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Therapeutic Potential of Multifunctional Tacrine Analogues

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Abstract: Tacrine is a potent inhibitor of cholinesterases (acetylcholinesterase and butyrylcho-linesterase) that shows limiting clinical application by liver toxicity. In spite of this, analogues of tacrine are considered as a model inhibitor of cholinesterases in the therapy of Alzheimer's disease. The interest in these compounds is mainly related to a high variety of their structure and biological properties. In the present review, we have described the role of cholinergic transmission and treat-ment strategies in Alzheimer's disease as well as the synthesis and biological activity of several recently developed classes of multifunctional tacrine analogues and hybrids, which consist of a new paradigm to treat Alzheimer's disease. We have also reported potential of these analogues in the treatment of Alzheimer's diseases in various experimental systems.

Keywords: Multifunctional tacrine analogues, tacrine hybrids, Alzheimer's disease, cholinergic transmission, neuroprotective activity, hepatoprotection.

1. INTRODUCTION

Alzheimer disease (AD) is irreversible and progressive neurodegenerative disorder, including mainly the elderly people. The most important risk factor for AD is age. It is estimated that the number of patients around the world will have tripled in the middle of this century [1]. AD is becoming an increasing challenge for both the health care system and society because the number of elderly people is still growing. Therefore, it is an urgent need for the development of new more effective drugs to be used in the treatment of AD. Although the knowledge on etiology, genetics and pathophysiological mechanism of AD has been extended, we still have not found effective treatment [2]. At the initial stages of the AD, there is growing deterioration of cognitive functions including memory, reasoning, speech, computational skills, praxis and information processing as well as executive function such as ability to plan, self-control, sequencing and monitoring of complex behaviours [3]. The current pharmacological therapy for AD based on the cholinergic hypothesis. With the exception of memantine, a partial antagonist of *N*-methyl-D-aspartate (NMDA) receptor, all drugs that are used today based on increasing the cholinergic transmission by inhibiting cholinesterases. Donepezil **1** (1996 year) [4] and galantamine **2** (2001 year) [5] are selective inhibitors of acetylcholinesterase (AChE), while rivastigmine **3** (2000 year) also inhibits butyrylcholinesterase (BChE) [6] (Fig. **1**). Unfortunately, inhibitors of cholinesterases are not able to stop the development of AD and can only improve cognitive function. Accordingly, it is highly desirable to develop new effective therapeutic strategies to stop or slow progression of AD [7]. Because of AD complexity, the cure paradigm is now shifted to the designing of a single chemical compounds having multiple biological activities and termed as "multifunctional drugs" [8]. The "multifunctionality" is the result of the interaction of chemical substances with various molecular targets. In case of traditional drugs, coformulation of two or more drugs in a single dosage, due to varied metabolism among the patients, can produce highly complex pharmacokinetics as well as pharmacodynamic relationships [9]. Moreover, drug-drug interactions could lead to severe side effects [10]. The advantages of multifunctional compounds including additive or synergistic therapeutic responses, prolonged duration of effectiveness, improved drugable characteristics and more predictable pharmacokinetics and pharmacodynamics relationships [11].

The present review describes the role of cholinergic transmission in Alzheimer's disease and current therapeutic strategies as well as sums up the synthesis of multifunctional tacrine derivatives, which despite inhibitory activity on AChE and BChE, showing additional function according to the current therapeutic approaches.

2. CHOLINERGIC TRANSMISSION AND ALZHEIMER'S DISEASE

The cholinergic system is one of the most important neurotransmitter systems in the brain. The major cholinergic innervation including the limbic system, which is associated with emotions, learning and memory as well as vegetative and survival behaviours [12-14]. Limbic system is anatomi-

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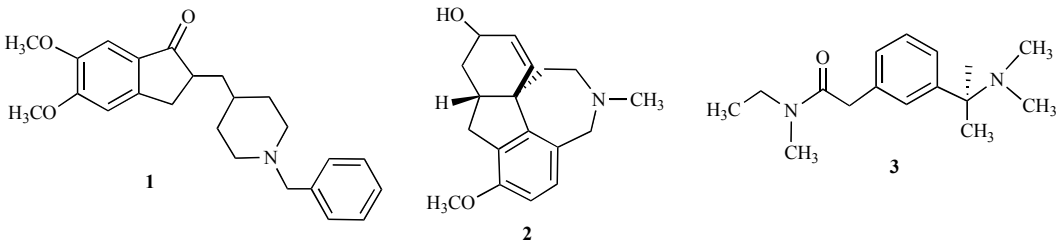


Fig. (1). Structures of drugs that are used in pharmacological therapy for AD: donepezil 1, galantamine 2 and rivastigmine 3.

cally composed of amygdala, anterior thalamus, hypothalamus, mammillary bodies, basal forebrain, hippocampus, orbitofrontal and parahippocampal cortices, septal area and cingulate [15]. The prime cerebral cortex does not contain intrinsic cholinergic neurons [16] and the majority of its cholinergic innervations receive from the basal forebrain (Fig. **2**) [14]. The nucleus basalis of Meynert is the major source of cholinergic inputs of the cerebral cortex as well as amygdala [17]. The cholinergic projections to hippocampus come from the medial septal nucleus and the vertical limb of the diagonal band of Broca. 90% of brainstem projections to the thalamus are cholinergic and they are considered to be essential for controlling selective attention [14]. In the stratium, the cholinergic innervations are predominantly intrinsic and come from ChAT (choline acetyltransferase)-positive striatum interneurons [18].

Acetylcholine acts throughout two classes of receptors: inotropic nicotinc receptors (nAChRs) and metabotropic muscarinic receptors (mAChRs) [20-21]. The muscarinic receptors are widely expressed in the central nervous system (CNS), and they are divided into two further groups. M1, M2 and M3 receptors are coupled to G_q proteins that activate phospholipase C and result in mobilization of intracellular Ca^{2+}, whereas M2 and M4 receptors are negatively coupled to adenylate cyclase *via* $G_{i/o}$ proteins and act to reduce cAMP levels [21]. Furthermore, mAChRs are located post- and presynaptically, which is related to complex functions in the brain. M2/M4 receptors can reduce glutamate release from corticostriatal and corticocortical synapses [22] or act as inhibitory autoreceptors on cholinergic terminals [23-24], whereas M1/M5 postsynaptic receptors increase excitability of cortical pyramidal neurons [25]. Additionally, M2 receptor acts as an inhibitory modulator on dopaminergic terminals and deficiency of M2 mAChR in mouse causes reduction in muscarinic-dependent antinociceptive responses [26]. Mice without M3 receptor show hypophagic and lean, suggesting its role in regulating food intake [27]. It is noteworthy that M5 mAChR-knockout mice are less sensitive to addictive drugs such as cocaine or morphine, so antagonists of this receptor could be candidates for the treatment of drug addiction [28].

Nicotinic receptors are nonselective, excitatory cation channels [20]. They consist of a large family of α- and β-subunits (α2-α7/ β2-β4) which forming a huge number of homomeric and heteromeric assemblies [29]. All nAChR subtypes display high penetrability to K^+ and Na^+ ions, whereas permeability to Ca^{+2} ions is diversified for different nicotinic receptors. The heteromeric α4β2 and homomeric α7 receptors are the most common expressed nAChR subtypes in the CNS [30]. Interestingly, of the 11 nAChR subtypes found in the brain, 9 have been detected in hippocampal CA1 neurons [31], while α7 subtype is the most abundant [31] and have been associated with memory formation[32]. However, the α4β2 receptors, despite their lower expression in the hippocampus, are also considered to play a significant role in memory process [33]. In addition, stimulation of nicotinic receptors can modulation of many different neurotransmitters release: glutamate, dopamine, GABA, norepinephrine or serotonin [34]. This process is often subtype-specific [35].

