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Therapeutic Potential Of Multifunctional Derivatives Of Cholinesterase Inhibitors

Abstract: The aim of this work is review of tacrine analogues from last three years, which were not included in the latest review work, donepezil and galantamine hybrids from 2015 and rivastigmine derivatives from 2014. In this account we summarize the efforts toward the development and characterization of non-toxic inhibitors of cholinesterases based on mentioned drugs with various interesting additional properties such as antioxidant, decreasing β -amyloid plaque aggregation, nitric oxide production, pro-inflammatory cytokines release, monoamine oxidase-B activity, cytotoxicity and oxidative stress *in vitro* and in animal model that classify these hybrids as potential multifunctional therapeutic agents for Alzheimer's disease. Moreover, herein, we have described the cholinergic hypothesis, mechanisms of neurodegeneration and current pharmacotherapy of Alzheimer's disease which is based on the restoration of cholinergic function through blocking enzymes that break down acetylcholine.

Keywords: tacrine, donepezil, galantamine, rivastigmine, multifunctional cholinesterase inhibitors, Alzheimer's disease.

1. INTRODUCTION

1.1. The Cholinergic hypothesis and therapy of Alzheimer's Disease

In the mid-1970s, studies based on a brain autopsy of patients with Alzheimer's disease (AD), revealed a significant deficits of the choline acetyltransferase (ChAT) in the limbic system and cerebral cortex and which is responsible for the acetylcholine (ACh) synthesis [1-3]. In further study, it has been determined that the nucleus basalis of Meynert in the basal forebrain, which is the source of cortical cholinergic innervation, undergoes neurodegeneration in Alzheimer's disease [4,5]. The aforementioned studies, as well as the demonstration that cholinergic antagonists impair memory [6], formed the basis for the cholinergic hypothesis of Alzheimer's Disease.

ACh is a major neurotransmitter in the brain, with activity through the cortex, basal forebrain and basal ganglia [7]. Cholinergic projection neurons are found in nuclei such as the medial habenula, the pedunclopontine and laterodorsal tegmental areas [8]. ACh acts through two classes of receptors: ionotropic nicotinic receptors (nAChRs) and metabotropic muscarinic receptors (M1-M5) [9]. They are located both pre- and postsynaptically throughout the brain being responsible for diverse brain activity. Cholinergic neurotransmission is involved in a number of psychic processes including walking and sleep, learning, memory, stress response, and affectivity [10,11]. Furthermore, its impact on other neurotransmitters greatly

expands the scope of activity. For instance, stimulation of M1/M5 receptors release neurotransmitter, dopamine, from striatal synaptosomes [12]. Moreover, nAChRs stimulate release of glutamate, gamma-aminobutyric acid (GABA), norepinephrine or serotonin [13]. Many studies have shown that the cholinergic lesion in Alzheimer's disease is associated with nicotinic and muscarinic receptors changes. Nordberg and Winblad [14] have determined decrease of postsynaptic nicotinic receptors on cortical neurons. Mash et al. [15] has shown decrease of M2 receptors (mostly presynaptic) in the cerebral cortex, however, level of M1 receptors remained unchanged. On the other hand, it has been suggested that M1 receptors may be dysfunctional in the cerebral cortex [16]. Interestingly, the gene expression level of $\alpha 7$ nicotinic receptor is increased in Alzheimer's disease patients compared to healthy controls, however, its impact on pathogenesis remains to be elucidated [17]. Current pharmacotherapy is based on the restoration of cholinergic function through blocking enzymes that break down acetylcholine. Suppression of acetylcholine breakdown by cholinesterase inhibitors sustain ACh activity at cholinergic synapses. Tacrine was the first cholinesterases inhibitor [both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] approved by the FDA for treatment of Alzheimer's disease [18]. Tacrine was shown to have efficacy in mildly to moderately impaired Alzheimer's patients, however, it did not affect the ultimate course of the disease [19]. Moreover, tacrine exhibited significant hepatotoxicity (in approximately 25 percent of patients) and

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quite low bioavailability after oral administration (about 17 percent) [19]. For the above reasons, use of tacrine was limited soon after its inception in therapeutic application [20].

However, tacrine structure is still widely used in medicinal chemistry for design hybrid or multitarget compounds without toxic side effects [21,22]. Medicines currently used in AD therapy include three cholinesterase inhibitors: donepezil, rivastigmine and galantamine [23]. Chemical structures of tacrine, donepezil, rivastigmine and galantamine are given in Figure 1. In 1996, donepezil was approved for mild to moderate AD therapy as a non-competitive, rapidly reversible acetylcholinesterase (AChE) inhibitor [24]. Rivastigmine is another drug, which was approved in 2000 for AD treatment [25]. Galantamine was approved in 2001 as a competitive, rapidly reversible potent AChE inhibitor [26]. Rivastigmine (the same as tacrine) is a non-competitive, pseudo-irreversible inhibitor of both AChE and BChE [25]. BChE regulates extracellular acetylcholine concentration and is mainly located on glial cells, whereas AChE plays a major role in cholinergic transmission and is located in pre- and postsynaptic membranes [27]. The activity of AChE rapidly declines with the death of cholinergic neurons with a simultaneous increase in BChE role in the regulation of cholinergic transmission [27]. Therefore, inhibition of both enzymes is a significant treatment benefit for rivastigmine. However, donepezil also acts not only at the neurotransmitter level. It inhibits various aspects of glutamate-induced excitotoxicity, induces a neuroprotective isoform of AChE as well as reduces early expression of inflammatory cytokines and oxidative stress effects [28]. Furthermore, some recent study indicate that treatment based on promotion of cholinergic function may

and clumsiness. A limitation of use is also its short half-life what requires multiply application by each patient [33]. Side effects occur with rivastigmine are nausea, vomiting, diarrhea, anorexia, weight loss and abdominal pain. Some neurological side effects are possible as well like headache, and syncope [34]. Also a donepezil revealed some lack of tolerance in patients manifested by dizziness, gastrointestinal symptoms (for example a nausea, vomiting, or constipation), insomnia, fatigue, sinus bradycardia and toxic effect toward the liver [35]. Any side effect of galantamine has not been proved. Its use is well tolerated and safe [36].

Unfortunately, there are also some limitations of cholinesterase inhibitors studies. Many investigations of neuropathology and cognitive impairment in multiple species have shown that Alzheimer disease is a uniquely human disease [37]. Therefore, commonly used experimental animal models contribute to a very poor success rate of AD clinical trials, which can be partially explained by the premature translation of successful pathology reduction in transgenic mice to humans [37]. Furthermore, the complexity of pathomechanisms of Alzheimer disease, and its incomplete understanding, makes it difficult to develop effective therapeutic strategy [38-40].

1.2. Other mechanisms of neurodegeneration in Alzheimer disease

Unfortunately, the current pharmacotherapeutic approach based on cholinesterase inhibitors has positive results only for a short period of time, usually 1-3 years, and is not able to completely arrest the progression of the disease [41]. This is due to the multifaceted, progressive and interactive pathophysiology of AD. Besides impairment of cholinergic neurotransmission, AD pathology includes aggregation of β -

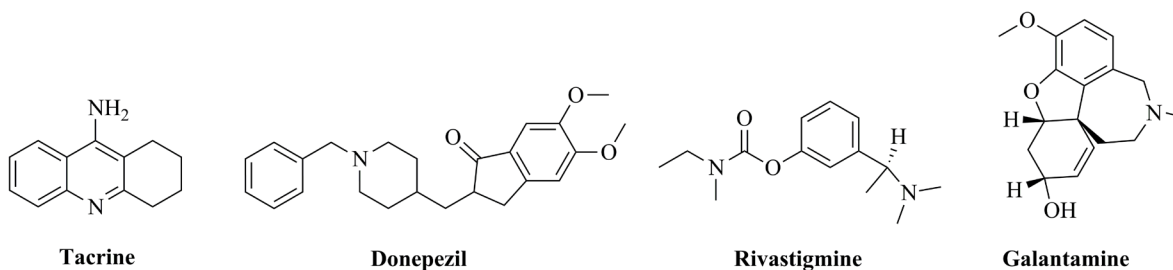


Fig. 1. Chemical structures of tacrine, donepezil, rivastigmine and galantamine.

also have more durable beneficial biological effects. It has been shown that use of donepezil is associated with substantially less regional cortical thinning and basal forebrain atrophy over time [29,30]. Moreover, study on the same population has found a 45% reduction in the rate of hippocampal atrophy after one-year donepezil treatment [31]. In another study, after 18 months of treatment with donepezil and galantamine, it has been shown decrease regional cerebral blood flow in the parietal cortex as well as an increase in the frontal and limbic cortices [32].

Although the current therapy of AD is based on the inhibition of cholinesterase, clinical limitations of its use should be mentioned. A serious side effects of tacrine are hepatotoxicity, nausea, vomiting, loss of appetite, diarrhoea

amyloid that leads to tau protein hyperphosphorylation, excess production of nitric oxide (NO) and reactive oxygen species (ROS), microglia and astrocyte activation and ultimately to neurotoxicity and cognitive dysfunction [42]. However, the complete mechanism of AD pathogenesis is still unclear which has a major impact on failure of clinical trials in AD treatment. Therefore, the develop of multi-target drugs to inhibit multiple factors involved in AD seems to be a promising therapeutic approach.

Senile plaques are one of neuropathological AD hallmarks present extracellularly [43]. They consist mainly of amyloid- β protein ($A\beta$), which is produced from its precursor called amyloid precursor protein (APP) [44]. APP is cut by β - and γ -secretases forming $A\beta$, which is mainly generated from

neurons, however, γ -secretase-dependent cleavage occurs in several cell types, and generates heterogeneity in $A\beta$, mainly producing $A\beta_{40}$ and $A\beta_{42}$ [44]. $A\beta_{42}$ is the predominant species deposited in AD brains as well as the most toxic [45]. According to the Amyloid Cascade Hypothesis the accumulation of $A\beta$ plaques in the brain of AD patients leads to a number of secondary pathological changes including altered ionic homeostasis, inflammation (microglial and astrocytic activation), accumulation of the hyperphosphorylated tau protein forming neurofibrillary tangles (NFTs), synaptic degeneration and neuronal cell death [46].

