Neuroprotective Effects of Cholinesterase Inhibitors: Current Scenario in Therapies for Alzheimer’s Disease and Future Perspectives

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Abstract. Alzheimer’s disease (AD) is a slowly progressive neurodegenerative disease conceptualized as a continuous process, ranging from mild cognitive impairment (MCI), to the mild, moderate, and severe clinical stages of AD dementia. AD is considered a complex multifactorial disease. Currently, the use of cholinesterase inhibitors (ChEI), such as tacrine, donepezil, rivastigmine, and galantamine, has been the main treatment for AD patients. Interestingly, there is evidence that ChEI also promotes neuroprotective effects, bringing some benefits to AD patients. The mechanisms by which the ChEI act have been investigated in AD. ChEI can modulate the PI3K/AKT pathway, which is an important signaling cascade that is capable of causing a significant functional impact on neurons by activating cell survival pathways to promote neuroprotective effects. However, there is still a huge challenge in the field of neuroprotection, but in the context of unravelling the details of the PI3K/AKT pathway, a new scenario has emerged for the development of more efficient drugs that act on multiple protein targets. Thus, the mechanisms by which ChEI can promote neuroprotective effects and prospects for the development of new drug candidates for the treatment of AD are discussed in this review.

Keywords: Acetylcholinesterase, butyrylcholinesterase, neurodegenerative diseases, neuroprotection, PI3K/AKT pathway

INTRODUCTION

Alzheimer’s disease (AD) is an irreversible neurodegenerative disease. AD is conceptualized as a...
(Aβ) and intracellular aggregation of hyperphosphorylated tau protein, respectively [5, 6].

The loss of cholinergic neurons in the brain leads to impairment of cholinergic transmission, being the main cause of a cognitive decline in patients with AD [6]. Due to the essential role of acetylcholine (ACh) in cognitive function, a cholinergic hypothesis was raised for the pathogenesis of AD, based on the progressive loss of limbic and neocortical cholinergic innervation and reduced ACh synthesis [7]. Since ACh is involved in several physiological processes (such as memory, attention, learning, sensory information, and other critical functions), the degeneration of cholinergic neurons in the brain leads to reduced ACh levels, affecting cholinergic transmission, generating cognitive deficits [1, 8]. Therefore, the acetylcholinesterase enzyme (AChE), which hydrolyzes the neurotransmitter acetylcholine, has become an important therapeutic target for AD [6]. Different AChE inhibitors (AChEIs) were developed based on the cholinergic hypothesis [8], and three drugs are currently available for the treatment of AD: donepezil, galantamine, and rivastigmine. However, the effectiveness of these drugs is limited since they reduce AD symptoms, but they are unable to stop disease progression [6, 9]. There are several literature reports showing that some AChEIs demonstrate neuroprotective effects that were unrelated to enzyme inhibition [1, 10, 11]. Thus, new therapeutic strategies are focusing not only on the amplification of cholinergic activity but also on modulating non-cholinergic functions; these strategies have been emerging to develop new disease-modifying agents for AD treatment [12].

This review addresses the mechanisms by which cholinesterase (ChE) inhibitors promote neuroprotective effects, which are capable of bringing benefits to patients with AD. In addition, we also show new perspectives in the development of potential drug candidates endowed with neuroprotective capacity, which have been designed for AD therapy.

**CHOLINESTERASE ENZYMES**

ChE is a ubiquitous class of serine hydrolases that cleaves choline esters. There are two forms of ChE (encoded by two distinct genes): AChE, which hydrolyzes the neurotransmitter acetylcholine, and butyrylcholinesterase (BuChE) [13, 14]. Although both enzymatic forms exhibit similar catalytic activities, they differ in ionic or hydrophobic interactions [14].

