

Pharmacochemical Strategies and Advances in Alzheimer's Disease Drug Development

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Abstract. Alzheimer's disease (AD) is one of the diseases that seriously threaten human life and health. Currently, the exact pathological mechanism of the disease is still unknown, and there are no drugs that can terminate or reverse the pathological process, which have limited the discovery of AD-related targets and the development of drugs that can effectively treat AD. However, with the deepening research on the molecular mechanism of the disease and the development of computers and artificial intelligence, some potential targets of AD have been discovered and their structures have been analyzed, and at the same time, the paradigm of drug discovery and development by chemical means has been continuously expanded, which has provided medicinal chemists with a variety of methods and strategies for designing new compounds and thus synthesizing new small molecules that can be effective in treating AD. In this paper, we summarize four widely accepted hypotheses on the pathogenesis of AD and the medicinal chemistry strategies for small-molecule drug discovery and development, based on the new research results in recent years.

Keywords: Alzheimer's disease, Pharmacochemical Strategies, pathological mechanism, Drug development.

1. Introduction

Alzheimer's Disease (AD) is a common neurodegenerative disease, one of the main types of dementia, which concentrates on the elderly and is commonly known as Alzheimer's disease, and its clinical symptoms are mainly cognitive dysfunction, mental-behavioral abnormalities, memory loss, and impairment of self-care [1]. It is estimated that about 55 million people worldwide suffered from dementia in 2019, and this number is expected to increase to 139 million by 2050, while at the same time, the annual cost of dementia reached 1.3 trillion dollars in 2019, and is expected to increase to 2.8 trillion dollars by 2030. As the population ages, dementia is emerging as one of the leading causes of death. Therefore, studying the pathological mechanisms and drug targets of Alzheimer's disease to develop new and more efficient small molecule drugs has become a hot research topic nowadays, which is of great significance to further alleviate the symptoms and reduce the burden on the families, healthcare professionals, and even the society as a whole [2].

Currently, researchers have put forward various hypotheses on the pathogenesis of Alzheimer's disease, including the A β hypothesis, the Tau protein hypothesis, the neuroinflammation hypothesis, and the neurotransmitter imbalance hypothesis. These hypotheses have provided an important theoretical framework for the study of Alzheimer's disease pathomechanisms and promoted the exploration of relevant drug targets, such as A β proteins, Tau proteins, neurotransmitters, and microglia, etc. ([3]). However, existing AD therapeutic drugs are still mainly focused on modulating excitatory neurotransmitters, which only serve to alleviate the disease and fail to stop its progression from the root cause. In addition, the complex pathological process of AD makes the identification of specific targets extremely difficult, and the yet to be elucidated pathomechanisms have also hindered the development of targeted drugs, compounded by the fact that the presence of the blood-brain barrier (BBB) may limit the transportation of drugs to the brain, further limiting the efficacy of the drugs [4].

Currently, the research hotspots of Alzheimer's disease include early diagnosis and treatment of AD, exploration of new pathogenic mechanisms and development of new drugs. Due to the limited efficacy of traditional AD drugs, and the high failure rate of research and development often due to

insufficient efficacy or adverse effects, the traditional therapeutic approaches targeting a single target can no longer meet the clinical needs [5]. Therefore, current research is mainly focused on drugs that can act simultaneously on multiple targets with synergistic effects. This paper integrates recent studies on the pathological mechanisms and drug targets of AD, and summarizes the strategies of single-target, multi-target, natural product-based drug design, repositioning, and computer- and artificial-intelligence-assisted drug design in AD drug discovery and development, aiming to provide an overview of the pathological mechanisms, drug targets, and medicinal chemistry strategies of AD.

2. Pathologic mechanisms and drug targets of Alzheimer's disease

2.1. Abnormal deposition of A β protein

Amyloid precursor protein (APP) in normal neuronal cells is cleaved by α - and γ -secretase to produce soluble peptides which can be recycled in the cell. However, when APP is abnormally cleaved by β -secretase (BACE) and γ -secretase, an insoluble peptide, β -amyloid (A β), is produced. β -amyloid plaques (ABP) formed by A β aggregation disrupt signaling between neurons, triggering inflammation, vasculopathy, and other pathological changes. The main therapeutic strategies based on this pathological trait are to reduce the production of A β or accelerate its metabolism [6].