Oxidative stress is a condition of imbalance between reactive oxygen species (ROS) production and antioxidant defences, resulting in excessive accumulation of ROS [47]. It has been suggested that oxidative imbalance may play an important role in the initiation and progression of Alzheimer Diseases [48]. It has been determined that the accumulation of $A\beta$ increases oxidative stress and lead to mitochondrial dysfunction and energy failure [49]. Moreover, $A\beta$ -induced oxidative imbalance may increase the levels of the products related to lipid peroxidation, protein oxidation and DNA/RNA oxidation [50]. It has been found decreased levels of antioxidants (uric acid, vitamin C and E) or antioxidant enzymes (e.g. superoxide dismutase, catalase) in patients with AD [51]. Besides, disturbances in metals levels, such as iron, zinc, and copper have been identified in AD patients [52]. There is evidence to support that these alterations lead to neuronal death through enhancing the ROS production, affecting cellular redox homeostasis (the depletion of glutathione), disruption of mitochondrial function as well as inducing intracellular free calcium, which is part of a signalling pathway [53,54]. Metal ions may also

accelerate protein aggregation and their high micromolar concentrations are found in amyloid plaques [55]. Moreover, overproduction of the nitric oxide (NO) inducing by $A\beta$ (through the activation of microglia) also contribute to the production of highly reactive oxidative species and secondary components of nitroxidative stress [56].

Many studies have shown that neuroinflammation playing a fundamental role in the progression of the Alzheimer disease [57]. Chronically activated microglia release many proinflammatory and toxic products, including cytokines reactive oxygen species and nitric oxide [58]. One of the more important proinflammatory cytokines in AD is tumour necrosis factor α (TNF- α), which plays both initiation and regulation role of the cytokine cascade during inflammation [59]. Mice lacking TNFR1 (TNF- α receptor) crossed with the transgenic AD model exhibit mitigated hippocampal microglial activation, reduced plaque deposition and improved performance in cognitive tasks [60]. Cyclooxygenase 2 (COX-2) is upregulated in many inflammatory disorders [61], however, it is not clear in case of AD. It has been detected increased levels of COX-2 mRNA and protein staining in AD tissue [62,63]. On the other hand, it has been found decreased COX-2 expression, particularly in late stage AD [64,65]. In case of COX-1, which is prominently expressed by microglia in human brain [66], contribution in neuroinflammation in AD has also not been established. Nevertheless, 5-lipoxygenase (5-LOX), producing pro-inflammatory leukotrienes, as well as COXs enzymes are considered to be potential novel targets for AD therapy [67]. The family of phosphodiesterase-4 (PDE4) enzymes regulating cyclic adenosine monophosphate (cAMP) signalling in neurons and glial cells represent another underexploited therapeutic targets [68]. PDE4B

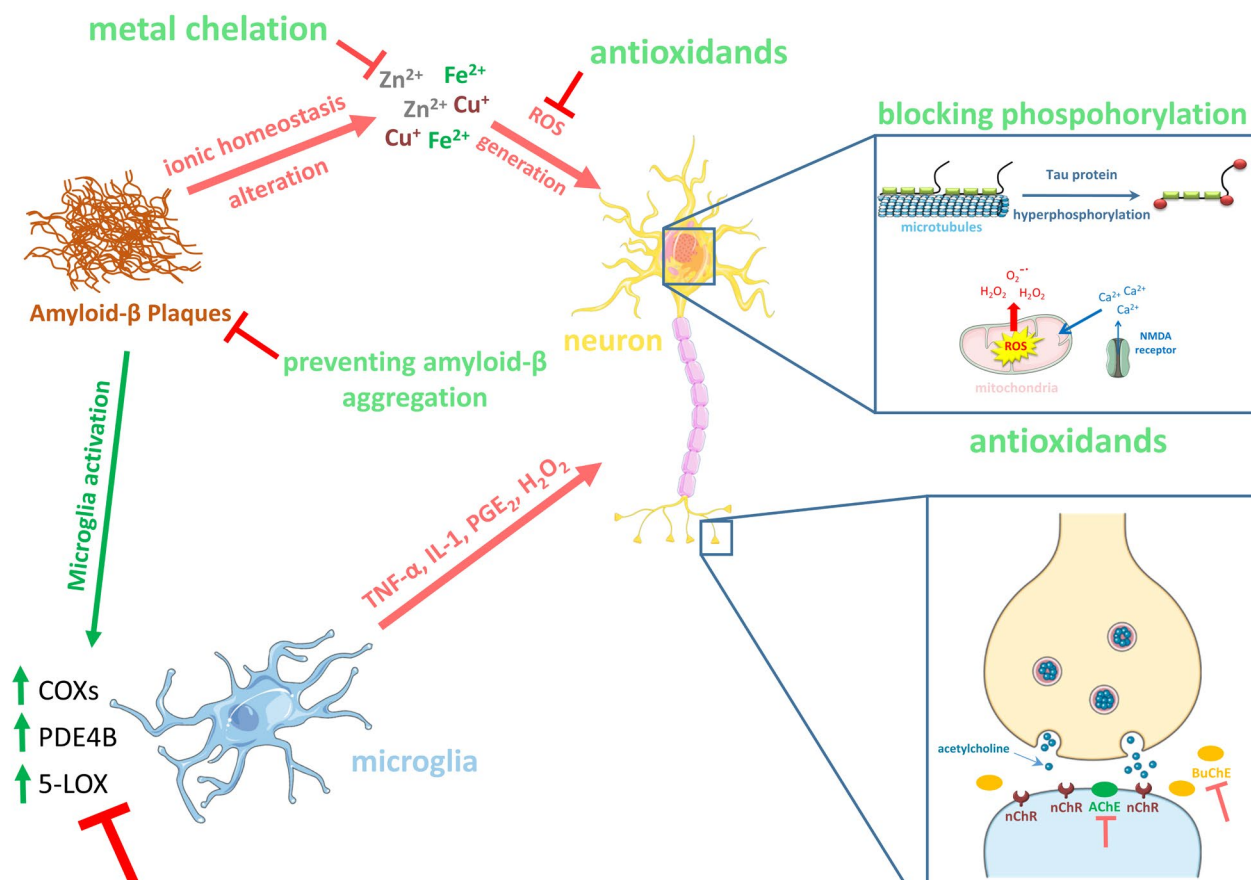


Fig. 2. Therapeutic strategies of multifunctional compounds for Alzheimer's disease.

(subtype of PDE4) is induced in macrophages, monocytes, and microglial cells by inflammatory stimulators including A β [69] and represents a novel therapeutic target, which may reduce neuroinflammation in AD [68].

Monoamine oxidase (MAO) is one of the main enzymes which catabolize catecholamines and serotonin and is widely distributed in the central nervous system in 2 main isoforms: A and B [70]. It has been determined that both A and B forms of MAO are altered in Alzheimer's disease [71], however, the mechanism by which they affect AD pathogenesis is not fully understood. The recent study has shown that MAO-B levels are increased in astrocytes as well as in pyramidal neurons in AD brain [72]. Moreover, the study also suggested that MAO-B regulates A β production in neurons via γ -secretase [72]. It has been shown that MAO-B inhibitor may be useful and reliable tool for the treatment of AD patients improving their cognitive functions and reduce behavioral alterations, without frequent or severe side effects [73].

Some metals like iron or copper play a significant role for a human health, because they have an impact on the proper course of the homeostasis process. The excess or deficiency of them is essential for an occurrence of several commonly known neurodegenerative disorders like e.g. AD. Hence, metal chelating activity is valuable and desirable property during searching for AD efficient treatment therapy [46]. Multitarget therapeutic strategies for AD is summarized in Figure 2.

2. MULTIFUNCTIONAL DERIVATIVES OF TACRINE, DONEPEZIL, GALANTAMINE AND RIVASTIGMINE

In the work published in 2019 [74], authors described new multifunctional tacrine derivatives. This article presents the latest analogues, which were synthesized and investigated in a span of the last three years. Moreover, herein we described donepezil and galantamine hybrids which were reported in last five years, as well as rivastigmine derivatives from the last six years. All structures showing multifunctional properties were included in Table 1.

2.1. Tacrine analogues

It has been proven that introduction of chlorine atom to the structure of tacrine and its derivatives has an effect on the increase of anti-cholinesterase activity and decrease in hepatotoxicity [75,76]. Ragab et al. [77] designed, synthesized and biologically evaluated a series of new 4-(chlorophenyl)tetrahydroquinoline derivatives. The results of biological investigation showed that all analogues had equivalent or much higher activity against cholinesterase and were less hepatotoxic than referenced drug tacrine. Moreover, *in silico* drug-likeness of the obtained derivatives were predicted and their practical logP were estimated showing ability of all of them to be promising hits/leads. In addition, the docking study was performed for the most

potent cholinesterase inhibitor in the series, showing its binding mode is similar to that representing by tacrine.

The first step against the main toxic effects of nerve agents is simultaneously administration of anticholinergic agents and oxime reactivators, such as pralidoxime (2-PAM), which can reactivate inhibited AChE [78,79]. 2-PAM is not active against every nerve agent, thus there is a need to look for more efficient compound. Novel series of tacrine-pyridinium hybrid reactivators linked by carbon chain were designed, synthesized and tested for their *in vitro* AChE inhibitory activity. The highest obtained results for AChE activity were 95%, 92% and 90% and 1 μ M concentrations of the oximes [80].

Wang et al. [81] synthesized a series of hybrids of tacrine and deferisirox as potential multifunctional drugs using in AD treatment. The most derivatives in the series were potent inhibitors of AChE from bovine serum (bAChE) metal ions chelators. The most effective compound, 1, showed in the addition an antioxidant activity, low cytotoxicity and protective properties against the injury caused by H₂O₂. Type of inhibition, based on molecular modeling, for all of compounds was mixed with the ability to bind to the catalytic anionic site (CAS) and the peripheral anionic site (PAS) of Torpedo californica variant of AChE (TcAChE).

Tacrine-isatin Schiff base hybrids displayed potent activity against AChE and BChE with the selectivity for AChE over BChE. The most potent inhibitors of AChE were derivatives 2a-c with IC₅₀ values of 0.42 nM, 0.62 nM, 0.95 nM, respectively. They were 92-, 62- and 41-fold stronger than referenced tacrine (IC₅₀ = 38.72 nM). The most active derivative against BChE was 2d with an IC₅₀ value of 0.11 nM, what was 56 times stronger than tacrine (IC₅₀ = 6.21 nM). All compounds in the series showed excellent metal chelating properties. Moreover, 2a,b were good inhibitors of AChE-induced amyloid-beta (A β) aggregation. Studies of kinetic and molecular modeling exhibited mixed-type inhibition for 2a [82].