AChE is primarily located at neuromuscular junctions and cholinergic synapses in the central nervous system (CNS), where it catalyzes the hydrolysis of ACh into choline and acetate, making it responsible for terminating ACh-mediated synaptic transmission with high catalytic efficiency [9, 13]. AChE is structurally composed of an active catalytic site (CAS), the peripheral anionic site (PAS), and the gorge region (Fig. 1) [9]. The CAS site is located at the bottom of the gorge region, which is about 5 Å wide and 20 Å deep, lined with up to 14 conserved aromatic residues, whose rings constitute ~70% of the gorge surface [15, 16]. CAS is the local where the hydrolysis reaction occurs and contains a catalytic triad (H440-E327-S200) and the critical aromatic residues, W84 and F330. The PAS is located near the entrance to the gorge, being composed of Y70, Y121, and W279 aromatic residues that are essential components of this site. PAS is a low-affinity binding site essential to forwarding ACh and to controlling substrate specificity at the gorge [9, 15, 16].

BuChE, also known as pseudocholinesterase or nonspecific cholinesterase, can hydrolyze larger substrates such as succinylcholine and acetylcholine, and, in particular, butyrylcholine [17, 18]. Similarly to AChE, BuChE is structurally formed by a 20 Å gorge lined with six aromatic amino acid residues, a CAS site, and a PAS site [19]; the catalytic triad is located at the bottom of the gorge, composed of S226, H466, and E353 [17]. Unlike AChE, in BuChE, the choline-binding site was replaced by Ala (A328), and the peripheral site was reduced to only two residues.

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**Fig. 1.** Schematic structure of AChE showing the gorge region, active catalytic site (CAS), and the peripheral anionic site (PAS).
of four aromatic residues that are found in the PAS site of AChE, thus exhibiting an inverse biochemical property [30].

Several studies indicate that AChE-Aβ complexes promote major neurotoxic effects when tested in vitro or in vivo, compared to treatment with Aβ peptides. Experiments performed in PC12 neuronal cell line demonstrated that treatments with AChE-Aβ complexes showed greater cytotoxicity than fibrillar Aβ complexes [24, 31, 32]. Interestingly, Reyes et al. (2004) found an increase in neurite network dystrophy, increased neuronal apoptosis, and a sustained increase in intracellular Ca²⁺ in hippocampal neurons from rats treated with AChE-Aβ complexes. There is evidence that AChE strongly stimulate mouse Aβ aggregation in vitro, resulting in Aβ-AChE complexes that are more toxic than Aβ fibrils [33], and altogether, these studies may suggest that AChE could play an important role in neurodegeneration [31]. Accordingly, AChEIs that selectively act at the PAS site of the enzyme also prevent the formation of AChE-AB complexes, as well as the acceleration of Aβ peptide assembly into filaments. Thus, AChEI seems to be an attractive and promising target for the development of new potential candidates for anti-AD therapeutic drugs [33, 34].

**TRADITIONAL CHOLINESTERASE INHIBITORS**

The various physiological alterations occurring in AD patients culminate in a reduction of ACh synthesis, as well as a decrease in cholinergic receptors, thus causing a reduction in cholinergic transmission [1]. Thus, ChEI was developed to block the catalytic action of the AChE, resulting in increased levels of ACh in the synaptic cleft and activation of cholinergic receptors [1, 5]. Thus, Tacrine 1, donepezil 2, rivastigmine 3, and galantamine 4 (Fig. 2) were the first drugs used in the clinic [34].

Tacrine, an acridine derivative, was the first drug approved by the Food and Drug Administration (FDA) in 1993 to treat AD [35]. It is a potent reversible and non-competitive AChE/BuChE inhibitor [35, 36]. Tacrine inhibits AChE through its interaction with the CAS site [6]. Despite being an excellent cholinesterase inhibitor, soon after its registration, tacrine was withdrawn from the market due to adverse side effects, such as hepatotoxicity and low bioavailability [1, 35-37]. Nausea, vomiting, loss of appetite, and diarrhea were also common side effects.
caused by regular tacrine administration [6]. However, its chemical structure has been widely used to design new compounds that are capable of inhibiting ChE, with lower side effects and toxicity [35].

