Tacrine hybrids as multi-target-directed ligands in Alzheimer's disease: influence of chemical structures on biological activities

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Abstract

Alzheimer's disease is a neurological disorder, which is the most common form of dementia affecting mostly elderly people. The number of patients is still rapidly increasing which results in negative impacts on population. The loss of cholinergic transmission is one of the signs of Alzheimer's disease; it is connected with cognition impairment and memory loss. Therefore, some drug therapies are based on restoring the cholinergic function. Therefore, drug therapies are based on the cholinergic hypothesis. Acetylcholinesterase inhibitors such as donepezil, galantamine, and rivastigmine are nowadays in medical use. Tacrine possesses a simple structure and strong activity, but despite these features, it was withdrawn from the market due to its side effects. Due to the multifactorial etiology of Alzheimer's disease, researchers have investigated multi-target-directed ligands based on tacrine structure to achieve better treatment efficacy. To broaden the therapeutic profile of the ligands, tacrine is bound to numerous moieties with various applications. The hybrids can be obtained in three ways: first, by conjugating; second, by fusing; third, by merging single molecules. The hybrids can target numerous disease pathways simultaneously. This is a promising concept, which will create multiple drugs that will minimize the symptoms of diseases in elderly populations. In this review, we will be describing differently synthesized series of tacrine compounds which were divided based on their biological activities.

Keywords Tacrine · Multifunctional drugs · Alzheimer's disease · Acetylcholinesterase · Hybrids

Introduction

Alzheimer's disease (AD) is an incurable, progressive neurodegenerative disorder which is characterized at the beginning by episodic and semantic memory loss and cognitive impairment. As the disease progresses, one may experience a decline in language functions, depression, apathy, and anxiety. In later stages, psychotic features and personality changes may develop (Hugo and Ganguli 2014; Lehrner et al. 2017). It is expected that, by 2030, 65.7 million, and, by 2050, 115.4 million people will be affected by this disease (Prince et al. 2013). Despite many years of research, the main cause of the disease is unknown and only symptomatic treatment is used. Pathological changes result in a loss of cholinergic neurons, decreased level of neurotransmitter

acetylcholine (ACh), increased β -amyloid ($A\beta$) and tau-protein deposition, and dyshomeostasis of bio-metals and oxidative stress (Mullan 1993; Querfurth and LaFerla 2010). There are a few hypotheses, which could explain the AD pathophysiology (such as the cholinergic, $A\beta$, or tau cascade hypotheses). Nowadays, $A\beta$ and tau cascade hypotheses are considered as more important than the cholinergic one. The cholinergic hypothesis was defined over 30 years ago as the first AD hypothesis. The damage of the cholinergic neurons is observed in the hippocampus, amygdala, frontal cortex, and other structures responsible for learning, memory, or conscious awareness. The main changes observed in cholinergic neurons are choline uptake, impaired acetylcholine release, and deficits in the expression of nicotinic and muscarinic receptors (Bartus et al. 1982; Terry and Buccafusco 2003). The second hypothesis is the $A\beta$ one. The basis to this hypothesis was given by Glenner and Wong 1984. They investigated the cerebrovascular amyloid derived from patients with Down syndrome (Glenner and Wong 1984). $A\beta$ is a peptide consisting of 37–43 amino acids in which isoforms 1–40 and 1–42 are the most common. The $A\beta_{1-42}$

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is considered as the one with the highest toxicity to neurons. A β derives from the amyloid precursor protein which is cleaved by several enzymes—beta-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), a β -secretase, and γ -secretase. The imbalance in production and A β aggregation and its accumulation in the brain may be one of the factors that causes AD (Grøntvedt et al. 2018; Haass and Selkoe 2007; Sanabria-Castro et al. 2017; Tanzi and Bertram 2005). Next hypothesis regards neurofibrillary tangles which occur during AD. The tangles are mostly consisted of hyper-phosphorylated and aggregated form of tau. Tangles may also occur as soluble protein in axons and do not cause any toxicity. Their main role is to stabilize microtubules. However, hyper-phosphorylated tau is insoluble, makes aggregates, and causes neurotoxicity. This results in cognition impairment (Khlistunova et al. 2006; Oddo et al. 2006; Querfurth and LaFerla 2010). The oxidative stress is mostly caused by A β , which is a generator of reactive oxygen species by causing mitochondria damaged (Good et al. 1996; Hensley et al. 1994). A β blocks mitochondrial enzymes (Reddy and Beal 2008). In results, electron transport, ATP production and mitochondrial membrane potential are being impaired and damaged mitochondria easily release free radicals (Good et al. 1996). In addition, higher levels of metal ions (iron, copper, and zinc) are associated with reactive oxygen species (ROS) damage and neurodegeneration. Iron ions increase tau phosphorylation and aggregation (Lovell et al. 1998; Yamamoto et al. 2004). The calcium regulation is in imbalanced in neurodegenerative disorders. The higher concentration of intracellular calcium leads to A β formation, its aggregation, and induction of apoptosis. A β by itself also increases calcium imbalance (Isaacs et al. 2006; Mullan 1993; Small 2009).

The most important risk factor is old age where AD is mostly diagnosed in people above 65. Comorbidities such as stroke, hypertension, heart diseases, hypercholesterolemia, smoking, and genetic factors have also their impacts (Alves et al. 2010; Cataldo et al. 2010; Hugo and Ganguli 2014). Currently, the most popular treatment is the inhibition of acetylcholinesterase (AChE)—which hydrolyzes the ACh to choline and acetate. The low levels of ACh result in the cognitive impairment and memory problems that occur decades after the beginning of the neurodegenerative process (Francis et al. 1999; Siegfried 1993). Treatment only alleviates the symptoms and barely stops the progression of disease. AChE inhibitors (AChEI) are donepezil, rivastigmine and galanthamine, which are used to treat mild-to-moderate AD. The *N*-methyl-D-aspartate (NMDA) receptor antagonist—memantine—is also used in symptomatic treatment.

The AChE possesses at least two substrate-binding sites: based on the bottom of the gorge, the active site; located at the entry of the gorge, the peripheral anionic site (PAS). The active site is built of two subsides: the catalytic

anionic site (CAS) where the ACh moiety is stabilized by Trp84, Glu199, and Phe330 (Sussman et al. 1991); by the second subside, the catalytic triad which is made of Ser200–His440–Glu327 which hydrolyzes ACh. The carbonyl oxygen of ACh is stabilized by oxyanion hole (made of peptic NH groups from Gly118, Gly119, and Ala201), whereas the acetyl group of ACh is connected with acyl-pocket (Trp233, Phe288, Phe290, and Phe331) of AChE (Harel et al. 1993). PAS influences on the catalytic activity and is made of the three principal amino acids—Trp279, Tyr70, and Asp72. In the surface of the gorge, the rings of 14 aromatic residues (Tyr70, Trp84, Trp114, Tyr121, Tyr130, Trp233, Trp279, Phe288, Phe290, Phe330, Phe331, Tyr334, Trp432, and Tyr442) are located (Colletier et al. 2006; Sussman et al. 1993). ACh is hydrolyzed by cholinesterases—AChE and butyrylcholinesterase (BChE). During the progression of AD, AChE level decreases, whereas BChE increases (Perry et al. 1978). The PAS of the AChE is responsible not only for hydrolyzing ACh, but also for aggregation of A β protein, its stabilization, and deposition. Accumulation of A β aggregates leads to calcium dysregulation, formation of reactive oxygen species, and neuronal cell membrane damage (Cai et al. 2011; Green and LaFerla 2008; Hardy and Selkoe 2002).