In 1976 has been reported by two independent teams that Alzheimer disease was associated with loss of cholinergic markers in the central cortex [36-37]. The cholinergic hypothesis of AD has been established by the later discovery of the loss of cholinergic cells in the septal nuclei and basal forebrain of patients with advanced AD [38]. The cholinergic hypofunction is currently considered to be linked to β-amyloid (Aβ) and tau pathologies [39]. Interestingly, the striatum cholinergic interneurons and cholinergic innervation of the thalamus (Fig. **2**) seem to be unharmed in AD patients [40]. The deficit of cholinergic system leads to many different cognitive symptoms including memory loss [41] and inhibitors of cholinesterases (acetylcholinesterase [AChE] and butyrylcholinesterase [BChE]) have shown some activity which prevents this symptoms [42]. The BChE is present in neurofibrillary tangles as well as senile plaques and is a non-specific enzyme playing an important detoxifying function through hydrolyses many different esters including xenobiotics *e.g.* cocaine, acetylsalicylic acid (aspirin) or heroin [43]. In the central nervous system, BChE is mainly linked with glial cells but also can exist in neurons. BChE regulates extracellular acetylcholine concentration, whereas AChE plays a major role in cholinergic transmission and is located in pre- and postsynaptic membranes. With the death of cholinergic neurons, the activity of AChE rapidly decline and this can lead to the increasing role of BChE in the regulation of cholinergic transmission [44]. Thus, BChE can constitute promising drug target in AD treatment. Besides, the involvement of M1 receptor in AD has been well known [45]. β-amyloid precursor protein (APP) (Fig. **3**) is an integral membrane protein cleaved by β-secretase (BACE1) and then by γ-secretase to generate βAPPs (the large secreted derivative and APP intracellular domain) as well as β-amyloid. Alternatively, α-secretase cleaves APP within the Aβ domain, which prevents Aβ production and generates αAPPs that have been exhibited neuroprotection activity [46-47]. Stimulation of M1 mAChR by agonist has reduced Aβ production and en-

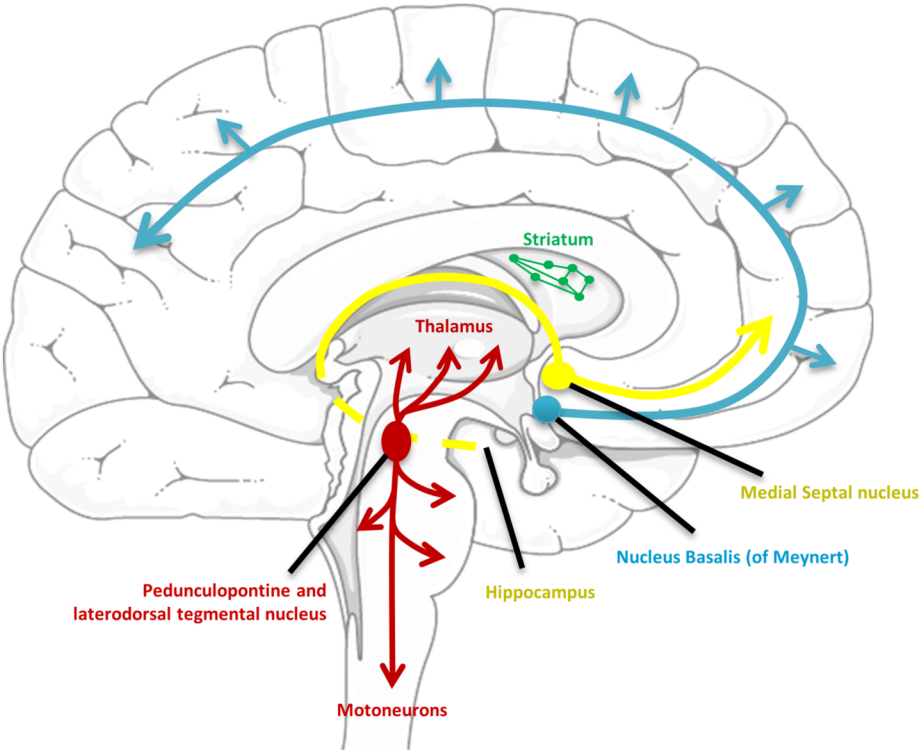


Fig. (2). Cholinergic innervation in the human brain. The figure shows major pathways: basal-forebrain cholinergic neurons, including the nucleus basalis (blue) and medial septal nucleus (yellow), and pedunculopontinelateral dorsal tegmental neurons (red). Green colour represents striatal interneurons (Perry *et al*., 1999; Pinto *et al*., 2011). Elements of this illustration were provided by Servier Medical Art (http://smart.servier.com/).

hanced αAPPs generation, by protein kinase C(PKC) activation [48-51]. PKC promote the activity of α-secretase and the trafficking APP to the cell membrane [52]. Some studies indicate ERK1/2 cascade activation as a result of M1 receptor stimulation, which could also modulate α-secretase activity [53]. In addition, M1 mAChR activation counteracts Aβ neurotoxicity (induces by disrupt Wnt pathway), inactivates GSK-3β, stabilizes β-catenin and induces the expression cyclin-D1 for neuron survival as well as Wnt-targeting genes engrailed [54]. Moreover, stimulation of M1 receptor has been found to protect human neuroblastoma cells against apoptotic factors, including DNA damage, oxidative stress, mitochondrial impairment and caspase activation [55]. It has been determined that β-amyloid induces the uncoupling of M1 receptor from G-protein, suppressing its function under pathological conditions of AD [56]. Fig. **2** sums up the M1 mAChR in Alzheimer disease. Despite the muscarinic acetylcholine receptor M1 another receptor from acetylcholine signals: α7nAChR offers therapeutic benefits in AD by its anti-inflammatory effects as well as promoting neuronal survival and synaptic plasticity [57].

3. TREATMENT STRATEGIES IN ALZHEIMER'S DISEASE

Unfortunately, current pharmacological therapy including cholinesterases inhibitors, do not represent a cure, because they do not arrest the dementia progression. Cholinesterases inhibitors are decreasing cholinesterase activity, resulting in higher acetylcholine level and brain function improved [59]. It is therefore not surprising, that a lot of novel strategies are being developed, however, neither of them have been improved to AD treatment [60]. In this review has been described selected treatment approaches, including strategies are being used to design multifunctional AD drugs (Fig. **4**).

Tau protein stabilizes the microtubules for proper functioning of neurons [61]. The hyperphosphorylation of tau, which is stimulated by amyloid peptide's aggregation, resulting in destabilization of the cytoskeleton and cells degeneration [61]. Furthermore, helically twisted filaments of hyperphosphorylated tau aggregate forming neurofibrillary tangles (NFTs), important pathogenetic factor of AD [62]. Tideglusib is irreversible GSK-3β inhibitor (enzyme involved in tau phosphorylation Fig. **3**), and has been shown reduce spatial memory deficits in preclinical studies [63]. Moreover, it has been determined that lithium and valproate have also inhibitory action on GSK3 and reduce tau pathology [64]. Some drugs with strong affinity for tau protein, like astemizole or lansoprazole, are able to indirectly reduce tau-tau interaction [65]. Another approach, connected with tau protein, focus on the heat shock protein 90 (Hsp 90), which is well documented to play a role in preventing tau degradation [66]. Curcumin has known to inhibit Hsp 90 [67]. In tau transgenic mice treated by curcumin has been observed decreases the tau pathology [68]. Interestingly, microtubule stabilizer paclitaxel has been determined to improve microtubule density, fast axonal transport as well as motor function in the experimental model of AD [69].