Donepezil is widely prescribed for mild, moderate, and severe AD and has been considered a first-line treatment against this disease since 1996 [38, 39]. It is a reversible, mixed inhibitor that exhibits competitive and non-competitive AChE activity [40, 41]. Donepezil is capable of simultaneously inhibiting both CAS and PAS sites of AChE [40]. It has a selective activity for AChE, but only a modest effect on BuChE, unlike other compounds such as rivastigmine and tacrine [41]. In addition to inhibiting AChE, donepezil also has activities at molecular and cellular levels in almost all stages involved in the pathogenesis of AD [6, 41]. However, adverse events can occur during its use, being significant at higher dosage (10 mg/d) compared to lower dosage forms (5 mg/d) [42]. Symptoms can range from appetite loss, vomiting, nausea, diarrhea, and rhinitis. However, gastrointestinal side effects are mainly observed [42].

Rivastigmine was approved for the treatment of patients with mild to moderate AD in 2000. It is a pseudo-irreversible carbamate-type of AChE and BuChE inhibitor, being selective for the CNS compared to peripheral tissues [8, 43, 44]. It is classified as a pseudo-irreversible inhibitor because it binds to AChE that cleaves the rivastigmine molecule. A covalent carbamoyl-AChE complex is formed, preventing the catalysis of acetylcholine and temporarily inactivating the enzyme [5, 43]. Rivastigmine was initially administered orally, but due to gastrointestinal adverse events [44], the transdermal patch emerged, providing a continuous and well-controlled administration, reducing fluctuations in plasma concentration, with minor side effects [8, 44].

Galantamine is an alkaloid belonging to the Amaryllidaceae family, isolated from the Galanthus woronowii plant and indicated for treating mild to moderate AD [45, 46]. It is a reversible competitive and selective inhibitor of AChE interacting with the anionic subsite and the aromatic gorge, having only slight activity in the inhibition of BuChE [5, 44, 46]. Galantamine has CNS selectivity with little effect on peripheral tissues [45]. Furthermore, it is an allosteric modulator of nicotinic acetylcholine receptors (nAChRs) [45]. Although its clinical efficacy is equivalent to that of donepezil [47], galantamine seems to cause more side effects, especially gastrointestinal symptoms, compared to other AChE drugs [46].

NEUROPROTECTIVE EFFECT OF TRADITIONAL ChE INHIBITORS

The mechanisms underlying the action of AChEIs drugs (donepezil, tacrine, rivastigmine, and galantamine) have been investigated not only for their effects on AChE inhibition but also for their ability to promote neuroprotection against cell damage. However, there still remains an immense challenge in the field of neuroprotection [11].

The Aβ cascade hypothesis, the most cited hypothesis in AD pathology, focuses on the abnormal processing of amyloid-β protein precursor (AβPP), leading to imbalance between the production and clearance of Aβ, generating an excess of Aβ aggregation and neurotoxicity [34, 48]. Several studies conducted in neuronal cultures treated with donepezil [49–51], tacrine [52, 53], rivastigmine [54], and galantamine [55–58] showed neuroprotective effects, such as increased cell viability, reduction of neuronal death and release of inflammatory mediators against Aβ toxicity. In animal models, donepezil was able to induce cognitive and behavioral improvements, as well as decrease the deposition of Aβ peptide and reduction of neuronal death [49, 59–62]. Other studies showed that donepezil, rivastigmine, and galantamine could promote neuroprotection and reduce Aβ deposition [63–66]. Furthermore, AD patients treated with donepezil
Fig. 3. Activities of traditional ChEI on multi-targets in AD may lead to various therapeutic effects, which altogether provide neuroprotection.

revealed improvements in cognitive functions and decreased levels of Aβ in peripheral blood cells [67].

Another hypothesis is based on tau protein hyperphosphorylation leading to the formation of intracellular neurofibrillary tangles (NFTs) in the brain of AD patients [68]. Glycogen synthase kinase-3β (GSK-3β) is one of the central kinases responsible for tau hyperphosphorylation. Studies suggest that the neuroprotective effects of donepezil would also be occurring through the inhibition of GSK-3β activity [50, 69]. Interestingly, donepezil, rivastigmine, and galantamine showed a neuroprotective effect against the toxicity of okadaic acid, an inducer of tau protein hyperphosphorylation, reducing neuronal death [70, 71]. Treatment of AD patients with donepezil for six months caused an increase in phosphorylated GSK-3β (inactive enzyme), which would reflect a reduction in tau protein phosphorylation [72].

