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**REVIEW ARTICLE**

# Combining *in vitro* and *in silico* Approaches to Find New Candidate Drugs Targeting the Pathological Proteins Related to the Alzheimer's Disease

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**Abstract:** ***Background*:** Alzheimer's disease (AD) as the most common cause of dementia among older people has aroused the universal concern of the whole world. However, until now there is still none effective treatments. Consequently, the development of new drugs targeting this complicated brain disorder is urgent and needs more efforts. In this review, we detailed the current state of knowledge about new candidate drugs targeting the pathological proteins especially the drugs which are employed using the combined methods of *in vitro* and *in silico*.

***Methods*:** We looked up and reviewed online papers related to the pathogenesis and new drugs development of AD. Then, articles up to the requirements were respectively analyzed and summaried to provide the latest knowledge about the pathogenic effect and the new candidate drugs targeting Aβ and Tau proteins.

***Results*:** New candidate drugs targeting the Aβ include decreasing the production, promoting the clearence and preventing aggregation. However these drugs have mostly failed in Phase III clinical trial stage due to the unsuccessful of reversing cognition symptoms. As to tau protein, the prevention of tau aggregation and propagation is a promising strategy to synthesize/design mechanism-based drugs against tauopathies. Some candidate drugs are under research. Moreover, because of the complex pathogenesis of AD, multi-target drugs have also shed light on the treatment of AD.

***Conclusion*:** Given to the consecutive failure of Aβ-directed drugs and the feasibilities of tau-targeted therapy, more and more researchers suggested that the AD treatment should be moved from Aβ to tau or focused on considering the soluble form of Aβ and tau as a whole. Moreover, the novel *in silico* methods also have great potential in drug discovery, drug repositioning, virtual screening of chemical libraries. No matter how many difficulties and challenges in prevention and treatment of AD, we firmly believe that the effective and safe drugs will be found using the combined methods in the immediate future with the global effort.

**Keywords:** Alzheimer's disease, amyloid β protein, tau protein, *in silico*, *in vitro*, AD new candidate drugs.

## 1. INTRODUCTION

Since the discovery of AD by Dr. Alzheimer in 1906, the disease has become the most common cause of dementia among older adults. AD, which is a progressive and irreversible neurodegenerative disorder, slowly destroys memory, thinking skill and eventually the ability to behavior. It is pathologically characterized by the amyloid beta (Aβ) deposition in the brain with subsequent formation of neuritic plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein [1, 2]. Virtual experiments using computer modeling show that if a therapeutic intervention delaying progression from mild to moderate dementia just by 2 years, changes in severity-specific prevalence could decrease by 3% for moderate and severe dementia [3]. Until now, because of lacking effective treatment strategies, AD has become a leading cause of the damage for the individual health and social economy. Drugs approved by the Food and

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Drug Administration (FDA, USA) are limited to acetylcholinerase (AChE) inhibitors (tacrine, galantamine, donepezil, rivastigmine) and an *N-methyl-D-aspartate* (NMDA) antagonist (memantine) [4, 5]. These five approved agents are used for symptomatic therapy which can only temporarily ameliorate thinking and memory problems. However, they cannot treat the underlying cause of AD or slow the rate of dementia, and thus fail to achieve a definite cure [6]. So, it is urgent to develop new drugs for the therapy of this complicated brain disorder with no identified cause. With the goal to prevent or effectively treat AD, the focus of drug discovery and development efforts has shifted toward disease-modifying therapies (DMTs) for AD. The main aim of DMTs is to affect the underlying pathological process by impacting one or more of the numerous characteristic changes of AD [7].

*In silico* is a coined phrase which is used to describe an experiment carried out in a computer. In recent years, this method has taken its place alongside the *in vitro* and the *in vivo* methods [8]. In fact, *in silico* biology is more than a computer game [9]. It depends on the usage of information to setup computational models or simulations which can be used to predict, hypothesize, and eventually provide discoveries or advances in medicine and therapeutics [10]. The project of *in silico* drugs ranges from the research of the structure-activity relationship until toxicology and pharmacokinetic studies. Methods are used for pharmacodynamics evaluation containing homology modeling and molecular docking. Homology modeling is dependent on the basis of the homology between amino acid sequences, which gathers useful information about the structure and function similarities [11]. Molecular docking relies on the prediction of bioactive conformation of ligand ( a small molecule) in a binding site of target protein (a macromolecule) [12]. Moreover, virtual screening mainly scores and ranks molecules in large chemical libraries according to their likelihood of having affinity for a certain target [13, 14]. Regarding *in silico* pharmacokinetic, both data-based approaches such as similarity searches quantitative structure-activity relationship (QSAR) and structure-based methods such as pharmacophore modeling and ligand- protein docking have been performed to describe the mode of drugs that interact with living system [15, 16]. Once the promising candidate drugs are found, *in vitro* tests are conducted to evaluate the biological activity. Given the rapid development of *in silico* approaches, it could be desired that biomedical investigations in virtual reality eventually lead to tremendous changes