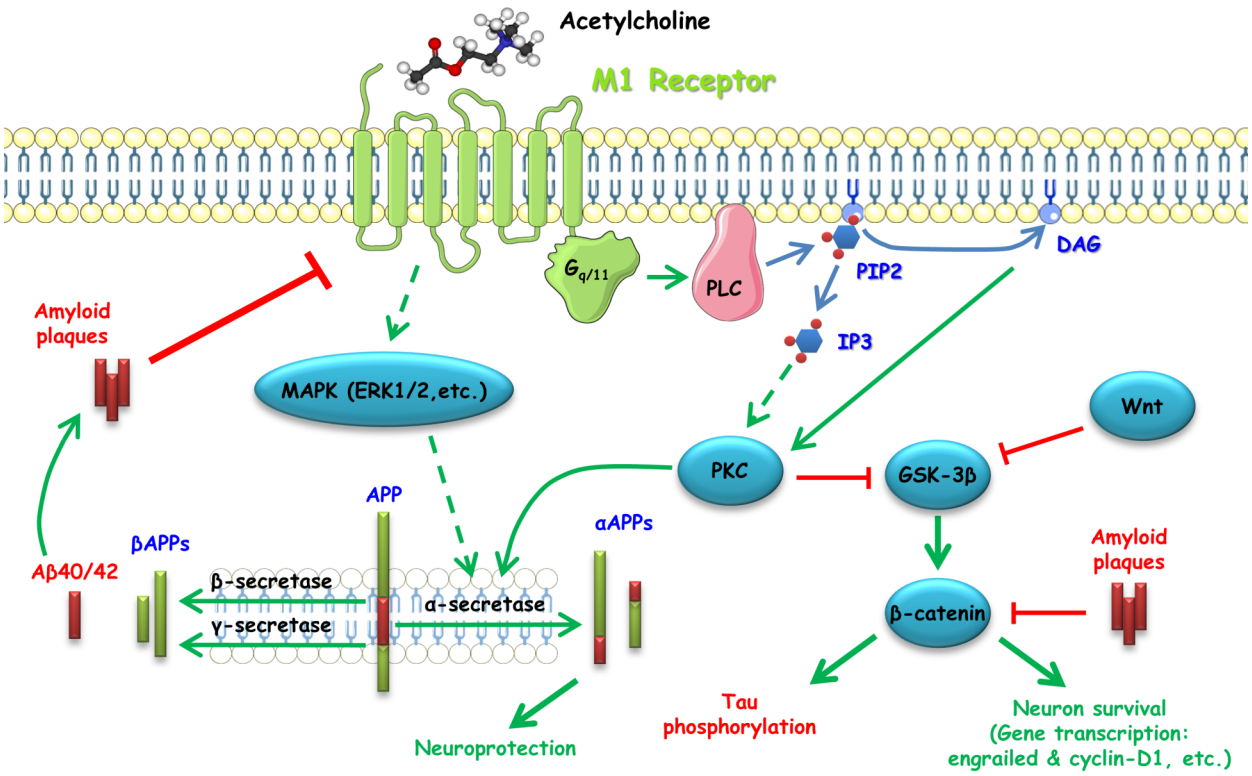


Fig. (3). The involvement of M1 receptor in AD. PLC- phospholipase C; PIP2- Phosphatidylinositol 4,5-bisphosphate; DAG- Diacylglicerole; IP3- Inositol trisphosphate; According with (Jiang *et al.*, 2014; Fisher, 2001). Elements of this illustration were provided by Servier Medical Art (http://smart.servier.com/).

The production of Aβ is modulated by three APP processing enzymes: α-, β-, and γ-secretase. This process has been described above in section 2 (Fig. **3**). The inhibition of β-secretase improved memory deficits and prevented cholinergic dysfunction in APP transgenic mouse model [70]. Some of β-secretase inhibitors have been approved to clinical trials [60], however long-term inhibition could led to potential side effects like hypomyelination and behavioural abnormalities [71-72]. In case of γ-secretase inhibitors, many side effects have also been noticed due to the involvement of γ-secretase in a number of physiological processes. The most notable substrate is Notch1, which regulates genes involved in the cell survival and development [73]. Consequently, γ-secretase inhibitors could led to many Notch-related adverse effects such as gastrointestinal and haematological toxicity, skin reaction and hair color change [74-77]. Second generation γ-secretase inhibitors, known as γ-secretase modulators, have been design in order to avoid influence on Notch protein and improve their safety profile [78]. Another target interfering with Aβ production is α-secretase. Its activation has been reported to be an effective approach for the reduction of β-amyloid production [79]. Agonists of muscarinic (Fig. **3**) as well as glutamate and serotonin receptors have been involved in α-secretase activity [45, 58].

Amyloid-β aggregates are exhibited high stability and resistance to disassociation and their process of aggregation and folding is still unclear [80]. Several small molecules, called anti-aggregate compounds, are able to bind to monomeric Aβ and preventing its oligomerization. Many of them are in various stages of clinical trials, including homotaurine, clioquinol and scyllo-inositol [81]. Interestingly, it has been determined that chelation of metal ions like Cu^{2+} and Zn^{2+}, involved in amyloid-β aggregation, prevents Aβ deposition [82]. Cu^{2+}/Zn^{2+} chelator, PBT2, and has reached the clinical trial stage [83]. It is noteworthy that a few cell surface receptor disturbs the formation of Aβ plaques and one of them is the receptor for advanced glycation end products (RAGE) [84].

It is generally accepted that deposition of β-amyloid increases nitro-oxidative stress in neurons and induces apoptotic cell death [85-86]. Moreover, Zn^{2+}, Cu^{2+}, Fe^{2+} and Fe^{3+} ions are contributing to nitro-oxidative stress [85-86]. Numerous studies are clearly shown elevated level of zinc in brain tissue (including the structure of the hippocampus and cerebral cortex) and copper ions in the blood of AD patients [85-86]. Additionally, despite the cholinergic transmission, disturbances in glutamate neurotransmission are also linked with the pathophysiological processes underlying AD [87]. Chronic activation of NMDA receptors by an increased level of glutamate ultimately leads to receptor-mediated excitotoxicity, which is connected with pathological influx of Ca^{2+} ions into postsynaptic neurons [87]. Consequently, memantine (an uncompetitive NMDA receptor antagonists) has been approved in the current pharmacological therapy [88]. Furthermore, the nitric oxide (NO) has been documented to protect the neurons from excitotoxicity through influence on NMDA receptor and caspase inhibition [89]. Therefore, modulators of NO biosynthesis consist promising pharma-

