (Harlow, UK), 77-LH-28-1, has shown great pharmacological profile and greater CNS penetration. Two M1 selective agonists, VU0357017 and VU0364572 from Vanderbilt Centre for Neuroscience Drug Discovery, (Nashville, TN, USA) have been selected and tested on cell line and animal models and found to be effective on many parameters. But a few of initial M1 agonists also failed after reaching clinical trial since they were not subtype selective.⁵⁸ EVP-6124, an $\alpha 7$ nicotinic receptor agonist developed by Elan Pharmaceuticals, currently in Phase II trial has shown positive outcomes in AD patients as a single molecule and as a combination product with an AChE inhibitor.⁵⁹

Tau protein

Tau is a microtubule associated protein which plays a vital role in the assembly and stability of the microtubules which is one factor in maintaining cell integrity. They are found in normal phosphorylated soluble form primarily in axons. These tau proteins become hyperphosphorylated and forms insoluble intracellular neurofibrillary tangles in neurons in

the case of AD. This condition disturbs the normal synaptic plasticity and causes neurodegenerative changes. Glycogen synthase kinase (GSK-3 β or Tau kinase 1) and cdk-5 are some of the major enzymes involved in hyperphosphorylation of tau.^{60,61} Thus, inhibiting GSK-3 β and bringing down the hyperphosphorylation of tau protein has been considered to be another beneficial therapeutic alternative. Many reputed pharmaceutical organizations like Eli Lilly (IN, USA), Roche (Basel, Switzerland), and GlaxoSmithKline (Harlow, UK) have tried and tested many small molecules as GSK-3 β inhibitors. Maleimide derivatives, oxadiazole, pyrimidine thiazolidinediones derivatives, benzimidazoles, imidazopyridines, and quinolones are some of the most common molecules which have been recognized for the purpose and have shown positive results *in silico* and further *in vitro* assays.^{62,63}

Beta-secretase enzyme

The β -secretase is the enzyme that initiates the generation of amyloid beta. It is an attractive drug target for lowering cerebral levels of APP for the treatment of AD. APP is subjected to degradation via amyloidogenic pathway or via the non-amyloidogenic pathway. APP is first cleaved either by α -secretase or β -secretase enzymes, and the resultant membrane attached fragments are processed by γ -secretase.⁴ The products of α -cleavage followed by γ -cleavage are highly soluble and nonamyloidogenic,⁵ whereas A β produced by β secretase mediated cleavage followed by γ -cleavage is biochemically insoluble and prone to polymerization into pathological fibrils. Besides, amyloidogenic APP cleavage leads to the synthesis of a fragment named APP intracellular domain that alters diverse cellular functions.⁶⁴ APP synthesized in the neuronal cell body, primarily undergoes axonal transport by being contained in transport vesicles. A β is secreted from the presynaptic terminals into the extracellular matrix, and thus fibrillary A β deposits in AD are formed outside neurons. FAD mutations on the APP gene either enhance β -cleavage relative to α -cleavage or alter the activity of γ -secretase to increase the ratio of amyloidogenic A β ₄₂–A β ₄₀, which forms fibrils less rapidly.⁴ This amyloid processing pathway makes beta-secretase (memapsin 2 or BACE1) an attractive target for the development of inhibitors against AD.

BACE 1 is a type 1 transmembrane aspartyl protease and is predominantly located in the intracellular acidic compartments. Their expression is found to be highest in neurons. Interestingly, over-expression and knockdown of BACE1 increases and decreases the A β production respectively.⁶⁴ BACE 1 has two aspartic acid residues in its active site (since it is an aspartyl protease) namely Asp32 and Asp228 present in the large hydrophobic cleft. Two conserved water molecules play an important role in maintaining the enzymatic stability and function.⁶⁵ The molecular docking based approach generated two first generation BACE1 inhibitors namely OM99-2 and OM00-3 which mimicked the natural substrate.⁶⁶ Some other reported inhibitors are the modified molecules based on the parent structure of hydroxyethylene (HE), hydroxyethyleneamine (HEA), carbinamine, macrocyclic, acylguanidine, aminoimidazole, and aminoquinazoline.⁶⁷ Synthetic coumarin derivatives were the first reported compounds which were computationally validated to be