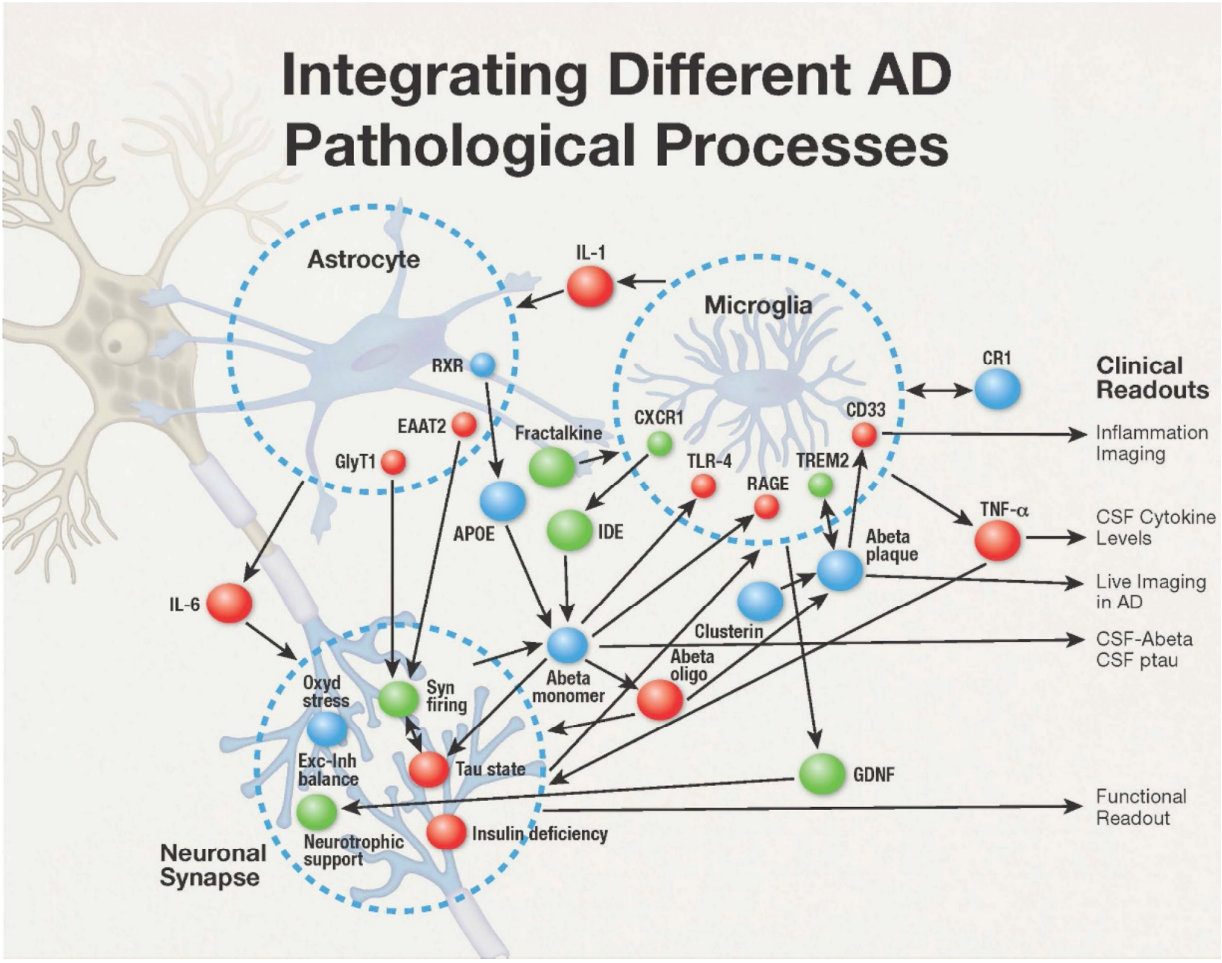


**Fig. (1).** Summary of different pathological processes (by no means exhaustive) occurring in the AD disease and how they putatively interact to lead to the same clinical phenotype. The figure was adapted from Geerts Hg *et al*. 2016 (doi: 10.1016/j.jalz.2016.04.008).

in the pharmaceutical research landscape by optimizing the drug development process, decreasing the number and cost of animal experiments, and smoothing the path to personalized medicine [17]. In this review, we are focusing on new drugs targeting the pathological processes using combined *in silico* and *in vitro* approaches.

