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Invited review

Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease

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A B S T R A C T

Alzheimer's disease (AD), the most common cause of adult-onset dementia is characterized by a progressive decline of cognitive functions accompanied by behavioral manifestations. The main class of drugs currently used for the treatment of AD are acetylcholinesterase/cholinesterase inhibitors (ChE-Is). The first ChE-I licensed for symptomatic treatment of AD was tacrine. The ChE-Is currently available in the market are donepezil, rivastigmine and galantamine as tacrine is no longer in use, due to its hepatotoxicity. According to mechanism of action the ChE-Is are classified as short-acting or reversible agents such as tacrine, donepezil, and galantamine, as intermediate-acting or pseudo-irreversible agent such as rivastigmine. Overall, the efficacy of the three ChE-Is available in the market is similar and the benefit of administration of these compounds is mild and may not be clinically significant. Due to gastrointestinal side effects of these drugs, medicinal chemistry and pharmaceutical delivery studies have investigated solutions to improve the pharmacological activity of these compounds. In spite of the limited activity of ChE-Is, waiting for more effective approaches, these drugs still represent a pharmacotherapeutic resource for the treatment of AD. Other approaches in which ChE-Is were investigated is in their use in combination with other classes of drugs such as cholinergic precursors, *N*-methyl-*d*-aspartate (NMDA) receptor antagonists and antioxidant agents. After many years from the introduction in therapy of ChE-Is, the combination with other classes of drugs may represent the chance for a renewed interest of ChE-Is in the treatment of adult-onset dementia disorders.

1. Introduction

The equilibrium of different neurotransmitters systems such as acetylcholine (ACh), noradrenaline, dopamine, gamma-aminobutyric acid, serotonin, and glutamate is essential for brain function (Watkins et al., 1994). Although the cholinergic system is not the only neurotransmitter system affected in adult-onset cognitive impairment, a deficient cholinergic neurotransmission function is involved in the pathophysiology of learning and memory impairment occurring in adult-onset dementia disorders including Alzheimer's disease (AD) (Amenta et al., 2001). Since early in the 70's a premature loss of basal forebrain cholinergic neurons was observed in the brain of AD patients, leading to the development of the cholinergic hypothesis of the geriatric memory dysfunction (Bartus et al., 1982). This hypothesis was supported by the neurochemical demonstration of a decrease of the ACh biosynthetic enzyme choline acetyltransferase (ChAT) in cognition-related brain areas such as the cerebral cortex and hippocampus in AD (Amenta et al., 2001).

The cholinergic neurotransmission plays a key role in impaired cognitive function in AD and in adult-onset dementia disorders. Treatments directed to counter amyloid-β accumulation, tau hyperphosphorylation, and immunotherapy were proposed, but failed to provide effects and therefore were discontinued in phase II or III clinical trials (Madav et al., 2019). At the present, the enhancement of the cholinergic neurotransmission still represents a main approach in the symptomatic treatment of cognitive and behavioral symptoms of mild and moderate stages AD. In line with this therapeutic strategy different molecules such as linopirdine, an agent increasing hippocampal ACh release, muscarinic ACh receptor agonists, such as xanomeline, and acetylcholinesterase (AChE) inhibitors like physostigmine and tacrine were used. ACh is hydrolytically degraded in the brain by two cholinesterases, AChE and butyrylcholinesterase (BuChE) (Nordberg et al., 2013). In the brain tissue of AD patients AChE is more abundant than BuChE, which contributes to the degradation of ACh in the hippocampus and cerebral cortex (Nordberg et al., 2013). It has been shown that AChE activity is reduced by 67% compared to the normal levels in the temporal lobe and hippocampus during the progression of AD, whereas an increase of the BuChE activity up to 165% of the normal levels is noticeable. Moreover, low levels of BuChE activity in the medial temporal cortex were related to slow cognitive decline (Perry et al., 2003).

At the beginning therapeutic strategies for enhancing impaired cholinergic neurotransmission were centered on the identification of inhibitors of AChE. Subsequent studies have identified the relevance of both AChE and BuChE in the pathophysiology of AD and have

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established the therapeutic interest of the inhibition of both AChE and BuChE (Lane et al., 2006). These studies have contributed to introducing the inhibition of as a therapeutic strategy in the treatment of AD. AChE and BuChE (cholinesterase) inhibitors (ChE-Is) prevent the degradation of the neurotransmitter by increasing the levels of brain ACh and therefore enhancing the deficient brain cholinergic neurotransmission. ChE-Is were the first drugs authorized in the US and in Europe for the specific indication for symptomatic treatment of AD. ChE-Is are classified as nonspecific when they inhibit AChE, BuChE, and other cholinesterases and specific when they inhibit AChE only. These drugs can be also classified as reversible, pseudo-irreversible, or irreversible based on the degree of enzyme inhibition (Giacobini, 1998).

The tetrahydroacridine derivative tacrine (Cognex®), a reversible inhibitor of both AChE and BuChE (Heilbronn, 1961). This compound showed positive effects on memory function in young and aged normal subjects and was the first molecule to enter in clinical trials for AD treatment (Fig. 1). Studies with tacrine started in 1984, but the drug was approved by the US Food and Drug Administration (FDA) for symptoms of AD and related dementias in 1993 (Watkins et al., 1994). Tacrine was withdrawn from the market in 2013 due to its hepatotoxicity. Tacrine is a nonspecific ChE-I featuring variable absorption, extensive distribution and central nervous system penetration. In spite of the potential interest of tacrine, its efficacy for symptoms of dementia remains controversial (Amenta et al., 2001).

In the mid-1970s physostigmine, a new ChE-I was developed (Fig. 1). Some studies demonstrated that these drugs provided temporarily modest improvement in symptoms of AD and stabilized or slowed for some time the decline of cognitive function and functional ability (van Dyck et al., 2000). Donepezil, a new AChE inhibitor structurally different from the above-mentioned compounds and derived from indanone was developed in 1983 by Sugimoto and co-workers at the Eisai Research Laboratory in Japan (Sugimoto et al., 2002) (Fig. 1). Donepezil was the second drug approved by FDA in 1996 for mild to moderate AD, and subsequently at the dose of 23 mg/day has received approval for moderate to severe AD in 2010 (English, 2012). Donepezil (Aricept®) is a highly selective reversible inhibitor of AchE acting centrally by increasing the bioavailability of ACh in the synaptic cleft (Szeto and Lewis, 2016).

In 2000, the FDA has approved the marketing of the oral formulation of rivastigmine (Exelon®) a pseudo irreversible carbamate-selective inhibitor of AchE and BuChE inhibitor for the treatment of mild-to-moderate AD and in 2006 for the treatment of mild-moderate Parkinson's dementia (Grossberg and Desai, 2003). It is a slowly reversible AchE and BuChE that is not metabolically metabolized in liver by the CYP-450 system. This property leads to fewer drug–drug interactions (Grossberg, 2003) (Fig. 1).

In the same year, Galantamine (Razadyne®), a selective reversible inhibitor of AchE and allosteric modulator of nicotinic cholinergic receptors, was introduced to the United States for the symptomatic treatment for AD (Fig. 1). Galantamine obtained authorization for being introduced in Swedish pharmaceutical market in 2000 (Amenta et al., 2001; Coelho and Birks, 2001). It increases ACh levels at the synapse improving cholinergic tone (Olin and Schneide, 2006).

Several studies have demonstrated that Huperzine A, a new alkaloid derived from the Chinese herb Huperzia serrata, is a potent, reversible, selective inhibitor of AchE and NMDA receptor antagonist (Yang et al., 2013) (Fig. 1). Preclinical studies and clinical trials have shown the potential effect of Huperzine A in treating AD, but until now, there is not enough evidence for recommending clinical use of the compound (Li et al., 2008). At the present Huperzine A is used in some countries as a dietary supplement with a concentration of up to 200 mcg.

Since their introduction in the pharmaceutical market in 1993, ChEIs play a role in managing the symptoms and possibly slowing the rate of progression of AD. However, the clinical relevance of their use and safety are discussed. In view of this, we have analyzed the main medicinal chemistry, in preclinical and clinical studies of ChEIs licensed for the treatment of AD.

Among the drugs for the symptomatic treatment of AD, four ChE-Is (tacrine, donepezil, rivastigmine and galantamine) were licensed to control the key symptoms of AD, namely memory, and cognitive impairment. This paper will limit his analysis to these four molecules, although currently only three of them are present in the pharmaceutical market.

2. Medicinal chemistry

2.1. Tacrine and its derivatives

The positive effects of tacrine (1,2,3,4-tetrahydroacridin-9-amine) on strong AChE binding was mitigated by its toxicity profile (Wlodek et al., 1996), and many efforts were done after its withdrawn from the market to decrease its side effects and to synthesize new derivatives of it. This strategy has lead to the development of several tacrine hybrids such as homo/heterodimer or hybrids of two moieties known for their anti-AD properties (Saxena and Dubey, 2019; Soukup et al., 2013; Kozurkova et al., 2011). These compounds contain condensed aromatic

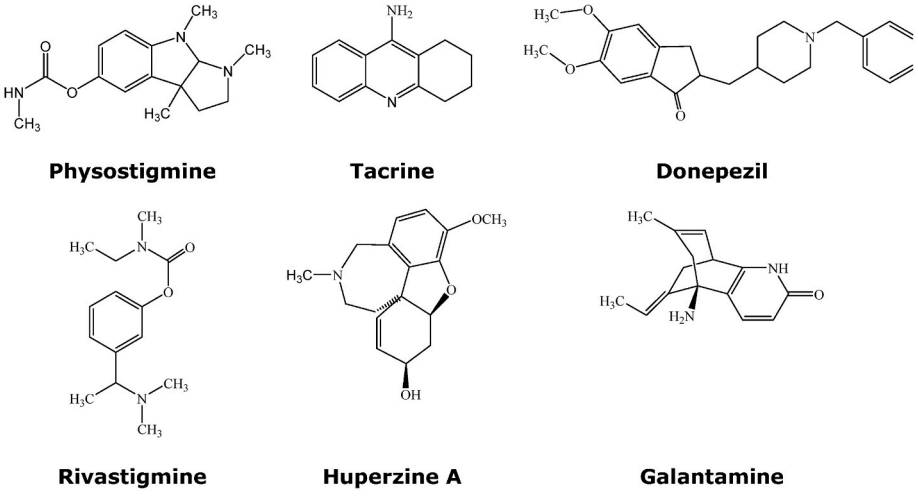


Fig. 1. Structure of tacrine, donepezil, rivastigmine, and galantamine.

cores and have quaternary ammonium or nitrogen included as a heteroatom. Most of the tacrine-based derivatives showed beneficial activities both *in vitro* and *in vivo* (Sameem et al., 2017; Lin et al., 2017; Ismaili et al., 2017) and their high ligand efficiency demonstrated that tacrine scaffold is an ideal starting point for designing and achieving potent and selective ligands.