Since A β is caused by abnormal cleavage of β -secretase and γ -secretase, these two enzymes are potential targets for the treatment of AD. By inhibiting the activity of these two enzymes and thus reducing the deposition of A β protein, the cognitive function of AD patients can theoretically be improved [7]. In addition to this, reducing the deposition of A β by accelerating its metabolism is also one of the main strategies to inhibit A β aggregation in the brain. Since the plaques formed by A β aggregation are the key factor that ultimately triggers the disease, the main research targets of this strategy are A β monomers and A β aggregates. Drugs currently being developed for this target include aducanumab ([8]), which targets A β aggregates, and lencanumab ([9]), which targets A β protofibrils, both of which bind to pathogenic proteins and then exert a scavenging effect through the immune system.

2.2. Tau protein hyperphosphorylation

Tau protein in neurons mainly plays a role in stabilizing the structure of microtubules, which are an important part of the cytoskeleton and a pathway for material transport. In the pathological process of AD, the formation of ABPs by A β aggregation triggers a series of pathways leading to the activation of kinases, which prompts tyrosine to transfer phosphoryl groups to Tau proteins, and the phosphorylated Tau proteins are detached from microtubules and aggregated to form neural protofiber tangles (NFTs), which ultimately lead to microtubule damage loss of signaling function and neuronal death [6].

In response to this pathological process, therapeutic strategies are divided into three aspects, the first is to inhibit the process of Tau protein phosphorylation thereby inhibiting the production of phosphorylated Tau proteins and ensuring the structural integrity of the microtubule, and glycogen synthase kinase-3 β (GSK-3 β), which is involved in the process of Tau protein phosphorylation, has been identified as a potential therapeutic target for AD [10]. The second is to inhibit Tau protein aggregation, and it has been suggested that G3BP2 can inhibit Tau protein aggregation by binding to the microtubule-binding region (MTBR) of Tau protein and thereby inhibiting Tau protein aggregation [11]. Finally, the metabolism of aggregated proteins is accelerated, and the removal of diseased proteins by immune means reduces the deposition of phosphorylated Tau proteins. The immune means are divided into active and passive immunization, active immunization is by stimulating the immune system to produce specific antibodies against AD antigens, while passive immunization is a direct method of treatment with specific antibodies targeting the causative agent, also known as the antigen [12]. Monoclonal antibodies and vaccines against both Tau proteins and A β are hot topics of research today, ACI-35 is a liposome-based vaccine that targets phosphorylated Tau proteins thereby reducing phosphorylated Tau protein aggregation [13].

2.3. Neuroinflammation

Microglia are immune cells in the CNS that are involved in neurodevelopment by phagocytosis and clearance of damaged neurons and synapses, and more inflammatory cytokines are expressed and produced in this cell than in other neuroglia, and are therefore considered to be a major source of inflammation in the brain [14, 15, 16]. Wang et al. [16] stated that neuroinflammation is not only a pathological outcome, but also an AD progression the active drivers of AD progression. In normal state, microglia can maintain CNS homeostasis by phagocytosis of abnormal proteins (e.g., A β), removal of cellular debris, and secretion of neurotrophic factors to maintain neuronal survival. However, in AD patients, A β , NFT and other damage-associated molecular patterns (DAMP) activate microglia through pattern recognition receptors (PRR) such as Toll-like receptor 4 (TLR4), prompting them to transform to a pro-inflammatory phenotype (M1), releasing a large amount of pro-inflammatory factors (e.g., IL-6, IL-12), leading to neuronal damage, synaptic loss and cognitive decline. In early AD, microglia may exhibit an anti-inflammatory phenotype (M2), which serves to clear A β and repair damage, but as the disease progresses, chronic inflammation contributes to the predominance of the M1 phenotype and exacerbates neurodegenerative lesions.