Drugs, which can act on various stages of the neurotoxic cascades, have been investigated and are called multi-target-directed ligands (MTDLs). MTDLs can target numerous pathways involved in the AD development. The hybrid comprises two or more molecules; each molecule belongs to different pharmacological and chemical classes. Single molecules are connected in three ways to obtain novel hybrids (Morphy et al. 2004; Morphy and Rankovic 2005). First, “conjugates” are MTDLs, which have two frameworks (each one possessing pharmacophore elements) and are connected by a linker group (no part of the linker should be found in the ligands). This method of obtaining MTDLs is very common and is described in detail in this review. The linker between two moieties impacts on hybrid’s inhibitory activity. In the second method, the size of the linker between frameworks decreases and ligands touch themselves by the same group in the linker. This type of strategy described and evaluated by Jerábek et al. (2017) fuses tacrine (THA) with resveratrol. In the third method, MTDLs are synthesized by merging both frameworks, where common structures combine together (Morphy et al. 2004; Morphy and Rankovic 2005). Fusing and merging strategies are recently regarded as better than conjugating, due to the fact that they receive lower molecular weight and decrease in structural complexity. These features should help hybrids in crossing the blood–brain barrier (BBB) (Rankovic 2015). Hybrids represent a new strategy to treat AD, enhancing efficacy and improving safety in regards to drugs that have only a single target. AChEI is bound to another moiety to synthesize a multifunctional hybrid, which

interacts with the AChE and provides additional properties. It can result in the increase of potency (Cavalli et al. 2008; Singh et al. 2016).

In our review, we focused on THA hybrids, which were synthesized in the last years. All hybrids were divided into five groups on the basis on their biological activities: THA derivatives with cholinesterase inhibition; with cholinesterase inhibition and A β antiaggregation properties; with cholinesterase inhibition and antioxidant properties; with cholinesterase inhibition, antiaggregation, and antioxidant properties; with cholinesterase inhibition, antioxidant, and non-hepatotoxicity properties. We provided detailed description of IC₅₀ values toward AChE and BChE, as well as information about type of inhibition, % inhibition of A β aggregation, impact of length of the linker on hybrids activities, or antioxidant assay results toward the most promising hybrid of each group. Moreover, we presented chemical structures with their modifications for all hybrids, which make easier to understand the connection between structure and activity.

Tacrine

THA **1** (Fig. 1) belongs to the group of acridine compounds and is a reversible, non-competitive AChEI. THA moiety interacts with AChE by getting stacked against Trp84. The Phe330 ring rotates to situate itself parallel to THA. THA is inserted between the Phe330 and Trp84.

Fig. 1 THA **1**

Fig. 2 Compounds **2a–b**, **3a–m**

The nitrogen from the THA ring forms a bond with the carbonyl oxygen of His440 (Wu et al. 2017). THA was approved by the FDA in 1993 for the treatment of AD. After 5 years, it was withdrawn from the market due to its hepatotoxicity caused by mediating the stimulation of reactive oxygen species production and glutathione depletion (Sameem et al. 2017). The liver toxicity effect is related to the formation of highly reactive metabolites (especially 7-hydroxytacrine and its metabolite quinone methide) which occurs during THA's metabolism. Reactive metabolites form covalent adducts with DNA or cellular proteins, or induce different redox cycling, which can result in liver damage and toxic effects (Patocka et al. 2008). Due to the good inhibitory activity, low molecular weight, and potential attenuates A β -induced neurotoxicity, THA has been widely investigated by the researchers worldwide, especially for the synthesis of MTDLs for AD treatment. Putting THA in the hybrids could improve the biological profile of THA and overcome some side effects (Meng et al. 2007; Minarini et al. 2013).

Tacrine derivatives with cholinesterase inhibition

Tacrine-trimethoxybenzene hybrids

Elsinghorst et al. synthesized trimethoxybenzene–THA hybrids **2a–b**, **3a–m** (Fig. 2) with different lengths of the linker. The highest inhibitory activity was presented by hybrids with the longest spacer [**3g**, and **3h**—electric eel AChE (eeAChE) IC₅₀ 3.25 nM, 2.73 nM, *Torpedo californica* AChE (TcAChE) IC₅₀ 17.2 nM, 5.99 nM, human AChE (hAChE) IC₅₀ 5.24 nM, 5.08 nM, human BChE (hBChE) IC₅₀ 0.293 nM, and 1.38 nM, respectively, THA

non-tested]. **3g** turned out to be a non-competitive inhibitor of eeAChE (Elsinghorst et al. 2006).

Tacrine-gallamine hybrids

Gallamine **4** (Fig. 3) was discovered in 1947 and has been used as a muscle relaxant due to its inhibition of muscarinic receptors with a selectivity for the M₂ subtype. Gallamine binds to the core region of the allosteric site of the receptor (Clark and Mitchelson 1976).

Elsinghorst et al. investigated novel gallamine–THA hybrids **4a–b**, **5a–b**, and **6a–b** (Fig. 3) as two binding site inhibitors. THA bound to the CAS and gallamine to the PAS of the AChE according to the molecular modeling study. Almost all the compounds showed lower IC₅₀ values toward AChE and BChE than THA and gallamine. The compound **4a** presented the best results towards eeAChE [IC₅₀ 0.467 nM, THA IC₅₀ 300 nM, and human BChE (hBChE) IC₅₀ 1.50 nM], while the compound **4b** showed the highest inhibitory activity towards hAChE (IC₅₀ 5.44 nM, THA hAChE IC₅₀ 926 nM, hBChE IC₅₀ 1.42 nM). The 6-chloro substitution in the THA moiety did not increase the inhibitory activity of the hybrids as it was observed for other hybrids. Increasing substitution of the gallamine-derived moiety diminished the inhibitory potency. The allosteric potency of hybrids was higher than the one observed for gallamine or THA. Novel compounds bound to the core and peripheral region of the allosteric site (Elsinghorst et al. 2007).

7-Methoxytacrine–adamantylamine hybrids

The 7-methoxytacrine (7-MEOTA) **7** (Fig. 4) is considered an active ChE inhibitor which causes minor side effects than THA (Gazova et al. 2017; Korabecny et al. 2011). Amantadine **8** (Fig. 4) is a drug used in the treatment of Parkinson's disease. Investigations showed that amantadine, whose analogue is memantine, was able to delay the onset and severity of dementia (Inzelberg et al. 2006; Robinson and Keating 2006).

Spilovska et al. evaluated novel heterodimers of 7-MEOTA–adamantylamine thioureas with different linker lengths **9a–g** (Fig. 4). 7-MEOTA and amantadine were less potent inhibitors than THA and novel heterodimers. There was no significant difference between compounds with a longer or shorter linking chain. The best inhibitory activity toward hAChE and hBChE was demonstrated by compound **9d** (IC₅₀ value of 0.47 μM for hAChE and IC₅₀ value of 0.11 μM for hBChE) which possessed five carbon atoms in the linker and whose potency was similar to the one presented by THA (AChE IC₅₀ 0.5 μM, BChE IC₅₀ 0.023 μM). Based on molecular modeling, this compound bound itself to the CAS and PAS of AChE (Spilovska et al. 2013).