El-Malah et al. [83] designed and synthesized new thienopyridine-tacrine analogues as potential cholinesterase inhibitors. Obtained derivatives have been evaluated for their *in vivo* brain AChE inhibitory activity. For the most promising compounds, *in vitro* AChE inhibition was investigated. The most potent in the series was hybrid 3 with an IC₅₀ value of 172 nM.

An example of bivalent reversible inhibitor of AChE is 5-amino-N1,N3-bis(2-(1,2,3,4-tetrahydroacridin-9-ylamino)ethyl)isophthalamide is compound 4. It was designed using molecular modeling and showed high affinity and ability to bind to CAS and PAS. The synthesis included using 9-alkyl(1,2,3,4-tetrahydroacridine) pharmacophore with appended functionality. Biological evaluation revealed high inhibition of AChE and BChE with IC₅₀ values of 0.54 \pm 0.06 nM and 32.49 \pm 1.2 nM, respectively (the value of selectivity ratio was 60.16 toward AChE). Moreover compound 4 was able to inhibit PAS and aggregation of A β induced by AChE. Rat hippocampal neurons studies showed low cytotoxic effect and ability to induce S-phase post-



treatment. Therefore, further detailed evaluation of this derivative as potential drug for AD treatment is proposed [84].

Li et al. [85] synthesized a series of novel tacrine-phenolic acid dihybrids and tacrine-phenolic acid-ligustrazine trihybrids and evaluated them towards cholinesterase inhibitory activity. The most interesting in the series was compound 5 and inhibited eeAChE, hAChE, eqBChE and hBChE with IC_{50} values of 3.9 nM, 65.2 nM; 24.3 nM and 48.8 nM, respectively. It was stronger towards AChE (mixed-type dual site inhibitor) and simultaneously weaker against BChE than referenced drug tacrine. It was not hepatotoxic and exhibited inhibition of $A\beta$ self-aggregation at level of 47% at 20 μ M, antioxidant properties towards PC12 cells from $CoCl_2$ -damage, what makes it promising multifunctional drug for patients with AD.

Quinoxalines are compounds showing anti-neuroinflammation, antitumoral, antimycobacterial and antifungal properties. Therefore, a series of novel quinoxalinetacrine derivatives was synthesized and their biological activity was investigated. Derivative 6 showed lower activity against AChE and BChE with IC_{50} values of $22.0 \pm 1.3 \mu$ M and $6.79 \pm 0.33 \mu$ M, respectively, but it was selective toward BChE. Furthermore this compound exhibited lower hepatotoxicity than tacrine and neuroprotective activity against rotenone plus oligomycin-A or okadaic acid [86].

Also, a series of tacrine analogues containing pyranopyrazole moiety were synthesized. These derivatives were investigated for their AChE and BChE inhibition. Most compounds in the obtained group showed high activity against AChE with IC_{50} values for the two strongest analogues of 0.044 μ M and 0.058 μ M [87].

Due to multifunctional nature of AD, it is important to design structures that could display multi-target-directed role. Example of this approach was described by Rajeshwari et al. [88]. Six selected hybrids containing two **pharmacophores**: tacrine and 2-phenylbenzothiazole exhibited, based on molecular studies, ability to bind to CAS and PAS of AChE. All compounds in the series proved excellent activity against AChE with IC_{50} values in the range of 0.06-0.27 nM. Studies carried out on SH-SY5Y (neuroblastoma cell line) cells treated with $A\beta_{1-42}$ peptides revealed neuroprotective activity against $A\beta$ -induced cell toxicity for three compounds: 7b,c,f, whereas 7c was the most potent. Results were expressed as the percentage of SH-SY5Y untreated cells and for mentioned compounds were $p < .005$.

A promising strategy in treatment of Alzheimer's disease is a combination of vanillina and tacrine. Because of many various biological properties of vanillin, e.g: antioxidant activity, $A\beta$ -amyloid inhibitory activity and capacity to inhibit AChE, it is using to obtain multi-target-directed ligands (MTDL). Antioxidant properties of novel series of tacrine-vaniline hybrids were tested by Scipioni et al. [89] using DPPH assay achieving IC_{50} value of 19.5 μ M, as well as FRAP and ORAC tests with results up to 1.54 and 6.4 Trolox equivalents, respectively. The kinetic studies revealed

inhibition of AChE at μ M range. Moreover compound 8 inhibited $A\beta_{1-42}$ amyloid similar to referenced curcumin. The investigation of neuroprotection for derivative mentioned above at 1 μ M proved it to protect cells of SH-SY5Y in the presence of hydrogen peroxide at 400 μ M.

Medrasi et al. [90] synthesized new pyrrolotacrine in Friedländer-type of reaction and investigated their ability to inhibit AChE. All compounds in the series showed potent or moderate anti-AChE activity with IC_{50} value of 0.2 μ M for the most potent derivative. Interestingly, the replacement of a fused cyclohexane ring by a fused cyclopentane ring didn't have the impact on improvement of AChE inhibition. Furthermore, the effect of presence of chlorine atom on amelioration cholinesterase inhibitory, which is observed by some authors is not observed in results reported by Medrasi et al. [91-93].

Deferiprone is well known metal chelating drug, which binds to iron in a 3:1 ratio, thus it decreases content of this element in body of animals and humans [94]. Some research proved that it attenuated the increase in AChE activity and decreased $A\beta$ and iron deposition, as well as neuroprotective and memory enhancing effects for deferiprone in rats treated with scopolamine which might be attributed to its iron chelating action and anti-oxidative effect [95]. A combination of tacrine and deferiprone provides good AChE inhibitory activity, inhibition of self-/Cu-induced $A\beta$ aggregation, radical scavenging capacity and metal chelating properties (Fe, Cu, Zn). Chand et al. [96] synthesized a series of tacrine-deferiprone hybrids. Besides advantages mentioned above, obtained analogues are characterized by neuroprotection in SH-SY5Y cells treated with $A\beta_{1-42}$ ascorbate/iron stressors (9a and 9b). The best results displayed derivatives with chloro-substitution and containing 2-hydroxypropyl linkers with IC_{50} values in the range of 0.64-1.01 μ M. One can include them in the potential of leading compounds in the search for effective therapy in Alzheimer's disease treatment.

Also, compound 10 is a promising multifunctional anti-Alzheimer's disease candidate. It was the most active in the series of tacrine-ferulic acid hybrids with IC_{50} against eeAChE and BChE of 37.02 nM and 101.4 nM, respectively. Moreover it didn't show relevant hepatotoxicity and it was able to inhibit $A\beta$ self-aggregation by 65.49% at 25 μ M and ameliorated the impairment of memory in mice models treated with scopolamine in the Morris water maze test [97].

Ökten et al. [98] designed and bioevaluated three disubstituted six or seven hydrocycle membered tacrine derivatives. Substituents were bromine atom or silyl. All of obtained compounds effectively inhibited four enzymes: AChE, BChE, Carbonic anhydrase isoenzymes I and II (hCA I and II) with IC_{50} values of 30.26 ± 6.71 – 117.54 ± 42.22 nM, 22.45 ± 5.81 – 77.41 ± 4.02 nM, 57.28 ± 22.16 – 213.41 ± 82.75 nM and 46.95 ± 11.32 – 274.94 ± 62.15 nM, respectively.

Also a series of eleven tacrine-1,2,3-triazole hybrids were synthesized through Cu(i)-catalyzed alkyne-azide 1,3-dipolar cycloaddition (CuAAC) reaction as potential drugs using in



Alzheimer's disease treatment. The most potent compound inhibited eeAChE and BChE in micromolar range, which gave slightly lower result than the referenced tacrine. Molecular docking studies revealed it can bind to both, CAS and PAS sites [99].

Cheng et al. [100] designed and synthesized a series of brominated phenylacetic acid-tacrine derivatives and evaluated their AChE and BChE inhibitory activity, capacity to inhibit self-induced and AChE-induced A β aggregation and hepatotoxic effect. Among all, compound 11 exhibited the highest AChE/BChE inhibition, lack of hepatotoxicity in comparison to referenced drugs tacrine and donepezil, as well as inhibition of mentioned above A β aggregation.

Multitarget small molecules (MTSM) are able to interact simultaneously with the different enzymatic systems or receptors playing important role in the pathology of AD, thus MTSM are one of the most promising approaches in searching for new anti-AD drugs. A novel series of tacrine and kojic acid hybrids, which are MTSM, were synthesized and their biological activity was evaluated. The most active compound in the series, 12, proved to be less hepatotoxic than tacrine and selective inhibitor of hAChE with IC₅₀ value of 4.52 μ M. Moreover it exhibited antioxidant activity and neuroprotection against A β ₁₋₄₀ at 3 μ M and 10 μ M concentrations in SH-SY5Y cells [101].

Next interesting group of tacrine analogues are tacrine-H₂S donor hybrids (THS). Hydrogen sulfide (H₂S) is an endogenous signaling gasotransmitter molecule. It is produced in the liver, as well as in brain by cystathionine betasynthase, 3-mercaptopyruvate-sulfurtransferase, cysteine aminotransferase and cystathionine γ -lyase [102]. The role of H₂S in brain is regulation of synaptic activity of neurons and glia by increasing the intracellular calcium ions, as well as it acts significant function in the development of long-term potentiation [103,104]. The decrease of level of H₂S promotes the progression of depression and it acts relevant role in the development of learning and memory deficits [105,106]. Some studies proved also that it slows down the progression of Alzheimer's disease in experimental models by targeting multiple pathophysiological mechanisms [107]. H₂S has also neuroprotective, hepatoprotective, and anti-inflammatory properties. From that perspective, the hybrids of H₂S-donors and tacrine could be efficient strategy in Alzheimer's disease treatment. An example of this approach is connection of tacrine and H₂S-releasing moieties (ACS81) to give compound 13. Authors reported that this hybrid besides excellent AChE inhibition, improved cognitive and locomotor activity in mouse model with AD in the step-through test and open field test, respectively. Moreover it induced the increase of amount of H₂S in hippocampus, decreased mRNA expression of the proinflammatory cytokines, TNF- α , IL-6, and IL-1 β and increased synapse-associated in the hippocampus of tested mice. Regarding the fact, tacrine was withdrawn from use due to its hepatotoxicity, it is important that obtained results for compound 13 showed its safety for liver. Without a doubt, this hybrid is a promising candidate against AD [108].