One of the first successful attempts on the synthesis of potential AChE inhibitors was performed by the development of a series of bis-tacrine analogues linked by an alkylene chain. Some of these dimeric molecules showed a greater potency and selectivity towards AChE than tacrine (Fig. 2a) (Pang and Brimijoin, 1997). The most active of the series was the heptylene linked bis- (6-chloro)-tacrine with a potency 3000 times higher than tacrine in inhibiting AChE in the rat. Many tacrine derivatives with good ability to inhibit AChE were synthesized. A series of benzoates (or phenylacetates or cinnamates) - tacrine hybrids (Fig. 2b) attracted particular attention. The most active compound of this series besides to an excellent AChE inhibitory activity exhibited also an interesting capacity to prevent Aβ aggregation with an IC_{50} value of 51.81 nM (Zhang et al., 2016). Hybrids tacrine - benzofuran derivatives showed very good activity for AChE inhibition (sub-micromolar range) and good capacity to inhibit Aβ aggregation with an efficacy depending on the linker size and substituent groups of each main moiety (Fig. 2c) (Fancellu et al., 2020).

2.2. Donepezil and its analogues

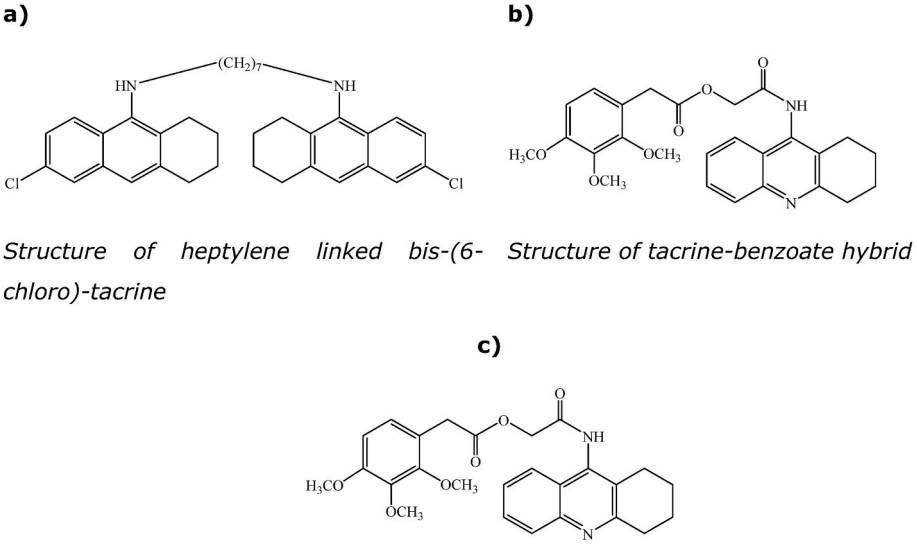
Donepezil (2-((1-Benzylpiperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one) possesses a high AChE inhibitory activity with an $IC_{50} = 5.7$ nM and a selective affinity of 1250 times greater for AChE than for BuChE. Many donepezil derivatives were synthesized conjoining donepezil with moiety like benzophenone, indanone, coumarin, 5,6-dimethoxy benzofuranone, benzylpyridinium-chalconoids and some of these are excellent AChE inhibitors (Alipour et al., 2014; Bautista-Aguilera et al., 2014; Samadi et al., 2013; Meng et al., 2012; Samadi et al., 2012; Bolea et al., 2011).

In the recent years, new theories dealing with the onset and AD progression based on Aβ and tau were proposed. These include, neurotoxic agents, oxidative stress, iron over load, and cholesterol levels in neuronal rafts triggering abnormal signaling cascades that promote tau hyper phosphorylation. Based on these new hypothesis, medicinal chemistry research was directed to the identification hybrid compounds able to treat AD by targeting the cholinergic neurotransmission, as AChE-Is, but at the same time capable to inhibit Aβ formation and deposition, and to decrease oxidative stress. Among donepezil derivatives, a series of 2-phenoxy-indan-1-one derivatives with an alkylamine side chain, which are able to targets AChE and BuChE and possess antioxidant activity was developed (Shen et al., 2008). The compound AP2238 showed an interesting ability to interact with AChE and, at the same time, to inhibit the pro-aggregation of Aβ (Piazzi et al., 2003). Few years later, a series of hybrid compounds containing a pharmacophoric fragment of donepezil and AP2238 were synthesized (Rizzo et al., 2010) (Fig. 3). Some of them displayed good anti-AChE activity and inhibited Aβ aggregation similarly to donepezil.

Starting from the observation that monoamine oxidases (MAOs), that catalyzing the oxidative deamination of monoamines, produce hydrogen peroxide implicated in the generation of oxygenated toxic radical species, a new class of compounds able to inhibit AChE and BuChE in nanomolar concentration and the MAO-A in the micromolar range was synthesized (Wu et al., 2017). The conjunction of donepezil with huprine leads to the synthesis of the hybrid AVCRI104P4 (Sola et al., 2015), which is a potential candidate for AD (Fig. 4). In fact, it advantageously displays inhibitory activities against AChE (low nanomolar range), BuChE, Aβ aggregation, and β-secretase BACE-1 (submicromolar or low micromolar range).

The combination of the *N*-benzyl-piperidine subunit of donepezil with the hydroxy-piperidine fragment of the AChE-I LASSBio-767 using an acylhydrazone linker lead to the synthesis of a new series of *N*-benzyl-piperidine-aryl-acylhydrazones hybrid derivatives (Fig. 5). The most active compound gave an AChE inhibition similar to donepezil, as well as an anti-inflammatory activity countering Aβ formation (Dias Viegas et al., 2018Dias et al., 2018; Fig. 5).

Recently, two new hybrids inclosing the indanone-piperidine moiety of donepezil and alpha-lipoic acid were synthesized (Terra et al., 2018; Amenta et al., 2018; Jacobson and Sabbagh, 2008). One of them showed interesting results since, even if it displayed a moderate inhibitory AChE activity with a more pronounced inhibitory activity on BuChE, showed a good antioxidant property more pronounced than alpha-lipoic acid itself (Terra et al., 2018).



Structure of heptylene linked bis-(6-chloro)-tacrine

Structure of tacrine-benzoate hybrid

Structure of the most active tacrine-benzofuran hybrid

Structure of tacrine derivatives.

Fig. 2. Structure of tacrine derivatives.

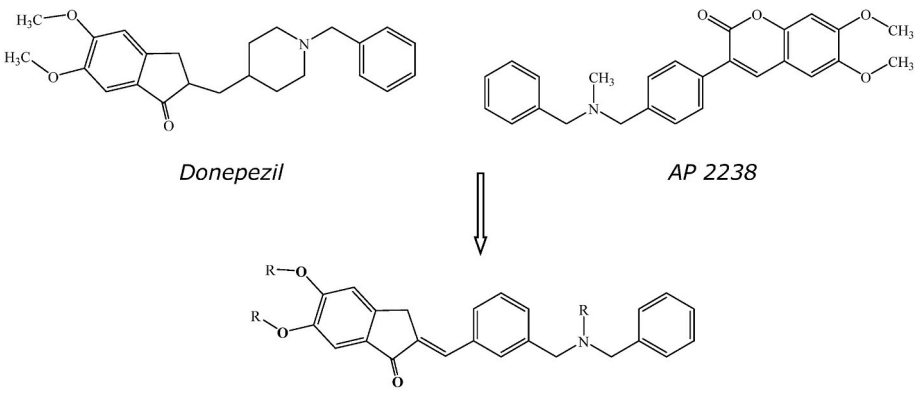


Fig. 3. General structure of hybrid compounds bearing a pharmacophoric fragment of donepezil and AP2238.

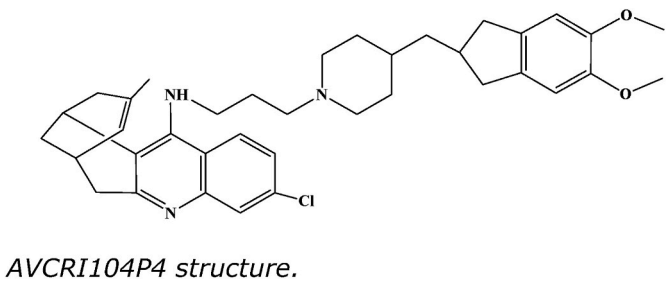


Fig. 4. AVCRI104P4 structure.

2.3. Rivastigmine and its analogues

Rivastigmine (3-[1-(Dimethylamino)ethyl]phenyl ethyl (methyl) carbamate), unlike some other ChE-Is, shows relatively low protein binding affinity, has a more selective action and less possibility of interactions with other drugs. The enzyme AChE exists in several isoforms and rivastigmine preferentially tends to inhibit the G1 than the G4 form (Enz et al., 1993; Desai and Grossberg, 2005). In general, G1 increases with the progression of AD and plays a major role in hydrolyzing ACh at cholinergic synapse. In view of this, rivastigmine's selective inhibition of G1 could be beneficial in the treatment of dementia of the Alzheimer's type (Jann, 2000; Eldufani and Blaise, 2019). This peculiar characteristic induced to explore the activity of rivastigmine derivatives (Onor et al., 2007). Among rivastigmine derivatives, particularly attention should be given to substituted 1,2,3,4 tetrahydroquinolin-6 (or-7)-yl carbamates. The most active compound of the series showed a potent AChE inhibition ($IC_{50} = 100$ nM), and was approximately 10 times more active than rivastigmine ($IC_{50} = 1030$ nM) in rat brain (Roy et al., 2015) (Fig. 6a).