According to the neuroinflammatory hypothesis, potential targets may exist in the following three processes. First, inhibition of microglia activation. Inflammatory response induced by neurological injury activates microglia in the resting phase (M0), and activated microglia exacerbate A β deposition, accelerated NFT formation, and neuronal damage, so repairing the damage and thus reducing the inflammatory response may be an effective therapeutic approach [16]. The second is to inhibit the pro-inflammatory response. Studies have shown that the targets for inhibiting pro-inflammatory responses may be nuclear factor κ B (NF- κ B) and NOD-like receptor heat protein structural domain-related protein 3 (NLRP 3), and targeting these two key pathways reduces pro-inflammatory factor release and inhibits the pathological processes of A β and Tau proteins [17,18]. Finally, receptors that modulate phenotypic variability of microglia are also one of the potential therapeutic targets; microglia clear damaged cells in the early stages of AD to maintain normal neuronal function, and modulation of their activation state can prolong their anti-inflammatory effects [16,19]. AL002c is a monoclonal antibody that agonizes myeloid triggered receptor 2 (TREM2) [20], while TREM2 is a lipid receptor expressed in microglia associated with the transition of microglia from a pro-inflammatory phenotype M1 to an anti-inflammatory phenotype M2 [21].

2.4. Neurotransmitter imbalance

Neurotransmitters are widely present in the brain as endogenous chemical messengers that transmit signals through synapses and neuromuscular junctions [22]. Neurons in the central nervous system can be categorized into cholinergic neurons, glutamatergic neurons, GABAergic neurons, etc., depending on the neurotransmitter. Neurotransmitters and their receptors have important roles in the regulation of synaptic plasticity, which is closely related to functions such as learning, cognition and memory. Therefore, abnormalities in the synthesis, storage, transportation and degradation of neurotransmitters may lead to neurotransmitter imbalance and trigger AD [23].

In the early stages of AD pathogenesis, pharmacologic modulation of neurotransmitter homeostasis will delay disease progression, and currently, the cholinergic, glutamatergic, and GABAergic systems are the more studied targets [24]. Acetylcholinesterase (AChE) is the main research target in the cholinergic system, and its inhibitors can inhibit the activity of AChE thereby increasing the concentration of inter-synaptic neurotransmitters, which in turn enhances the activity of the cholinergic system, and ultimately acts as a therapeutic effect, for example, Donepezil, Galanthamine, etc. [23]. It has been pointed out that AChE has two important structural domains, the catalytically active site (CAS) and the peripheral anionic site (PAS), and simultaneous action on these two sites enhances the inhibitory activity of drugs on AChE [31]. In addition, the role of butyrylcholinesterase (BuChE) in the later stages of AD is gradually being emphasized, and therefore, the development of drugs that target both AChE and BuChE is also a therapeutic strategy [46]. Glutamatergic receptors can be affected by A β and thus produce neurotoxicity, NMDA receptors are

a subtype of ionotropic glutamate receptors, and NMDA receptor antagonists such as memantine can resist neurotoxicity caused by A β [24]. GABA is an inhibitory neurotransmitter in the central nervous system (CNS), and it has been shown that some GABAergic drugs, such as human lignocaine, can modulate the effects of GABA by modulating the GABAergic system to improve cognitive and memory deficits in patients [25].

3. Medicinal chemistry strategies and advances in Alzheimer's disease drug development

3.1. Single-target drugs

Single-target drugs (STDs) are drugs that exert their therapeutic effects by specifically acting on a particular molecular target (e.g., protein, enzyme, receptor, or gene). Donepezil is a second-generation reversible acetylcholinesterase inhibitor, Gaonkar et al. used methyl 4-piperidinecarboxylate as a starting material, and N-benzyl-4-piperidinecarboxaldehyde was obtained by alkylating, reducing and Swern oxidizing it, and then an intermediate product of condensation with 5,6-dimethoxy-1-indanone, which was finally reduced and salted to obtain donepezil hydrochloride [26].