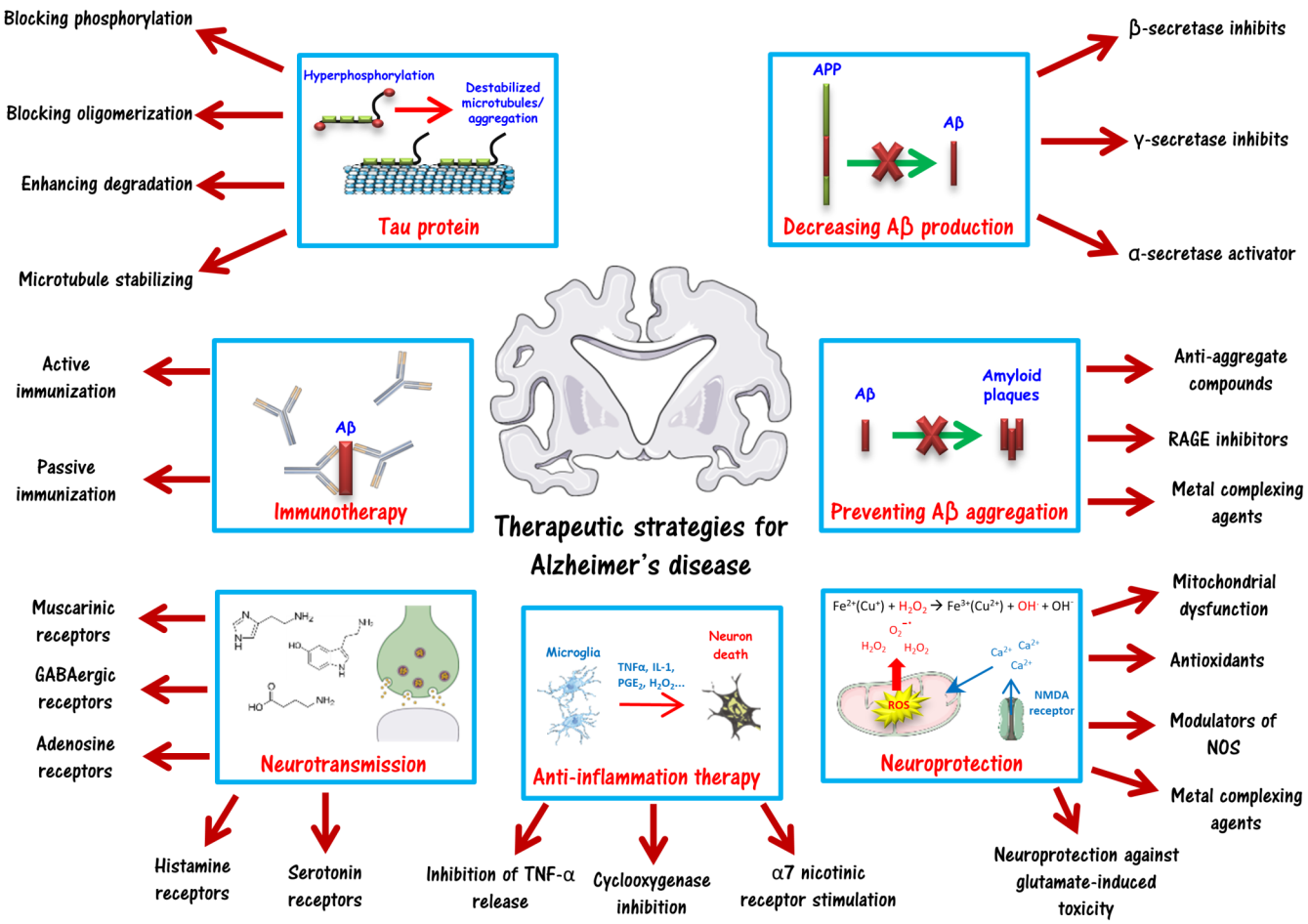


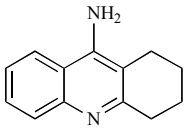
Fig. (4). The Experimental therapeutic strategies for Alzheimer's disease. Elements of this illustration were provided by Servier Medical Art (http://smart.servier.com/).

cotherapeutic strategies. Glutamate exerts also its toxic effects through non-receptor-mediated oxidative toxicity [90]. Moreover, Selkoe [91] indicated synaptic dysfunction in early disease leading to the loss of communication within neural circuits important for cognitive functions.

Besides, current investigations indicated a crucial role of mitochondrial dysfunction in the early pathology of AD as a trigger of this process [92-96]. Monteiro-Cardoso *et al.* [97] determined, by used mice model, that cardiolipin profile changes and increase of diacyl- and lyso-phosphatidylcholine lipids are associated with the early synaptic mitochondrial dysfunction in AD. A significant increase in oxidative damage in mtDNA and cytochrome oxidase has been also demonstrated in AD model [98-99]. Interestingly, APP caused mitochondrial dysfunction and impaired energy metabolism in a Tg mouse model of AD through the accumulation of full-length APP in the mitochondrial compartment in a membrane-arrested form [100]. Furthermore, it has been shown that APP accumulates exclusively in the protein import channels of mitochondria, resulting in inhibition of nuclear-encoded cytochrome oxidase's subunits entrance, which decreases its activity and increases H_2O_2 levels [101].

It has been described that some natural antioxidants as well synthetic compounds provide protection in AD [102]. Combining vitamin E with memantine has been improved in clinical trials [103]. It has been reported that CoQ10 exhibits potential neuroprotective effects through suppression of ROS production and stabilization of mitochondrial function [104]. Peptide, SS-31, is also a mitochondrial targeted ROS scavenger therapy [105].

Despite cholinergic and glutamatergic transmission also others neurotransmissions consist potential target for AD treatment. Areas of CNS related to learning and memory show the high level of serotonin receptors: 5-HT_{1A}, 5-HT_4, 5-HT_6 and 5-HT_7 [106]. As blockade of 5-HT_6 receptor cause increase acetylcholine release into the synaptic cleft, some 5-HT_6 antagonists have been improved to clinical studies and shown positive results [107, 108]. However, both agonist and antagonist of may improve memory and learning in animal model [109]. A number 5-HT_4 agonistic substances have also exhibited cognitive benefits [110]. Furthermore, brain regions concerned with cognitive functions show high concentration of histamine receptor, H_3 and its inhibition, as with 5-HT_6 receptor, enhances the release of acetylcholine as well as GABA, dopamine and noradrenaline [111]. Novel H_3 antagonists have reached phase II trial [112]. In case of GABAergic transmission, $GABA_B$ antagonist $GABA_A$ modulator have shown promising results [113].



4

Fig. (5). Structure of tacrine 4.

One of the most promising approaches is β-amyloid-directed immunotherapy, including passive immunization (injection of pre-prepared antibodies) and active immunization (the immune system is stimulated to produce its own antibodies). The first option is currently the most widely developed approach [114]. However, many side effects, caused by the first humanized monoclonal antibody directed against Aβ, contributed to its clinical trials terminated [115].

Unfortunately, most of the compounds from all experimental therapeutic strategies with positive phase II trials, have not succeeded in phase III due to lack of therapeutic efficacy and/or serious adverse effects. A lack of in-depth understanding of AD pathomechanisms result of a huge number of experimental strategies which, in addition to those mentioned above, including anti-inflammation therapy, targeting intracellular signalling cascades, cholesterol-lowering drugs, gonadotropin hormones, neurogenesis, epigenesis, caspase inhibitors, inhibition of calcium channels and glucagon-like peptide-1 (GLP-1) receptor agonist [60, 62, 116].

4. TACRINE

One of the first drugs which has been approved for the Alzheimer's disease (AD) treatment was tacrine (9-amino-1,2,3,4-tetrahydroacridine) **4** (Fig. **5**) [117]. Tacrine (TAC) is derivative of acridine, which in the 1993 year was approved by the U.S. FDA in the hydrochloride form as a drug for use in Alzheimer's disease as acetylcholinesterase as well as butyrylcholinesterase inhibitor [117]. Unfortunately, some side effect, including hepatotoxicity and poor bioavailability after oral administration, led to its withdrawal from clinical [118, 119]. Moreover, Melo *et al*. [120] described tacrine-induced disruption of mitochondrial function and bioenergetics in rat brain. In spite of this tacrine structure has been widely and successfully used in medicinal chemistry for design hybrid or multitarget compounds without toxic side effects.

5. NEW MULTIFUNCTIONAL ANALOGUES AND HYBRIDS OF TACRINE

In recent years a series of tacrine analogues have been reported in the literature as potential human AChE and BChE inhibitors [121-123]. Current modifications of tacrine structure have been mainly focused on the replacement of the benzene ring by a heterocyclic ring as well as tacrine-based molecular hybridizations [124-127]. In this part of article, it has been described multifunctional analogues of tacrine which have been reported in the last three years. All chemical structures of tacrine hybrids and their additional function are summarized in Table **1**.

For example, Hamulakova *et al*. [128] and Janočková *et al*. [129] synthesized a series of substituted heterodimers consisting of tacrine and acridine molecules and homodimers containing two tacrine moieties linked by aliphatic or alkylene-thiourea. *In vitro* studies demonstrated that four compounds in the series were especially potent against AChE with IC_{50} values of 3-8 nM and three of them were the most effective inhibitors against BChE with IC_{50} values of 0.4-20 nM [128, 129].

Khoobi *et al*. [130] synthesized and described the tetracyclic tacrine analogues containing pyrano[2,3-c]pyrazole *e.g*. (**1a-e**). They found that the most effective compound **1d** bearing a 3,4-dimethoxyphenyl group was more active than the reference drug tacrine and significantly protected neurons against oxidative stress, as potent as quercetin at low concentrations. A similar tacrine analogs family has been independently and almost simultaneously described by Marco-Contelles' team [131].

Interestingly, `novel multifunctional compounds were designed by linking caffeic acid (CA), ferulic acid (FA) and lipoic acid (LA) with tacrine, which exhibited potent activity against different pathologies of AD. The authors suggested that the compound **2** possessed a good ability to inhibit the β-amyloid (Aβ) self-aggregation, sub-micromole AChE/ BChE inhibitory, modest β-secretase (BACE1). Furthermore, this analogue might be a promising lead multi-targeted ligand [132].

A novel series of tetrahydroacridine derivative with 4-dimethylaminobenzoic acid moiety were synthesized by Bajda *et al*. [133] The kinetic studies of the most active compound **3** revealed the competitive type of AChE inhibition and displayed inhibitory potency against AChE-induced $A\beta_{1-42}$ aggregation. Moreover, all target compounds were more potent inhibitors of human AChE than tacrine. The most active compound revealed IC_{50} value of 19 nM.