A recent promising strategy for rational designing of selective central AChE-Is is represented by some bio-oxidizable pro-drugs, which on crossing blood-brain barrier (BBB) get oxidized in central nervous system where they activate the central cholinergic system. The most active compound of the series resulted to be inactive against AChE ($IC_{50} > 1$ nM) in the peripheral system and inactivate the AChE with an IC_{50} of 20 nM (Bohn et al., 2015) (Fig. 6b).

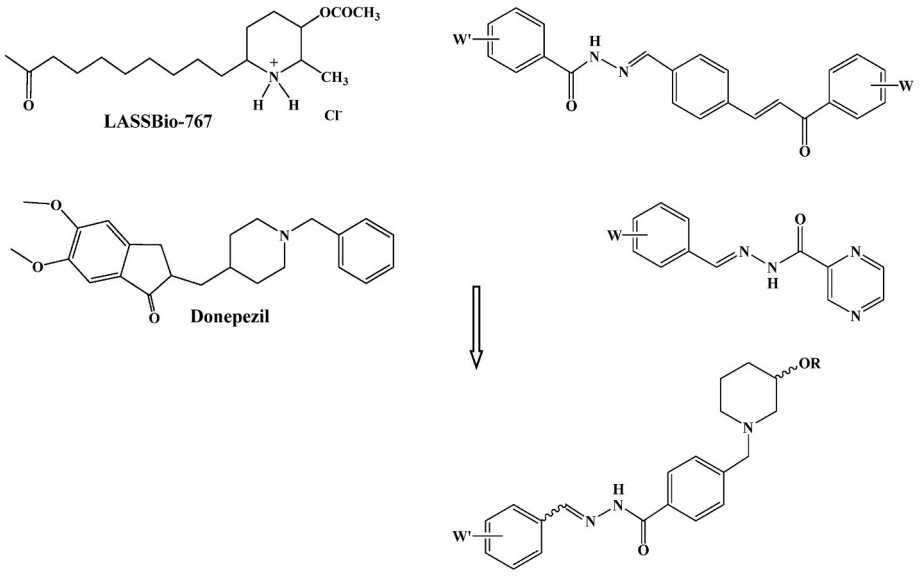


Fig. 5. General structure of hybrid *N*-benzyl-piperidine-aryl-acylhydrazones.

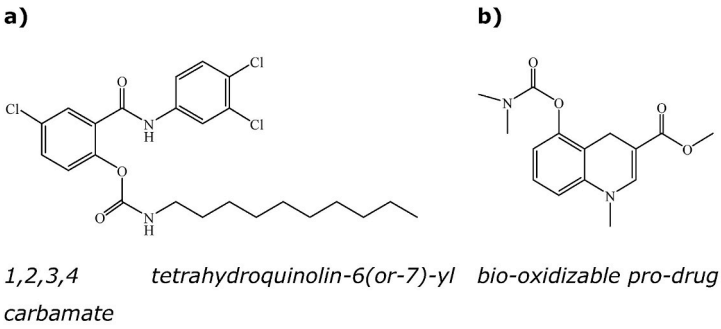


Fig. 6. Structures of the most active compounds of the series a) tetrahydroquinolin-6 (or-7)-yl carbamate and b) bio-oxidizable pro-drugs.

2.4. Galantamine and its analogues

Galantamine ((4aS,6R,8aS)-5,6,9,10,11,12-Hexahydro-3-methoxy-11-methyl-4aH-[1] benzofuro [3a, 3,2-ef][2]benzazepin-6-ol) is a heterocyclic phenantridine derivatives, it was isolated from the bulbs and the flowers of Galanthus woronowii. This alkaloid, belonging to the Amaryllidaceae family, contains several diverse structural types (Fig. 7).

A characteristic that makes galantamine suitable for AD treatment is a selective activity for AChE in the central nervous system with little effect on peripheral tissues. The interesting galantamine biological activity combined with its limited availability from natural sources has increased the interest in approaches to its total synthesis. A great number of research groups succeeded in the preparation and biological evaluation of galantamine structural analogues and derivatives developed to improve the biological profile of the natural product (Rinner et al., 2017). For this purpose, Memogain® a pro-drug of galantamine was developed (Maelicke et al., 2010). The bioavailability of Memogain has more than 15-fold higher, in the brain, than the same dose of galantamine. Since Memogain is enzymatically cleaved to galantamine, it is able to produce a more pronounced cognitive improvement than the same doses of galantamine, without exhibiting any significant levels of gastrointestinal side effects (Fig. 8).

A series of indole analogues of galantamine with a good AChE inhibition potency and the most active compound showed an IC_{50} of 11 nM was also developed (Atasanova et al., 2015Atanasova et al., 2015; Bautista-Aguilera et al., 2014) (Fig. 9).

Moreover, new derivatives with an increased water solubility and/or a multitargeted therapeutic approach were synthesized. Even "modest" changes to the galantamine framework, as in case of the oxygenated derivatives, completely altered the binding profile of the native molecule and therefore these compounds were totally inactive (Buckler et al., 2017).

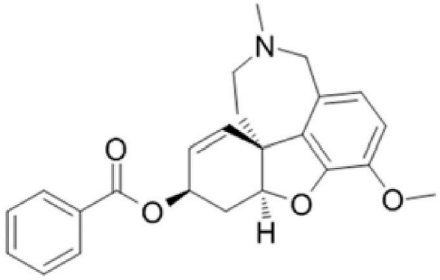


Fig. 8. Structure of memogain.

3. Preclinical studies

Several preclinical studies have investigated the pharmacological profile of ChE-Is and their activity in animal or other preclinical models (Jacobson and Sabbagh, 2008). In the description below, ChE-Is are classified as short-acting or reversible agents such as tacrine, donepezil, and galantamine, as intermediate-acting or pseudo-irreversible agent such as rivastigmine.

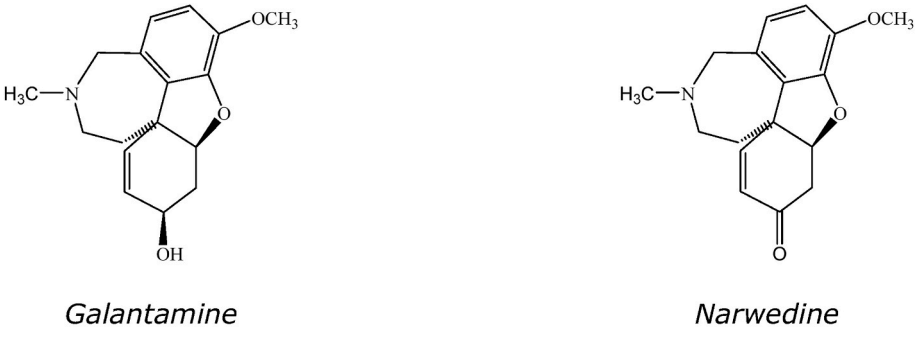
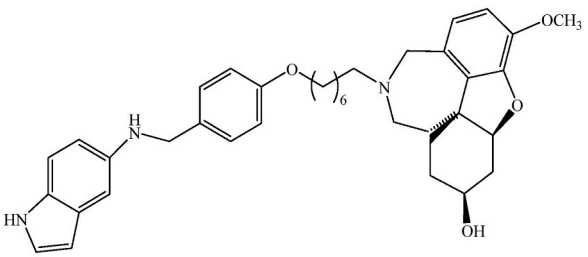


Fig. 7. Structures of galantamine and narwedine.



Structure of the most active galantamine-indole.

Fig. 9. Structure of the most active galantamine-indole.

3.1. Short acting or reversible agents

3.1.1. Tacrine

Tacrine was developed as an antibacterial agent, but demonstrated weak bactericide potency and displayed a respiratory stimulation activity on analeptic animals sedated by morphine (Korabecny et al., 2014). Tacrine, in fact, is a potent ChE-I (IC_{50} 125 ± 23 nM) that potentiates cholinergic transmission in the brain and at the periphery. It is about 100 times more potent to inhibiting BuchE than AChE, which could explain the respiratory stimulation in morphine-treated animals (Korabecny et al., 2014).

The mechanism of action of tacrine is not fully known, but it is suggested that the drug is an anti-AChE agent which reversibly binds with and inactivates cholinesterases. This inhibits the hydrolysis of ACh released from functioning cholinergic neurons, leading to an accumulation of the neurotransmitter at cholinergic synapses. Tacrine modulates different neurotransmitter systems by interacting with muscarinic and nicotinic receptors displaying 100-fold higher affinity towards muscarinic receptors. It increases the release of ACh by inhibiting presynaptic M_1-receptors and both isoforms of monoamine oxidases MAOs. Moreover, tacrine blocks the potassium channel (Reid and Sabbagh, 2008). The pharmacological profile of tacrine was reviewed by several studies (Nordberg et al., 2013; Jarrott, 2017).

Intraperitoneal administration of tacrine in the rat produced dose-dependent increases in salivation and tremor (ED_{50} 15 mmol/kg), with a most sustained effect on tremor, being these effects the result of a selective central nervous system activity. *In vivo* microdialysis studies in the cerebral cortex have shown that tacrine produces, a 30-fold, increase in extracellular ACh, which remained elevated for more than 2 h after its administration (Snape et al., 1999).

3.1.2. Donepezil

Donepezil has several pharmacological properties consistent with the modulation of different neurotransmitter systems such as α_1 adrenergic receptors, improvement of neuronal plasticity, reduction of the pro-inflammatory cytokine, and improvement of cerebral blood flow. Moreover, it decreases the level of amyloid precursor protein (APP) and excitotoxic injury, modulates the cholinergic effects and oxidative stress, influences the AChE isoform expression and interacts with nicotinic receptors regulation in cerebral cortex. (Jacobson and Sabbagh, 2008).