Small molecule therapeutic diagnostic agents targeting biomarkers are one of the current directions in the development of AD drugs, and it has been pointed out that the administration of drugs at a later stage of the disease may be the reason for the high failure rate of AD drug development, and at the same time, the deposition of A β , which is a therapeutic target and a marker of the neurophysiopathologist of AD, occurs a few years before the clinical symptoms, so the development of probes targeting A β for the early diagnosis and monitoring of AD is an important research [27]. Li et al. ([28]) modified the chemically unstable diketone structure of curcumin with pyrimidine as an acceptor and constructed an "electron donor-electron acceptor-electron donor (D-A-D)" conjugated scaffold using N-dimethylaniline or triphenylamine as a donor together with a linker group to design two types of JP-1 and JP-2 conjugates (Fig. 1). JP-1 and JP-2 were designed as molecular NIRF probes. JP-1 was synthesized by firstly synthesizing PTS-PEG with tetraethylene glycol and p-toluenesulfonyl chloride as starting materials, while 2, 4-pentanedione and urea were catalyzed by HCl to obtain 2-hydroxy-4,6-dimethylpyrimidine (Compound 1), and then reacting Compound 1 with PTS-PEG to get M-PEG. 4-N,N-dimethyl-4-(2-thienyl)aniline was synthesized by coupling bromo-N,N-dimethylaniline with 2-thiopheneboronic acid via Suzuki coupling, and then 5-[4-(dimethylamino)phenyl]-2-thiophenecarboxaldehyde was obtained by Vilsmeier formalization, and then it was condensed with M-PEG to obtain JP-1, and JP-2 was only the replacement of 4-bromo-N,N-dimethylaniline by 4-bromo-N,N-dimethylaniline in this synthesis process. -dimethylaniline to 4-bromotriphenylamine in this synthesis. In conclusion, it was shown that both compounds, JP-1 and JP-2, have the potential to be used as small molecule models for the development of bifunctional probes for imaging and inhibition of aberrant A β deposition in AD, and that JP-2 has better biological properties, greater fluorescence quantum yield, and enhanced responsiveness compared to JP-1.

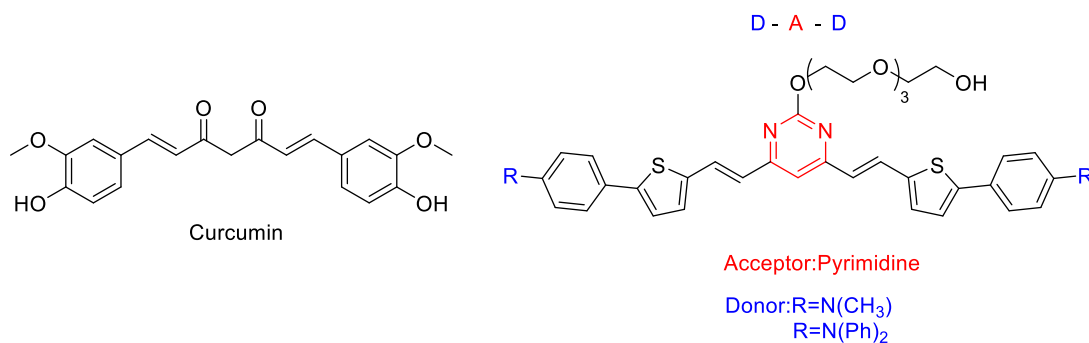


Figure 1. Structure of curcumin and molecular design concept for the D-A-D probes

3.2. Multi-target drugs

Multi-target drugs are drug molecules designed to produce synergistic efficacy by targeting multiple targets that play a role in the pathological process. Tacrine was the first acetylcholinesterase inhibitor approved for AD treatment, and although it has been discontinued due to hepatotoxicity, drug design based on its parent nucleus is one of the current research hotspots [29].