Fig. 3 Gallamine **4** and compounds **5a–b**, **6a–b**, **7a–b**

Fig. 4 7-Methoxytacrine **7** and Amantadine **8** and compounds **9a–g**

Tacrine derivatives with cholinesterase inhibition and A β antiaggregation properties

Tacrine-indole heterodimers

Munoz-Ruiz et al. synthesized THA–indole hybrids **10a–y** (Fig. 5) linked by the methylene tether of different length. The compounds were dual-binding site of AChE inhibitors. The linker was variously modified by the following factors, such as a propionamide chain, additional *N*-Me function in the central part of the methylene linker, and replacement of the amide group by a carbamate moiety like in rivastigmine. Researchers assayed on the bovine erythrocyte AChE (beAChE) inhibitory potency, hBChE, and hAChE.

Almost all heterodimers showed the higher potency than THA (beAChE IC₅₀ 167 nM and hBChE IC₅₀ 24 nM). **10c** (beAChE IC₅₀ 0.02 nM, hAChE IC₅₀ 70 pM, and hBChE IC₅₀ 2.9 nM) and **10d** (beAChE IC₅₀ 0.06 nM, hAChE IC₅₀ 20 pM, and hBChE IC₅₀ 0.1 nM) were the most active hybrids. They contained chlorine atom in position 6, two methylene groups at the indole ring, and tether length of 6–7 methylenes. The hybrids were rather selective toward AChE than BChE. The compounds also proved ability to inhibit A β aggregation. The compounds **10c** and **10d** were strong antiaggregating molecules; they noticeably inhibited the AChE-induced A β aggregation (inhibition of A β with and without AChE at the concentration of 100 μ M for **10c**, **10d**, THA—98%, 49%, and 7% and 96%, 65%, and 0%, respectively) (Muñoz-Ruiz et al. 2005).

Compound	R ¹	R ²	R ³	X	Y	Z	Compound	R ¹	R ²	R ³	X	Y	Z
10a	H	H	H	-(CH ₂) ₅ -	-(CH ₂) ₂ -	CH	10m	Cl	H	H	-(CH ₂) ₆ -		CH
10b	Cl	H	H	-(CH ₂) ₅ -	-(CH ₂) ₂ -	CH	10n	Cl	H	H	-(CH ₂) ₇ -		CH
10c	Cl	H	H	-(CH ₂) ₆ -	-(CH ₂) ₂ -	CH	10o	Cl	H	H	-(CH ₂) ₈ -		CH
10d	Cl	H	H	-(CH ₂) ₇ -	-(CH ₂) ₂ -	CH	10p	Cl	H	H	-(CH ₂) ₇ -	-CH ₂ -	CH
10e	Cl	H	H	-(CH ₂) ₈ -	-(CH ₂) ₂ -	CH	10q	Cl	Br	H	-(CH ₂) ₇ -	-CH ₂ -	CH
10f	Cl	H	H	-(CH ₂) ₉ -	-(CH ₂) ₂ -	CH	10r	Cl	H	H	-(CH ₂) ₅ -	-(CH ₂) ₃ -	CH
10g	Cl	H	H	-(CH ₂) ₁₀ -	-(CH ₂) ₂ -	CH	10s	Cl	H	H	-(CH ₂) ₆ -	-(CH ₂) ₃ -	CH
10h	H	H	H	-(CH ₂) ₃ NMe(CH ₂) ₃ -	-(CH ₂) ₂ -	CH	10t	Cl	H	Me	-(CH ₂) ₇ -		CH
10i	Cl	H	H	-(CH ₂) ₃ NMe(CH ₂) ₃ -	-(CH ₂) ₂ -	CH	10u	Cl	H	H	-(CH ₂) ₇ -		N
10j	Cl	CN	H	-(CH ₂) ₆ -	-(CH ₂) ₂ -	CH	10w	Cl	H	H	-(CH ₂) ₅ -	-(CH ₂) ₂ O-	CH
10k	Cl	H	H	-(CH ₂) ₆ -	-(CH=CH)-	CH	10x	Cl	H	H	-(CH ₂) ₆ -	-(CH ₂) ₂ O-	CH
10l	Cl	H	H	-(CH ₂) ₅ -		CH	10y	Cl	H	H	-(CH ₂) ₇ -	-(CH ₂) ₂ O-	CH

Fig. 5 Compounds **10a–y**

Tacrine–donepezil hybrids

Donepezil **11** (Fig. 6) has a big structure which can form various interactions with residues of the AChE. Due to its dual-binding site character, donepezil possesses A β antiaggregation activity. Its benzyl moiety binds to CAS and its indanone structure connects with PAS of AChE (Kryger et al. 1999).

Camps et al. evaluated novel donepezil–THA hybrids **12a–h** (Fig. 6). According to the molecular modeling study, they bound to the PAS (donepezil moiety), CAS (THA or 6-chlorotacrine moiety), and mid-gorge-binding sites of AChE. The length of the linker between moieties has an impact on the arrangement of the hybrids inside the gorge of the enzyme. All of the novel hybrids were very potent inhibitors of human erythrocytes AChE and human serum BChE with IC₅₀ values in the sub and nanomolar range. The most active inhibitors of human erythrocytes, AChE, were those with the indanone system, a chlorine atom on the THA moiety, and a three-methylene linker, especially the

compound **12d** (IC₅₀ 0.27 nM, hBChE IC₅₀ 66.3 nM, THA hAChE IC₅₀ 205 nM, and donepezil hAChE IC₅₀ 11.6 nM). Novel hybrids were less potent toward hBChE than hAChE. All of them showed higher potency than THA. Moreover, the compounds **12c–h** (at the concentration of 100 μ M) presented their ability to inhibit AChE–A β _{1–40} aggregation. All the hybrids showed significant A β antiaggregation properties, and were more potent than THA or donepezil. The most potent toward hAChE, **12d**, showed 46.1% inhibition of A β antiaggregation. The strongest was **12f**, which inhibited A β antiaggregation in 65.9% (THA—only 7% inhibition) (Camps et al. 2008).

Tacrine–pyrano(3,2-c)quinoline–6-chlorotacrine hybrids

A pyrano(3,2-c)quinoline scaffold comprising 5-phenylsubstituted derivative resembles a 6-phenylphenanthridinium group in propidium **13** (Fig. 7). Therefore, this compound may be considered as AChEI (Bourne et al. 2003).