The cortical neurodegeneration of AD is also attributed to glutamate-induced neurotoxicity [10]. Several studies have shown that donepezil protects neurons against glutamate-induced toxicity by reducing Ca²⁺ influx, caspase-3 activation, and neuronal death [73–75]. There are reports showing that tacrine and galantamine can also reduce glutamate-induced cell death [10, 74, 76].

Under conditions of tissue injury, such as ischemia, the generation of oxidative stress can cause damage to all major cellular molecules [77]. On the other hand, under oxygen-glucose deprivation, assays performed in primary cultures of rat cortical neurons showed that donepezil caused protective effects against ischemic damage; however, galantamine, tacrine, and rivastigmine did not promote the same effect [75]. A protective effect of galantamine against oxidative damage has been observed in neuronal cultures [56, 78, 79]. Furthermore, \textit{in vitro} pre-treatment with donepezil could protect endothelial cells against damage induced by hydrogen peroxide [77]. In the last decade, several reports in the literature have focused on the ability of ChEI to induce neuronal recovery by increasing neurogenesis and neurotogenesis. Several studies demonstrated that donepezil could promote neuronal differentiation and neurite growth \textit{in vitro} and \textit{in vivo} [80–84]. Similar effects were also found for tacrine, galantamine, and rivastigmine [85–90].

The importance of ChEIs is mainly centered on their mechanisms related to neuroprotection, and its capability of acting on different targets or processes in AD (Fig. 3). The ChEIs action occurs by modulating the cell survival pathway to promote neuroprotective effects. Most of these effects induced by ChEI are related to the stimulation of nAChRs, downregulation of NMDA receptors, and consequently, induction of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) and mitogen-activated protein kinase (MAPK) signaling pathways [10, 73, 91]. PI3K/AKT pathway plays a significant functional impact on synaptic plasticity, neuronal polarity, neurotransmission, use-dependent translation, metabolic control and stress responses, including DNA repair in the brain [92, 93]. Thus, the PI3K/AKT pathway has been increasingly investigated regarding its role on the induction of neuroprotective effects.

**PI3K/AKT PATHWAY MEDIATING THE MULTI-TARGET EFFECTS IN AD**

PI3K/AKT pathway provides a link between several pathological processes in AD, such as ageing “itself”, glucose metabolism, Aβ aggregation, tau hyperphosphorylation, synapse loss, oxidative stress, inflammation, and neuronal death [94]. The PI3K/AKT pathway can be induced by AChEI through signaling by nAChRs reported \textit{in vitro} studies [95–98]. To date, about seventeen subunits
of nAChRs have been described [99]. In mammalian brain cells, the most commonly found nAChRs are the α4β2 heteromeric (α4β2-nAChR) and the α7 homomeric subunit–α7 nicotinic ACh receptor (α7-nAChR) [100], whose importance in AD treatment has been widely examined. For example, the stimulation of α7-nAChRs contributes to memory formation and consolidation [96] and cellular recovery after Aβ-induced neurotoxicity in mice [97]. On the other hand, the blockade of α7-nAChRs induces cognitive deficits in mice [98]. In addition to inhibiting AChE, there is evidence that ChEI interacts with α7-nAChRs, which in conjunction with tyrosine kinase Fyn and Janus-activated kinase 2 (JAK2), lead to the activation of PI3K signaling cascade and induction of neuroprotective effects [95, 101]. PI3K is a plasma membrane-associated kinase protein composed of a p85 regulatory subunit and a p110 catalytic subunit. Upon activation, PI3K catalyzes the conversion of phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which further activates several downstream proteins, such as AKT [102]. Activated AKT modulates key biological processes that are essential for maintaining cellular metabolism and cell survival [103].