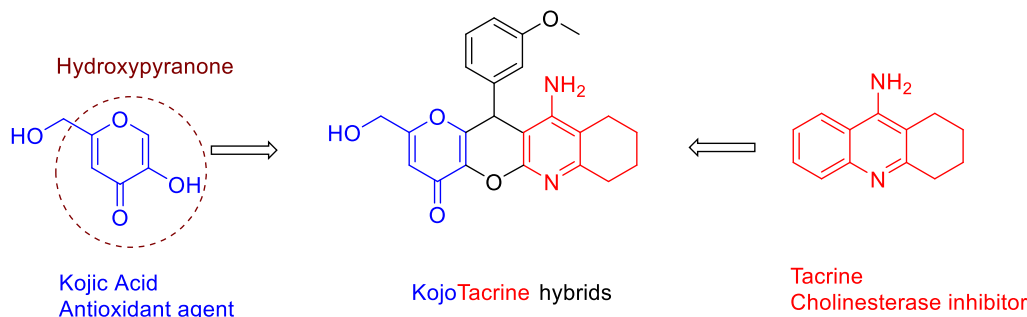


Figure 2. Design of Kojo Tacrine hybrids

It has been shown that hydroxypyranone/pyridinone possesses anti-inflammatory, antioxidant and metal chelating properties, therefore, modifying it as a backbone or fusing it with other active moieties such as tacrine pharmacophore is an idea for designing multi-targeted AD drugs. Dgachi et al. ([30]) designed a multitargeted small molecule compound, KT2d (Fig. 2), with anti-oxidant activity and neuroprotective effects by using Kojic acid, which has a hydroxypyranone backbone with antioxidant properties, and a pharmacophore of tacrine, 5,6,7,8-tetrahydro-4-quinolinamine to design the multi-targeted small molecule compound KT2d with antioxidant capacity, cholinesterase inhibitory activity and neuroprotective effects and verified that it possesses lower hepatotoxicity than tacrine. Targeting different sites of acetylcholinesterase is also one of the ideas of multi-target drug design, Carlier et al. [31] designed compounds that can inhibit two important structural domains, the catalytically active site (CAS) and the peripheral anionic site (PAS) on acetylcholinesterase, by linking the structural fragments of tacrine and Huperzine-A with different lengths of methylene groups. Among them, bis (7)-cognitin, which is formed by linking seven methylene groups, is the most active. In addition, it has been shown that upregulation of glycogen synthase kinase-3 β (GSK-3 β) leads to increased phosphorylation of Tau proteins. Jiang et al. [32] identified the co-crystal structure of pyrimidinothiazole-based ATP-competitive GSK-3 β inhibitors with the ATP-binding site of the structural domains of GSK-3 β , and found that methoxyl and amide compounds located on the thiazole ring of the GSK-3 β inhibitor are not active. By analyzing the co-crystal structure of the GSK-3 β inhibitor and the ATP binding site of the GSK-3 β structural domain, they identified the solvent-exposed region of the GSK-3 β inhibitor where the methoxy and amide in the thiazole ring are the binding sites, and introduced the tacrine moiety into this region as the access site for the linking chain of the alkyl diamine, which led to the design of the bifunctional inhibitor of GSK-3 β /AChE (Fig. 3), and proved that it had an inhibitory effect on the pathological process of AD.