The cognitive effects of different doses of donepezil on hippocampal-dependent memory deficits after lesions of different brain areas were investigated. At a 0.1 mg/kg dose, donezepil was ineffective (Xu et al., 2002). A daily dose of donepezil (0.75 mg/kg), in aged male Fisher rats, starting 4 days before testing and continuing for 15 days showed an improved water-maze acquisition and retention compared to controls (Hernandez et al., 2006). Doses of 0.25 and 0.5 mg/kg of donepezil produced a significant improvement in water-maze performance (Abe et al., 2003). At the dose of 3.0 mg/kg donepezil improved the acquisition in the water maze task in entorinal cortex of ibotenic acid-lesioned rats compared to untreated lesioned animals (Spowart-Manning and van der Staay, 2005). The donezepil dose of 0.695 mg/kg/day for 3 weeks before testing for the subsequent 2 weeks by subcutaneous administration did not improve radial-arm-maze performance in aged rats (Barnes et al., 2000). A donepezil acute high dose (3.0 mg/kg/day) given before testing improved radial arm maze tasks in male Wistar rats with experimental cerebral ischemia and receiving intracerebroventricular Aβ infusion (Iwasaki et al., 2006). Collectively preclinical studies with donezepil suggest that the compound has positive effects on hippocampus-dependent memory tests (Yuede et al., 2007).

The donepezil neuroprotective effects demonstrated by *in vivo* or *in vitro* experiments in AD models, are probably not related to the inhibition of AChE induced by the compound (Kim et al., 2017). The donepezil neuroprotective mechanism occurs by mitigating the Aβ-induced toxicity via α7nAChRs and the PI3K-Akt pathway. Other studies have shown that donepezil can prevent systemic inflammation in the brain and spleen by suppressing IL-1β and cyclooxygenase-2 expression (Kim et al., 2017). Anti-amnesic and neuroprotective effects against Aβ-induced toxicity were also demonstrated (Meunier et al., 2006). Moreover, donepezil showed a protective effect against oxygen-glucose deprivation induced-injury in rats. These results can lead to the assumption that, in AD, this compound may protect the cortical neuronal cells from the progressive degeneration (Zhou et al., 2001). These properties suggest that donepezil could counter the progressive degeneration of brain neurons and the hippocampal atrophy, contributing to maintain functional brain activity.

3.1.3. Rivastigmine

Rivastigmine is a pseudo-irreversible carbamate noncompetitive inhibitor of both AChE and BuChE. This profile makes the compound quite interesting for the treatment of AD. In fact these two enzymes involved in the catabolism of ACh have a role in the formation of neurofibillary tangles and neuritic plaques, which represent hallmarks in the pathophysiology of AD (Mesulam et al., 2002). At the dose of 12 mg/day, rivastigmine inhibits brain AChE and BuChE by the 61.7% and 61.8% respectively, whereas the percentage of inhibition of peripheral BuChE is approximately 33%. This indicates a good selectivity of the compound for the brain cholinergic system. In preparations of rat striatum rivastigmine dose-dependently inhibited AChE with an IC_{50} of 32 ± 2 μM (Enz and Gentsch, 2004). In AChE knockout mice, rivastigmine increased by 30-folds hippocampal ACh suggesting that the increase of ACh levels elicited by the compound is mediated though the inhibition of BuChE (Ogura et al., 2000).

To clarify the importance of BuChE in regulating brain cholinergic function a microdialysis study was done in rat cerebral cortex. This investigation has demonstrated that rivastigmine at the dose of 0.6 mg/kg inhibits both AChE and BuChE by 40% and 25%, respectively. In 5-week-old imprinting control region mice with cognitive dysfunction induced by amyloid-β peptide, treatment with at 0.03, 0.1, and 0.3 mg/kg rivastigmine significantly ameliorated cognitive dysfunction demonstrating that dual AChE/BuChE inhibition may represent a therapeutic strategy in AD (Cerbai et al., 2007; Furukawa-Hibi et al. 2001Furukawa-Hibi et al., 2011). The effect of rivastigmine to inhibit preferentially AChE/BuChE in the central nervous system than at the periphery results in decreased the locomotor activity without creating sedation in mice. Moreover, in rats, cats, and monkeys rivastigmine had minimal effects on heart rate and blood pressure (Enz et al., 1993).

In the primary neuronal culture model, rivastigmine preserved neuronal morphology as well as pre-synaptic protein markers and enhanced the expression of neuronal Aβ precursor protein (Bailey and Lahiri, 2010). Rivastigmine increased neuronal Aβ precursor protein in wild-type rats to a similar extent than in the *in vitro* model. These findings suggest that changes in metabolic activity resulting from rivastigmine treatment are associated with increased neuronal survival, accompanied by changes in the relative levels of the predominant isoforms of neuronal Aβ precursor proteins (Bailey and Lahiri, 2010).

Preclinical studies collectively suggest that rivastigmine could induce benefits in cognition, global function, and behavioral symptoms in AD.

3.1.4. Galantamine

Galantamine is a tertiary alkaloid with a profile of selective reversible, competitive inhibitor for AChE rather than BuChE (Darvesh et al., 2003). It also interacts allosterically with nicotinic acetylcholine receptors to potentiate the action of agonists at these receptors (Maelicke, 2000). This potentiating effect may contribute to the clinical effectiveness of galantamine, since the severity of cognitive impairment in AD is related to the loss of nicotinic receptors (Perry et al., 2000). On the other hand, cholinergic stimulation promotes the proliferation and survival of neural precursor cells (Mohapel et al., 2005).

Some studies have shown that galantamine stimulates in the hippocampus the proliferation of neural progenitor cells in the subgranular zone via activation of the M_1 muscarinic receptor and the survival of the newly divided cells in the granule cell layer via activation of the α7 nicotinic receptor. It has been suggested that insulin-like growth factor 2 is involved in the effects of galantamine on survival of 2-wk-old immature cells in the granule cell layer (Kita et al., 2014).

Studies conducted on the 5XFAD mouse model of AD showed that galantamine counters plaque formation and behavioral decline (Bhattacharya et al., 2014). In transgenic mice, galantamine attenuates amyloid-β deposition and neuroinflammation (Wu et al., 2015). Based on these findings it was suggested that galantamine may represent a promising compound for multi-target anti-AD therapy because of the combining effects of AChE inhibitory activity and the countering deposition of amyloid-β.

The effects of galantamine on learning and memory were assessed by passive avoidance behavioral studies in sodium nitrite-induced hypoxic rats. At mg/kg 0.5 and 1.0 mg/kg oral doses galantamine induced a dose-dependent improvement of learning and long-term memory retention tests, but increased latency reactions ($P < 0.05$). The effect of galantamine was attributed to allosteric ACh potentiation mediated by the activation of nicotinic receptors (Dimitrova and Getova-Spassova, 2006).

Recent studies have demonstrated that galantamine has an *in vitro* and *in vivo* anti-inflammatory activity. Galantamine treatment prevented activation of microglia and astrocytes and countered neuroinflammation by inhibiting inflammatory signaling molecules (NF-κB and p65) and cytokines (TNF-α, IL-1β and IL-6) in the hippocampus of lipopolysaccharide-exposed mice. This effect is associated with ACh binding to α7 nicotinic receptors suppressing the activation of NF-κB and inhibiting the production of pro-inflammatory cytokines (Liu et al., 2018). Moreover, galantamine displays an antioxidant, and cholinomimetic activity and possesses anti-inflammatory properties that might be beneficial for inflammatory bowel disease.

In the treatment of AD galantamine is administered orally. A main problem with this route of administration is the poor brain bioavailability that the compound reaches, accompanied with relevant peripheral side effects primarily gastrointestinal. In view of this, more effective drug delivery approaches were investigated. These studies have investigated pharmaceutical systems allowing to reach high brain deposition of the compound after nasal administration. Carrier systems investigated included nanoparticles, liposomes, lipid nanocarriers and hydrogel, in-situ gelation (Mishra, 2019; Alexander et al., 2015, 2016). The purpose of the different systems was to enhance drug naso-mucosal permeability for increasing brain drug bioavailability with parallel reduction of peripheral side effects (Alexander et al., 2011). The more promising compound obtained by these studies was a thiolated chitosan nanoparticle galantamine. This nanoparticle was compared in terms of nasal and oral delivery by pharmacodynamic studies and biochemical analysis of AChE activity in Swiss albino mice brain. TRhe obtained results revealed a significantly greater nasal than oral delivery ($p < 0.05$) (Alexander et al., 2011, 2015; 2016).

In spite of some preclinical and clinical evidence of an increased galantamine bioavailability after intranasal administration of it, intranasal galantamine formulations were not introduced in the pharmaceutical market. This may be due to the inconsistent results obtained in preclinical and clinical studies with this compound in clinical trials.

4. Clinical studies

Several clinical studies were published on the activity of ChE-Is on cognitive, functional and behavioral symptoms in AD. Using MEDLINE as an index of the biomedical journal literature and as entries "Alzheimer's disease and acetylcholinesterase inhibitors" 7740 papers published from 1979 to 2018 were retrieved of which 924 clinical trials. With the entries "Alzheimer's disease and cholinesterase inhibitors" 7188 papers published between 1979 and 2019 were retrieved of which 912 clinical trials.

The majority of the studies indicate that ChE-Is induce an improvement of the cognitive function scales. This improvement was observed in mild to moderate stages of the disease, whereas some studies reported an activity in the severe stage of AD. The improvement was found primarily at 24 weeks of treatment, whereas the results obtained at 1 and 2 years of treatment are uncertain.

Table 1 lists the names of the three ChE-Is (donepezil, rivastigmine and galantamine) available in the market for the symptomatic control of the AD, their main mechanism of action, pharmaceutical form and recommended dosage.

The main results of the clinical trials with tacrine and with other AChE-I/ChE-I available in the market are summarized below.

4.1. Tacrine

Tacrine was the first AChE inhibitor introduced into clinical use for treating of AD and was approved for use in the United States in 1993 with the indication of the symptomatic treatment of mild-to-moderate dementia of the Alzheimer's type. The compound was marketed in capsules of 10, 20, 30 and 40 mg under the brand name Cognex® with the typical dose being 20–40 mg four times daily. Clinical trials with tacrine were performed in 2706 patients with AD and in 9861 patients with AD in a treatment investigational new drug (TIND) program. Clinical effects of tacrine were assessed in more than 190,000 AD patients in the United States receiving tacrine during the first 2 years following marketing approval (Gracon et al., 1998). In terms of cognitive function analysis, the effect of tacrine was not statistically different from placebo for the Mini Mental State Examination (MMSE) score. A barely statistical significance in favor of treatment for the AD Assessment Scale-cognitive (ADAS-Cog) scale was observed. Behavioral disturbances assessed by the ADAS noncognitive scale, did not show difference between tacrine and placebo (Jarrott, 2017).