Gniazdowska et al. [109] designed and synthesized group of radioconjugates – tacrine derivatives labeled with technetium-99m and gallium-68, which are potential diagnostic radiopharmaceuticals. Authors tested lipophilicity, stability in the presence of an excess of standard amino acids cysteine or histidine, human serum and in cerebrospinal fluid. Based on investigation of lipophilicity, Gniazdowska et al. selected for molecular docking and biodistribution studies, as well as cholinesterases inhibition assays only two compounds, but both of them were good BChE and AChE inhibitors. Unfortunately, they showed the ability to cross the blood-brain barrier into the brains of rats, but in insufficient level, what may be due to an insufficient lipophilicity related to used hydrophilic chelators (Hynic and DOTA). Enhanced utility of PET and SPECT imaging for both conjugates may be reached after some modifications in administration procedure like change into the central nervous system injection or by insertion through the catheter (for PET) and proper adjustment of detection system for applied activity dose (for SPECT).

BChE, besides AChE, plays important role in AD. In the central nervous system it is mainly present in glial cells and neurons and it regulates extracellular acetylcholine concentration. With the decrease of AChE activity which occurs with the death of cholinergic neurons, the role of BChE in cholinergic transmission increases [110]. Due to this fact, a series of tacrine-phenolic heterodimers were designed and synthesized as potent BChE inhibitors using in Alzheimer's disease treatment [111]. A series includes different modifications of the nature (imino, amino, ether), various length of the linker, as well as number and position of the substituents on the aromatic ring. All of them proved to be strong BChE inhibitors from the nanomolar to subnanomolar range. The most active was compound 14 and it showed IC₅₀ value of 0.52 nM against human BChE, what was 85-fold more active than referenced drug tacrine. What is important for multifunctional nature of AD, this compound can inhibit the self-aggregation of A β ₄₂ with lack of neurotoxicity up to 5 mM, low hepatotoxicity and stability under physiological conditions. Moreover, it exhibited neuroprotection in rats neurons in a serum and K⁺ deprivation model.

Lopes et al. [112] designed, synthesized and evaluated cholinesterase inhibitory activity of tacrine and carbohydrate-based moieties hybrids as potential agents against AD. The synthesis was based on the reaction between tacrine and the appropriate sugar-based tosylates according to the mechanism of nucleophilic substitution. Most of the D-xylose analogues showed potent AChE and BChE inhibitory activity with IC₅₀ values of 2.2 nM and 4.93 nM, respectively, for the most active compound. Most hybrids in the series were selective for AChE over BChE (the highest IC₅₀ ratio that was noticed was 7.6). Only two derivatives showed selectivity for BChE. Mostly, described derivatives showed lack of hepatotoxicity according ProTox-II. Furthermore, on the basis of molecular modeling studies it was found that designed hybrids are able to interact with the entire binding cavity and the main contribution of the



linker is to enable the most favorable positioning of the two moieties with CAS, PAS, and hydrophobic pocket to provide optimal interactions with the binding cavity.

Cheng et al. [113] designed and synthesized a series of dual AChE and BChE inhibitors for AD treatment by connecting tacrine with indole-3-acetic acid (IAA). Some compounds in the series exhibited dual inhibitory activity in nanomolar range. Based on molecular modeling analysis for these derivatives, the capacity to bind to catalytic active site and peripheral anionic site of cholinesterase was found. The preliminary SAR analysis suggested the optimal length of diaminoalkyl linker between tacrine and IAA is six atoms of carbon.

M1 muscarinic acetylcholine receptors (M1 mAChRs) play important, selective role for verbal memory mechanisms [114,115]. Stimulation of them is able to improve cognitive deficit and M1 agonists can advance verbal memory, enhance cholinergic transmission, as well as have impact on amyloid precursor protein (APP) processing, what in results prevents A β -aggregation and slows the progression of Alzheimer's disease [116-119]. The series of tacrine-benzyl quinolone carboxylic acid (tacrine-BQCA) derivatives was synthesized and their hAChE/hBChE inhibitory activity was evaluated. The indirect aim was increase of cholinergic transmission by inhibiting of cholinesterase and the direct aim was supporting cholinergic transmission via M1 mAChR activation. The series consisted of three groups of compounds that differed in tacrine moiety (7-methoxytacrine, 6-chlorotacrine or unsubstituted tacrine). After the introduction of BQCA, a positive modulator of M1 muscarinic acetylcholine receptors the impact on M1 mAChRs was investigated via Fluo-4 NW assay on the Chinese hamster ovarian (CHO-M1WT2) cell line. All of the derivatives in the series were potent hAChE and hBChE inhibitors with activity in micromolar and nanomolar range. Unfortunately, the agonist effect towards M1 mAChRs, which should be provide by introduction of BQCA moiety wasn't revealed. Investigation of permeation BBB in vitro was positive for compounds 15a,b [120].

Phosphodiesterase 4 (PDE4) is the primary cAMP-specific hydrolase which is involved in the process of memory consolidation and a PDE4D inhibitor can affect on hippocampus and dependent on it memory tasks [121,122]. Pan et al. [123] designed, synthesized and bioevaluated a novel series of tacrine-pyrazolo[3,4-b]pyridine hybrids as potential cholinesterase and phosphodiesterase 4D (PDE4D) inhibitors. The inhibitory activity depended on the length of carbon chain between tacrine and pyrazolo[3,4-b]pyridine. The most active compounds in the series inhibited AChE with IC₅₀ value of 0.125 μ M, as well as it showed a desired balance of AChE and BChE and PDE4D inhibition activities, with IC₅₀ values of 0.449 and 0.271 μ M, respectively.

Also, a novel series of phosphorus and thiophosphorus tacrine analogues was designed and synthesized in reaction between appropriate chlorophosphates or chlorothiophosphates and N1-(1,2,3,4-tetrahydroacridin-9-yl)propane-1,3-diamine. Their inhibitory activity and

modeling studies were investigated. Two compounds in the series proved to be more active than tacrine, whereas the most active was 3-times stronger than referenced drug. All of the synthesized derivatives proved to be less toxic against SH-SY5Y cell line than tacrine [110].

2.2. Donepezil analogues

Łozińska et al. [124] synthesized a novel series of donepezil-melatonin hybrids as selective inhibitors of BChE. The synthesis was based on coupling reaction of alkylamine-substituted N-benzylpiperidine derivatives and with a carbonate analogue of N-acetylserotonin. The modification of the linker between two main moieties has been carried out by introduction of carbamate bond connected to the aromatic system of the melatonin. It led to improvement of the affinity toward BChE, as it was observed in the case of tacrine and melatonin derivatives [125].

Also, a novel series of donepezil-hydrazinonicotinamide hybrids was synthesized and bioevaluated. All of the obtained compounds revealed a higher affinity to AChE in comparison to BChE, higher selectivity toward AChE than to BChE and they were less active against AChE and more active against BChE than referenced drug donepezil [126].

Next interesting group of donepezil derivatives are hybrids of donepezil, chromone and melatonin as potential multifunctional drugs against AD. The most promising compound in the series, 16, exhibited potent hBChE inhibition with IC₅₀ values of 11.90 \pm 0.05 nM, but also a moderate inhibition of hAChE, human monoamine oxidase A (hMAO A), and monoamine oxidase B (MAO-B) with IC₅₀ of 1.73 \pm 0.34 μ M, 2.78 \pm 0.12 μ M, 21.29 \pm 3.85 μ M, respectively. This analogue revealed also strong antioxidant properties (3.04 TE) [127]. It should be mentioned, it is a selective BChE inhibitor, what is significant in the case of patients with moderate to severe forms of AD [128].

Benchekroun et al. [129] a novel series of donepezil and ferulic acid derivatives as potential multitarget anti-AD agents. All of the obtained compounds were characterized by strong antioxidant effect with the oxygen radical absorbance capacity values in the range 4.80–8.71 TE, what is much higher than results observed for referenced melatonin and ferulic acid. One of the most active hybrids, selective against eqBChE with strong antioxidant properties proved to be compound 17.

Donepezil – trolox hybrids were designed, synthesized and bioevaluated as multifunctional drugs for AD treatment. They exhibited moderate to good inhibition of hAChE and MAO-B with IC₅₀ values of 0.54 μ M and 4.3 μ M, respectively, for the most active compound in the series, 18. Moreover, it showed A β ₁₋₄₂ inhibitory activity, great antioxidant effect (IC₅₀ = 41.33 μ M by DPPH method, 1.72 and 1.79 TE by ABTS and ORAC methods) and ability to chelat Cu²⁺ ions. It did not show significant toxicity in HepG2, PC12, and BV-2 cells (murine microglia cell line), as well as it is able to protect cells from oxidative stress



caused by H₂O₂, rotenone, and oligomycin-A. Furthermore, oral administration of this analogue improved cognition and spatial memory against scopolamine-induced acute memory deficit, D-galactose (D-gal) and AlCl₃ induced chronic oxidative stress in mice model without acute toxicity and hepatotoxicity [130]. This derivative revealed ability to cross BBB in vitro.

A promising strategy in searching for new drugs for AD is represented by a novel series of donepezil – butylated hydroxytoluene (BHT) hybrids. Most of the compounds displayed moderate to good inhibition of AChE/MAO-B and excellent antioxidant activities. The most active in the series was compound 19 and it proved to be a multifunctional agent with cholinergic, antioxidant and neuroprotective activity. It inhibited eeAChE and hAChE with IC₅₀ values of 0.075 μM and 0.75 μM, respectively. Moreover, it exhibited inhibitory activity against hMAO-B with IC₅₀ value of 7.4 μM, ability to inhibit self-induced and hAChE-induced Aβ aggregation, antioxidant properties with IC₅₀ value of 71.7 μM using DPPH method, as well as 0.82 and 1.62 TE by ABTS method and ORAC method, respectively. Also, the neuroprotective effects on PC12 cells treated with H₂O₂ and against Lipopolysaccharides (LPS)-stimulated inflammation on BV-2 cells were tested. In addition, compound 19 could cross BBB in vitro and showed good liver metabolic stability in RLM (rat liver microsomes) and the passive avoidance test showed for this derivative dose-dependently reversed scopolamine-induced memory deficit in mice model but without acute toxicity [131].