Notoriously, AKT plays an important role in the regulation of cell death through the activation of proteins, such as the B-cell lymphoma 2 (Bcl-2) protein family [104]. Bcl-2 protein family includes Bcl-2-associated death promoter (BAD), Bcl-2 Associated X-protein (BAX), Bcl-2, and B-cell lymphoma-extra large (Bcl-xL) proteins that localize within the mitochondrial membrane [105]. Whereas Bad and Bax are pro-apoptotic proteins that can trigger the release of cytochrome C or caspase, thus inducing apoptosis, Bcl-2 and Bcl-xL are anti-apoptotic proteins that contribute to cell survival [105]. Furthermore, AKT also acts on different processes, such as stress response, antioxidant defense and autophagy, through the inhibition of FOXO transcription factors [106, 107]. There are reports showing that Aβ oligomers alter the PI3K-AKT signaling [108, 109], thus suggesting this pathway as a potential therapeutic target for new drugs. Actually, studies in AD patients demonstrated a downregulation of PI3K and its downstream targets [110, 111].

Of particular interest in AD, AKT normally inhibits GSK-3β and activates the nuclear factor (erythroid-derived 2)-like 2 (NRF2) protein, which is the main transcription regulator of antioxidant genes [112]. As a consequence of GSK-3β activation, there is an increase in Aβ production, hyperphosphorylation of tau protein, and consequently, NFTs formation [113, 114]. In this context, GSK-3β seems to be an interesting, promising target for designing new drugs, under the hypothesis that its inhibition might impact several alterations in AD; some of them have been associated with reduced levels of Aβ, tau protein, and neuronal death [115, 116]. An interesting finding is that activated GSK-3β promotes NRF2 downregulation [117]. Since the role of the NRF2 gene is related to the antioxidant system, NRF2-deficient mice present increased levels of oxidative stress, phosphorylated tau, Aβ accumulation [118], and also significant cognitive deficits [119]. Interestingly, studies on mouse AD models showed that drugs targeting NRF2 are potential neuroprotectors and can ameliorate cognition in mice [120, 121].

Another key protein in the PI3K/AKT pathway is the mammalian target of rapamycin (mTOR), which is phosphorylated and activated by AKT. mTOR is the catalytic subunit of two multi-protein complexes: mTOR complex 1 (mTORc1) and 2 (mTORc2) [122]. mTORc1 plays a crucial role in preventing the initiation of autophagy, which is an essentially biological process responsible for the clearance of misfolded and aggregated proteins, such as Aβ and tau, two proteins considered as hallmarks of AD. Enhanced mTORc1 activity is associated with reduced Aβ clearance, accumulation of Aβ aggregates [123, 124], tau protein and increased NFT formation [125, 126] due to dysfunctional autophagy. mTORc1 can also upregulate AβPP processing, enhancing Aβ formation [127]. On the contrary, mTOR inhibitors have ameliorated cognitive function [124], learning capacity and memory [123], thus suggesting that mTOR might be a potential target for AD treatment. mTORc2 can phosphorylate AKT and induce AKT signaling. Additionally, mTOR plays a role in the regulation of synaptic plasticity and neurotransmission [128]. It has been demonstrated that the coordination between mTORc1 and mTORc2 is necessary for the correct functioning of AKT signaling [122].