The use of tacrine was accompanied by a high incidence of cholinergic side effects. In 29% of treated patients alanine aminotransferase elevation three times above normal, in 28% nausea and vomiting, in 14% diarrhea, in 9% dyspepsia or anorexia, and in 7.5% myalgia were observed (Wagstaff and McTavish, 1994). Treatment of AD patients with tacrine was associated with asymptomatic serum aminotransferase elevation in almost half of patients in general within 6–8 weeks of starting therapy. The unfavorable side effects and the inconvenience of the four-times/day administration, as well as the availability of other ChE-Is led to the withdrawal of tacrine from the market in 2013.

4.2. Donepezil

Donepezil (Aricept®) is a reversible AChE-I increasing brain ACh concentrations and enhancing cholinergic neurotransmission. It was approved in 1996 for the treatment of mild to moderate dementia of the Alzheimer's type. In 2004, the approval was extended to 5 and 10 mg oral solutions and disintegrating tablets and in 2010 a film-coated tablet containing 23 mg donepezil was approved in the USA for the treatment

Table 1
Main cholinesterase inhibitors available in the pharmaceutical market. Indications, pharmaceutic forms and recommended dosage.

Name	Indication	Mechanism of action	Pharmaceutic form	Recommended dosage	Countries of license
Donepezil Hydrochloride	Alzheimer's disease-dementia (Mild to moderate/Moderate to severe)	AChE-I	*Tablet* 5 mg 10 mg 23 mg *Tablet, oral disintegrating* 5 mg 10 mg	*Mild to moderate AD* 5 mg oral at bed time initially, may increase to 10 mg/day after 4–6 weeks if warranted *Moderate to severe AD* 5 mg oral at bed time initially, may increase to 10 mg/day after 4–6 weeks; may further increase to 23 mg/day after 3 months if warranted	38
Galantamine Hydrobromide	Alzheimer's disease-dementia (Mild to Moderate)	AChE-I BuChE-I	*Tablet* 4 mg 8 mg 12 mg *Tablet, extended-release (ER)* 8 mg 16 mg 24 mg *Oral solution* 4 mg/mL	*Initial* Conventional: 4 mg oral every 12hr ER: 8 mg oral in the morning *Maintenance* Conventional: Titrate to 8–12 mg oral every 12hr; Increase by 4 mg every 12 h at no less than 4 week intervals ER: 16–24 mg oral in the morning; increase by 8 mg/day at no less than 4 week intervals	34
Rivastigmine Tartrate	Alzheimer's disease - dementia (Mild to Moderate oral and transdermal/ Moderate to severe transdermal)	AChE-I BuChE-I	*Capsule* 1.5 mg 3 mg 4.5 mg 6 mg *Transdermal patch* 4.6mg/24hr 9.5mg/24hr 13.3mg/24hr	*Oral, Indicated for mild-to-moderate AD* Initial: 1.5 mg oral every 12hr Increase by 1.5 mg/dose every 2 weeks; not to exceed 6 mg oral every 12hr Maintenance: 3–6 mg oral every 12hr (higher end may be more beneficial) *Transdermal, Indicated for mild, moderate, and severe AD* Initial: Apply 4.6 mg every 24hr Dose titration: May increase dose to 9.5 mg every 24hr after a minimum 4 weeks if well tolerated; after an additional 4 weeks, may further increase to 13.3 mg patch if needed *Mild-to-moderate AD:* Effective dosage range is 9.5–13.3 mg/24 h *Moderate-to-severe AD:* Effective dose is 13.3 mg/24 h. Replace with new patch every 24hr	33

of severe AD. (Multum, 2019).

Donepezil has a 100% bioavailability, reaches a plasma peak in 3–4 h, is metabolized by hepatic P-450 enzymes CYP2D6, CYP3A4 and has a half-life of about 60–90 h. This allows to administer the compound once daily dosing due to its long half life (Shigeta and Homma, 2001; Atri, 2019). Extensive evidence indicates that donepezil at dosages of 5 and 10 mg/day improves cognition and global clinical function in the short term (up to 24 weeks) and long term (for up to about 1 year) in patients with mild to moderate AD. Improvements in the activities of daily living have also been observed with donepezil 10 mg/day. Adverse events associated with donepezil are mainly cholinergic. Donepezil is considered as a first-line treatment in patients with mild to moderate AD and its activity was analyzed in several clinical trials.

A recent Cochrane meta-analysis has included 30 studies involving 8257 participants (Birks and Harvey, 2018). Twenty-eight studies reported results allowing a meta-analysis. Donepezil was also associated with better function measured with the ADCS-ADL-sev, (MD: 1.03, 95%, CI: 0.21 to 1.85, No.733 participants, 3 studies). In behavioral symptoms no differences were reported between donepezil and placebo (MD: −1.62, 95%, CI: −3.43 to 0.19, No. 1035 participants, 4 studies) or by the BEHAVE-AD scale (MD 0.4, 95% CI -1.28 to 2.08, 194 participants, 1 study) as well as for Quality of Life (QoL) (MD -2.79, 95% CI -8.15 to 2.56, 815 participants, 2 studies). During these clinical trials, donepezil tablets at a dosage of 5 or 10 mg were used. The major part of trials lasted approximately 24 weeks and only few trials were continued for 52 weeks. Two studies have tested a slow-release oral formulation of 23 mg/day donepezil (English, 2012).

Since AD is characterized by longtime evolution, a limit of these clinical trials is in the relatively short time of observation. However, the results showed that the adverse effects reported are mainly mild and there are modest, but significant benefits in the treatment of cognitive symptoms in mild-to-moderate stages of AD. In contrast, no relevant effect of donepezil on Quality of Life (QoL) was demonstrated in these clinical studies. More data are required from longer-term clinical studies examining measures of disease progression or time to needing full-time care (Birks and Harvey, 2018). In general, when the dose of donepezil increased, side effects occur more frequently leading some patients treated to discontinue treatment (Adlimoghaddam et al., 2018).

It is a matter of discussion if AchE-Is/ChE-Is may represent a disease-modifying therapy for AD. The identification of disease-modifying therapies is of particular relevance for AD to treat the growing number of individuals with the disease or at immanent risk for it (Cummings and Fox, 2017). Among the five biomarkers of AD progression proposed, three are represented by imaging biomarkers (amyloid PET, structural MRI, and FDG PET) and the evidence they can provide is crucial for obtaining disease-staging information. Imaging biomarkers over fluid biomarkers distinguish the different phases of the disease both temporally and anatomically (Pini et al., 2016; Márquez and Yassa, 2019). The demonstration that a given treatment may slow the rate of brain shrinkage in cerebral areas particularly affected by AD can be considered a neuroprotective/disease modifying activity of this treatment.

4.3. Rivastigmine

Rivastigmine (Exelon®) is a low reversible dual AChE/BuChE inhibitor. In the cerebral cortex, AChE is present in the nerve synaptic junctions, whereas BuChE is located in the glial cells and modulates cholinergic neurotransmission (Mesulam et al., 2002; Wright et al., 1993; Mesulam and Geula, 1991). Rivastigmine was approved to treat mild to moderate AD in over 40 countries in North and South America, Asia, and Europe. It is available in capsules (1.5 mg, 3 mg, 4.5 mg, and 6 mg doses twice a day), oral solution (2 mg/ml), and transdermal patch formulation (10 cm^2) (4.6 mg, 9.5 mg, 13.3 mg, and 13.3 mg/24 h patch applied to the skin once day). The adsorption of capsules is rapid and

complete within 1 h and transdermal patch within 30–60 min. The maximum rivastigmine plasma concentration is reached in 1.5 h for capsule and in 3 h for transdermal patch. Dose titration is needed when initiating treatment. Initial dosing recommendations are 1.5 mg per oral route twice a day with a maximum oral dose of 12 mg/day. The transdermal patch 13.3 mg/24 h is approved for all stages of AD disease, including severe.

Rivastigmine has a good BBB penetration and is extensively metabolized via cholinesterase-mediated hydrolysis and is excreted by kidneys (Multum, 2019). The principal metabolite of rivastigmine has at least 10-fold lower activity against AChE compared with the parent drug. Different from other AChE inhibitors, the hepatic cytochrome P-450 (CYP-450) system is not involved in the metabolism of rivastigmine. Rivastigmine has a short pharmacokinetic half-life, whereas the plasma concentration of rivastigmine in patients with AD is 30%–50% higher than in healthy elderly patients.

Oral and patch rivastigmine, were significantly better than placebo in delaying functional impairment based on network meta-analysis (NMA) of 19 trials with 7445 patients (Mercier et al., 2007; Corey--Bloom et al., 1998; Rosler et al., 1999). Using imaging techniques such as PET and magnetic resonance imaging (MRI) it was demonstrated that treatment of patients with mild-to-moderate AD with rivastigmine (3, 6, or 9 mg/day) for 6 months, significantly increased brain hippocampal metabolism. This increment was of 32.5% in rivastigmine responders ($P < 0.03$) compared with the non-significant decrease in rivastigmine non-responders 6.4% and those treated with placebo 4.1% (Potkin et al., 2001). A 13-week, randomized, open-label study in 56 patients with mild-to-moderate AD has shown that rivastigmine inhibits both AChE and BuChE. The compound decreased AChE and BuChE in the cerebrospinal fluid with an inhibition of 42.6% ($P < 0.001$) versus baseline of 21.8%, and of 45.4% ($P < 0.001$) versus baseline of 9.3%, respectively (Parnetti et al., 2011). Another study, the trans dermal Exelon in AD (IDEAL) trial, has investigated comparatively the effect of rivastigmine patches versus rivastigmine capsules and placebo. IDEAL was a 6 months double-blind trial assessing the influence of 10 cm^2 (9.5 mg/day) or of 20 cm^2 (17.4 mg/day) rivastigmine patches, rivastigmine 6 mg capsules and placebo on 1195 patients with mild-moderate AD. The study has shown that the 10 cm^2 patch had an efficacy similar to that of the capsules, with a very low side effects not significantly different compared to placebo. The 20 cm^2 patch induced a greater cognitive improvement compared to the 10 cm^2 patch with a similar tolerability profile (Farlow et al., 2013).