Dias et al. [132] designed, synthesized and evaluated biological activity of novel series of feruloyl – donepezil hybrids as potential multifunctional drugs using in AD. The most active analogue in the series was compound 20. It inhibited eeAChE with IC₅₀ value of 0.46 μM. Moreover, it proved its multifunctional profile in the investigation of anti-inflammatory properties in vivo in mice model, antioxidant activity in SH-SY5Y cell line and ability to chelate Cu²⁺ and Fe²⁺ ions. Also, a neuroprotection studies revealed it protected SH-SY5Y cells against the late neuronal death caused by Aβ₁₋₄₂ oligomers. Molecular docking studies showed non-competitive mechanism of inhibition that can bind to PAS of AChE.

Several studies suggest that Aβ deposits and neurofibrillary tangles provide cause neuroinflammation, which contribute to early and late stage of the AD neurodegeneration [133,134]. Therefore, drugs designed to treat the different neuroinflammations are needed. Dias Viegas et al. [135] reported synthesis and biological evaluation of a series of N-benzyl-piperidine-arylacetylhydrazone derivatives as donepezil hybrids, that can possess anti-inflammatory properties, as well as can be active against neurodegeneration in patients brains with AD. Two compounds in the series, 21a and 21b, were good AChE inhibitors, exhibited the best ADME (Absorption, Distribution, Metabolism, Excretion) profile in silico, anti-inflammatory and neuroprotective activity against AβO-induced neuroinflammation and neurodegeneration in vitro

in THP-1 cells (human acute monocytic leukemia cell line) and in vivo in mice model. They inhibited tumor necrosis factor alpha (TNF-α), which is secreted by activated microglia cells and as a result it makes a progression of AD. It suggests, they could prevent the signal transduction pathway mediated by TNF type 1 receptor (TNFR1) at brain level, what is important regarding the fact that TNFR1 also is involved in cognitive decline [136,137]. Also, they showed inhibitory activity against cyclooxygenase-1 and -2 (COX-1 and COX-2, respectively), which play significant role in the mechanisms of CNS inflammation [138].

Next interesting group are deoxyvasicinone-donepezil hybrids. They exhibited from moderate to excellent inhibitory activity against hAChE, BACE1, and Aβ₁₋₄₂ aggregation. Among all, compounds 22a and 22b revealed low cytotoxicity and neuroprotective properties against Aβ₁₋₄₂-induced damage in SH-SY5Y cells. Derivative 22a inhibited hAChE, BACE1 and Aβ₁₋₄₂ aggregation with IC₅₀ values of 56.14 nM, 0.834 μM and 13.26 μM, respectively. In the same investigation, the results obtained for compound 22b are 3.29 nM, 0.129 μM and 9.26 μM, respectively. Moreover, these compounds should be able to penetrate the blood-brain barrier [139].

Valencia et al. [140] described neurogenic and neuroprotective hybrids by connecting flavonoid-related structures and a donepezil fragment to obtain new 4-chromone- and 4-quinolone – N-benzylpiperidine hybrids (DFHs). They exhibited nanomolar affinities for the sigma-1 receptor (σ₁R). Also, they inhibited hAChE, 5-lipoxygenase (5-LOX), MAO-A and MAO-B. Based on kinetic studies, the new DFHs can bind with the two main sites of the AChE gorge (CAS and PAS). Hybrids derived from 4-chromone series inhibited hAChE with IC₅₀ value in the range from the nanomolar to the low micromolar. What is interesting, derivatives from 4-oxo-1Hquinoline series were not as potent hAChE inhibitors as 4-chromone analogues. It may be caused by existence of a tautomeric equilibrium in the azaheterocycle what can impede the interaction with the enzyme. Moreover, most of the compounds in the series can cross BBB, based on in vitro PAMPA-BBB model. Also, they can scavenge free radical species and protect neuronal cells against mitochondrial oxidative stress.

Also, a novel series of donepezil derivatives obtained by connecting donepezil by a 1–2 methylene bridge with a substituted planar heterocyclic structure were designed, synthesized and bioevaluated as potential drugs for patients with AD. AChE inhibitory activity of obtained compounds, as it was anticipated, was lower in comparison to referenced drug, donepezil. The reason for this was the weak binding of the benzylpiperazine or N-benzylpiperidine portion with the CAS of AChE. Introduction of a heterocyclic planar group contributed to a moderate inhibitory activity against Aβ self-aggregation, while the inhibition of the Cu²⁺-induced Aβ aggregation was particularly enhanced for compounds with metal chelation capacity. Four compounds in the series, 23a-d, were able to protect SH-SY5Y cells from the Aβ-induced toxicity [141].



Chandrika et al. [142] described synthesis and biological tests of two series of bifunctional donepezil derivatives against AD. They contained 1,3- or 1,4-chalcone-donepezil hybrids. The second group possessed higher eeAChE and efBChE inhibitory activity than 1,3-chalcone-donepezil hybrids, with several exceptions. Some compounds showed better inhibition of bioA β ₄₂ oligomer assembly in comparison to donepezil, but any of them was able to dissociate preformed oligomers. Moreover, combination studies proved a covalent linkage between chalcones and donepezil is needed for the prevention of bioA β ₄₂ oligomerization.

Coumarin-donepezil hybrids were synthesized and their biochemical properties were tested as potent ChE and MAO-B inhibitors. Most of the compounds displayed potent inhibition of AChE, BChE and selective activity against MAO-B. The most active derivative in the series, 24, inhibited eeAChE, eqBChE, hAChE, hBChE and hMAO-B with IC₅₀ values of 0.87 μ M, 0.93 μ M, 1.37 μ M, 1.98 μ M and 2.62 μ M, respectively. Cytotoxicity investigation did not reveal toxic effect on SH-SY5Y cell line. Furthermore, molecular modeling studies proved it to be mixed-type inhibitor, able to bind to CAS, PAS and mid-gorge site of AChE, and it revealed a competitive mechanism of inhibition of MAO-B. In addition it was able to penetrate BBB in vitro [143].

Synthesis of 2-acetylphenol-donepezil hybrids is another approach in searching for multi-target-directed molecules against AD. reported by Zhu et al. [144]. They evaluated their inhibitory activity against AChE, BChE, MAO-A and MAO-B. Compound 25a revealed the highest inhibition of eeAChE with IC₅₀ value of 2.9 μ M and is able to bind to CAS and PAS of AChE. In addition, it was a selective Cu²⁺ chelator in a 1:1 ratio and can pass the BBB in vitro. The most active against MAO-B was derivative 25b with IC₅₀ value of 6.8 μ M., also with ability to cross the BBB. The structure-active-relationship indicated that the presence of O-alkylamine moiety contributed decrease potency of hMAO-B inhibition.

2.3. Galantamine analogues

The binding site of AChE is deep and narrow. PAS is located at the entrance of the binding gorge and takes part in an interaction with A β , forming as a result the amyloid plaques. That is why blocking of PAS is important for protecting from AChE-induced A β aggregation. Galantamine fits perfectly to the binding gorge of AChE, but unfortunately it is too short to fully fill it, so galantamine derivatives that can bind to both CAS and PAS are needed [145-149]. Stavrov et al. [150] synthesized a series of galantamine and camphane hybrids as dual-site binding AChE inhibitors. Camphane is a bulky fragment that can be located on the wide gorge entrance, what short galantamine is not able to fill. Some compounds in the series exhibited 191- and 369-times higher AChE inhibition than referenced drug galantamine. Moreover, they bind to PAS of AChE, do not show neurotoxic effect and can pass the BBB.

2.4. Rivastigmine analogues

Phosphodiesterases 9 (PDE9) are group of enzymes which can hydrolyze the intracellular second messenger cyclic guanosine monophosphate (cGMP). cGMP can regulate pleiotropic cellular functions in health and disease [151,152]. One of eleven isoforms of PDE9 is phosphodiesterase 9A (PDE9A). Its concentration is high in the cortex, basal ganglia, hippocampus, and cerebellum of brain. It has been proved that the inhibition of phosphodiesterases (PDEs) increase the performance of cognition functions in animals organisms. The inhibition of PDE9A is relevant for AD, because of restoring proteins associated with memory and attenuating neuronal injuries in hippocampal area by the reduction of amount of cGMP in brain to active NO/cGMP/PKG/CREB signaling, with simultaneous increase of brain-derived neurotrophic factor (BDNF) [151]. Yu et al. [153] as the first one designed, synthesized and evaluated biological activity of series of pyrazolopyrimidinone-rivastigmine hybrids to inhibit simultaneously PDE9A and BChE. Most of the analogues in the series proved to be inhibitors of both PDE9A and BChE. The most potent were compounds 26a and 26b and inhibited PDE9A with IC₅₀ values of 14 nM and 17 nM, respectively and BChE with IC₅₀ values of 3.3 μ M and 0.97 μ M, respectively. The results obtained for BChE proved these two derivatives to be stronger inhibitors than referenced rivastigmine. In addition, they were not toxic.

Also, a series of novel 4'-aminochalcone-rivastigmine hybrids were synthesized and their biological properties were evaluated as multitarget drugs against AD. In the whole series, the highest multifunctional activity exhibited compound 27. It inhibited AChE with mixed type of inhibition with IC₅₀ value of 4.91 μ M, binding both CAS and PAS. In addition, it was able to inhibit self-induced and Cu²⁺-induced A β ₁₋₄₂ aggregation by 89.5% and 79.7% at 25 μ M, respectively, MAO-B with IC₅₀ value of 0.29 μ M. The antioxidative properties of 27 were at the level of 2.83-fold of Trolox. Moreover, it could chelate metals and cross the BBB in vitro [154].

A novel series of chalcone and tacrine derivatives were reported by Wang et al. [155] for AD treatment. Most of the analogues in the series, exhibited selective hBChE inhibition. The most potent in the series was compound 28, which inhibited hAChE and hBChE with IC₅₀ values of 0.87 μ and 0.36 μ M, respectively, what was better or similar to results obtained for referenced rivastigmine. Due to multifunctional nature of AD, the series were designed as molecules with wide spectrum of biological activity. Compound 28 prevented as well from reactive oxygen species (ROS) in SH-SY5Y cell line, possessed low cytotoxicity and is in the required 10 druggability ranges from in silico ADMET prediction.