To counterbalance the PI3K-AKT pathway, other proteins, such as phosphatase and tensin homolog (PTEN) protein and Sdc homology domain-containing inositol 5′-phosphatase 1 (SHIP1) are required as negative regulators of AKT signaling; these proteins induce the dephosphorylation of PIP3 into PIP2 [129]. In addition, PH domain and leucine-rich repeat protein phosphatase (PHLPP) and protein phosphatase 2A (PP2A) are also downregulators of
Fig. 4. Schematic representation of PI3K/AKT signaling pathway. ChEI binding leads to the stimulation of α4 and α7 nicotinic acetylcholine receptors (nAChRs); subsequently, occurs the activation of tyrosine kinase Fyn and Janus-activated kinase 2 (JAK2), leading to the activation of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K). PI3K converts phosphatidylinositol (3,4)-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which activates protein kinase B (AKT). mTOR Complex 2 (mTORC2) also activate AKT signaling. PI3K/AKT pathway regulates several cellular functions, such as inhibition of Glycogen synthase kinase-3β (GSK-3β) that affects tau hyperphosphorylation, inhibition of Forkhead box O (FOXO) and Bcl-2-associated death promoter (BAD) proteins, which are cell survival regulators, such as B-cell lymphoma 2 (Bcl-2) and Bcl-xL. AKT also activates the mTOR Complex 1 (mTORC1) autophagy regulator and NRF2, which promotes antioxidant response. In AD, the Aβ also acts by inducing GSK-3β activity, increased NFT formation. The PI3K/AKT signaling pathway can be regulated in several ways. The Phosphatase and tensin homolog (PTEN) protein and Src homology domain-containing inositol 5'-phosphatase 1 (SHIP1) induce the dephosphorylation of PIP3 into PIP2, being negative regulators of PI3K/AKT signaling. PH domain and leucine-rich repeat protein phosphatase (PHLPP) and protein phosphatase 2A (PP2A) are also downregulators of AKT protein.

AKT by dephosphorylating AKT [129]. Inhibition of PTEN in AD mouse models is associated with several consequences, such as reduced expression of Bax protein, decreased apoptosis, lower levels of DNA fragmentation, reduced endoplasmic reticulum stress [130], and rescue of normal synaptic function and cognition [131]. Noteworthy, reduced activation of PTEN and increased AKT phosphorylation might be involved in neuritogenesis and neurodifferentiation [84]. Accordingly, targeting PTEN to induce AKT activation might be a potential approach to be investigated in AD therapy (Fig. 4).

Over the years, the accumulation of literature information pointed out the increasing importance of the PI3K/AKT pathway in the pathogenesis of AD. Altogether, several key proteins acting on this pathway can be selected as potential targets for the development of multi-target-directed ligands (MTDLs), thus affecting simultaneously different stages of the signaling cascade [132]. Thus, several compounds,
that act in the PI3K/AKT signaling pathway, have been developed for the treatment of AD. DL0410, a novel dual cholinesterase inhibitor, is a MTDL small molecule [132]. Studies have shown that DL0410 improves cognitive deficits, and also mitochondrial function; besides, the activation of the AKT/GSK-3β and MAPK/ERK signaling pathway promotes the reduction of Aβ that can result in the induction of synaptic transmission [132–134].

Treatment with EVP-6124 (encenicline), an α7-nAChR partial agonist, in patients with mild to moderate AD at Phase I (NCT00766363) and II (NCT01073228) led to improvements in cognitive and clinical functioning, being safe and well-tolerated [135]. However, Phase III studies (NCT01969136) have been suspended due to clinical hold. Another potent and selective α7-nAChR antagonist, ABT-126, showed cognitive efficacy in animal models [138]. ABT-126 was investigated in Phase I (NCT00867399) and Phase II (NCT00948909) studies, demonstrating to be well-tolerated and safe, but a significant cognitive improvement was not observed in patients with mild to moderate AD [136].

Phase I (NCT00948259) and II (NCT01350362) studies with NP031112, a GSK3 protein inhibitor, carried out in patients with mild and moderate AD demonstrated that the treatment was well tolerated, but without any clinical benefit [137, 138]. Another selective inhibitor of GSK3, AZD1080, tested in animal studies significantly reduced tau phosphorylation, reversing cognitive deficits [139].

Hence, in the context of unravelling the details of the PI3K/AKT signaling pathway regarding its role linked to AD pathology and neuroprotective effects caused by certain therapeutic drugs, data in the literature on the outcome of clinical applications are still scarce; but in spite of this, a new scenario emerges towards the development of more efficient drugs, which act on multiple protein targets. However, the consequences of inhibiting multiple targets must be investigated with caution, since the effects on more than one target may likely extend to different cellular processes, and these must be balanced against the desirable effects in terms of targeting the inherent changes to AD pathology.