Efficacy, safety, and tolerability of 13.3 versus 4.6 mg/day rivastigmine patches were also investigated for 24 weeks in 716 patients with severe AD in the prospective, randomized, double-blind ACTION study. In this trial, 356 patients were randomized to 13.3 mg/day and in 360 patients 4.6 mg/day rivastigmine patches. Since this study was performed in severe stage patients, at the end of 24 weeks both treatment groups resulted deteriorated. The 13.3 mg/day patch revealed a greater efficacy than the 4.6 mg/day patch indicating the clinical relevance of the high-dose treatment primarily in terms of benefits on cognition. The overall incidence of side effects was similar between the two groups suggesting that a higher dose of rivastigmine does not affect negatively tolerability to the drug (Farlow et al., 2013). In summary, rivastigmine provides benefits in terms of cognitive function and activities of daily living and in psychological symptoms in mild to moderate dementia.

A Cochrane review on the clinical efficacy of rivastigmine has analyzed comparatively 13 trials with a duration between 12 and 52 weeks and testing the efficacy of capsule forms with a dose of up to 12 mg/day and transdermal patch formulations delivering 4.6, 9.5 and 17.7 mg/day of the active principle. Studies made confirmed the safety and efficacy of rivastigmine 6–12 mg/day orally or 9.5 mg/day transdermally versus placebo. Analysis of seven trials including 3450 with mild to moderate AD with a mean age of about 75 years have shown that after 26 weeks of treatment rivastigmine compared to placebo was associated with better outcomes for cognitive function. Cognitive functions were assessed with the AD Assessment Scale-Cognitive (ADAS-Cog) score (mean difference (MD) −1.79; 95% confidence interval (CI) −2.21 to −1.37, n = 3232, 6 studies) and the Mini-Mental State Examination (MMSE) score (MD 0.74; 95% CI 0.52 to 0.97, n = 3205, 6 studies). Activities of daily living (SMD 0.20; 95% CI 0.13 to 0.27, n = 3230, 6 studies) and clinician rated global impression of changes were also analyzed. A small proportion of patients treated with rivastigmine did not experience changes or deterioration (OR 0.68; 95% CI 0.58 to 0.80, n = 3338, 7 studies). A benefit from rivastigmine on the outcome of clinician's global assessment was also observed. The drug influenced positively the cognitive functions in AD patients, whereas no differences were found between the rivastigmine group and placebo group in behavioral changes or impact on carers (Birks and Grimley Evans, 2015).

4.4. Galantamine

Galantamine (Razadyne®) is a selective competitive and reversible inhibitor of AChE, that elevates ACh levels in the cerebral cortex by slowing the neurotransmitter degradation and modulates allosterically nicotinic ACh receptors. This modulation further increases ACh in presynaptic nerve terminals. Galantamine increases also glutamate and serotonin levels. Galantamine is available in an oral formulation (4 mg; 8 mg; 12 mg; 4 mg/ml; 16 mg; 24 mg) in immediate release (IR), requiring twice daily assumption and extended release (ER), requiring once a day administration. The initial dose proposed for treatment is 8 mg/day with an increase, as maintenance dose, up to 16 mg/day twice a day after 4–8 weeks (Seltzer, 2010). IR and ER forms of galantamine showed comparable safety profiles and the rate of discontinuation of the IR form was non-significantly increased compared to the ER form (Mohammad et al., 2017).

The galantamine maximum concentration in plasma is reached in 1 h with a half-live of about 7 h and a good BBB penetration. The compound is metabolized in the liver via CYP2D6 to O-desmethyl-galantamine and 3A4 to galantamine-*N*-oxide. Galantamine metabolites are not considered clinically relevant (Farlow, 2003; Scott and Goa, 2000). Hepatotoxicity might occur as a result of an idiosyncratic metabolism to a toxic or immunogenic intermediate. Several placebo-controlled clinical trials, have shown in patients treated with galantamine no increase in the rate of serum enzyme elevations compared to those receiving placebo (Alzheimer's disease agents: Livertox 2012).

To improve medication adherence and limit side effects of immediate-release (IR) and extended-release (ER) forms were developed. A randomized trial presented that the two forms showed a comparable safety profile and the rate of discontinuation of the IR form was non-significantly increased compared to the ER form (Mohammad et al., 2017). Galantamine is licensed for the treatment of mild to moderate dementia of the AD type. It enhances central cholinergic function and inhibits AChE, but there is no evidence that galantamine alters the course of the underlying dementing process.

The cognitive effects of 21–26 week galantamine treatment versus placebo were assessed in clinical trials and confirmed by meta-analysis. Memory, language skills, and reasoning capabilities were assessed using the ADAS-cog scale (Raskind et al., 2000; Tariot et al., 2000; Wilcock et al., 2000; Rockwood et al., 2010; Wilkinson and Murray, 2001; Brodaty et al., 2005). The clinician's interview-based impression of change plus caregiver information (CIBIC-plus) which provides a global assessment of behavior, thinking, and the ability to carry out daily activities (such as eating, dressing, shopping and managing finances) was also used to investigate galantamine activity. A 2006 meta-analysis of 10 trials with 6805 patients on the efficacy of galantamine in AD did not find additional cognitive benefit above 16 mg daily. A dose-dependent increase in adverse effects above this dose was noticeable. The effect size was modest averaging 3 points on the ADAS-Cog scale at six months. A mild benefit on activities of daily living was also observed. A more

recent meta-analysis including 4074 participants confirmed the cognitive benefits (2.95 points on ADAS-Cog) but did not show any effect on activities of daily living. The trials that showing benefit on activities of daily living were of longer duration suggesting that longer treatment duration is necessary for demonstrating measurable benefit (Jiang et al., 2015).

A clinical trial on 2033 patients, pooled from multiple studies has shown that chronic galantamine treatment reduces behavioral symptoms (agitation, anxiety, disinhibition, and aberrant movements) measured by the Neuropsychiatric Inventory (NPI) (Kavanagh et al., 2011). Long-term treatment with galantamine delays a patient's nursing home placement and caregiver burden, making it a cost-effective treatment. Nevertheless, gastrointestinal side effects often caused treatment discontinuation. To sum-up, chronic administration of galantamine to patients affected by AD leads to an improved cognitive function and delays the development of behavioral changes associated with the disease.

5. Combination of ChE-Is with other drug classes in the treatment of adult-onset dementia disorders

Considering the complex nature of AD pathophysiology and the different symptomatology that characterizes this disorder, it is improbable that a single class of drugs, can solve the relevant problems posed by the presence of adult-onset cognitive dysfunctions. As mentioned in Table 1, ChE-Is were licensed for the symptomatological treatment of mild-moderate forms of AD. Other indications in line with this first one were added subsequently. ChE-Is so far represent the current standard treatment for AD. *N*-methyl-*d*-aspartate (NMDA) receptor antagonists and antioxidant agents were also licensed and investigated respectively for the treatment of adult-onset dementia disorders. The collection of further evidence about the activity of these drugs in different clinical conditions could provide insights about their relevance in the treatment of AD. Another AD is represented by the association of ChE-Is with other drug classes. Among the association approaches investigated in clinical trials, cholinergic precursors, *N*-methyl-*d*-aspartate (NMDA) receptor antagonists and antioxidant agents were those more extensively studied.

Cholinergic precursors belonging to the class of choline-containing phospholipids used alone or in association with ChE-Is represented the first attempts in the treatment of AD. The use of these compounds was based on the hypothesis that cholinergic precursors may enhance deficient cholinergic neurotransmission. Clinical trials did not confirm an activity of this class of compounds (Amenta et al., 2014). The cholinergic precursors most largely used in the early studies, such as choline and phosphatidylcholine (lecithin), were probably not effective at enhancing brain levels of ACh. Other phospholipids involved in choline biosynthetic pathways, such as CDP-choline (citicoline), choline alphoscerate and phosphatidylserine enhanced ACh bioavailability or release in animal models and improved cognitive function in patients with AD (Amenta et al., 2001). The activity of citicoline in association with ChE-Is in AD was recently reviewed (Piamonte et al., 2020). The choline-containing cholinergic precursor more extensively investigated in the clinical trial ASCOMALVA was choline alphoscerate (alpha-glyceryl-phosphorylcholine). The results of the ASCOMALVA trial published in different studies have shown that the addition of choline alphoscerate to standard treatment with the ChE-I donepezil induces an improvement in cognitive and functional tests compared to patients treated with donepezil alone (Amenta et al., 2014; Carotenuto et al., 2017; Traini et al., 2020). Moreover, in a study lasting for 4 years, the association between choline alphoscerate and donepezil has shown to counter to some extent the loss in volume occurring in some brain areas of AD patients (Fig. 10). The observation of parallel less pronounced decrease in cognitive and functional tests in patients with the same treatment suggests that the morphological changes observed may have functional relevance.