As it was mentioned above, a relevant decrease of amount of H₂S is observed in brains of patients with AD. Also, a series of rivastigmine connected with ulforaphane (SFN) and erucin (ERN) were synthesized as H₂S donors with antioxidant and neuroprotective activity. Compound 29 proved to be a new well-balanced anti-inflammatory and



antioxidant agent in BV-2 cells. Moreover, it could induce the expression of proteins (i.e. GSH) involved in the antioxidant defense in SH-SY5Y cell line, decrease ROS levels and NO-release in microglia BV-2 cells what was not observed for referenced drug, rivastigmine [156].

Another approach is synthesis and evaluation of AChE/BChE inhibitory potential, metal chelating activity and neuroprotection against hydrogen peroxide induced PC12 cell injury of novel scutellarein rivastigmine derivatives. The most interesting compound in the series was mixed-type inhibitor, compound 30 which inhibited AChE and BChE with IC_{50} values of 0.57 and 22.6 μ M, respectively and could bind to both CAS and PAS. It showed antioxidative activity at the level of 1.3-fold of Trolox. In addition, it was selective metal chelator and neuroprotector against mentioned above H_2O_2 -induced PC12 cell injury. Moreover it could pass the BBB *in vitro* and showed relevant neuroprotective effects in scopolamine-induced cognitive impairment in mice model [157].

Nesi et al. [158] combined a rivastigmine with natural antioxidant moieties such as gallic acid (GA), lipoic acid (LA) and 2-chromonecarboxylic acid (CCA) to get potential cholinesterase inhibitors with neuroprotective activity. LA derivatives 31a-c revealed the highest BChE inhibitory activity with IC_{50} values in the range of 340 – 378 nM and pleiotropic activities. Analogues 31d-f were able to protect from the self-mediated $A\beta$ aggregation with percentages of inhibition from 53% to 59%. Compounds 31e,f showed neuroprotective effect in HT22 cells against glutamate-induced neuronal death.

Rivastigmine-curcumin hybrids showed high AChE/BChE inhibitory activity with sub-micromolar IC_{50} values. The most active compound 32 was 20-times stronger (IC_{50} = 0.097 μ M) than referenced rivastigmine and prevented the $A\beta$ self-aggregation in TEM assay. In addition, the hydrolysate of 32 revealed potent ABTS⁺ scavenging and was able to chelate Cu^{2+} ions in moderate range [159].

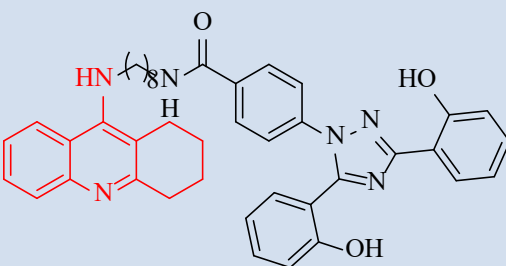
On the basis of rivastigmine and caffeic or ferulic acid, a novel group of compounds was designed, synthesized and

evaluated as MTDLs. The most interesting compound in the series was 33 and inhibited cholinesterase, prevented $A\beta$ self-aggregation, protected HT22 cells from glutamate and H_2O_2 induced cell death, scavenged free radicals and chelated Cu^{2+} ions [160].

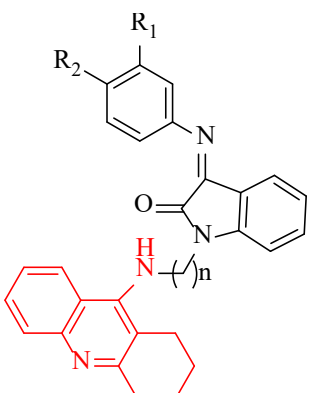
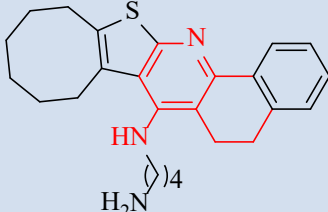
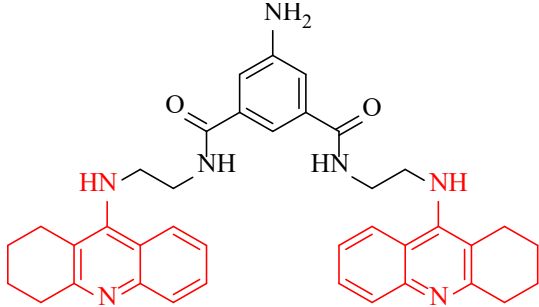
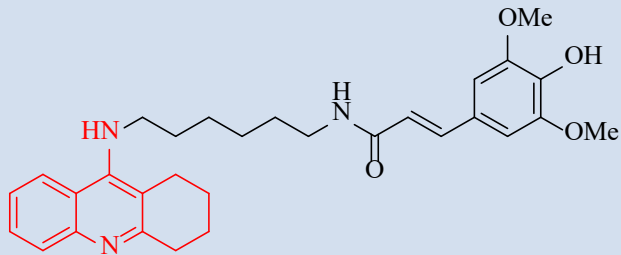
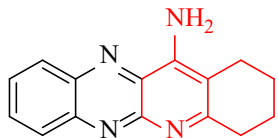
A reversible hAChE/hBChE inhibitor was an analogue of apigenin and rivastigmine, compound 34, which inhibited these enzymes with IC_{50} values of 6.8 mM and 16.1 mM, respectively. Moreover, it demonstrated remarkable antioxidant activity (ORAC = 1.3 eq), it exhibited neuroprotection of H_2O_2 -induced PC12 cells and $A\beta_{1-42}$ -induced SH-SY5Y cells, hepatoprotection of H_2O_2 -induced LO2 (normal human hepatocytes cell line) cells and it was selective metal chelator. It also inhibited Cu^{2+} -induced $A\beta_{1-42}$ aggregation (78.9%), hAChE-induced $A\beta_{1-40}$ aggregation (73.6%) and self-induced $A\beta_{1-42}$ aggregation (77.9%). It could also disaggregate Cu^{2+} -induced $A\beta_{1-42}$ aggregation (64.6%) and pass the BBB *in vitro*. Compound 34 was selected to *in vivo* studies and improved dyskinesia recovery ratio and response efficiency on $AlCl_3$ -mediated AD zebrafish, and showed remarkable neuroprotective activity against $A\beta_{1-40}$ -induced vascular injury. It also did not exhibit acute toxicity at dose up to 2000 mg/kg, and could improve scopolamine-induced memory impairment. The metabolism of 34 *in vitro* revealed that 4 metabolites in rat liver microsome metabolism, 2 metabolites in human liver microsome metabolism, and 4 metabolites in intestinal flora metabolism, which offered supports for the preclinical study of this derivative, what makes it promising MTDL against AD [161].

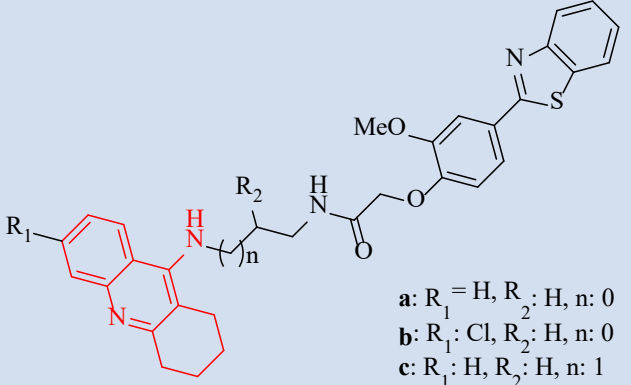
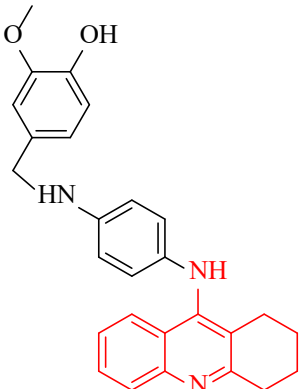
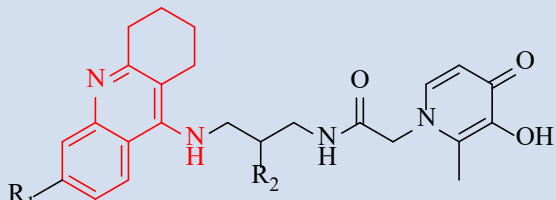
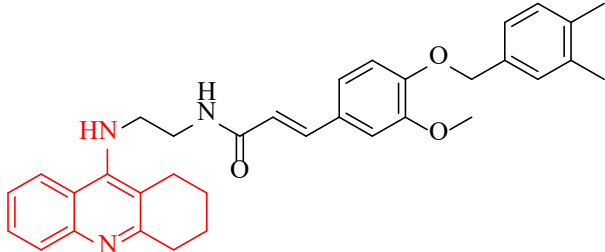
Krátký et al. [162] reported synthesis of series of 4-chlorophenyl N-substituted carbamates 5 from isocyanates and evaluated obtained compounds towards eeAChE/eqBChE inhibitory activity. They demonstrated not selective inhibition. Only some of them were more active than referenced drug, rivastigmine. Based on SAR analysis, it was found out neither the halogenation of the salicylanilide nor the presence of 2-phenylcarbamoyl moiety is required for the potent inhibition of AChE.

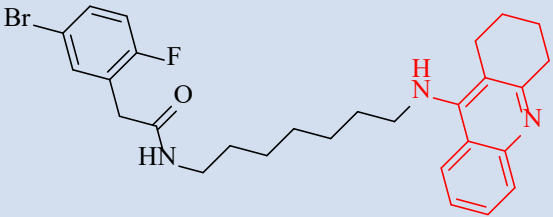
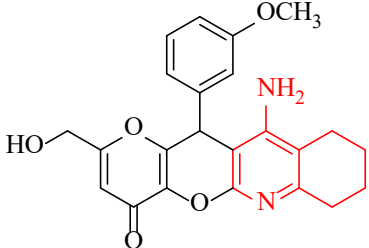
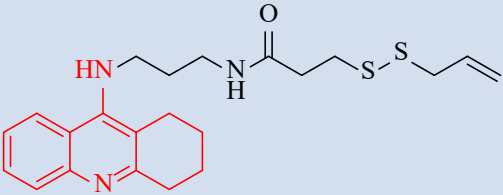
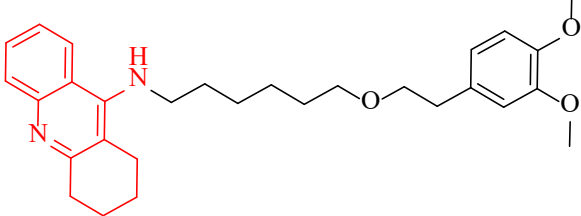
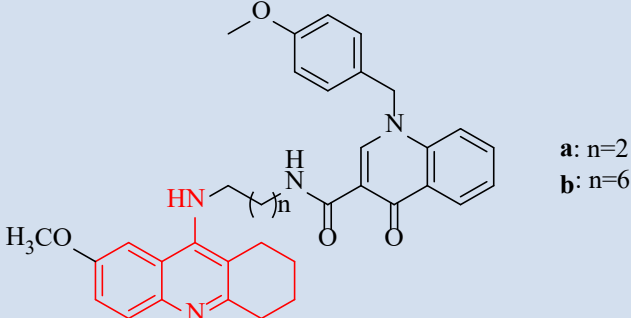
Table 1. Structures of tacrine, donepezil and rivastigmine derivatives and their biological activities.