## 2. AD PATHOGENESIS

AD is inherently a complex and multifactorial brain disorder, and individual patients display a wide variety of pathologies, depending on age, life history, comorbidities, and genotypes (Fig. **1**) [18]. In order to explain this complicated syndrome, several hypotheses, including the cholinergic hypothesis, Aβ hypothesis, tau proteins hypothesis, and neuroinflammation hypothesis, have been proposed during the last two decades [19]. Numerous AD researches have presented substantial evidence that accumulation of abnormally folded Aβ and tau proteins in amyloid plaques and neuronal tangles are directly associated with neurodegerative processes in patients' brain [20]. According to the Aβ hypothesis, the amyloid precursor protein (APP) is commonly cleaved by the action of β-secretase and γ-secretase producing two types of Aβ peptides named Aβ40 and Aβ42. The Aβ40 contain 40 amino acids while the Aβ42 is longer by two amino acid at the C-terminus [21]. As a consequence of the imbalance in production and clearance of Aβ peptides, they aggregate into soluble oligomers and coalesce to form fibrils insoluble beta-sheet conformation and are finally deposited in diffuse senile plaques [22]. It has been reported that Aβ42 oligomers would increase tau hyperphosphorylation and lead to oxidative damage, which further generate toxic effects on synapses and mitochondria and attract microglial [23]. During the course of AD, the hyperphosphorylated tau proteins aggregate to form neurofibrillary tangles and neuropil threads. Tau, a microtubule binding protein principally found in axons, is to stabilize microtubules [24]. These misfolded and aggregated proteins bind to pattern recognition receptors on microglia and astrocyte and induce an innate immune response releasing inflammatory mediators [25]. These inflammatory mediators play significant role in the processes of disease progression and deterioration [26]. Moreover, the accumulation of Aβ and tau at the synapse may result in synapse dysfunction, loss and the propagation of pathological proteins through synaptic connections which has important contribution to dementia in AD [27]. So, the present article primarily reviews the current drug discovery and development targeting the AD pathological proteins Aβ and tau using the *in silico* and *in vitro* methods.

## 3. TARGETING THE Aβ PROTEIN

The formation and aggregation of Aβ peptides into fibrillar plaques around neurons in the brain is the hallmark of AD [28]. But how the Aβ directly cause or just contribute to AD is not well known. Hotltzman and Musiek claimed that Aβ acts as an initiator of other downstream processes especially tau aggregation. Aβ seems to be necessary, but not sufficient to cause AD. Its primary role may play in the very early stage of AD [29]. In transgenic mouse model, the accumulated Aβ oligomers in the absence of fibrillar plaques could also induce cognitive impairment, neuroinflammation and synaptic alteration [23, 30, 31]. In human, The Aβ oligomers appear to aggregate with age and relate with development of tau pathology [32, 33]. The Aβ oligomers provide a substantive molecular basis for the origin, treatment and diagnosis of AD [34]. Genetic mutation of the APP and presenilin (PS1 and PS2) induces Aβ overproduction and subsequently accumulation into plaques in the brain of AD patients [35, 36]. From Fig. (**2**), we observed that the Aβ42 differed from Aβ40 only in two residues Ile 41 and Ala42 at C-terminus. The researchers using the combined *in silico* and *in vitro* approaches found that the hydrophobic residue at the position 42 is the major contributor to the increased fibril formation rates and neurotoxicity [37]. Although the cause of Aβ oligomers is not clear, factors mentioned above can affect the formation. Supplementary to the vitro and *in vivo* studies, computer stimulations are important tools to provide more useful information on structure, stability and the self-assembly of fibril mechanism of the Aβ proteins and the molecular mechanism of inhibitors [38, 39].

### 3.1. New Candidate Drugs for Decreasing Aβ Production

In the brain, the membrane APP is cleaved by β-secretase forming the N-terminus and by γ-secretase forming the C-terminus to produce the Aβ peptide (Fig. **3**). The α-secretase cleaves APP at the side within Aβ that decreases its production. Therefore, the inhibition of β and γ-secretase and the activation of α-secretase are considered as prime therapeutic strategy to reduce the concentration of Aβ peptide in patients of AD [40]. Especially the β-secretase with 501 amino acids which is widely named as β-site amyloid precursor protein cleaving enzyme 1 (BACE1), is the first and rate-limiting step in Aβ production [41]. In the *in silico* fragment-based molecular design approach, an x-ray crystal structure of the BACE-1 enzyme was used to design new potential ligand structures *via* the precise docking of molecular fragment into the chosen regions of the target site. These fragments are then joined together in ways dictated by the user to produce synthetically approachable ligand scaffolds which are predicted to show good affinity for the targeted enzyme. Subsequently, the *in vitro* cell viability assay is used to evaluate the potential toxicity of designed inhibitors with high binding affinity [42, 43]. In order to discover nonpeptide BACE1 inhibitors, the researchers applied the *de novo* fragment-based molecular design program SPROUT which is based on upoetamide scaffold. The results showed that the compound 15 (C6F5), the most potential within this series of inhibitors, was cell-active and had relatively low toxicity [44]. Kiso and his colleagues used *in-silico* conformational structure-based design to formulate and synthesize non-peptidic and small-sized BACE-1 inhibitors which possessed a heterocyclic scaffold at P2 position. They validated that the σ-π interaction of an inhibitor with the BACE-1-Arg235 side chain played key role in the inhibition of BACE-1. Therefore, they also designed and synthesized a series of peptides that were modified at the P2 position and found that some of these peptides exhibited a potent BACE-1 inhibitory effects despite their structural similarity to the BACE1 substrate [45]. Using R-group search and molecular docking to study 3D-QSAR and binding mode of BACE-1 inhibitors, the results shows that the following residues ASP93, THR133, GLN134, ASP289, GLY291, THR292, THR293, ASN294,