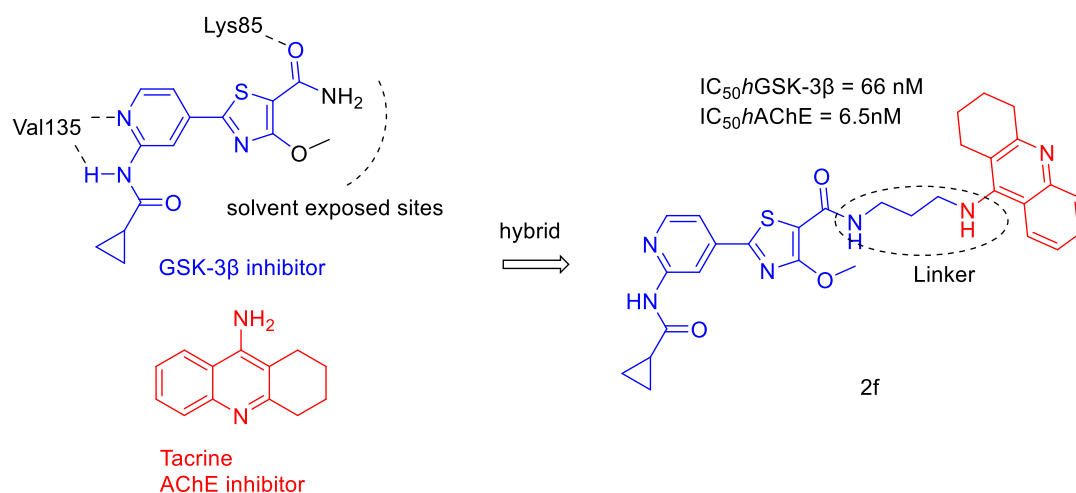


Figure 3. Design strategy of dual GSK-3β/AChE inhibitors

3.3. Natural product-based drug design

Natural products show remarkable potential in the development of AD drugs due to their multi-target, multi-pathway, high efficiency and low toxicity [33]. Incorporating natural products into multi-target drug design strategies is a promising design idea. Chalcone and its derivatives have anti-inflammatory, monoamine oxidase (MAO-B) inhibiting, Aβ aggregation inhibiting and neuroprotective effects, but the inability to inhibit cholinesterase nor metal chelating effects limit their role in AD therapy. Chen et al. [34] introduced hydroxyl and carbamate fragments into the neighboring position of the carbonyl side chain of chalcone and its derivatives to obtain carbamate derivatives (Fig. 4) with the functionality of metal chelating and inhibitory effects on acetylcholinesterase and butyrylcholinesterase, and demonstrated that they indeed expand the targets of chalcone action.

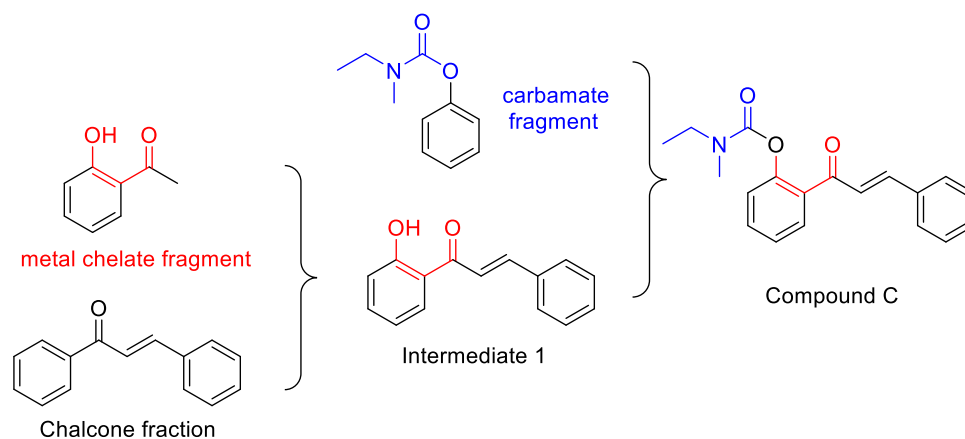


Figure 4. Design strategies for multi-target compound C

Galantamine (Galantamine) is a natural product mainly extracted from plants of the family Staphylinidae [35], which not only inhibits the activity of acetylcholinesterase, but also serves as a metamorphic enhancer of nicotinic acetylcholine receptors [24]. However, due to the difficulty of its extraction, its isolation by asymmetric synthesis of galantamine instead of its extraction from plants has become the main research direction [36]. As early as 1962 Barton et al. achieved the total synthesis of galanthamine by oxidative coupling [37]. Some researchers used isovanillin as a starting material and obtained the racemate of narvidin by bromination, reductive amination, carbamoylation, oxidative coupling with potassium ferricyanide, carbonyl protection and hydrogenolysis of bromine, and then reduced by a selective reducing agent, lithium tri-sec-butyl borohydride, to obtain levogalanthamine [38]. This process is harsh and costly; therefore, Liu et al. [39] simplified the steps in the synthesis of naftalantine by using a non-carbonyl protected method for the direct reduction of

the formyl group. Song et al. [40] abandoned the strategy of protecting the amine group with ethyl formate, and used formaldehyde to perform the "one-pot method". Wang et al. [38] proposed a chemical-enzymatic method for the preparation of levogalantamine. By replacing lithium aluminium hydride with catalytic amounts of palladium and triphenylphosphine in the debromination step of nalvidin and using sodium formate as the hydrogen-transferring reagent, a short-chain carbonyl reductase SAR capable of stereoselectively reducing Levon avidin for the preparation of levogalanthamine was successfully screened by screening a carbonyl reductase library to provide a reaction with higher stereoselectivity and yield.