dual inhibitors of AChE and BACE1.^{68,69} Using docking studies, some dual inhibitors of AChE and BACE1 have been generated using HE, HEA, and hydroxymethylcarbonyl as the scaffolds and two compounds even exhibited excellent activity in cell based assays.⁷⁰ In another computational study, flavonols and flavones namely quercetin, kaempferol, myricetin, morin, and apigenin have been validated to be potent BACE 1 inhibitors.⁷¹ The most effective peptidomimetic BACE1 inhibitors have been the statine-based structures with great binding efficacy and IC₅₀ values.⁷²

In silico identified therapeutic molecules validated through *in vitro* and *in vivo* studies

Flavonoid derivatives have been tested *in vitro* on rat AChE and shown better inhibitory activity than the marketed drug rivastigmine, while a few demonstrated inhibitory activities similar to donepezil.³³ Nordihydroguaiaretic acid, a phenolic lignin isolated from *Larrea tridentates*, has been shown to be a cholinesterase inhibitor similar in activity to the marketed drugs and even has an additional antiaggregation effect on A β .⁷³ Many novel carbamates designed and synthesized chemically have shown better AChE inhibitory activity than the already present rivastigmine.³⁵ One group has demonstrated that certain pyridopyrimidine derivatives have approximately 2–2.5 folds higher AChE inhibitory activity than the drug galantamine, demonstrated through the *in vitro* enzyme assay.³⁶ Certain pyridonepezil derivatives have been shown to be better and selective AChE inhibitors than the reference compound donepezil.³⁷ One recent study proposed that derivatives of 4-hydroxycoumarins displayed significant AChE inhibitory activity.⁷⁴ Novel piperidine derivatives demonstrated to have dual inhibitory activity against both AChE and A β aggregation in *in vitro* assay.⁷⁵ In a major breakthrough study, 6-chloro-pyridonepezils have shown to have dual inhibitory activity against AChE at both the catalytic site and PAS.³⁸ Significant dual site inhibitory effect was also shown by certain piperzine derivatives.³⁹ All the above enzymatic assays were done according to the established Ellman's method which uses the thiocholine (released from AChE degradation) reduced Ellman's reagent for spectrometric analysis.³⁹

HE was used as scaffold and its novel derivatives have been shown to have a dual inhibition action against BACE1 and AChE both.⁷⁰ Flavonols and flavones especially myricetin and quercetin exhibited very good cell-free BACE1 inhibitory effect. Neuronal BACE1 secretion and extracellular A β concentration was significantly reduced after administration of myricetin and quercetin.⁷¹ In another study, modified benzodiazepine molecules displayed a good BACE1 inhibition in cell based assay.⁷⁶ Orally effective HEA derivatives were shown to have a significant BACE1 inhibition in a preclinical animal model.⁷⁷ TAK-070, a novel nonpeptide BACE1 inhibitor compound was developed by Takeda Pharmaceuticals Japan, demonstrated significant A β lowering activity in a mouse model.⁷⁸ Another compound AZD3293, a BACE1 inhibitor by Astra Zeneca (London, UK), is presently in a Phase 1 clinical trial.⁷⁹ Merck & Co., Kenilworth, NJ, USA has also brought an MK-8931 against BACE1 into a Phase 3 clinical trial.⁸⁰

The M1 muscarinic agonist AF267B (NGX267 by Torrey Pines Therapeutics, Inc., CA, USA) decreased A β levels and

Table 1 Available drugs, targets, and clinical trial molecules for Alzheimer's disease