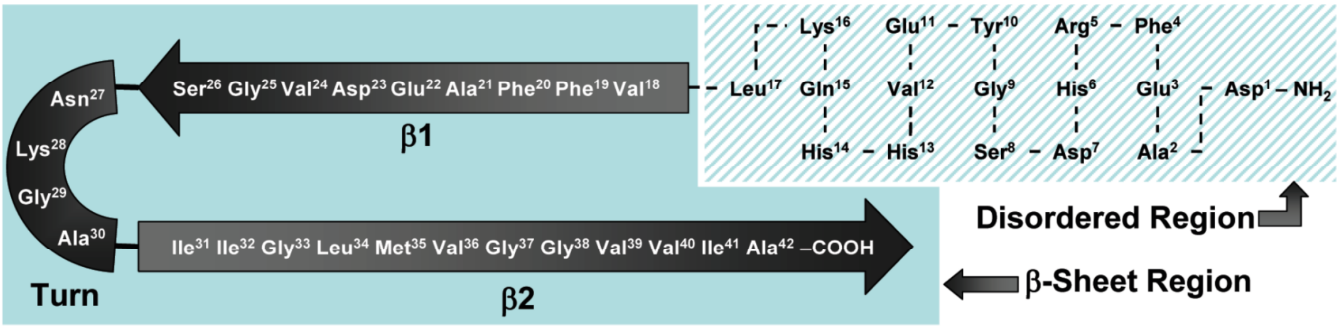


**Fig. (2).** Amino acid sequence of human amyloid beta 1-42 peptide (A_1-42) and schematic representation of a molecule of A_1-42 in a hair-pin shape. The residues 1-17 comprise the disordered region. The residues 18-42 comprise the β_-sheet region. The figure was adapted from Masman M F *et al*. 2009 (doi: 10.1021/jp901057w).