3.4. Repositioning strategies for drugs

Drug repositioning (DRP), also known as "new use of old drugs", refers to the strategy of using the pharmacokinetic characteristics, safety data, and preclinical study results of existing drugs to explore their new indications, which is characterized by a short R&D cycle, low R&D costs, and a high success rate ([41]). Currently, only four cholinesterase inhibitors are approved for AD and tacrine has been withdrawn from the market due to its hepatotoxicity, so some researchers have been working on the discovery of novel cholinesterase inhibitors. Zhou et al. [42] (Fig. 5) firstly obtained the crystal structures of 1,615 approved and marketed drugs and the complexes of human AChE, BuChE and inhibitors from the ZINC database and the RCSB PDB database, and the original structures of the drugs were hydrogenated, charged, and generated tautomers and chiral isomers by LigPrep in Schrödinger software. It was shown that positively charged ligands can bind to cholinesterase through the attraction of the anionic site in the periphery of cholinesterase and the formation of cation- π interactions with the aryl ring in the active site, so they screened a 664 conformation by Ligandfilte-ring targeting positively charged amines. Then they constructed a pharmacophore model including positively charged and aromatic ring pharmacophore groups and exclusion volume generated by acceptor atoms by studying the crystal structure of the ligand and cholinesterase complex with the Phase module in Schrödinger software, and obtained a further 48 conformations by virtual screening based on the pharmacophore. They then screened four drug molecules by molecular docking through the Glide module, further narrowed it down to three drug molecules by BBB permeability prediction through the QikProp program, and finally screened mitoxantrone with potent cholinesterase inhibitory activity by in vitro activity testing and pointed out its structural similarity to cholinesterase by comparing it with co-crystallized ligands donepezil and compound 2. The lack of π - π stacking of specific amino acids within the active site, etc., provided clues for structure optimization using mitoxantrone as a lead compound.

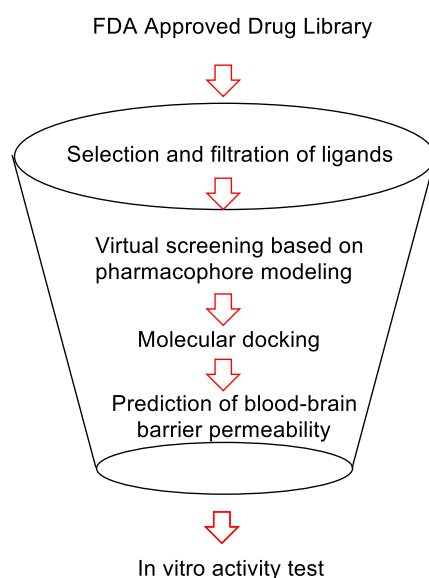


Figure 5. Flowchart of the repositioning strategy study

3.5. Computer-aided drug design (CADD) and artificial intelligence drug design (AIDD)

With the rapid development of computational science and artificial intelligence, it has been realized that CADD and AIDD have great potential to improve the efficiency of target discovery, accelerate the R&D process, and reduce the cost of R&D. CADD provides assistance in the design of drug structures and the discovery of lead compounds through the visualization of the three-dimensional structures of ligands, proteins, and protein-ligand interactions [43]. It has been shown that HDAC6 can play a therapeutic role in AD by inhibiting the phenomenon of oxidative stress. Qin et al. [44] used B-4 and E-15k as lead compounds to molecularly dock with different receptor proteins (HDAC1, HDAC2, HDAC3, HDAC6), and the comparison yielded that HDAC6 has a larger and more hydrophobic pocket of activity, so they used saturated carbon chains to connect the lead compounds with pyrazole through a target structure-based drug design strategy. Therefore, they designed a series of tricyclic compounds (Fig. 6) with potentially better selectivity and inhibitory properties by linking the benzene and pyrazole rings in the structure with saturated carbon chains to increase the size and hydrophobicity of the compounds through a target structure-based drug design strategy, and by adjusting the size of the A and B rings. Finally, compounds 17-b with good efficacy and safety were screened by in vitro activity evaluation, and the conformational relationships of such compounds were summarized to provide a reference for structure optimization.