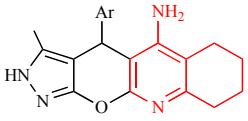
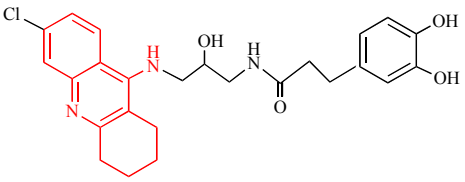
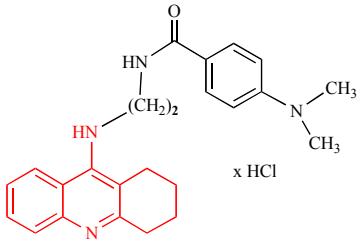
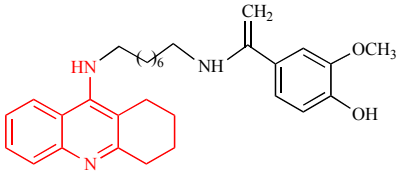
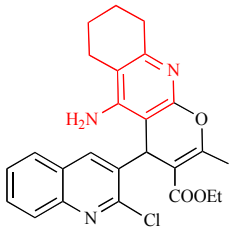
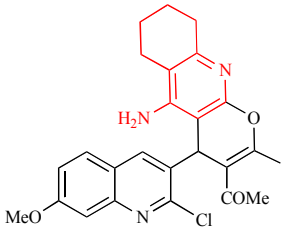
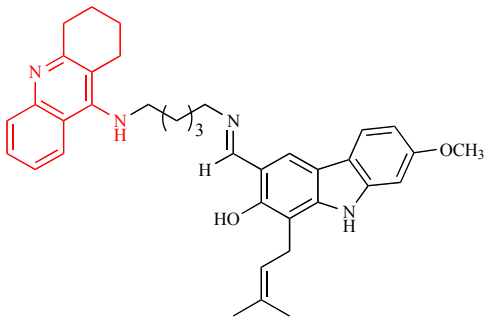
Eckroat *et al*. [134] synthesized a series of 6-chlorotacrine analogs with linkers varying in terminal functional group (-NH_2, -CH_3, -OH) and length (number of carbon atoms 4-12). These derivatives were evaluated for AChE inhibition and were compared to tacrine and 6-chlorotacrine-mefenamic acid hybrids. These 6-chlorotacrine with linkers were significantly more potent than tacrine and were often more potent than similar 6-chlorotacrine-mefenamic acid hybrids.

Also, a series of heterobivalent tacrine derivatives were designed. The compounds containing hydroxyl group showed potent peroxyl radical absorbance activity. Compound **4** exhibited higher self-induced Aβ aggregation inhibitory activity than curcumin, which could become a multifunctional agent for further development of the treatment of AD [135].

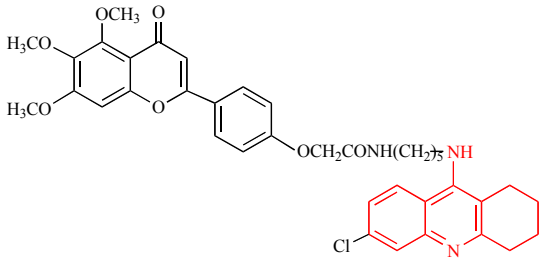
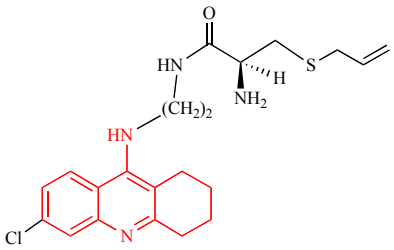
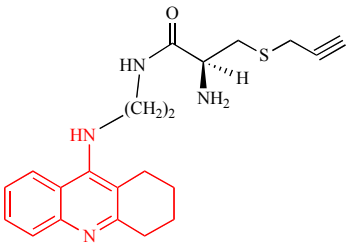
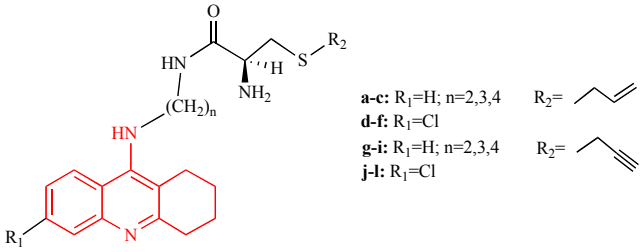
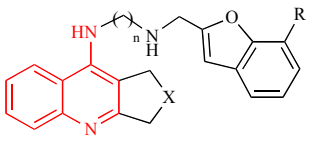
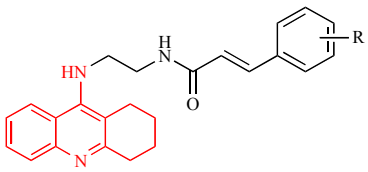
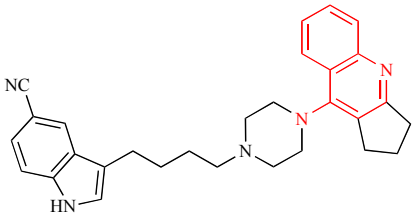
New tetrahydroquinoline derivatives were characterized for their *in vitro* and *in vivo* AChE inhibitory activity and hepatotoxicity. In the course of investigation, the compounds mentioned above displayed AChE inhibition level comparable to or slightly higher than tacrine. Among these compounds, the 2-chlorotetrahydroquinoline derivative emerged with hepatotoxicity results comparable to saline [136].

A new tacrine analogues bis(7)tacrine and cystamine-tacrine dimer were investigated as the promising agents for

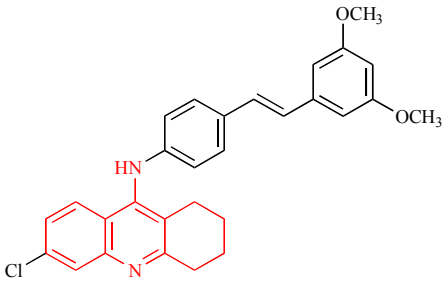
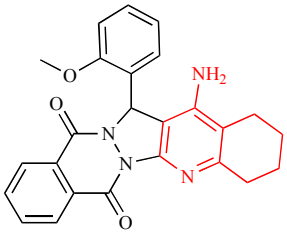
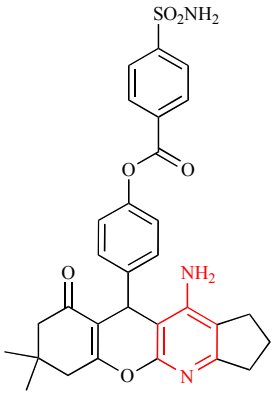
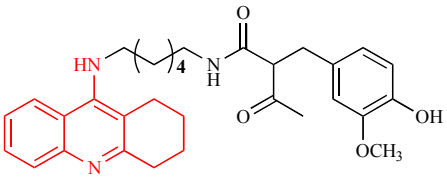
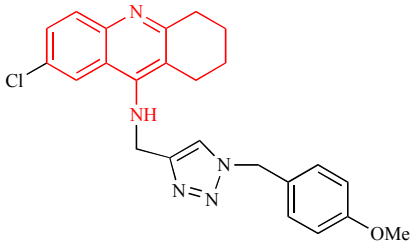
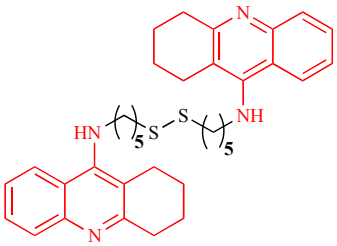
Table 1. Selected multifunctional tacrine's derivatives.