Fig. 6 Donepezil **11** and compounds **12a–h**

Fig. 7 Propidium **13** and compounds **14a–j**

Camps et al. synthesized and tested novel pyrano(3,2-c)quinoline–6-chlorotacrine hybrids **14a–j** (Fig. 7) as potent dual-binding site AChEI connected through an amido-containing oligomethylene linker. Being a dual-binding inhibitor, 6-chlorotacrine interacted with the CAS and pyrano(3,2-c)quinoline moiety with the PAS of AChE which was evaluated in the molecular modeling study. Novel compounds exhibited inhibition potency toward hAChE (**14c** IC₅₀ 7.03 nM) and hBChE (**14c** IC₅₀ 331 nM), although their inhibitory activities (except **14c**) were slightly lower than that of 6-chlorotacrine (hAChE IC₅₀ 8.32 nM and hBChE IC₅₀ 916 nM). The length of the linker and the position of the amino group had an impact on potency. Due to the inhibition of the PAS of AChE, inhibitors diminished the AChE-induced Aβ-40 aggregation (**14i**, 6-chlorotacrine—45.9% and 8.5% of inhibition, respectively, at the concentration of 100 μM). They might inhibit self-induced Aβ aggregation (**14i**, 6-chlorotacrine—49.1%, 7.1% of inhibition, respectively, at the concentration of 50 μM) and β-secretase activity (**14j**, 6-chlorotacrine—77.8%, not active, respectively, at the concentration of 2.5 μM) (Camps et al. 2009).

Compound	n	X	Y	Compound	n	X	Y
15a	3	N	S	15e	3	N	O
15b	4	N	S	15f	4	N	O
15c	5	N	S	15g	3	C	O
15d	6	N	S	15h	4	C	O

Fig. 8 Compounds **15a–h**

Fig. 9 Flurbiprofen **16** and compounds **17a–e**

Tacrine–phenylbenzoheterocycle hybrids

Huang et al. designed phenylbenzoheterocycle–THA hybrids **15a–h** (Fig. 8) connected through the carbon spacers. All the compounds presented inhibitory activity toward eeAChE and equine serum BChE (esBChE). Almost all of them showed higher potency toward eeAChE than THA (IC₅₀ 0.311 μM). The most potent inhibitor was **15b** with 4-carbon linker (eeAChE IC₅₀ 0.017 μM and esBChE IC₅₀ 0.122 μM). However, various lengths of the linker did not have an impact on the inhibitory activity. The structure of the benzoheterocycle affected the inhibitor potency. The activity of benzoheterocycle has changed in direction indole > benzothiazole > benzofuran > benzoxazole. This trend was observed with AChEI, not with butyrylcholinesterase inhibitors. Compounds demonstrated Aβ aggregation inhibitory activity (**15b**, **15f**, **15h**, curcumin—51.8%, 57.6%, 62.8%, and 18.7% of the inhibition at the concentration of 20 μM, THA not tested). The molecular modeling study presented that the inhibitors interacted with the PAS (benzothiazole moiety), the CAS (THA moiety), and the gorge of the AChE (Huang et al. 2012).

Tacrine–flurbiprofen hybrids

Flurbiprofen **16** (Fig. 9) is a non-steroidal antiinflammatory drug (NSAID). Moreover, it was identified as a compound which lowers the production of Aβ40/42 due to its allosteric modulation of presenilin-1 (Eriksen et al. 2003).

Chen et al. synthesized a series of THA–flurbiprofen hybrids **17a–e** (Fig. 9) connected by the alkylenediamine linker. Among all hybrids, **17d**, the longest linker chain, presented the best inhibitory activity toward eeAChE (IC₅₀ 19.3 nM, IC₅₀ BChE 3.7 nM). Its result was better than those of THA (AChE IC₅₀ 61.7 nM and BChE IC₅₀ 9.0 nM). **17d** demonstrated its binding to the CAS through THA moiety and interaction with the PAS of AChE through the benzene ring of flurbiprofen. Moreover, **17d** was tested in the Aβ

inhibition assay (31% reduction of A β formation at the concentration of 0.25 μ M) and could inhibit the formation of A β due to its ability to inhibit presenilin (Chen et al. 2013).

Tacrine–levetiracetam hybrids

Levetiracetam **18** (Fig. 10) is an antiepileptic drug which improves memory performance in patients with amnesic mild cognitive impairment and also in hAPPJ20 or APP/PS1 mouse models of AD (Sanchez et al. 2012; Shi et al. 2013).

Sola et al. synthesized heptamethylene-linked levetiracetam–huprine and levetiracetam–6-chlorotacrine hybrids **19a–b**, **20** (Fig. 10). The hybrids showed inhibitory potency against human recombinant AChE in nanomolar range (**19a** IC₅₀ 3.1 nM, **19a** hBChE IC₅₀ 135 nM, THA hAChE IC₅₀ 317 nM, and 6-chlorotacrine hAChE IC₅₀ 5.9 nM). Inhibition of A β 42 was tested in the inhibition assay at the

concentration of 20 μ M. **20** had the best inhibition—36.4%, where THA was below 10%. The C57BL6J mice that were treated with **20**, a dose of 5 mg/kg, provide (based on results from acute toxicity study) a 43% inhibition of mouse brain AChE after 10 min of administration. A β burden was reduced in APP/PS1 mice's brains which treated with **20** (A β burden—below 0.6% area; THA—0.8% area) (Sola et al. 2015).

Tacrine–multialkoxybenzene hybrids

Zhang et al. evaluated novel series of benzoates (or phenylacetates or cinnamates)–THA hybrids **21a–n**, **22** (Fig. 11). All inhibitors showed IC₅₀ values in nanomolar range when the strongest hybrid was the compound **21c** with a trimethoxyphenylacetate group (eeAChE IC₅₀ 5.63 nM, esBChE IC₅₀ 364 nM, THA eeAChE IC₅₀

Fig. 10 Levetiracetam **18** and compounds **19a,b** and **20**

Fig. 11 Compounds **21a–n** and **22**

73.36 nM, and esBChE IC₅₀ 14.45 nM). Compounds confirmed the inhibitory activity of A β aggregation. Compound **21j** with a hydroxybenzoate group exhibited the best inhibition of A β aggregation with IC₅₀ 17.36 nM (**21c** IC₅₀ 51.81 nM and THA IC₅₀ 12.21 nM). The spacer length between THA and phenyl moiety as well as the electron density on aromatic ring that was connected with THA through the acetyl group were significant factors for AChE inhibition but lower important for A β self-aggregation. Molecular modeling study and Lineweaver–Burk plot presented that **21c** targeted both the CAS and PAS of ChEs simultaneously (Zhang et al. 2016).

Fig. 12 Compounds **23a–f**

Compound	n	R	R ¹	Compound	n	R	R ¹
25a	5	H	H	25h	5	H	OMe
25b	6	H	H	25i	6	H	OMe
25c	5	6-Cl	H	25j	5	6-Cl	OMe
25d	6	6-Cl	H	25k	6	6-Cl	OMe
25e	5	6-Cl	H	25l	5	6,8-diCl	OMe
25f	5	6,8-diCl	H	25m	5	H	OH
25g	6	6,8-diCl	H				

Fig. 13 Melatonin **24** and compounds **25a–m**

Tacrine–fluorobenzoic acid hybrids

Czarnecka et al. evaluated a novel series of THA–fluorobenzoic acid hybrids **23a–f** (Fig. 12). The compounds with butyl linker had higher activity than others with ethyl or propyl tether. Among them, compound **23c** showed the highest activity toward eeAChE IC₅₀ 41.37 nM and esBChE IC₅₀ 1.39 nM, higher than the reference compound THA (eeAChE IC₅₀ 80.82 nM and esBChE IC₅₀ 19.55 nM). Kinetic characterization of AChE inhibition revealed mixed-type inhibitor behavior of compound **23c**. In the A β inhibition assay, compound **23c** confirmed its inhibitory activity with 77.32% inhibition of A β aggregation at the concentration of 50 μ M. In the molecular modeling study, it was confirmed that THA bound to the PAS of AChE and the fluorobenzoic moiety bound to the CAS of AChE (Czarnecka et al. 2017).