NEXT-GENERATION ChE INHIBITORS

There is a growing interest in the development of potential MTDL drugs for the treatment of neurodegenerative diseases, such as AD. This comes from the perception that for this disease, the pathophysiology may result from an impairment of a complex intracellular network, mainly involving important signaling cascade, such as the PI3K/AKT pathway, as aforementioned. In this context, relying on a single target or process will probably not achieve the desired result, mainly due to the existence of cross-signaling mechanisms, as well as a positive and negative control of molecular pathways. Thus, interventions towards more than one step of this intricate molecular signaling network have great potential to overcome the pathological changes that occur in AD, although there are many limitations and the need to control biological responses and undesirable effects.

Although all initial AChEI drugs have been used as scaffolds to propose novel therapeutic compounds, a significant amount of these potential drugs relied on tacrine 1 to guide the research on anti-AD drug discovery. It is still of interest nowadays, even though this compound was discontinued in 2013 due to its hepatotoxicity [6, 140]. Some novel tacrine-like hybrids were recently reported [6, 140, 149–152, 141–148]. Compounds with the highest ChE inhibition are represented in Fig. 5, with their IC50 displayed in Table 1. It is worth mentioning that modifications in the tetrahydroamninacrine system have been proposed not only to increase the effectiveness of ChE inhibition, but also aiming to reduce hepatotoxic effects [6, 45, 140]. Besides, many structures are endowed not only to achieve ChE inhibition, but also to obtain antioxidant properties, metal chelation, blockage of Aβ self-aggregation, inhibition of BACE1, HDAC, or NMDA receptors, aiming to prevent neurodegeneration [141–143, 147, 149, 151, 153]. Although there is a great interest in developing rivastigmine and galantamine analogues [45, 140], significant efforts regarding the anti-cholinergic approach rely mostly on tacrine and donepezil modifications. Thus, here we focused on these kinds of structures.

Donepezil-based derivatives were also designed and synthesized as AChEI. Their structure modification mainly relied upon replacing dimethoxy indanone fragment, responsible for the interaction with the PAS site, while preserving the benzylpiperidine moiety, which fulfills the CAS site [140, 154]. The hybrid molecular strategy was also employed, leading not only to novel selective ChEIs but also MTDLs, which possess other non-cholinergic effects, such as inhibiting MAO 19 [155]. BACE-1 20 and 21 [156, 157], chelating metal and diminishing ROS production 27 [158], or even inhibiting Aβ aggregation...
Fig. 5. Chemical structure of tacrine and most potent tacrine-based hybrids as ChE inhibitors described in the last years. The color pink highlights the 1,2,3,4-tetrahydroakridin-9-amine rings as derivatives in each structure.

Table 1
Inhibitory activities (IC₅₀ nM) of AChE and BuChE and selectivity index of novel tacrine-like compounds

| Compounds | IC₅₀ (nM) | Ratio of BuChE/ AChE IC₅₀ | Multi-targeted ligand | Ref |
|-----------|----------|---------------------------|----------------------|-----|
| Tacrine   | 174.00   | 32.00                     | 5.40                 | —   | [151] |
| 5         | 150.00   | 36.00                     | 0.24                 | BACE1 inhibition, Neuroprotection against glutamate toxicity | [145] |
| 6         | 49.00    | 93280.00                  | 1903.67              | Neuroprotection and Aβ aggregation inhibitory activities | [146] |
| 7         | 3.90     | 24.30                     | 6.23                 | Inhibition of Aβ₁₋₄₂ self-aggregation and fibril formation, Antioxidant activity, Neuroprotection | [147] |
| 8a        | 580.00   | >66050                    | >113.88              | —   | [148] |
| 8b        | 440.00   | >61020                    | >138.68              | —   | |
| 9         | 3050.00  | 3190.00                   | 1.05                 | Moderate-to-potent calcium channel blockers | [149] |
| 10        | 2.20     | 4.93                      | 2.24                 | —   | [150] |
| 11        | 0.12     | 361.52                    | 3012.67              | HDAC inhibition, Cu²⁺ chelating properties | [151] |
| 12        | 26.00    | 9.00                      | 0.35                 | —   | [152] |
| 13        | 259.00   | —                         | —                    | Antioxidant activity | [141] |
| 14        | 22.15    | 6.96                      | 0.31                 | —   | [142] |
| 15        | 10.00    | 57.00                     | 5.70                 | Antioxidant activity | [143] |
| 16a       | 13.00    | 21.00                     | 1.62                 | —   | [144] |
| 16b       | 2.00     | 110.00                    | 55.00                | —   | |
| 17        | 33.40    | 62.00                     | 1.86                 | NMDA receptors inhibition | [153] |
Table 2