No.	Structure	Additional Functions	Refs.
1	 **a:** Ar = 2-fluorofenyl **b:** Ar = 3-metoksyfenyl **c:** Ar = 2,5-dimetoksyfenyl **d:** Ar = 3,4-dimetoksyfenyl **e:** Ar = 2,3-dichlorofenyl	Protecting neurons from oxidative stress as potent as quercetin at low concentrations	[130]
2		Good ability to inhibit the β-amyloid (Aβ) self-aggregation	[132]
3	 x HCl	Inhibitory potency against AChE-induced $A\beta_{1-42}$ aggregation	[133]
4		Neuroprotection through antioxidant activity	[135]
5		Neuroprotective activity against Aβ-aggregation on exposure to $A\beta_{1-40}$, as well as $A\beta_{1-40}$ aggregation-dependent tau-oligomerization and phosphorylation in ^{396}Ser	[138]
6		Neuroprotective activity against Aβ-aggregation on exposure to $A\beta_{1-40}$, as well as $A\beta_{1-40}$ aggregation-dependent tau-oligomerization and phosphorylation in ^{396}Ser	[138]
7		Antioxidant activity, ability to improve short-term and long-term memory deficit in mice induced by scopolamine	[159]

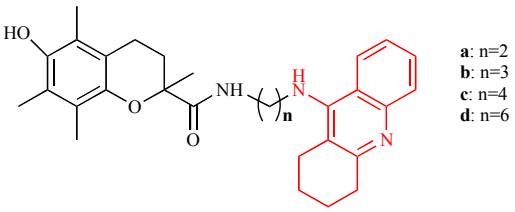
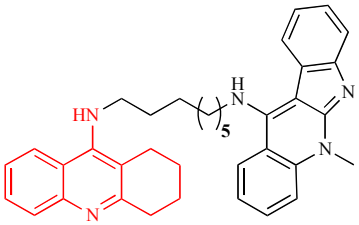
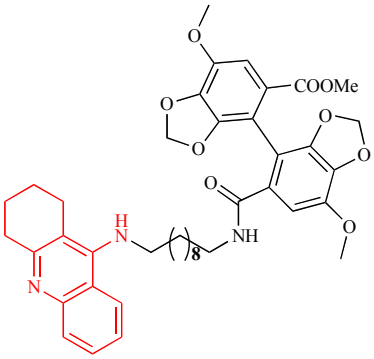
(Table 1) contd….

No.	Structure	Additional Functions	Refs.
8		Inhibition of self-induced $A\beta_{1-42}$ aggregation	[160]
9		Ability to improve the inhibition of Aβ aggregation in the presence of Cu (II)	[161]
10		Ability to improve the inhibition of Aβ aggregation in the presence of Cu (II)	[161]
11	 **a-c:** R_1=H; n=2,3,4 R_2= **d-f:** R_1=Cl **g-i:** R_1=H; n=2,3,4 R_2= **j-l:** R_1=Cl	Neuroprotective activity to Aβ- and ROS-induced cellular toxicity, inhibition of Aβ aggregation	[163]
12	 **a**: n=6; X=CH_2; R=H **b**: n=6; X=CH_2; R=OMe **c**: n=6; X=$(CH_2)_2$; R=OMe **d**: n=7; X=CH_2; R=H **e**: n=7; X=$(CH_2)_2$; R=H **f**: n=7; X=$(CH_2)_3$; R=OMe	Inhibition of hAChE- and self-induced Aβ aggregation	[164]
13	 **a**: R=4-OBn **b**: R=3-OMe-4-OBn	Ameliorating scopolamine-induced cognition impairment in comparison to tacrine	[168]
14		Improvement of scopolamine-induced cognition impairment of amnesia mice and mitigate depressive effects in tail suspension test	[169]

(Table 1) contd….

No.	Structure	Additional Functions	Refs.
15		Anti-inflammatory and immune-modulatory activity in neuronal and glial AD cell models	[171]
16		Neuroprotective activity against H_2O_2-induced damage in PC12 cells, inhibition of AChE-induced and self-induced Aβ-amyloid aggregation	[173]
17		Neuroprotection through antioxidant activity	[174]
18		Neuroprotection through antioxidant activity, metal ions-chelating activity	[176]
19		Improvement of impairment induced by scopolamine, neuroprotective effect against H_2O_2-induced cell death in PC12 neurons	[177]
20		Antiproliferative activity against six human tumor cell lines	[178]

(Table 1) contd….

No.	Structure	Additional Functions	Refs.
21	 **a**: n=2 **b**: n=3 **c**: n=4 **d**: n=6	Free radical scavenging activity	[179]
22		Inhibition of $A\beta_{1-42}$ self-aggregation, excellent potential to cross blood-brain barrier	[182]
23		Improvement of the cognitive performances of scopolamine-treated ICR mice	[183]

the treatment of Alzheimer's disease with the aid Molecular Dynamics (MD) simulations. Different interactions of both target cholinesterases with the target ligands have been demonstrated and the roles of the important residues in the active sites have been investigated. Integration of bis(7)tacrine and cystamine (as an antioxidant) has introduced cystamine-tacrine dimer with a very good inhibition performance that simultaneously affects several pathogens of Alzheimer's disease [137].

García-Font *et al.* [138] designed, synthesized and evaluated a new, racemic, diversely functionalized 2-chloroquinolin-3-yl substituted PyranoTacrines (PTs) **5, 6**. Compound **6** (IC_{50} = 0.48 ± 0.05 mM) are potent, mixed-type (**6**: K_i = 0.0142 ± 0.003 mM), and selective AChE inhibitors, binding at both catalytic and peripheral anionic sites of the enzyme. Compounds **5** and **6** are neuroprotective agents at low µM concentrations upon decreased viability of SHSY5Y cells (SHSY5Y) induced by oxidative stress, and stimulators of GSK3β-dependent tau phosphorylation. Molecules **5** and **6** effectively counteract Aβ-aggregation on exposure to $A\beta_{1-40}$, as well as $A\beta_{1-40}$ aggregation-dependent tau-oligomerization and phosphorylation in ^{396}Ser, which could be ascribed to the anti-aggregating properties shown *in vitro* [138].

Understanding the structure of AChE allowed to obtain very valuable guidance in the design of inhibitors of the enzyme - a potential candidate has the ability to bind to AChE in two active sites (catalytic site and peripheral). The hybrid strategy for drug development may lead to the formation of new compounds with improved biological activity. Hybrid molecules are defined as chemical entities, with two or more structural domains; their different biological functions and dual activity indicate that a hybrid molecule acts as two distinct pharmacophores, with independent modes of action [139].

In the literature, tacrine hybrids are characterized as a new paradigm to treat Alzheimer's disease [140]: homodimers hybrids, heterodimers hybrids, tacrine-4-oxo-4*H*-chromene [141], tacrine-melatonin hybrids [142, 143], tacrine-imidazole hybrids [144, 145], tacrine-xanomeline hybrids [146], tacrine–donepezil hybrids [147], tacrine–oxoisoaporphine hybrids [148], tacrine–huperzine hybrids [149], tacrine–ebselen hybrids [150,151], tacrine–lipoic acid hybrid [152], tacrine–ferulic acid hybrid [153], tacrine–trolox hybrids [154], tacrine-*m*-(trimethylammonio)trifluoroacetophenone hybrids *e.g.* [155] and tacrine-6 hydrazinonicotinamide [156].

Several tacrine-8-hydroxyquinoline hybrids with neuroprotective, cholinergic, antioxidant, and copper-complexing properties are described in the scientific publications [157]. Recently, tacrine-allyl/propargylcysteine-benzothiazole trihybrids have been presented [158].

New tacrine-carbazole hybrids were synthesized as potential multifunctional agents for the treatment of AD. Derivative **7** revealed the most potent inhibitory activity against

AChE and antioxidant action as well as exhibited the ability to improve short-term and long-term memory deficit in mice induced by scopolamine. Furthermore, tacrine-carbazole hybrids indicated a neuroprotective effect against oxidative stress induced by H_2O_2 and $A\beta_{1-42}$ toxicity [159]. Also, a series of 5,6,7-trimethoxyflavone-6-chlorotacrine hybrids were synthesized. Compound **8** exhibited the strongest AChE inhibitory activity with IC_{50} value of 12.8 nM and potent inhibition of self-induced $A\beta_{1-42}$ aggregation with inhibition ratio of 33.8% at 25 μM. Furthermore, **8** could cross the blood-brain barrier (BBB) *in vitro* [160]. Two hybrid compounds, **9** and **10** are able to improve the inhibition of Aβ aggregation in the presence of Cu(II) and this is slightly more relevant for the allyl derivative **9**, a stronger copper chelator, than for the propargyl **10**. Moreover, the presence of the chloro atom in the tacrine moiety and the size of the chain length between the two NH groups appeared also to improve the inhibition capacity for Aβ aggregation [161, 162].