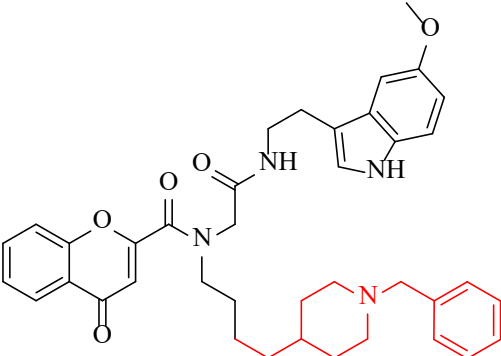
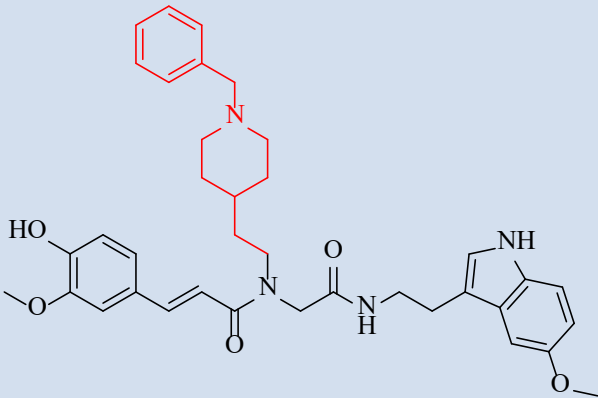
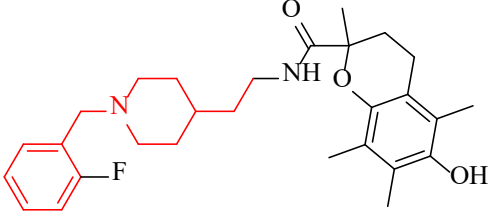
No.	Structure	Additional functions	Reference
1		Antioxidant activity, protective properties against the injury caused by H_2O_2	[81]

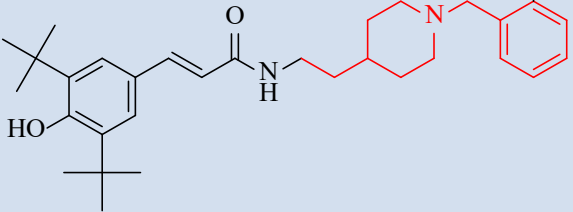
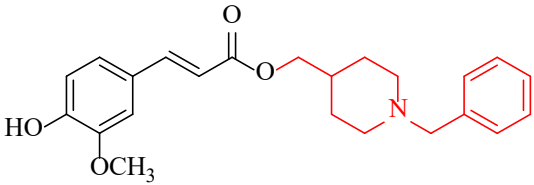
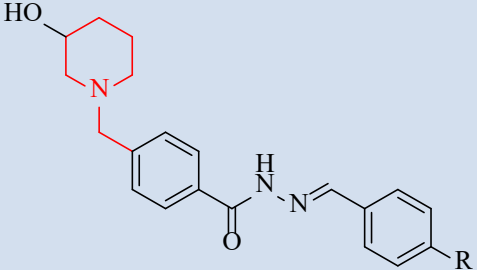
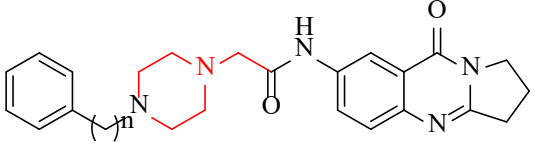


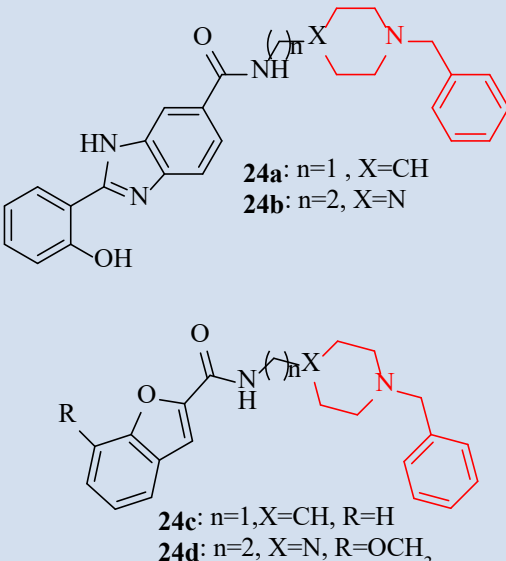
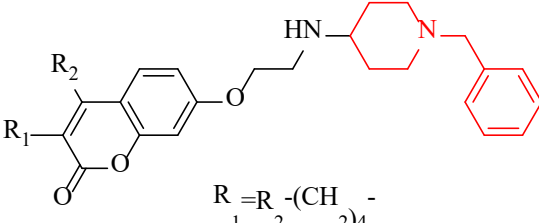
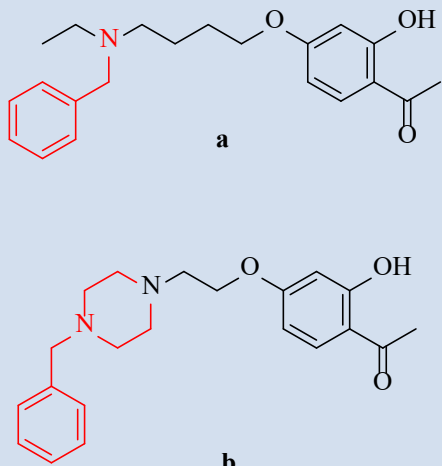
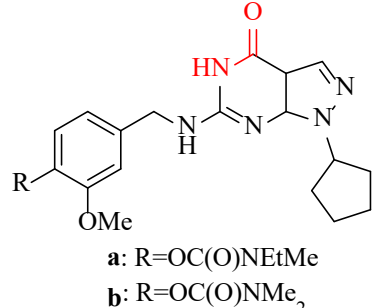
2	 <p> $\mathbf{a: n=6, R_1=Cl, R_2=H}$ $\mathbf{b: n=6, R_1=NO_2, R_2=H}$ $\mathbf{c: n=6, R_1=H, R_2=OCH_3}$ $\mathbf{d: n=5, R_1=CH_3, R_2=H}$ </p>	<p>2a-d: excellent metal chelating properties. 2a,b: inhibitory potency against AChE-induced Aβ aggregation</p>	[82]
3		Promising <i>in vivo</i> AChE inhibition profile	[83]
4		Inhibitory potency against PAS and AChE-induced A β aggregation, ability to induce S-phase post-treatment	[84]
5		Inhibitory potency against A β self-aggregation at level of 47% at 20 μ M, antioxidant properties towards PC12 cells from CoCl ₂ -damage	[85]
6		Neuroprotective activity towards SH-SY5Y cells against rotenone plus oligomycin-A or okadaic acid	[86]

7	 <p> a: R₁ = H, R₂ : H, n: 0 b: R₁ : Cl, R₂ : H, n: 0 c: R₁ : H, R₂ : H, n: 1 d: R₁ : Cl, R₂ : H, n: 1 e: R₁ : H, R₂ : OH, n: 1 f: R₁ : Cl, R₂ : OH, n: 1 </p>	7b,c,f: neuroprotective activity on SH-SY5Y cell line	[88]
8		Antioxidant properties, inhibition of Aβ ₁₋₄₂ aggregation, neuroprotection of SH-SY5Y in the presence of H ₂ O ₂	[89]
9	 <p> a: R₁=Cl, R₂=H b: R₁=H, R₂=OH </p>	Inhibition of self-/Cu-induced Aβ aggregation, radical scavenging capacity and metal chelating properties (Fe, Cu, Zn), neuroprotection in SH-SY5Y cells treated with Aβ ₁₋₄₂ ascorbate/iron stressors	[96]
10		Inhibition of Aβ self-aggregation amelioration, the impairment of memory in mice models treated with scopolamine in the Morris water maze test	[97]

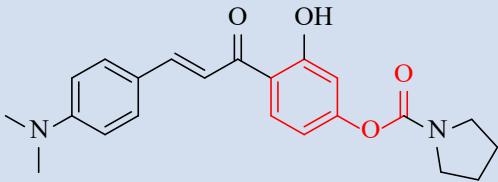
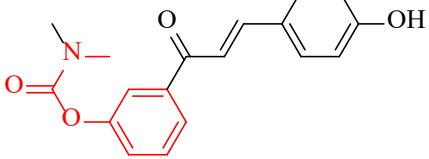
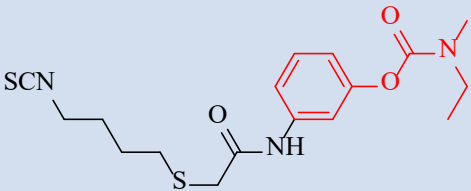
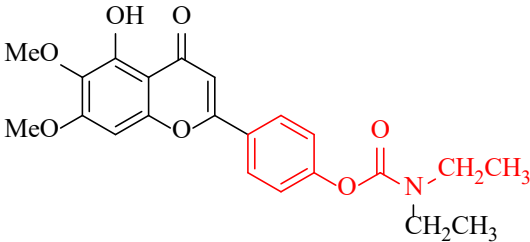
11		Inhibition of A β aggregation	[100]
12		Antioxidant activity and neuroprotection against A β ₁₋₄₀ in SH-SY5Y cells	[101]
13		Improvement of cognitive and locomotor activity in mouse model with AD in the step-through test and open field test, increase of amount of H ₂ S in hippocampus, decrease mRNA expression of the proinflammatory cytokines, TNF- α , IL-6, and IL-1 β and increase synapse-associated in the hippocampus of tested mice	[108]
14		Inhibition of A β ₄₂ self-aggregation, neuroprotection in rats neurons in a serum and K ⁺ deprivation model	[111]
15	 <p>a: n=2 b: n=6</p>	Ability to cross the BBB <i>in vitro</i>	[120]