Tacrine derivatives with cholinesterase inhibition and antioxidant properties

Tacrine–melatonin hybrids

Melatonin **24** (Fig. 13) is a neurohormone which takes part in circadian rhythms of many physiological functions, e.g., sleep timing (Brzezinski 1997). It has antioxidant activity and is able to scavenge of ROS (Reiter 1998).

Rodriguez-Franco et al. evaluated novel melatonin–THA hybrids **25a–m** (Fig. 13). Hybrids presented inhibitory activity of IC₅₀ from subnanomolar to picomolar range. The addition of chlorine atom into the position 6 of the THA moiety

improved the inhibitory activity. The most potent compound toward hAChE was **25g** (IC_{50} of 8 pM, hBChE IC_{50} 7.8 nM, THA hAChE IC_{50} 350 nM, and hBChE IC_{50} 40 nM). Their oxygen radical absorbance capacity was higher than that of melatonin. **25e** was four times, when **25g** was 2.5 times more active than trolox (Rodriguez-Franco et al. 2006).

Tacrine–mefenamic acid hybrids

Mefenamic acid **26** (Fig. 14) is a non-steroidal antiinflammatory drugs. Apart from its ability of cyclooxygenase inhibition, it interacts with AChE and decreases the occurrence of free radicals and A β -induced neurotoxicity (Joo et al. 2006).

Bornstein et al. evaluated novel series of THA and 6-chlorotacrine–mefenamic acid hybrids **27a–t**, **28a–t**, **29a–f**, **30a–f** (Fig. 14). Hybrids were non-competitive or mixed-type inhibitors of AChE. Hybrids interacted with AChE by PAS (mefenamic acid), CAS (THA moiety), and spanning the active-site gorge through a methylene-based linker according to the molecular modeling study. Most of the hybrids presented higher inhibitory activity toward eeAChE AChE than THA (IC_{50} 52.4 nM). Among the series of compounds (**29a–f**, **30a–f**), hybrid **30c** (IC_{50} AChE 0.495 nM) possessed the highest inhibitory activity towards AChE. Among the series of compounds **27a–t**, **28a–t**, the hybrids with 6-chlorotacrine moieties showed higher inhibitory activity than non-halogen counterparts (**28m** IC_{50} AChE 0.418 nM). Due to the fact that mefenamic acid has anti-inflammatory activity, the ROS inhibition assay was performed. All compounds presented good inhibitory activity, where hybrid **28m** had the best inhibition toward ROS (IC_{50} 0.009 nM and THA IC_{50} 183 nM) (Bornstein et al. 2011).

Tacrine–(hydroxybenzoyl-pyridone) hybrids

Chand et al. tested novel THA–(hydroxybenzoyl-pyridone) hybrids **31a–c** (Fig. 15). The molecular modeling study was performed on eeAChE (BChE was not tested). The THA moiety interacted with the bottom of the gorge of the enzyme, binding to the CAS by π – π interaction with the

Compound	n	R ¹	R ²
31a	1	OMe	H
31b	1	H	OMe
31c	2	OH	H

Fig. 15 Compounds **31a–c**

aromatic ring of Trp84 and Phe330. The benzoyl ring of inhibitors placed at the entrance of the gorge was bound with Tyr70 and Trp279 of the PAS. It was proved that compound with an alkyl spacer of 4 methylene carbon atoms established stronger interaction with Asp275 residue than compound with two methylene carbon atoms in the linker. The eeAChE inhibitory activity test confirmed that compound **31c** was the strongest inhibitor (IC_{50} 0.57 μ M) among others, but slightly weaker than THA (IC_{50} 0.31 μ M). The DPPH test showed that radical scavenging capacity of novel inhibitors was better (EC_{50} 204–249 μ M) than that of THA (EC_{50} > 1000 μ M) (Chand et al. 2016).

Tacrine–1,2,3-triazole hybrids

Najafi et al. evaluated a new series of THA–1,2,3-triazole hybrids **32a–o** (Fig. 16). Almost all compounds showed good inhibitory activity toward eeAChE and esBChE. Compound **32l**, which had chlorine in 7-position of the acridine moiety and 4-methoxyphenyl connected to 1,2,3-triazole ring, was the most active inhibitor toward eeAChE— IC_{50} 0.521 μ M and esBChE IC_{50} 1.853 μ M; however, it was weaker than a

Fig. 14 Mefenamic acid **26** and compounds **27a–t**, **28a–t**, **29a–f**, **30a–f**

Compound	R ¹	R ²	R ³	Compound	R ¹	R ²	R ³
32a	H	H	H	32j	H	H	4-OMe
32b	Cl	H	H	32k	Cl	H	4-OMe
32c	H	Cl	H	32l	H	Cl	4-OMe
32d	H	H	4-Cl	32m	H	H	4-F
32e	Cl	H	4-Cl	32n	Cl	H	4-F
32f	H	Cl	4-Cl	32o	H	Cl	4-F
32g	H	H	4-Me				
32h	Cl	H	4-Me				
32i	H	Cl	4-Me				

Fig. 16 Compounds **32a–o**

reference drug, THA (eeAChE IC₅₀ 0.048 μ M and esBChE IC₅₀ 0.010 μ M). Compound **32k** with chlorine in 6-position of the acridine ring and 4-methoxyphenyl connected to 1,2,3-triazole moiety showed less eeAChE inhibitory activity (IC₅₀ 0.737 μ M) in comparison with the compound **32l**. Molecular modeling and kinetic studies presented that compounds **32l** and **32j** bound both to the PAS and CAS of the AChE and BChE. The tested compounds **32d**, **32j**, and **32l** showed no important antioxidant activities in the DPPH test. The compound **32l** showed neuroprotective activity at 50 and 100 μ M (cell viability 73.45% and 74.46%, respectively) against H₂O₂-induced cell death in PC12 neurons (Najafi et al. 2017).

Tacrine derivatives with cholinesterase inhibition, antiaggregation, and antioxidant properties

Tacrine–carbazole hybrids

Carvedilol **33** (Fig. 17) is a vasodilating blocker with antioxidant properties. It is used in the treatment of mild-to-moderate hypertension. Carvedilol also has a modulatory action on the NMDA receptors as a low-affinity antagonist. Its carbazole moiety has antioxidant properties and may act as an inhibitor of A β fibril formation (Howlett et al. 1999; Lysko et al. 1998).

Rosini et al. designed and investigated novel carvedilol–THA hybrids **34a–d** (Fig. 17). Compounds presented their inhibitory activity toward hAChE in the nanomolar range (compound **34c** IC₅₀ 1.54 nM and hBChE IC₅₀ 189 nM) and their potency was higher than that of THA (hAChE IC₅₀ 424 nM). Hybrids were able to bind to the CAS of AChE by tetrahydroacridine moiety and PAS through carbazole moiety as to the molecular modeling study. The compounds were also able to inhibit A β self- and AChE aggregation (**34c**, THA 63.2% and 7% at 100 μ M concentration; 25.5% and 5% at 10 μ M concentration, respectively) and antagonized NMDA receptors. The compound **34a** was capable of neuronal cell protection against ROS (IC₅₀ 23 μ M; trolox IC₅₀ 49.55 μ M) caused by oxidative stress (Rosini et al. 2008).