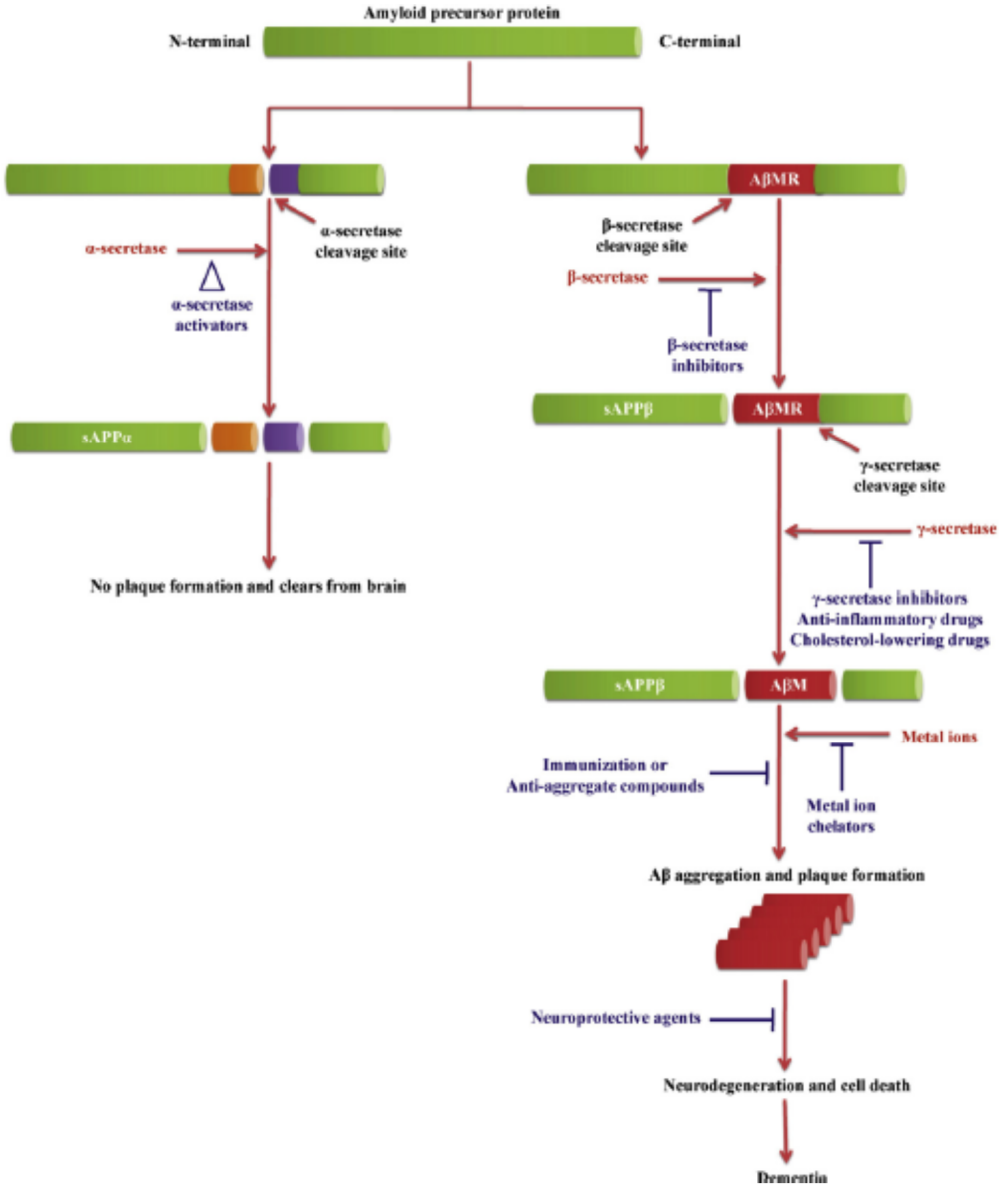


**Fig. (3).** Depiction of amyloid related potential targets along with various therapeutic strategies. The symbol (⊢) indicates the inhibitory effect of therapeutic molecules while (∆) indicates activating effect. AβMR: Aβ monomer region; AβM: Aβ monomer; sAPP: soluble amyloid precursor protein. The figure was adapted from Awasthi M *et al*. 2016 (doi: 10.1016/j.jns.2016.01.008).

ARG296 and SER386 of BACE-1 are tightly interacted with the inhibitors [46]. More than 300000 small molecules were docked and about 15000 prioritized applying the linear interaction energy model with evaluation of solvation by continuum electrostatic method. Then 88 compounds were tested *in vitro*, and 10 of these compounds shared a triazine scaffold [47]. This *in silico* high-throughput screening approach is a cost-effective alternative to high-through *in vitro* screening campaigns. In conclusion, the computer-based *in silico* approaches are taken not only to design, synthesize, and screen the candidate drugs and the lead compounds, but also to study the structure, intermolecular interactions and binding sites of the protein [48-51]. γ-secretase, an integral membrane protein complex, can cleave hundreds of type-1 transmembrane proteins such as the Notch receptor and APP [52]. With a direct route from the membrane to nucleus, the Notch signaling pathway plays role in many different developmental and homestatic processes [53]. The effective γ-secretase complex is a 1:1:1:1 heterotetrameric composed of presenilin 1 (PS1), nicasrin, PEN-2 and APH1 with a mass of 174 kDa [54]. These complexes are bilobed. The head contains nicastrin ectodomain. The membrane-embedded base has a central channel and a lateral cleft. Perhaps this section is initial substrate docking site. Upon the inhibitor binding, its structure will widespread change including rotation of the head and closure of the lateral cleft [55]. Molecular dynamics simulation study reveals potent entry path into γ-secretase/PS1 [56]. By molecular descriptors and machine learning (random forest) methods, the virtual screening of γ-secretase inhibitors against the ZINC database discovered 386 potential hit candidates [57]. Because of α-secretase cleaving within the Aβ domain, its activation can possibly prevent the production of Aβ and prompt the generation of soluble fragments of APP to protect neurons. The M1-agonist talsaclidine is thought to activate α-secretase and inhibit β and γ-secretase to reduce CFS-levels of Aβ42 in 40 AD patients [58].