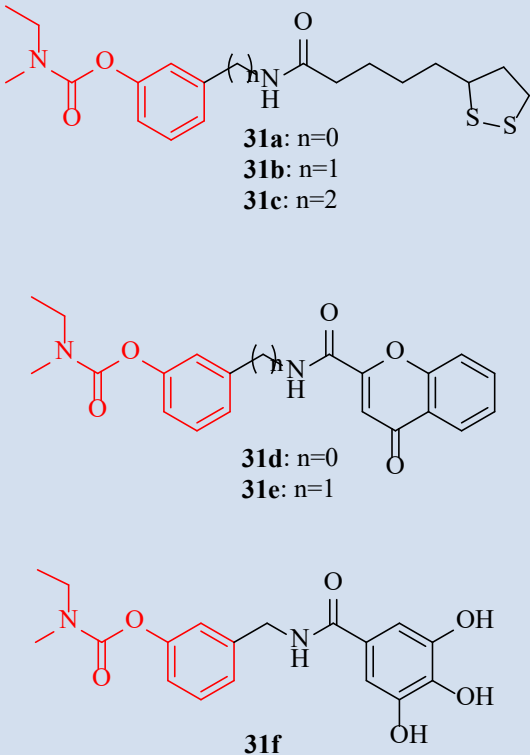
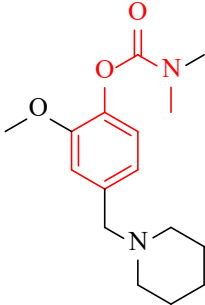
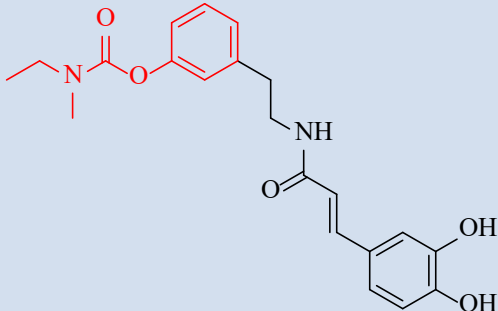
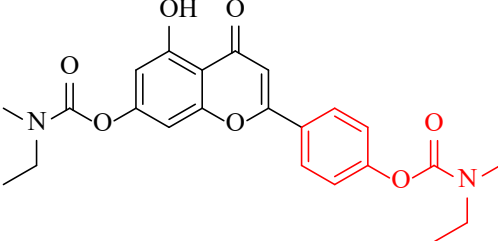
16		Inhibition of hMAO A, and MAO-B, strong antioxidant properties	[127]
17		Strong antioxidant effect with the oxygen radical absorbance capacity	[129]
18		Inhibition of MAO-B and A β_{1-42} aggregation, antioxidant effect, ability to chelat Cu ²⁺ ions, to protect PC12 cells from oxidative stress caused by H ₂ O ₂ , rotenone, and oligomycin-A , to protect BV-2 cells from LPS-Stimulated Inflammation, improvement of cognition and spatial memory against scopolamine-induced acute memory deficit, D-galactose (D-gal) and AlCl ₃ induced chronic oxidative stress in mice model without acute toxicity and hepatotoxicity, ability to cross the BBB <i>in vitro</i>	[130]

19		<p>Inhibitory activity against hMAO-B, self-induced and hAChE-induced Aβ aggregation, antioxidant properties, the neuroprotective effects on PC12 cells treated with H₂O₂ and against LPS-stimulated inflammation on BV-2 cells, ability to cross the BBB <i>in vitro</i>, good liver metabolic stability in RLM, dose-dependently reversed scopolamine-induced memory deficit in mice model but without acute toxicity</p>	[131]
20		<p>Anti-inflammatory properties <i>in vivo</i> in mice model, antioxidant activity in SH-SY5Y cell line and ability to chelate Cu²⁺ and Fe²⁺ ions, protection of SH-SY5Y cells against the late neuronal death caused by Aβ₁₋₄₂ oligomers</p>	[132]
21	 <p>a: R=Cl b: R=F</p>	<p>ADME profile <i>in silico</i>, anti-inflammatory and neuroprotective activity against AβO-induced neuroinflammation and neurodegeneration <i>in vitro</i> in THP-1 cells and <i>in vivo</i> in mice model</p>	[135]
22	 <p>a: n=4 b: n=2</p>	<p>Inhibitory activity against BACE1 and Aβ₁₋₄₂ aggregation, neuroprotective properties against Aβ₁₋₄₂-induced damage in SH-SY5Y cells</p>	[139]

23	 <p> 24a: n=1, X=CH 24b: n=2, X=N 24c: n=1, X=CH, R=H 24d: n=2, X=N, R=OCH₃ </p>	Inhibitory activity against A β self-aggregation and Cu ²⁺ -induced A β aggregation, ability to protect SH-SY5Y cells from the A β -induced toxicity	[141]
24	 <p> $R_1 = R_2 = -(\text{CH}_2)_n-$ </p>	Inhibitory potency against MAO-B, ability to cross the BBB <i>in vitro</i>	[143]
25	 <p>a</p> <p>b</p>	Selective Cu ²⁺ chelator in a 1:1 ratio, ability to cross the BBB <i>in vitro</i> , activity against MAO-B	[144]
26	 <p> a: R=OC(O)NEtMe b: R=OC(O)NMe₂ </p>	Inhibition of PDE9A	[153]



27		Ability to inhibit self-induced and Cu ²⁺ -induced Aβ ₁₋₄₂ aggregation, inhibitory activity against MAO-B, antioxidative properties, ability to chelat metals and to cross the BBB <i>in vitro</i>	[154]
28		Prevention from ROS in SH-SY5Y cell line	[155]
29		Anti-inflammatory and antioxidant activity in BV-2 cells, the induction of proteins expression (i.e. GSH) involved in the antioxidant defense in SH-SY5Y cell line, decrease of ROS levels and NO-release in BV-2 cells	[156]
30		Antioxidative activity, selective metal chelator and neuroprotector against H ₂ O ₂ -induced PC12 cell injury, ability to pass the BBB <i>in vitro</i> , relevant neuroprotective effects in scopolamine-induced cognitive impairment in mice model	[157]

31	 <p>31a: n=0 31b: n=1 31c: n=2</p> <p>31d: n=0 31e: n=1</p> <p>31f</p>	<p>31a-c: pleiotropic activities</p> <p>31d-f: protection from the self-mediated Aβ aggregation,</p> <p>31e,f: neuroprotective effect in HT22 cells against glutamate-induced neuronal death</p>	[158]
32		Prevention of A β self-aggregation in TEM observation, ABTS ⁺ scavenging, and ability to chelate Cu ²⁺ ions	[159]
33		Prevention of A β self-aggregation, protection of HT22 cells from glutamate and H ₂ O ₂ induced cell death, scavenging free radicals and Cu ²⁺ ions chelation	[160]
34		Antioxidant activity, neuroprotection of H ₂ O ₂ -induced PC12 cells and A β ₁₋₄₂ -induced SH-SY5Y cells, hepatoprotection of H ₂ O ₂ -induced LO2 cells, selective metal chelator,	[161]



		activity against Cu ²⁺ /hAChE/self-induced Aβ ₁₋₄₂ aggregation, disaggregation of Cu ²⁺ -induced Aβ ₁₋₄₂ aggregation, ability to pass the BBB <i>in vitro</i>	
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CONCLUSION

Despite extensive knowledge of AD, the etiology and cause of it is still not clear, what has an impact on the lack of the effective therapy which could stop the progression of disease. Many years of work on obtaining an active, multifunctional and free of side effects substance that could treat AD has resulted in the publication of numerous scientific papers that are a source of valuable knowledge for the world of science.

Herein, we have supplied the previous review article with the latest tacrine analogues from 2017, as well as with donepezil and galantamine from 2015. Moreover, we have reviewed rivastigmine derivatives from last six years. We clearly described tacrine conjugates with e.g. deferasirox, pyridinium, thienopyridine, phenolic acid, vanillina, deferiprone or also indole-3-acetic acid. Many of them exhibited promising additional therapeutic properties, like protection against Aβ-aggregation, oxidative stress or ability to chelate metal ions. Described donepezil hybrids include e.g. multtarget donepezil-ferulic acid agents or connection with natural moiety such as melatonin with chromone, which showed activity against hAChE, hBChE, hMAO-A, MAO-B. Among galantamine derivatives must be mentioned compounds that can bind to both CAS and PAS. The binding site of AChE is deep and narrow and galantamine is too short to fully fill the binding gorge of AChE, so its analogues must be able to bind to both CAS and PAS. In the literature one can find alantamine and camphane hybrids as dual-site binding AChE inhibitors. Among the rivastigmine hybrids we described were connections with e.g. pyrazolopyrimidinone, 4'-aminochalcone or with natural antioxidant moieties such as GA, LA and CCA to get potential cholinesterase inhibitors with neuroprotective activity.

Conclusively, the results reported by many research groups, mentioned by us in this work, support a role of tacrine, donepezil, galantamine and rivastigmine as chemical scaffolds for further structural modification and the design of new drugs for AD treatment. Due to the multifactorial nature of AD, multi-target compounds are of greatest importance at this point. Current science focuses not only on the inhibition of cholinesterase, but also on other factors responsible for the pathogenesis and symptoms of AD. It is desirable that the proposed substances among others could inhibit Aβ aggregation, would have neuroprotective and antioxidant properties and would be able to chelate metal ions.

As the most interesting anti-AD candidates, that target most of the therapeutic strategies showed in the Fig. 2, we type compounds 2, 4 and 7 among tacrine analogs, 18, 19, 22a and

22b among donepezil derivatives and 27, 30, 31a-c among rivastigmine hybrids. They revealed their multifunctional nature and an excellent cholinesterase inhibitory activity, what is required for potential anti-AD agents. The compounds mentioned above are worth expanded research and could lead to a strong cholinesterase inhibitor with interesting biological properties in the future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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