S. No.	Drug target	Molecules/drugs	Mechanism of action	Current status
1.	AChE	Tacrine, donepezil, rivastigmine, galantamine	Acetylcholinesterase inhibitor	Generic form marketed by many companies
2.	Beta-secretase	MK-8931, TAK-070, AZD3293,	β -site amyloid precursor protein cleaving	Phase III trial; Phase I trial
3.	Muscarinic (mAChR)/nicotinic (nAChR) receptor	AF267B, AF102B, 77-LH-28-1, VU0357017, VU0364572, EVP-6124	activators of specific mAChR (M_1 & M_4) & nAChR (α_7 & $\alpha_2\beta_4$)	Preclinical trials; Phase II clinical trial
4.	N-methyl D-aspartate (NMDA)	Memantine, nameda, axura & akatinol, ebixa, & abixa	N-methyl D-aspartate (NMDA) antagonist	Generic form marketed by many companies
5.	Tau aggregation	LMTX	Inhibition of aggregation of tau	Phase III trial by TauRx Therapeutics

prevented its further aggregation in preclinical rabbit and mouse models. Long term treatment with AF102B in AD patients decreased the level of A β in cerebrospinal fluid.⁸¹ In the search for novel agonists for M1 receptors, allosteric sites have become inevitable targets. Since these sites are highly conserved, the ligands have to be very specific in structure and action. These allosteric agonists should enhance the binding of orthostatic site agonists. A few examples of such M1 allosteric agonists developed and are either in preclinical or clinical trial are AC-42, AC-260584 (piperidine derivative from Acadia Pharmaceuticals Inc., CA, USA in preclinical trials), 77-LH-28-1 (GlaxoSmith Kline, Harlow, UK), TBPB, and BCQA (Merck & Co. Inc.).⁸¹ Besides the BACE1 inhibitors, certain γ -secretase inhibitors like begacestat, BMS-708163 and PF-3084014 have also made it to clinical trials.⁸¹ In case of tau hyperphosphorylation and aggregation, methylene blue (Rember, TauRx Therapeutics Ltd, Singapore) has shown great results in Phase 2 trials by preventing aggregation and it also decreased A β levels in a mouse model. Lithium chloride, in controlled range, has shown to be a good GSK-3 inhibitor.⁸¹ Another synthetic pyrimidine derivative [6-(4-pyridyl) pyrimidin-4(3H)-one] has been shown very effective *in silico* and *in vivo* mouse assay with great CNS penetration.⁶² TauRx Therapeutics has also announced Phase 3 trials of its investigational drug LMTX (TRx 0237, TauRx Therapeutics Ltd, Singapore) which is chemically methylthionium chloride where this drug help inhibiting the tau aggregation in the neurons.^{82,83} (Table 1).

Discussion

The aim of molecular docking is the accurate prediction of the structure of a ligand within the constraints of a receptor binding site and to correctly estimate the strength of binding. To explore effective drugs for the treatment of AD, different compounds against known and novel targets of AD could be designed and investigated using molecular docking. Dual or multiple inhibitors that inhibits two or more targets of AD may also be investigated. Currently there is no treatment to prevent or cure AD but several approved drugs can treat some of the symptoms and cause a modest and temporary improvement in memory. Targeting the direct cause of neuronal degeneration would constitute a rational strategy and hopefully offer better prospects for the treatment of

AD. Several molecules for the above discussed targets have been withdrawn even from the clinical trials either due to their ineffectiveness in human trials or their nonspecificity for receptors. The brain, being the most complex organ, is difficult in terms of its structural accessibility and the presence of the blood-brain barrier and thus difficult for many *in vitro* molecules to be effective *in situ*. Therefore, special attention should be paid for the development of effective ligands against the potent targets of AD. In a nut shell, molecular modeling and docking would be a promising aspect for novel drug design and would shorten the time span of drug discovery that could be further explored as possible therapeutic interventions for AD.

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