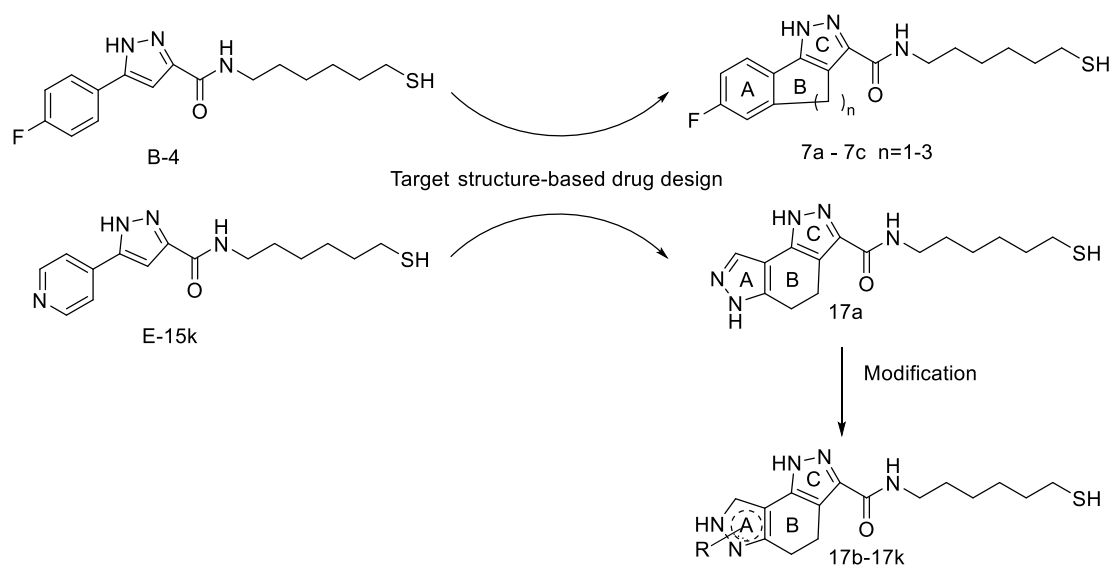


Figure 6. Design of tricyclic compounds based on surface-recognized regions by structure-based drug design strategies

Most diseases are closely related to proteins, and AIDD provides assistance in structure-based drug design by efficiently predicting protein 3D structures. AlphaFold3 is an artificial intelligence program developed by Google capable of predicting the 3D structures of proteins. Yao et al. ([45]), by combining a novel Mendelian randomization method (MR-SPI) with AlphaFold3, identified seven plasma proteins (CD33, CD55, EPHA1, PILRA, PILRB, RET, and TREM2) that are significantly associated with AD risk and predicted their 3D structural changes due to missense mutations, in addition, the Therapeutic Target Database and Drug Bank database, which revealed that some of the proteins such as CD33 and RET have been identified that they have some FDA-approved drugs such as Givolizumab and Platinib. This study provides a reliable method for the discovery of potential AD targets, the exploration of molecular mechanisms of pathogenesis and the discovery of potential AD repurposing candidates.

4. Summary

Single-target drugs have the advantages of high selectivity and low off-target rate, but with the deepening research on the molecular mechanism of diseases, researchers have found that single-target

drugs have limited efficacy for complex diseases such as cancer or AD. Therefore, multi-targeted drugs have gradually become a hot research topic. Compared with single-targeted drugs, multi-targeted drugs can not only increase the efficacy but also reduce the toxicity, however, their R&D process still faces many challenges, such as potential off-target problems, how to optimize the affinity of the drug for different targets, how to improve the penetration of the drug into the BBB, and how to make the multi-targeted drugs with large molecular weight have good physicochemical properties. In addition, natural products show great potential in the development of AD drugs due to their multi-target properties. Drug repositioning strategies, on the other hand, can significantly shorten the R&D cycle, reduce the R&D cost and increase the success rate, providing a new way for AD drug development.

This article systematically summarizes the pathomechanisms, drug targets, drug development strategies and their progress of AD, providing readers with a more systematic and comprehensive AD-related results. Although the pathogenesis of AD has not been fully elucidated, and there is no treatment that can effectively reverse the pathological process, with the in-depth study of early AD biomarkers, early diagnosis and treatment of AD is expected to become a reality. Meanwhile, the rapid development of computational science and artificial intelligence has brought important breakthroughs in the exploration of AD pathogenesis, drug target discovery, and drug development, and we believe that we will be able to truly solve the challenges posed by AD in the near future.

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