### 3.2. New Candidate Drugs for Promoting Aβ Clearance

The imbalance between Aβ monomer production and clearance in AD patients has been regarded as the base of Aβ plaque formation. Undoubtedly, enhancing the clearance of Aβ monomer and oligomers from the central nervous system is also a promising treatment approach. The clearance system mainly includes the following methods [59]. Firstly several key enzymes participating in the Aβ degradation have been identified including neprilysin, and insulin-degrading enzyme [60]. Then it is more challenging and difficult to find candidate drugs to activate these enzymes. If we could not stimulate the degradation, we may try to move the Aβ out from the brain. Two potential targets have been reported to modulate Aβ transport at the blood-brain barrier. One is the receptor for advanced glycation end products (RAGE) mediating the influx of Aβ into the brain. The other one is the low-density lipo-protein receptor-related protein (LRP-1) regulating efflux of Aβ from the brain [61, 62]. Finally, both active and passive immunization approaches have been used to clear the monomeric and aggregated Aβ to inhibit their pathological processes. However, the new drug development studies are prone to focus on the Aβ immunological strategies. Nevertheless, the removal of the high concentration Aβ peptides to avoid the adverse effects remains challenging [25, 63]. Up-regulation of P-glycoprotein (P-gp) which is a member of the ATP binding cassette transport family could increase the clearance of Aβ. Around 125 indian medicinal plants have been screened to find their binding affinity towards the Pgp receptor. Then researchers designed and optimized the bioactives under ligand based pharmacophore development, virtual screening, molecular docking and molecular dynamics stimulation studies to make sure acceptable ADME properties [64]. Bexarotene is approved by the U.S. Food and Drug Administration to treat non-Hodgkin's lymphoma. It has been reported that bexarotene would boost the clearance of Aβ, which is validated by the *in silico* study especially in the early stage of AD [65]. The transgenic mice were immunized with human Aβ all lifelong protecting them against cognition impairment [66].

### 3.3. New Candidate Drugs for Preventing Aβ Aggregation

Several researches have shown that the Aβ dimer, oligomers and protofibrils do more harm to AD patients than the plaques [67]. The dimers can block the synaptic Long-Term Potentiation, enhance long-term-depression and reduced dendritic spine density in normal rodent hippocampus [68]. Intracerebroventricular passive immunization with anti-oligo Aβ antibody significantly decreased Aβ and almost completely restored SNP-25 immunoreaction up to 8 weeks postinjection in transgenic mice brain [69]. So the agents prevent Aβ aggregation would be a potential and more effective therapy for AD patients. Structural isomorphs of Aβ Gly25-Ser26 dipeptide induce distinct Aβ42 conformational dynamic and assembly characteristics, which provide useful therapeutic strategies targeting formation of Aβ oligomers and high-order assemblies [70]. A replica exchange molecular dynamics (REMD) simulation was performed with Aβ10-35 dimer, trimer, and tetramers. If the side of the oligomer increased from a trimer to a tetramer, the number of configurations was decreased. So the detailed structures of the oligomers intermediate their folding and aggregation [71]. The polyphenol (-)-epigallocatechin gallate (EGCG) could inhibit Aβ aggregated into unstructured, off-pathway, oligomers [72]. Recently all-atom REMD study revealed that EGCG buried in the interface between the Aβ42 peptides and bind mostly to the hydrophobic residues of the central hydrophobic core and C-terminal region, and also bind to the N-terminal amino acids [73]. Molecular dynamic researches of the interactions between inhibitors and oligomers revealed that the inhibitor acts not only by hampering the addition of successive layers at the ends of the oligomers but also by affecting the structure and stability of oligomers [74]. What to be noted is that Aβ protein has two primary Aβ alloforms Aβ40 and Aβ42. The Aβ42 is more strongly involved in the disease. Structure studies found that the C-terminal region played key roles in Aβ42 oligomerization while the Aβ40 oligomer formation was mainly triggered by intermolecular interactions among the central hydrophobic regions [75].

In conclusion, therapeutic drugs which target the Aβ have been succeed in reducing production and aggregation but have mostly failed in Phase III clinical trial stage due to the