Inhibitory activities (IC$_{50}$ nM) of AChE and BuChE of donepezil-like compounds

| Compounds | IC$_{50}$ (nM) | Multi-targeted ligand | Ref |
|-----------|----------------|-----------------------|-----|
|           | AChE | BuChE |                              |     |
| Donepezil | 30.00 | 4660.00 | Aβ aggregation inhibition; BACE1 inhibition; MAO B inhibition; | [142, 155, 157] |
| 18        | 1420.00 | 0.25 | — | [162] |
| 19        | 350.00 | 460.00 | MAO inhibition | [155] |
| 20        | 4.10 | — | BACE-1 inhibition, metal chelating | [156] |
| 21        | 16.00 | — | BACE-1 inhibition | [157] |
| 22        | 1670.00 | — | Aβ aggregation inhibition, metal chelating | [159] |
| 23        | 0.36 | — | Antioxidant activity | [163] |
| 24        | 1147.00 | — | — | [164] |
| 25        | — | 510.00 | weak Aβ aggregation inhibition | [165] |
| 26        | 0.27 | — | Aβ antiaggregating activity | [160] |
| 27        | 120.00 | — | Aβ aggregation inhibition, metal chelating | [158] |
| 28        | 14.00 | 3690.00 | Neuroprotection against Aβ$_{42}$ | [12] |
| 29        | >10,000 | 0.17 | — | [161] |

Fig. 6. Donepezil and some of the most potent ChE inhibitors derived from donepezil lately developed. Highlighted in blue are the indanone fragments, in red benzylpiperidines moieties, and in pink the 1,2,3,4-tetrahydroakridin-9-amine rings in each structure.

22, 26, 27, 28, and 29 [12, 158–161]. Nevertheless, the hybridization of donepezil with tacrine was also attempted, originating potent compounds (Table 2) that conserved indanone, piperidine, and tetrahydroaminoacrine moieties 26 and to structures that interchanged one of these moieties to an isostere, for example, 27, which replaced the indanone group to a benzofuran, as demonstrated in Fig. 6.

As mentioned above, despite ChE inhibition is still considered an important strategy for anti-AD therapy, interest in novel MTDLs designed for AD treatment is a crucial aspect in the search for effective outcomes. Despite the neuroprotective effects reported for ChEIs, the effectiveness of drugs traditionally used in the clinic is modest, and there is an immense challenge in the search for more effective drugs capable of slowing the progression of AD, in addition to controlling the symptoms of the disease (Fig. 7). Thus, the search for understanding the molecular mechanisms of action of these compounds remains, highlighting the relevance of designing new drugs capable of reaching multiple targets in AD.
CONCLUSIONS

AD is considered a complex multifactorial disease. The use of cholinesterase inhibitors (donepezil, rivastigmine, galantamine) is currently the main treatment modality. Several in vitro and in vivo studies have shown that traditional ChEI is provided with cholinergic activities as well as activity against various AD molecular targets, but their effects in reducing AD progression are still limited. In the field of therapeutic strategies for AD patients, it is extremely important to understand the signaling pathways and cellular mechanisms that are relevant for inducing neuroprotection. In this context, a substantial progress has been observed and, for the short-term future, the expected amount of information may provide the essential basis for the development of new highly effective multi-target-directed ligand drugs designed to improve cognitive functions and reduce AD neurodegeneration.

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