Tacrine–nimodipine hybrids

1,4-Dihydropyridines selectively inhibit L-type voltage-dependent Ca²⁺ channels (VDCC). Nimodipine **35** (Fig. 18) belongs to the VDCC group; therefore, it may protect cells from neuronal death caused by Ca²⁺ overload and focal cerebral ischemia (Cano-Abad et al. 2001; Sobrado et al. 2003). Calcium dysfunction is one of the factors which triggers the development of AD (Kruman et al. 1998; Mattson et al. 1992).

Fig. 17 Carvedilol **33** and compounds **34a–d**

Fig. 18 Nimodipine **35** and compounds **36a–n**

Marco-Contelles et al. evaluated novel hybrids based on THA and nimodipine called tacripyrines **36a–n** (Fig. 18). Compounds possessed inhibitory activity toward hAChE and hBChE. Half of them presented higher potency toward AChE than THA, although their inhibitory activity for BChE was rather weak. The best inhibitors were **36h** (hAChE IC₅₀ 71 nM; hBChE IC₅₀ > 100,000 nM), **36j,l** (hAChE IC₅₀ 58 nM and 45 nM, respectively, both hBChE IC₅₀ > 100,000 nM) compounds (THA hAChE IC₅₀ 147 nM and hBChE IC₅₀ 36 nM). Hybrids showed their potency to inhibit pro-aggregating action of AChE and self-aggregation of Aβ (the best was compound **36k**—30.7% of inhibition of hAChE-induced aggregation and 34.9% of inhibition of self-aggregation at the concentration of 50 μM). This compound was also investigated in molecular modeling tests. It showed binding to the PAS, but not to the CAS of AChE. Hybrids also showed higher efficiency against free radicals than THA and nimodipine (**36k**—55.78% of protection;

nimodipine—36.03%; THA—0%) (Marco-Contelles et al. 2009).

Tacrine-*o*-hydroxy- or *o*-aminobenzylamine hybrids

Mao et al. designed and synthesized *o*-aminobenzylamine–THA hybrids **37a–g**, **38a–e** and *o*-hydroxybenzylamine–(7-chlorotacrine) hybrids **37h–n** (Fig. 19). The longer the methylene linker, the better results could be obtained. Hybrids with two, three, or four carbon spacers had the lowest inhibitory activity. Regarding *o*-aminobenzylamine–THA hybrids, **37f** possessing an eight carbon linker had the highest potency (eeAChE IC₅₀ 6.17 nM; esBChE IC₅₀ 6.25 nM) **38c**, possessing a nine carbon spacer (eeAChE IC₅₀ 0.55 nM, hAChE IC₅₀ 3.52 nM, esBChE IC₅₀ 2.65 nM, THA eeAChE IC₅₀ 109 nM, and esBChE IC₅₀ 15.8 nM), which demonstrated the strongest inhibition activity toward eeAChE and hAChE. **38c** exhibited good

Fig. 19 Compounds **37a–n**, **38a–e**

inhibition of A β aggregation (39.4% of inhibition at the concentration of 20 μ M and curcumin 52.2% of inhibition). This inhibitor was also tested in molecular modeling study, and it occupied CAS, the mid-gorge sites, and PAS of AChE simultaneously. Most of them showed good antioxidative properties (by measuring the oxygen radical absorbance capacity of fluorescein); THA was not tested. The antioxidant activities diminished as the length of the carbon linker got longer. **43c** was a moderate antioxidant (1.22 of Trolox equivalents) (Mao et al. 2012).

Tacrine–2-aminobenzothiazole hybrids

The 2-aminobenzothiazole moiety appears in many biomolecules. Its derivatives strongly interact with A β and inhibit the peptide aggregation (Soto-Ortega et al. 2011). They play neuroprotective roles and may be used as brain-imaging agents in Alzheimer's disease (Alagille et al. 2011).

Keri et al. tested novel THA–2-aminobenzothiazole hybrids **39a–e** (Fig. 20). Hybrids showed strong inhibitory activities toward AChE with IC₅₀ values in the submicromolar-to-low-micromolar range, although slightly lower than THA. BChE inhibition was not determined. The geometry, length, and aromaticity of the X moiety-influenced activity. Compound **39a** demonstrated the best inhibitory activity (IC₅₀ 0.34 μ M and THA IC₅₀ 0.19 μ M) towards eeAChE. It contained the shortest linker and a simple phenyl group attached to the BTA moiety. The presence of a methylene group or linear aliphatic Y spacer resulted in the reduction of the inhibitory activity. All the compounds were able to inhibit A β self-aggregation and **39b** showed the strongest

inhibition of the process (61.3% at the concentration of 50 μ M; THA was not determined). β -Sheet secondary structure of the A β can interact with the 2-aminobenzothiazole moiety in **39b**. A 2-aminobenzothiazole moiety composed of bi-aryl heterocyclic scaffold interacted better with an abnormal β -sheet conformation of the A β and, thus, provided the inhibition of the fibrillogenesis. In the DDPH, all compounds showed comparable to THA antioxidative properties (EC₅₀ > 10,000 μ M). Compounds **39d** and **39e** appeared to protect neuronal SHSY-5Y cells from the cell death induced by A β . The molecular modeling study presented that hybrids interacted with the CAS (THA moiety) and PAS (2-aminobenzothiazole moiety) of the AChE (Keri et al. 2013).

Tacrine–(β -carboline) hybrids

β -Carbolines are derived from the biogenic amines tryptamine and serotonin by condensation with aldehydes or R-ketoacids. They are potent and specific inhibitors of dual-specificity tyrosine phosphorylation-regulated kinase 1A; moreover, they are proved to inhibit AChE and the phosphorylation of tau protein on multiple sites associated with tau pathology in AD (Adayev et al. 2011; Frost et al. 2011).

Lan et al. evaluated a novel series of THA–(β -carboline) hybrids **40a–q** (Fig. 21). The inhibitor **40c** with 6 carbon atoms in the linker had the highest inhibitory activity toward eeAChE—IC₅₀ 40.6 nM and esBChE IC₅₀ 93.6 nM. Then, the series of hybrids with various moieties (modified β -carboline ring) but with the same length of tether were synthesized. Among them, the highest inhibitory activity toward eeAChE had **40l** (IC₅₀ 21.6 nM and esBChE IC₅₀ 39.8 nM) with tetrahydro- β -carboline moiety when THA eeAChE IC₅₀ 260 nM and esBChE IC₅₀ 50.5 nM. The most potent inhibitory activity toward hAChE also had inhibitor **40l** (IC₅₀ 63.2 nM), seven times stronger than THA (IC₅₀ 467 nM). In the kinetic study of AChE inhibition, compound **40l** was a mixed-type inhibitor. Molecular modeling study showed that THA moiety of **40l** bound to the CAS of AChE and the tetrahydro- β -carboline moiety interacted the PAS. Moreover, compound **40l** had good inhibition of A β aggregation (65.8% at 20 μ M; curcumin—42.3% of inhibition). In the ABTS antioxidant assay, **40l** showed the most potent antioxidant activity (1.57 trolox equivalent; curcumin—1.83). In the neuroprotection assay, **40l** provided cell viability of 73.67% at the concentration of 10 μ M (Lan et al. 2014).