Another association approach investigated was that of ChE-I with memantine, a *N*-methyl-*d*-aspartate (NMDA) receptor antagonist. Memantine binds preferentially to NMDA receptor-operated cation channels with low to moderate affinity and inhibits the prolonged influx of Ca^{2+} ions, which represents the basis of neuronal excitotoxicity. It has been postulated that overactivation of NMDA receptors may lead to neurodegeneration and loss of synaptic function via chronic "excitotoxicity" (Adler et al., 2014). Memantine, by acting as an uncompetitive NMDA receptor antagonist with moderate binding affinity, prevents the pathologic influx of Ca^{2+} ions, not interfering with physiologic signals relevant for learning and memory processes. An excessive activation of glutamate receptors leads to degeneration of cholinergic neurons in AD. Preclinical studies have shown that the co-administration of donepezil and memantine exhibits synergistic effects on spatial memory in mouse models of AD, suggesting a complementary activity of memantine and donepezil. These studies have led to speculate that the association of

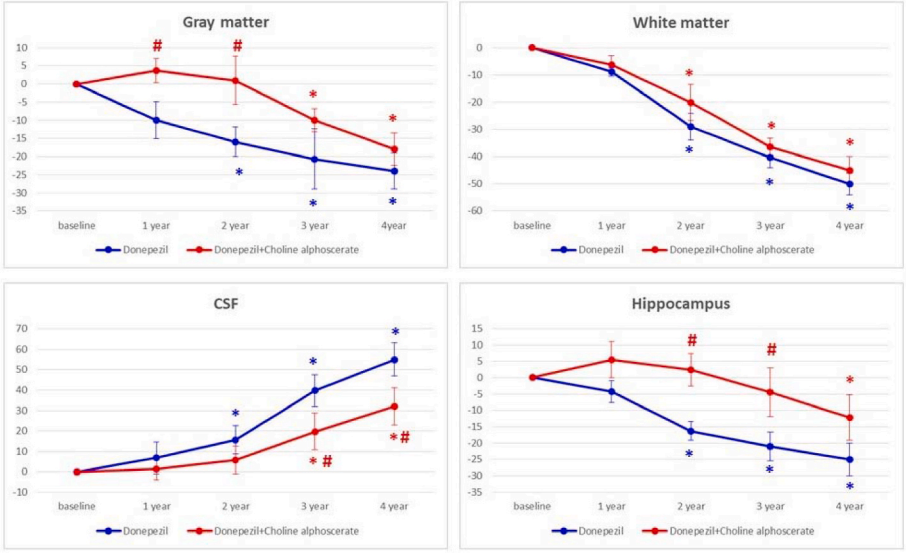


Fig. 10. *Changes in the percentage of gray and white matter, cerebrospinal fluid (CFS) and hippocampus volumes in the two groups of patients treated with donepezil (*10 mg/*day) plus placebo or with donepezil + choline alphoscerate (*1200 mg/*day) over the four years of observation.* The data are means of the percentage variation± S.E.M. *p < 0.05 versus baseline; #p < 0.05 versus donepezil and placebo.

memantine and CheI-s may result in greater clinical benefits than single drugs alone (Brewer, 2013). On the other hand, the association of the two drugs did not affect the respective pharmacological or pharmacodynamic properties. A negative point of the association principle is an increase of the pill burden induced by the administration of two pills (memantine plus ChE-I) in patients in general receiving many medicines daily. In December 2014, the US Food and Drug Administration has licensed Namzaric™ (28 mg memantine extended-release (ER)/10 mg donepezil), a once-daily, fixed-dose combination (FDC) of memantine ER and donepezil for patients with moderate-to-severe AD (Grossberg et al., 2013, Greig, 2015; Boinpally et al., 2015, http://www.fda.gov.). Clinical trials have shown that the FDC capsule containing memantine ER and donepezil is bioequivalent to co-administered commercially available memantine ER and donepezil, and it can be taken with or without food. The availability of two drugs in one capsule reduces pill burden, simplifies medication management facilitating caregiving, and improves patient safety for those who have swallowing difficulties. A further positive aspect of Namzaric™ use is that capsule contents can also be sprinkled onto soft foods to facilitate drug intake (Deardorff and Grossberg, 2016).

Another approach in the pharmacotherapy of AD is represented by the association of ChE-Is with antioxidants. Neurodegenerative pathologies including AD, Parkinson's disease (PD) and Huntington's disease are associated with an increased oxidative stress in nerve cells. On this basis, various antioxidant drugs such as Vitamin E, selegiline and Ginkgo Biloba were used as an adjuvant treatment of AD. It was found that the nuclear factor-erythroid 2-related factor 2 (Nrf2) induces the transcription of antioxidant response elements (ARE) (Johnson et al., 2008; Tufekci et al., 2011). Antioxidant enzymes, such as superoxide Dismutase (SOD), glutathione peroxidase (GPx), glutathione-*s*-transferase (GSTr), catalase (CAT), heme-oxygenase-1 (HO-1), NADPH-quinoneoxidoreductase (NQO-1), phase II enzymes of drug metabolism and heat shock proteins (HSP), are involved in the transcription of ARE. Both free antioxidants and anti-oxidative enzymes may protect cells from oxidation and inflammation and can reverse the chronic oxidative stress (Tufekci et al., 2011). This evidence collectively suggests that a possible protection by antioxidants against neurodegenerative diseases, such as AD and PD, is correlated to the activation of Nrf2. In spite of the theoretical advantage of the association between antioxidants and ChE-Is, administration of the two drugs together did not show obvious clinical benefits in the long term.

A different associative treatment consisted in the association between ChE-Is and ozone therapy as integrative treatment with ChE-Is. The mechanism of action of ozone therapy is based on activation of Nrf2 via moderate oxidative stress and suppression of NFB and inflammatory responses.

In the last years an emerging approach in the treatment of several neurological diseases such as AD, PD, epilepsy, and multiple sclerosis consisted in the use of cannabidiol (CBD). Cannabinoids were identified in Cannabis sativa L. 113 and the phytocannabinoid CBD accounts for up to 40% of the plant extract (Campos et al., 2012). CDB has no euphorigenic or psychedelic properties (Russo, and Guy, 2006; Rong et al., 2017) and in preclinical and clinical studies has shown a neuroprotective activity in several pathologies. The biological effects of CDB are mediated by the interaction with cannabinoid receptors (CB1 and CB2 receptors) and other components of the endocannabinoid system, with which it interacts (Karl et al., 2017). In the central nervous system, the CB1 receptor is the most abundant G protein-coupled endocannabinoid receptor and it can convert extracellular stimuli into downstream intracellular signaling pathways. Activation of CB1 and CB2 receptors by CBD may induce multiple signaling pathways, such as the PKC, PI3K/Akt, and ERK pathways, to promote the growth of neurites. It was demonstrated that in the case of neuron damage, CBD inhibits the release of Glu to generate a protective response, which may be triggered by CBD-induced retrograde signal transmission between synapses in the cannabinoid receptors. Moreover, CBD inhibits the production of neurofibrillary tangles caused by AB stimulation through the upregulation of Wnt/b-catenin pathway (Li et al., 2020). Until now the mechanism of pharmacological activities of CBD is not yet clear and further studies are necessary.

6. Conclusions

ChE-Is activity is characterized by the inhibition of AChE, the enzyme primarily responsible for breakdown of ACh in the nervous system. This allows to prolong the action of the deficient neurotransmitter in the brain. ChE-Is were the first drugs licensed for symptomatic treatment of AD and three compounds belonging to this therapeutic class are currently in clinical use in many countries worldwide. These include donepezil (Aricept), rivastigmine (Exelon), and galantamine (Razadyne). A fourth AChE inhibitor, tacrine, is no longer in use, due to its hepatotoxicity (for a review, see Joe and Ringman, 2019).

Overall, the benefits of the ChE-Is on cognition are modest. A meta-analysis of 80 trials that reviewed outcomes on MMSE scores of various ChE-Is in multiple forms of dementia found a mean effect size of 1.08, 1.0, and 1.10 points on the MMSE at 3, 6, and 12 months of treatment respectively (Knight et al., 2018).

There is no clear evidence of the optimal duration of ChE-I therapy, as the majority of clinical trials were limited to 6 months of observation. However, the three AChE/ChE-Is on the market are safe and maintain cognitive benefits over some years (Joe and Ringman, 2019) The 2000 CE trial, a randomized study including 565 patients with mild-moderate AD treated with donepezil for up to four years, has shown a cognitive benefit of about 0.8 points on the MMSE scale over the course of the study (Courtney et al., 2004). On the other hand, the DOMINO-AD trial, which included 295 patients with moderate to severe AD stable on donepezil, has reported that discontinuation increased the probability of nursing home placement within the first year (Howard et al., 2015). The AChE-I donepezil maintains the efficacy in severe dementia. An open label study of 97 patients living in assisted living facilities found that donepezil was well tolerated and the magnitude of benefit was similar to that reported for populations suffering from mild to moderate disease dwelling in the community (Rosenblatt et al., 2010).

Several studies have investigated if ChE-Is have any disease modifying effect, but the obtained results were inconsistent. A recent meta-analysis of seven trials enrolling 1708 participants have found a small but significant benefit in favor of AChE-I therapy, more pronounced for donepezil on brain atrophy (Kishi et al., 2015). Another meta-analysis of 10 trials including 3092 patients at different stages of AD comparing immediate versus delayed (~6 months) initiation of AChE-I and memantine treatment did not find significant differences on cognition or functional status between early or delayed starting of treatment (Tsoi et al., 2016). Overall there is no enough evidence that ChE-Is have a clinically meaningful disease modifying activity. Other approaches in which ChE-Is consisted in their use in combination with other classes of drugs such as cholinergic precursors, *N*-methyl-*d*-aspartate (NMDA) receptor antagonists and antioxidant agents. The combination with other classes of drugs may represent the chance for a renewed interest of ChE-Is in the treatment of adult-onset dementia disorders.

AD is considered as a leading cause of global deaths worldwide and many drugs targeting the production, aggregation, and clearance of Aβ plaques are under study, but failed to give conclusive clinical outcome. Lacking appropriate and documented treatments for AD, in spite of the modest clinical effects elicited by AChE/ChE-Is and waiting for better therapeutic strategies, these drugs should continue to be considered in the therapeutic armamentarium of AD.

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Declaration of competing interest

The authors declare no conflict of interest.

References

Abe, Y., Aoyagi, A., Hara, T., Abe, K., Yamazaki, R., Kumagae, Y., Naruto, S., Koyama, K., Marumoto, S., Tago, K., Toda, N., Takami, K., Yamada, N., Ori, M., Kogen, H., Kaneko, T., 2003. Pharmacological characterization of RS-1259, an orally active dual inhibitor of acetylcholinesterase and serotonin transporter,in rodents: possible treatment of Alzheimer's disease. J. Pharmaceut. Sci. 93, 95–105. https://doi.org/10.1254/jphs.93.95.

Adler, G., Mueller, B., Articus, K., 2014. The transdermal formulation of rivastigmine improves caregiver burden and treatment adherence of patients with Alzheimer's disease under daily practice conditions. Int. J. Clin. Pract. 68, 465–470. https://doi.org/10.1111/ijcp.12374.

Adlimoghaddam, A., Neuendorff, M., Roy, B., Albensi, B., 2018. A review of clinical treatment considerations of donepezil in severe Alzheimer's disease. CNS Neurosci. Ther. 1–13. https://doi.org/10.1111/cns.13035.

Alexander, A., Ajazuddin, M., Patel, R.J., Saraf, S., Saraf, S., 2016. Recent expansion of pharmaceutical nanotechnologies and targeting strategies in the field of phytopharmaceuticals for the delivery of herbal extracts and bioactives. J. Contr. Release 241, 110–124. https://doi.org/10.1016/j.jconrel.2016.09.017.