Tacrine hybrids with natural-based cysteine derivatives **11a-l** were evaluated for some representative biological properties, including AChE activity and Aβ aggregation inhibition, as well as for their neuroprotective activity to Aβ- and ROS-induced cellular toxicity. The most promising results were achieved by compounds **11d** for the AChE inhibition and **11l** for the remarkable prevention of superoxide production and Aβ-induced cellular toxicity [163].

Tacrine-benzofuran hybrids **12a-f** displayed significant inhibition of human β-secretase-1 (hBACE-1). Hybrids **12e** showed the most interesting profile as a subnanomolar selective inhibitor of AChE (IC_{50} = 0.86 nM) and a good inhibitor of both β-amyloid aggregation (hAChE- and self-induced, 61.3% and 58.4%, respectively) and hBACE-1 activity (IC_{50} = 1.35 μM). *In vivo* studies confirmed that **12e** significantly ameliorates performances of scopolamine-treated ICR mice and did not exhibit significant hepatotoxicity [164].

A novel promising prophylactic approach was described and it is exploitable in the case of organophosphate (OP) intoxication. The toxicity of compounds was analogous, *i.e.* pralidoxime > 4-PA > obidoxime > novel prophylactic agent > 7-MEOTA, where pralidoxime represents the less toxic agent. It is evident that if 7-MEOTA does not exert any sign of hepatotoxicity *in vivo*, the same effect could also be anticipated in the novel prophylactic agent, exceeding the IC_{50} value of 7-MEOTA 100-times [165].

A preliminary evaluation of pro-cognitive effects of newly-developed 7-MEOTA-donepezil like hybrids and *N*-alkylated tacrine derivatives was presented by using an animal model of pharmacologically-induced cognitive deficit [166]. The potential of novel inhibitors was proved. Learning and memory of male Wistar rats were tested with using a water maze and step-through passive avoidance task. Therefore, further detailed evaluation of these compounds as potential drugs for Alzheimer's disease treatment is proposed [166].

The selection of a suitable carrier plays an important role in the greater effectiveness of drugs. Due to good biocompatibility, biodegradability and low toxicity of polymeric nanoparticles, especially poly(*n*-butylcyanoacrylate) (PBCA) and chitosan, these nanoparticles are considered as efficient carriers in drug delivery to the brain [167]. The results of the study indicate greater compatibility of tacrine with PCBA than chitostan. In addition it was proved that interaction between tacrine and PCBA is higher with increasing the length of that polymer. It has to be noticed that the methodology applied for modeling the polymer/tacrine system can be extended to various other traditional or new drugs and different polymer drug carriers [167].

Chen *et al*. [168] synthesized tacrine-cinnamic acid hybrids and determined their AChE and BChE inhibitory activity, inhibition of β-amyloid self-aggregation, cytoprotective effects against hydrogen peroxide and antiproliferative activity in PC-12 cells. Two compounds (**13a** and **13b**) in the series were selected for *in vivo* assays. The compound **13b** indicated ameliorating scopolamine-induced cognition impairment in mouse model and hepatotoxicity decrease in comparison to tacrine.

About 50% of patients with Alzheimer's disease suffer from depression at the same time. Li *et al*. [169] reported synthesis and biological evaluation of vilazodone-tacrine hybrids as potential multitarget-directed compounds for the treatment of AD with depression. The hybrid **14** in the series of 30 compounds showed tolerable hepatotoxicity and satisfactory inhibition of the human Ether-a-go-go-Related Gene (hERG). In addition **14** could across the blood-brain barrier *in vivo*. It has been confirmed that oral intake of 30 mg/kg **14**×HCl could improve scopolamine-induced cognition impairment of amnesia mice and mitigate depressive effects in tail suspension test.

O-heterocycles and N-heterocycles also deserve attention because of their versatile biological activities such as antimycobacterial, antibacterial, antifungal, antitumor, anti-inflammatory, cytotoxic and AChE inhibitory properties [170]. Due to their activities, Mahdavi *et al*. [170] synthesized a novel pyrazolo[4′,3′:5,6]pyrano[2,3-*b*]quinolin-5-amines as a potential drugs in the treatment of AD. Subsequently, they evaluated the *in vitro* AChE and BChE inhibitory activities of these tacrine derivatives using Ellman's method and compared with rivastigmine as reference drug. Among the series, two compounds possessed high activity against AChE as well as BChE (128 times better than reference rivastigmine).

Another approach to searching for a novel multi-target-directed ligands (MTDLs) against Alzheimer's disease is combination of tacrine and resveratrol. The preparation of multi-targeted hybrid is possible because of antioxidant and anti-neuroinflammatory properties of resveratrol. Two compounds in the series of eight hybrids, showed AChE inhibitory activity at micromolar concentrations and decreased Aβ self-aggregation *in vitro*. Moreover **15** indicated anti-inflammatory and immune-modulatory activity in neuronal and glial AD cell models. In addition, for all of the mentioned compounds is predicted to be blood-brain barrier permeable and low cytotoxic on primary neurons [171].

Eghtedari *et al*. [172] synthesized and determined the biological activity of new tacrine analogues namely 5-

amino-2-phenyl-4*H*-pyrano[2,3-*b*]quinoline-3-carboxylates as inhibitors of AChE and BChE. Most of the derivatives in series were characterized by potent AChE and BChE inhibition. Among them, the most promising compound exhibited IC_{50} values of 0.069 μM and 1.35 μM against AChE (five times more potent in comparison to tacrine) and BChE, respectively. Moreover, one of them displayed lower cytotoxicity on human liver cells than reference tacrine. It has also been found that introduction of chloro/bromo substituent at ortho or meta position of the 4-phenyl ring ameliorated AChE/BChE inhibitory properties of this compound.

A new multifunctional lead for the treatment of Alzheimer's disease is compound **16** involved in the series of tacrine derivatives containing a pyrazolo[1,2-*b*]phthalazine structure. **16** compound exhibited very high inhibition of AChE with IC_{50} value of 49 nM and was seven times more potent compared to tacrine. This derivative was highly selective against AChE over BChE [173]. In addition, this compound showed much lower hepatotoxicity than tacrine in HepG2 cells and neuroprotective activity against H_2O_2-induced damage in PC12 cells. Additional function is inhibition of AChE-induced and self-induced Aβ-amyloid aggregation.

The next important and interesting group of compounds represents sulfonamides. Because of their broad spectrum of biological activity, they are used in the pharmaceutical industry as anticancer, antimicrobial, antiobesity, antiglaucoma and AChE inhibitory drugs [174]. Ulus *et al*. [174] designed, synthesized and evaluated AChE and BChE inhibitory activity of novel series of tacrine analogues containing sulfonamide group. The series consisted of fifteen compounds and all of them showed inhibitory activity on AChE as well as BChE. The most potent inhibitor against AChE demonstrated an IC_{50} value of 0.009 μM, 220 times greater than galantamine (IC_{50}=2.054 μM) and 6 times greater than tacrine (IC_{50}=0.055 μM). The most promising compound against BChE exhibited IC_{50} value of 2.250 μM, found to be 8 times more potent than galantamine (IC_{50}=18.130 μM) and slightly stronger than donepezil (IC_{50}=2.680 μM). Moreover, all of the analogues demonstrated more effective BChE inhibition in comparison to galantamine. In addition, the antioxidant activity of mentioned above compounds was evaluated. **17** exhibited greater ABTS cation radical scavenging ability (IC_{50}=94.390±2.310 μM) compared to all of the newly obtained derivatives.