Tacrine hybrids with natural-based cysteine

Keri et al. tested novel hybrids with neuroprotective and antioxidant roles, hybrids conjugating THA, and 6-chloro-tacrine with S-allylcysteine **41a–l** (Fig. 22). The IC₅₀ values were ranged in low-micromolar (THA derivatives) and

Fig. 20 Compounds **39a–e**

Fig. 21 Compounds 40a–q

Fig. 22 Compounds 41a–l

high-nanomolar (6-chlorotacrine derivatives). 6-Chlorine atom filled the hydrophobic pocket of enzyme better and provided higher inhibitory potency. **41d** showed the highest inhibitory activity toward *TcAChE*— IC_{50} 0.3 μ M and *THA* IC_{50} 0.19 μ M. The molecular modeling study presented that the spacer with three carbon atoms in the alkylene chain turned out to bind better with both the CAS and PAS of *AChE*. Regarding two groups of inhibitors, the length of the spacer and the cysteine moiety had only a little effect. The 6-chlorotacrine hybrids possessed the best inhibition with two carbon atoms in the linker, independently of the cysteine substituent group, allyl (**41d** *TcAChE* IC_{50} 0.30 μ M) or propargyl (**41j**, *TcAChE* IC_{50} 0.56 μ M). Similarly, for the non-substituted *THA* hybrids, the one with three (**41b** *TcAChE* IC_{50} 0.88 μ M) or four (**41i** *TcAChE* IC_{50} 1.21 μ M) carbon atoms, respectively, had the highest inhibitory activity. Hybrids decreased A β 42 self-aggregation in the range of 8.5–14.7% at concentration 40 μ M (**41e**—14.7%,

THA not tested). In the DDPH test, the antioxidant activity of hybrids was better than of *THA* (**41g**, *THA* EC_{50} 238 μ M, > 1000 μ M, respectively) (Keri et al. 2016).

Tacrine derivatives with cholinesterase inhibition, and antioxidant and non-hepatotoxicity properties

Tacrine–ebselen hybrids

Ebselen **42** (Fig. 23) is an organoselenium compound with antiinflammatory properties, antioxidant activity, and neuro-protective effects in preclinical studies (Schewe 1995; Yamagata et al. 2008). In vivo studies revealed that selenium-deficient diet in Tg2576 transgenic mice increased the total

Fig. 23 Ebselen **42** and compounds **43a–i**

area of A β plaques in comparison to mice which were put on a selenium diet (Haratake et al. 2013).

Mao et al. evaluated novel THA–ebselenium hybrids **43a–i** (Fig. 23). Compound **43i** (AChE IC₅₀ 2.55 nM and BChE IC₅₀ 2.80 nM) with 5-carbon linker presented the highest inhibitory activity toward eeAChE and esBChE when THA IC₅₀ was 109 nM and 15.8 nM. Hybrids with shorter carbon spacer were less potent AChE and BChE inhibitors. Hybrid **43i** was a mixed-type inhibitor of AChE. It interacted with the both CAS and PAS of AChE regarding to the molecular modeling study. Compounds were evaluated for their antioxidant activity. Compound **43e** and **43i** possessed similar hydrogen peroxide scavenging activity as ebselen (about 75%) at the concentration of 100 μ M when

THA had almost no activity at this concentration. In the hepatotoxicity assay (on human hepatic stellate cells), hybrid **43i** at the concentration of 10 and 5 μ M provided higher cell viability than THA (110–100%; THA—90%) (Mao et al. 2013).

Tacrine–trolox hybrids

The best-known natural antioxidant is vitamin E. It has capacity to absorb reactive oxygen species. To achieve a better absorption, there was synthesized a water-soluble analogue of vitamin E, trolox **44** (Fig. 24) (Scott et al. 1974). Trolox is known as a compound that protects cells against

Fig. 24 Trolox **44**, compounds **45a–u**, and compounds **46a–g**

oxidative stress (Quintanilla et al. 2005; Thiramatrakul et al. 2014).

Nepovimova et al. synthesized novel THA–trolox hybrids **45a–u** (Fig. 24). Hybrids were tested for their inhibitory activity towards human recombinant AChE. The inhibitors block enzyme in micro and submicromolar ranges (from hAChE IC_{50} 13.29 to 0.08 μ M, from hBChE IC_{50} 1.30 to 0.02 μ M, THA hAChE IC_{50} 0.32 μ M, hBChE IC_{50} 0.08 μ M, and trolox did not inhibit AChE). **45p**, **45t**, and **45u** presented the best inhibitory activity (hAChE IC_{50} 0.08 μ M, hBChE IC_{50} 0.77, 0.70, 0.54 μ M, respectively). Hybrids were divided into three groups, differing in the substitution on THA heterocycle. For the 7-methoxytacrine derivatives (**45a–g**), the IC_{50} values ranged in micromolar scale and were more potent (except **45d**) than prototype, 7-methoxytacrine. Non-substituted hybrids provided better results than the first group. In the third group, the addition of chlorine atom into tetrahydroacridine scaffold led to increased inhibitory activity. None of novel inhibitors showed a better potency than 6-chlorotacrine. The best inhibitor, **45u** (with 6-chlorotacrine, 8-carbon linker, and trolox moiety), presented the mixed-type inhibition and bound to the AChE in inverse way in comparison with the other dual-binding-site inhibitors of AChE—the trolox moiety interacted with the CAS, whereas THA binds to the PAS of the enzyme. In DDPH, **45u** displayed good antioxidant activity (EC_{50} 44.09 μ M), however, slightly lower than trolox (EC_{50} 16.20). Only compounds **45h** and **45u** showed Pe values above the limits (Pe 4.4×10^{-6} cm s $^{-1}$, 5×10^{-6} cm s $^{-1}$, respectively), which indicated that they could cross the BBB. The cytotoxicity effect on HepG2 cell line was investigated. Derivative **45u** showed nontoxic properties at concentration 0.5 μ M, when IC_{50} of THA was 19.37 μ M and that of 6-chlorotacrine—7.13 μ M. The metabolic assay was carried out to explain the missing in vitro hepatotoxic effect of **45u**. This compound did not convert into toxic hydroxylated derivative of THA; more than 85% remained unchanged after 1 h of incubation in human liver microsomes system. In the in vivo study, **45u** was evaluated for its potential acute toxicity properties and LD $_{50}$ value. Conversely to the in vitro study, **45u** had LD $_{50}$ value above 500 mg/kg when 6-chlorotacrine only 7.5 mg/kg. It can be caused due to the differences in metabolism and pharmacokinetics in animal body in comparison only to one type of cells in the in vitro studies (Nepovimova et al. 2015).