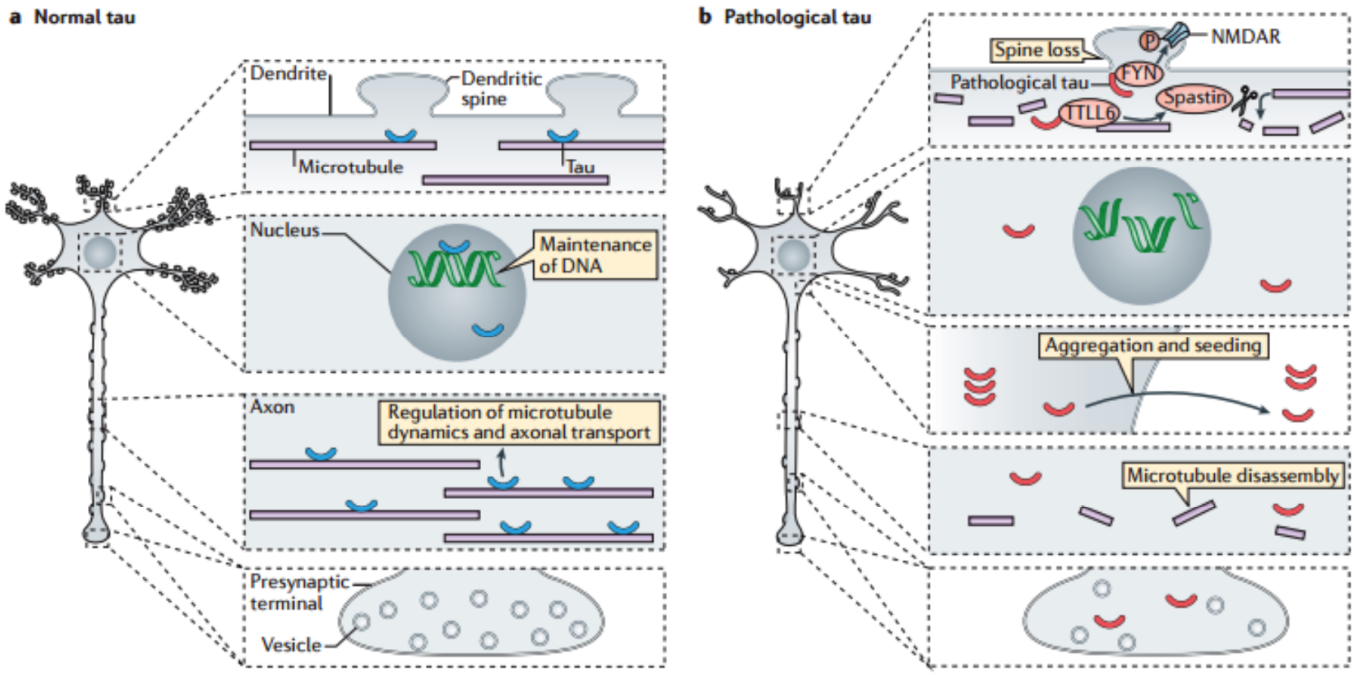


**Fig. (4).** Function of tau. a, Physiological functions of tau protein in normal, healthy neurons. b, In pathological conditions, loss of function, toxic gain of function and mislocalization lead to tau-mediated neurodegeneration. The figure was adapted from wang YP *et al.* 2016 (doi: 10.1038/nrn.2015.1).

unsuccessful of reversing cognition symptoms [76]. The possible reason for failure maybe that Aβ initiates pathology at the early stage of the disease and only early anti- Aβ would be effective [29]. Together with decreasing number of Aβ to delay the disease progression, therapeutic drugs will be still needed to restore network-level and circuit-level function of patients with AD [77]. Several methodological issues can be attributed to the non-meaning clinical results. Perhaps it is timely to reconsiderate the Aβ hypothesis, which takes the amyloid plaques as the heart of AD pathogenesis. Especially after solanezumab's failure in phase III clinical trial on Nov 23th 2016, there is hugely increasing controversial around the Aβ hypothesis [78, 79]. Sloanezumab, developed by Eli Lilly, is a promising humanized amyloid antibody [80]. It binds to the central, more hydrophobic region of the human Aβ peptide (against the amyloid beta 13-28 residues) and preferred to bind to soluble amyloid beta, but not to fibrillar amyloid beta [81, 82]. However, the unbelievable and gloomy failure is a wake-up call to look elsewhere for an answer and therapy to AD.

## 4. NEW CANDIDATE DRUGS TARGETING THE TAU PROTEIN

Tau is a microtubule-associated protein and an important regulator of microtubule. The hyperphosphorylated tau is a vital component of neurofibrillary tangles (NFT), which is a typical characteristic of AD patients [83]. In the AD brain, three different types of tau could be observed: normal phosphorylated, soluble and hyperphosphorylated, and hyperphosphorylated insoluble aggregates [84]. The AD related hyperphosphorylated tau disrupts the microtubules by segregatin the binding of normal tau and could bind to other neuronal microtubule-associated protein leading to aggregation [85]. tau, mainly an axonal protein, becomes mislocalized (missorted) into the somatodendritic compartment, which likely plays an important part in the pathology [86]. The aggregation and missort of tau proteins gain toxic function and lose to function normally [87], which is central to many human neurodegeneration diseases. Although tauopathy is the dominant of AD, the first tau aggerates begin self propagating and spread to distant brain regions [88]. Neuropathological studies of AD suggested that a close Association between tau deposits, decreased cognitive function, and neurodegenerative changes [89]. In fact, tau is usually regarded as the secondary AD pathogenesis (Fig. **4**) and drug target. Interest in developing new drugs targeting the tau protein is on the rise recently, partly attributed to the consecutive failure in Aβ therapeutic. The prevention of tau aggregation and propagation is a promising strategy to synthesis/design mechanism-based drugs against tauopathies. 200000 compounds were screened through *in silico* methods to identify potential hits to inhibit tau aggregation. A new phenylthiazolul-hydrazide (PTH) compound identified as possible hit was then designed and synthesized into 49 similar structures, representing a lead structure. These lead structures possessed strong interaction with the tau protein. The *in vitro* N2A cell model studies showed a low toxicity [90]. The main tau proline-directed protein kinases are primary glycogen sythase kinase-3β (GSK-3β), cyclin-dependent-like kinase-5 (CDK5) and calcium/calmodulin-activated protein kinase II (CaMKII) and so on [91]. For the reason of increased activities in the AD brain and the involvement in tauopathy, these enzymes are also potential therapeutic targets against AD. Oral administration of the novel 2-(alkymorpholin-4-yl)-6-(3-fluoropyridin-4-yl)-pyrimidin-4(3H)-ones inhibited tau