Koti Reddy *et al*. [175] designed, synthesized and evaluated the biological activity of twenty four tacrine analogues. The three of them exhibited high inhibitory activity against AChE with IC_{50} values of 12.97±0.47 nM, 5.17±0.24 nM and 7.14±0.78 nM, respectively. *In silico* studies confirmed the ability of these derivatives to bind strongly to the enzyme active site and protect from enzyme-substrate interactions. The hepatotoxicity was evaluated on HepG2 cells and cytotoxicity was tested using HEK-293 cells. One analogue showed much lower cytotoxicity and hepatotoxicity than tacrine. Even concentration of 300 μM did not decrease cell viability.

A promising strategy in the treatment of Alzheimer's disease is combination of tacrine and curcumin. Curcumin is polyphenolic compound with anti-inflammatory, antimicrobial and anti-carcinogenic properties [176]. In addition, there is an evidence that curcumin has a potential therapeutic role in AD due to binding to Aβ-amyloid and decrease its aggregation. Furthermore, that compound could chelate metal ions and eliminate ROS. Liu *et al*. [176] synthesized and evaluated biological activity of multifunctional tacrine-curcumin hybrids. *In vitro* studies revealed high cholinesterase inhibition of the obtained compounds. The different selectivity on AChE and BChE of some hybrids was the result of structural difference. Among the series, **18** was the most potent against AChE with IC_{50} value of 0.08±0.03 μM and showed higher inhibition than tacrine. Moreover, the hybrids possessed antioxidant, neuroprotective and metal ions-chelating activity.

Najafi *et al*. [177] synthesized and determined biological activity of novel tacrine-1,2,3-triazole hybrids. Most of the compounds in the series indicated high AChE and BChE inhibitory activity. **19** was the most powerful against AChE with IC_{50} value of 0.521 μM. Another compound showed the highest inhibition against BChE with IC_{50} values of 0.055 μM. Both of them exhibited lack of cytotoxicity and bound simultaneously to the peripheral anionic site and catalytic sites of the AChE and BChE in molecular modeling and kinetic studies. Moreover, result from Morris water maze test indicated that **19** improved memory impairment induced by scopolamine in rats and exhibited neuroprotective activity.

Another modifications of tacrine are tacrine-based homo- and heterodimers designed, synthesized and evaluated by Roldan-Pena *et al*. [178] as potential drugs using in Alzheimer's disease and as antiproliferative agents. Symmetrical homodimers containing dichalcogenide or selenide antioxidant linker were the most potent inhibitors of AChE in the series with IC_{50} values of 1.2-26.3 nM (up to 19 times stronger than tacrine). Among them, **20** was the most interesting compound with multipotent profile and lack of neurotoxicity on immortalized mouse cortical neurons at 50 μM concentration.

Moreover, obtained compounds demonstrated *in vitro* antiproliferative activities against six human tumor cell lines with GI_{50} values of 0.12-0.95 μM for the most potent analogues and were up to 306 times more effective compared to 5-fluorouracil and up to 162 times greater than cisplatin.

The various biological activity of tacrine, trolox and β-carboline analogues influence their potential application in the treatment of Alzheimer's disease. Based on the topology of the active site of AChE and BChE, Teponnou *et al*. [179] designed and synthesized tacrine-trolox and tacrine-tryptoline hybrids with various linker chain lengths. The compounds with trolox moiety (**21a-d**) exhibited AChE/BChE inhibition and free radical scavenging activities with IC_{50} values of 17.37-2200 nM, 3.16-128.82 nM and 11.48-49.23 μM, respectively. It has been confirmed that inhibitory activity increased with chain length and free radical scavenging activity was not affected by varying linker chain length. Among compounds with tryptoline moiety, one showed the highest AChE/BChE inhibitory activity with IC_{50} value of 17.37 nM and 3.16 nM, respectively.

Spilovska *et al*. [180] designed, synthesized and evaluated biological activity of novel series of 6-chlorotacrine-scutellarin hybrids as a potential drugs using in Alzheimer's

disease treatment. *In vitro* studies consisted of assessing the inhibition of human AChE and BChE, antioxidant activity, hepatotoxicity and ability to cross the blood-brain barrier. One of them proved to be a very potent AChE inhibitor with IC_{50} value of 1.63 nM. It has been shown that analogues with shorter linkers exhibited higher inhibitory activity than derivatives with longer linker chain. Simultaneously, none of the compounds displayed antioxidant properties, improvement of BChE inhibition (the strongest one against BChE showed IC_{50} value of 174 nM) and less hepatotoxicity in comparison to tacrine. Among the series, only two compounds indicated potential to cross the BBB.

Boulebd *et al*. [181] synthesized and evaluated biological activity of ten (benz)imidazopirydyno tacrines compounds. One of them demonstrated lack of hepatotoxicity and moderate, but completely selective inhibition of AChE with IC_{50} value of 0.50±0.03 μM. Furthermore, two of them indicated less hepatotoxicity in comparison to tacrine with temperate AChE inhibition with IC_{50} values of 0.53±0.03 μM and 0.31±0.02 μM, respectively, and BChE inhibition with IC_{50} values of 1.84±0.10 μM and 2.41±0.30 μM, respectively. Three above-mentioned compounds were identified for further development of anti-AD agents.

A novel tacrine-neocryptolepine heterodimers were synthesized and their biological activities were evaluated by Wang *et al*. [182]. Among the compounds in the series, **22** demonstrated the highest inhibition of AChE and BChE with IC_{50} values of 0.95±0.04 nM and 2.29±0.14 nM, respectively. Importantly, it is one of the strongest known AChE inhibitors. In addition, this analogue showed a moderate inhibition of $A\beta_{1-42}$ self-aggregation, excellent potential to cross blood-brain barrier and lack of hepatotoxicity *in vitro* at concentrations at which the cholinesterases are inhibited.

Novel hybrids of tacrine and bifendate were synthesized and evaluated as potential compounds using in Alzheimer's disease treatment [183]. They revealed inhibitory activity against cholinesterase and self-induced β-amyloid aggregation. Moreover a Lineweaver–Burk plot and molecular modeling study indicated that these analogues are able to bind at the catalytic active site and peripheral anionic site of AChE. Among the series, **23** showed much lower hepatotoxicity against human hepatocyte cell line (HL-7702) and cytotoxicity against PC12 and HepG2 cells in comparison to tacrine. In addition, **23** affects the improvement of the cognitive performances of scopolamine-treated ICR mice.

Despite the multifunctional activity, recent analogues of tacrine do not resolve all pathophysiological problems. The mitochondrial dysfunction (including lipids profile changes), inflammation process connected with microglia activity or the glutamatergic neuronal networks connectivity remain beyond the reach of tacrine's derivatives or they have not been determined in biological investigation of new compounds. Therefore, new multifunctional tacrine's analogues as well as implementation of recent pathophysiological knowledge in biological activity screening are required.

CONCLUSION

This review summarizes current knowledge of tacrine analogues with promising therapeutic properties. This article covers medicinal chemistry, pharmacological and biological literature space on the subject. The authors herein described conjugated with natural moieties such as melatonin, hydroxyquinoline or thioflavine that protect cells against oxidative stress, and NO-donor-tacrine hybrids that showed hepatoprotective properties. Furthermore, diverse hybrid molecules designed by exploring pharmacophores of marketed drugs are reported in the literature. Compounds that are inhibitors of cholinesterases structured hybrid have activity in inhibiting cholinesterases activity, and additionally have antioxidant properties and prevent aggregation Aβ (β-amyloid). Consequently, they are potentially neuroprotective drugs with improved biological activity. The effect of tacrine, donepezil, galantamine and rivastigmine on acetylcholinesterase inhibition in *Dugesia tigrina* has been described which can be used as a model for the investigation of AChE inhibitory substances. *Dugesia tigrina* are low maintenance with respect to manipulating them under laboratory conditions and are low cost in comparison to other models of test animals. Immune related abnormalities observed in the periphery as well as in the brain of AD patients, in relation to known risk factors of AD such as genetics, type-2 diabetes or obesity, aging, physical inactivity and hypertension have also been discussed.

The current targetable pathways have been described for designing anti-Alzheimer's agents. The authors herein provide a summary update and perspective on realized and potentially druggable pharmacological targets for this CNS disorder.

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