Xie et al. synthesized novel series of THA–trolox hybrids **46a–g** (Fig. 24). All compounds showed AChE inhibitory activity in nanomolar range. Compound **46d** possesses the highest inhibitory activity towards hAChE (IC_{50} 23.5 nM and THA IC_{50} 435.1 nM) and hBChE (IC_{50} 20.5 nM and THA IC_{50} 23.2 nM). In the DDPH assay, hybrids showed that they were strong antioxidants (**46d** IC_{50} 48.7 μ M), similar to trolox (IC_{50} 35.6 μ M). Kinetic studies and molecular

modeling provided information about the mixed-type inhibition by **46d**. It was proved that **46d** could easily cross the BBB (8.87×10^{-6} cm s $^{-1}$). The hepatotoxicity test was performed in rats. Animals were treated with the highest tolerated dose of THA or equimolar dose of **46d**. **46d** did not change the level of liver enzymes (ALAT and ASPAT) and was comparable to control; in result, it had a little hepatotoxicity and was less toxic than THA (Xie et al. 2015).

Conclusions

The research for an effective drug against AD is still ongoing. The current treatment is only symptomatic and cannot stop the progression of disease. The promising way concerns MTDLs based on THA moiety. The THA modification leads to improve its chemical properties. In our comprehensive review, compounds are divided into the groups: THA derivatives with cholinesterase inhibition; with cholinesterase inhibition and A β antiaggregation properties; with cholinesterase inhibition and antioxidant properties; with cholinesterase inhibition, antiaggregation, and antioxidant properties; with cholinesterase inhibition, antioxidant and non-hepatotoxicity, properties; with cholinesterase inhibition, A β antiaggregation, and non-hepatotoxicity properties. To summarize: (1) THA moiety is important structure in MTDLs' development. It mostly binds to CAS; however, for example in THA–trolox hybrids, THA moiety can bind also to PAS. It is possible to obtain dual-binding site hybrids, which can have good inhibitory activity. (2) The length of the spacer influences the inhibitory activity. The highest inhibition is found for hybrids with 5–9-carbon linker. (3) The substitution at 6 or 6 and 8 positions of THA ring by chlorine leads to increase inhibitory activity towards AChE. (4) The most effective inhibitors were: THA–mefenamic acid hybrid (IC_{50} 0.495 nM with 8-carbon linker and 6-chlorotacrine moiety); THA–gallamine hybrids (IC_{50} 0.467 nM with 5-carbon linker and THA moiety); THA–donepezil hybrids (IC_{50} 0.27 nM with 3-carbon linker and 6-chlorotacrine moiety); THA–indole heterodimers (IC_{50} 0.02 nM with 6-carbon linker and 6-chlorotacrine moiety); THA–o-aminobenzylamine hybrids (IC_{50} 0.55 nM with 9-carbon linker and THA moiety); THA–benzofuran hybrids (IC_{50} 0.86 nM with 7-carbon linker and THA moiety); THA–melatonin hybrids (IC_{50} 8 pM with 6-carbon linker and 6,8-dichlorotacrine moiety). (5) Linking many activities of drugs together has led to the development of MTDLs. Novel hybrids possessed many properties such as: inhibition of A β aggregation, low cytotoxicity effect on different cell lines, low hepatotoxic properties, reduction of oxidative stress, and increase in the BBB permeability.

While talking only about advantages of MTDLs therapy, it also faces some problems. Novel hybrids might possess

too high-molecular-weight mass due to two or three connected smaller moieties. Therefore, hybrids may violate Lipinski's rule of 5 and present low CNS penetration. For novel compounds, it is always crucial to obtain good results in ADMET calculations and later in the *in vivo* pharmacokinetic studies. It is also important to notice the reason for the different concentrations of inhibitor in *in vitro* assay. In the AChE-A β inhibitions assays, researchers use higher concentrations of AChE, A β , and inhibitor than those present in human brain. The high level of AChE concentration is essential to accelerate the aggregation process of A β . It is important for analytical purposes. The inhibitor/AChE concentration ratio from AChE inhibition assay and from A β -inhibition test should be compared. The obtained values should be of the same magnitude, which can indicate that high (in μM) and low (in nM) concentrations of inhibitor give both inhibitory activities (Marco-Contelles et al. 2009). Similar situation is observed in comparison between *in vivo* and *in vitro* tests. Both assays may differ due to the differences in pharmacokinetics and metabolism, where also biotransformation is an important process, for example, in toxicity assays. Therefore, concentration of drugs used in *in vitro* and *in vivo* assays may vary—for example, like for 7-MEOTA 7, which has some toxicity for the cell in the *in vitro* assays, but does not show acute or chronic hepatotoxicity in the *in vivo* assays (Nepovimova et al. 2015). Moreover, in the *in vivo* assays, it is important to assess LD_{50} values of novel inhibitors before performing another test. It is crucial to obtain concentration that is general not toxic for animals and will enable to perform *in vivo* tests.

The last drug against AD, which was approved by the FDA in 2003, was memantine. Since 2003, over 200 compounds reached Phase II, but only 50 went to the Phase III. However, none of them have passed so far Phase III. It might be caused by wrong strategy, inadequate doses of novel drugs, lack of therapeutic efficacy, the serious side effects or just lack of diagnostic criteria, and markers of the disease which could set the clinical endpoints and efficacy standards. Clinical trials are conducted at late stages of AD, when therapeutic drugs are aimed at the early pathological processes. For example, anti-A β agents should be tested at first stages of disease. Moreover, the dosing needs to be more aggressive due to the large burden of pathological changes in the brain even in mild stage of AD. Some clinical trials failures have proper explanations. For example, solanezumab slowing (in around 15%) of cognitive decline, but more likely binds to monomers than to A β oligomers (more toxic). It caused a small yield of clinical benefit. Dimebon and R-flurbiprofen did not provide good pharmacokinetics and convincing efficacy. Currently, in III Phase are a few types of novel drugs: amyloid antibodies (anti-A β oligomers agents)—crenezumab, aducanumab and gantenerumab, acetylcholinesterase inhibitor—octohydroaminoacridine succinate, the combination of

donepezil and choline alphoscerate, and the MTDLs, such as AVP-786 (NMDA antagonist and P-glycoprotein inhibitor) and TRx-00237 (inhibitor of monoamine oxidase, nitroxide production, and Tau aggregation) (Bachurin et al. 2017; Hung and Fu 2017; Stower 2018). Many novel compounds have being excessively tested in clinical trials (in 2016, anti-A β drugs were investigated in the largest number). So far, A β is considered as the best target for AD treatment. The A β hypothesis is acknowledged and inspires researchers to develop new drugs. Numerous compounds affect A β pathology pathway and might reduce effects (such as oxidative stress) which is caused by A β . These compounds focus on reducing production of A β (beta and gamma secretase inhibitors), increasing clearance (monoclonal antibodies, which bind to soluble A β or senile plaques) and preventing A β aggregation. Among described hybrids, almost all were tested against A β aggregation and showed their inhibitory properties. Therefore, MTDLs are highly promising compounds for treating AD. They might act against A β and toxicity which A β caused. Nevertheless, better understanding the pathophysiology of AD will be important to obtain compounds with advantageous properties and finally to combat the disease.

Original research on novel tacrine derivatives has been still carrying out. Since the original drafting of our review, a few important publications have appeared: Cen et al. 2018; Chioua et al. 2018; Hamulakova et al. 2018; Hepnarova et al. 2018; Hiremathad et al. 2018; Jiang et al. 2018; Li et al. 2018; Liu et al. 2018; Skibiński et al. 2017; Wu et al. 2018.

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