phosphorylation in mice. Molecular docking studies found that this compound has a higher affinity than the prototype drug UDA-680 [92]. The potential tau therapeutic strategies primarily include kinase inhibitors and phosphastase activators, immunotherapies, small molecular inhibitors of protein aggregation, and microtubule-stabilizing agents. Among all thees above mentioned therapeutic targets, the microtubule stabilization approach seems to be the most advanced and ready human trial due these drugs are used in cancer therapy [93]. Although the treatment of tauopathies is promising and induced accumulating interest, it still faced considerably severe challenge [94].

## 5. NEW CANDIDATE MULTI-TARGET DRUGS

Due to the complex pathogenesis of AD, the available therapy for AD is limited and the efficacy remains unsatisfactory. These drugs that regulate a single target can only relieve symptoms instead of curing or preventing the neurodegeration [95]. One possible way to get out of this dilemma is the multi-target drugs (MTDs), which target several factors of the disease pathology [96]. Until now, enlargement of biological target for potential therapeutic has been identified containing the above discussed Aβ, tau, receptors (cholinergic, glutamatergic) and enzymes (AchE, BuChE, BACE1, monoamine oxidase A/B) [97]. The key MTD design methods include structure-based, *in silico*, and data-mining [98]. *In silico* techniques are used in computational pharmacology to better understand and predict how drugs affect biological system and in turn instruct clinical use [99]. ASS2324 is a multi-target directed propargylamine and is able to bind to all the AChE/BuChE and MAO A/B enzymes. As leading-compound, it entered in pre-clinical studies for AD and could inhibit Aβ-aggregation and possessing antioxidant and neuroprotective properties [100]. With the development of computational methods, integration of various cheminformatic, QSAR, virtual screening and docking protocols successfully applied in multi-target drugs design for AD such as novel donepezil-indolyl hybrids, N-Methyl-N-((1-methyl-5(1-(2-methylbenzuyl)piperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-yn-1-amine, and donepezil-pyridyl hybrids, as multi-target inhibitors of AChE/BuChe/MAO-A/MAO-B [101, 102]. Clausenalanstium, a small molecule compound originally isolated from the traditional Chinese herbal medicine, has been demonstrated that its multi-target actions, which include mild elevation of intracellular $Ca^{+}$ concentrations, regulation of the cholinergic system and synaptic plasticity, and activation of cellular and molecular signaling pathways participated in learning and memory [103].

## CONCLUSION

Alzheimer's disease is a multifactorial and complicated syndrome with a progressive loss of memory and cognition, for which there is still no cure. Given to the consecutive failure of Aβ-directed drugs and the feasibilities of tau-targeted therapy, Gold suggested that AD treatment should be moved from Aβ to tau [104]. In addition, more and more researchers prefer to regard the soluble form of Aβ and tau together, independent of their accumulation into plaque and tangles, as the main cause leading to normal neurons into the structure and function loss state. Aβ is the upstream of the tau in AD pathogenesis and induce the transformation of tau from a normal state to a toxic state, and there are also studies which testified that toxic tau improved the toxicity of Aβ through the feedback loop [105, 106]. The novel *in silico* methods also have great potential in drug discovery, drug repositioning, virtual screening of chemical libraries [107-110]. As *in silico* is a relatively new approach, there is still a long way to go, which includes selecting appropriate simulation, model and avoiding false-positive, false-negative results. Undoubtedly, *in vitro* experiments mainly use related cells to further testify the pharmacological activity and toxicity. The combined method of *in silico* and *in vitro* has already been used in new drugs discovery for AD as partly summarized in this review. No matter how many difficulties and challenges are in the prevention and treatment of AD, we firmly believe that the effective and safe drugs will be found using the combined method in the immediate future with the global effort.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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