Alexander, A., Ajazuddin, M., Swarna, M., Sharma, M., Tripathi, D.K., 2011. Polymers and permeation enhancers: specialized components of mucoadhesives. Stamford J. Pharm. Sci. 4, 91–95. https://doi.org/10.3329/sjps.v4i1.8878.

Alexander, A., Saraf, S., Saraf, S., 2015. A comparative study of chitosan and poloxamer based thermosensitive hydrogel for the delivery of PEGylated melphalan conjugates. Drug Dev. Ind. Pharm. 41, 1954–1961. https://doi.org/10.3109/03639045.2015.1011167.

Alipour, M., Khoobi, M., Moradi, A., Nadri, H., Homayouni Moghadam, F., Emami, S., Hasanpour, Z., Foroumadi, A., Shafiee, A., 2014. Synthesis and anti-cholinesterase activity of new 7-hydroxycoumarin derivatives. Eur. J. Med. Chem. 82, 536–544. https://doi.org/10.1016/j.ejmech.2014.05.056.

Amenta, F., Buccioni, M., Dal Ben, D., Lambertucci, C., Martì Navia, A., Ngouadjeu Ngnintedem, M.A., Ricciutelli, M., Spinaci, A., Volpini, R., Marucci, G., 2018. Ex-vivo absorption study of lysine R-lipoate salt, a new pharmaceutical form of R-ALA. Eur. J. Pharmaceut. Sci. 15 (118), 200–207. https://doi.org/10.1016/j.ejps.2018.03.025.

Amenta, F., Carotenuto, A., Fasanaro, A.M., Rea, R., Traini, E., 2014. The ASCOMALVA (association between the cholinesterase inhibitor donepezil and the cholinergic precursor choline alphoscerate in alzheimer's disease) trial: interim results after two years of treatment. J Alzheimers Dis. 42 (Suppl. 3), S281–S288. https://doi.org/10.3233/JAD-140150.

Amenta, F., Parnetti, L., Gallai, V., Wallin, A., 2001. Treatment of cognitive dysfunction associated with Alzheimer's disease with cholinergic precursors. Ineffective treatments or inappropriate approaches? Mech. Ageing Dev. 122, 2025–2040. https://doi.org/10.1016/S0047-6374(01)00310-4.

Atanasova, M., Stavrakov, G., Philipova, I., Zheleva, D., Yordanov, N., Doytchinova, I., 2015. Galantamine derivatives with indole moiety: docking, design, synthesis and acetylcholinesterase inhibitory activity. Bioorg. Med. Chem. 23 (17), 5382–5389. https://doi.org/10.1016/j.bmc.2015.07.058.

Atri, A., 2019. Current and future treatments in Alzheimer's disease. Semin. Neurol. 39 (2), 227–240. https://doi.org/10.1055/s-0039-1678581.

Bailey, J.A., Lahiri, D.K., 2010. A novel effect of rivastigmine on pre-synaptic proteins and neuronal viability in a neurodegeneration model of fetal rat primary cortical cultures and its implication in Alzheimer's disease. J. Neurochem. 112, 843–853. https://doi.org/10.1111/j.1471-4159.2009.06490.x.

Barnes, C.A., Meltzer, J., Houston, F., Orr, G., McGann, K., Wenk, G.L., 2000. Chronic treatment of old rats with donepezil or galantamine: effects on memory, hippocampal plasticity and nicotinic receptors. Neuroscience 99, 17–23. https://doi.org/10.1016/S0306-4522(00)00180-9.

Bartus, R.T., Dean, R.L., Beer, B., Lippa, A.S., 1982. The cholinergic hypothesis of geriatric memory dysfunction. Science 217, 408–414. https://doi.org/10.1126/science.7046051.

Bautista-Aguilera, O.M., Esteban, G., Bolea, I., Nikolic, K., Agbaba, D., Moraleda, I., Iriepa, I., Samadi, A., Soriano, E., Unzeta, M., Marco-Contelles, J., 2014. Design, synthesis, pharmacological evaluation, QSAR analysis, molecular modeling and ADMET of novel donepezil-indolyl hybrids as multipotent cholinesterase/monoamine oxidase inhibitors for the potential treatment of Alzheimer's disease. Eur. J. Med. Chem. 75, 82–95. https://doi.org/10.1016/j.ejmech.2013.12.028.

Bhattacharya, S., Haertel, C., Maelicke, A., Montag, D., 2014. Galantamine slows down plaque formation and behavioral decline in the 5XFAD mouse model of Alzheimer's disease. PloS One 9, e89454. https://doi.org/10.1371/journal.pone.0089454.

Birks, J.S., Grimley Evans, J., 2015. Rivastigmine for Alzheimer's disease. Cochrane Database Syst. Rev. 4, CD001191. https://doi: 10.1002/14651858.CD001191.pub3.

Birks, J.S., Harvey, R.J., 2018. Donepezil for dementia due to Alzheimer's disease. Cochrane Database Syst. Rev. 6 https://doi.org/10.1002/14651858.CD001190.pub3.

Bohn, P., Gourand, F., Papamicael, C., Ibazizene, M., Dhilly, M., Gembus, V., Alix, F., Mihaela Liliana, Ţ., Marsais, F., Barré, L., Levacheet, V., 2015. Dihydroquinoline carbamate derivatives as "biooxidizable" prodrugs for brain delivery of acetylcholinesterase inhibitors: [11C] radiosynthesis and biological evaluation. Chem. Neurosci. 6, 737–744. https://doi.org/10.1021/cn5003539.

Boinpally, R., Chen, L., Zukin, S.R., McClure, N., Hofbauer, R.K., Periclou, A., 2015. A novel once-daily fixed-dose combination of memantine extended release and donepezil for the treatment of moderate to severe Alzheimer's disease: two phase I studies in healthy volunteers. Clin. Drug Invest. 35, 427–435. https://doi.org/10.1007/s40261-015-0296-4.

Bolea, I., Juárez-Jiménez, J., de Los Ríos, C., Chioua, M., Pouplana, R., Luque, F.J., Unzeta, M., Marco-Contelles, J., Samadi, A., 2011. Synthesis, biological evaluation, and molecular modeling of donepezil and N-[(5-(benzyloxy)-1-methyl-1H-indol-2-yl)methyl]-N-methylprop-2-yn-1-amine hybrids as new multipotent cholinesterase/monoamine oxidase inhibitors for the treatment of Alzheimer's disease. J. Med. Chem. 54 (24), 8251–8270. https://doi.org/10.1021/jm200853t.

Brewer, L., Bennett, K., McGreevy, C., Williams, D., 2013. A population based study of dosing and persistence with anti-dementia medications. Eur. J. Clin. Pharmacol. 69, 1467–1475. https://doi.org/10.1007/s00228-013-1483-y.

Brodaty, H., Corey-Bloom, J., Potocnik, F.C., Truyen, L., Gold, M., Damaraju, C.R., 2005. Galantamine prolonged-release formulation in the treatment of mild to moderate Alzheimer's disease. Dement. Geriatr. Cognit. Disord. 20 (2–3), 120–132. https://doi.org/10.1159/000086613.

Buckler, J.N., Taher, E.S., Fraser, N.J., Willis, A.C., Carr, P.D., Jackson, C.J., Banwell, M. G., 2017. The synthesis of certain derivatives and analogues of (−)- and (+)-Galanthamine and an assessment of their capacities to inhibit acetylcholine esterase. J. Org. Chem. 82, 7869–7886. https://doi.org/10.1021/acs.joc.7b01062.

Campos, A.C., Moreira, F.A., Gomes, F.V., Del Bel, E.A., Guimarães, F.S., 2012. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 367, 3364–3378. https://doi.org/10.1098/rstb.2011.0389.

Carotenuto, A., Rea, R., Traini, E., et al., 2017. The effect of the association between donepezil and choline alphoscerate on behavioral disturbances in alzheimer's disease: interim results of the ASCOMALVA trial. J Alzheimers Dis. 56 (2), 805–815. https://doi.org/10.3233/JAD-160675.

Cerbai, F., Giovannini, M.G., Melani, C., Enz, A., Pepeu, G., 2007. N1phenethyl-norcymserine, a selective butyrylcholinesterase inhibitor, increases acetylcholine release in rat cerebral cortex: a comparison with donepezil and rivastigmine. Eur. J. Pharmacol. 572 (2–3), 142–150. https://doi.org/10.1016/j.ejphar.2007.06.053.

Coelho, F., Birks, J., 2001. Physostigmine for Alzheimer's disease. Cochrane Database Syst. Rev. (2), CD001499. https://doi.org/10.1002/14651858.CD001499.

Corey-Bloom, J., Anand, R., Veach, J., 1998. A randomized trial evaluating the efficacy and safety of ENA 713 (rivastigmine tartrate), a new acetylcholinesterase inhibitor, in patients with mild to moderately severe Alzheimer's disease. Int. J. Geriatr. Psychiatr. 1, 55–65. https://doi.org/10.3329/sjps.v4i1.8878.

Courtney, C., Farrell, D., Gray, R., 2004. AD2000 Collaborative Group. Long-term donepezil treatment in 565 patients with Alzheimer's disease (AD2000): randomised double-blind trial. Lancet 363, 2105–2115. https://doi.org/10.1016/S0140-6736(04)16499-4.

Cummings, J., Fox, N., 2017. Defining disease modifying therapy for alzheimer's disease. J. Prev. Alzheimers Dis. 4 (2), 109-115. https://doi.org/10.14283/jpad.2017.12.

Darvesh, S., Walsh, R., Kumar, R., Caines, A., Roberts, S., Magee, D., Rockwood, K., Martin, E., 2003. Inhibition of human cholinesterases by drugs used to treat Alzheimer disease. Alzheimer Dis. Assoc. Disord. 17 (2), 117–126. https://doi.org/10.1097/00002093-200304000-00011.

Deardorff, W.J., Grossberg, G.T., 2016. A fixed-dose combination of memantine extended-release and donepezil in the treatment of moderate-to-severe Alzheimer's disease. Drug Des. Dev. Ther. 10, 3267–3327. https://doi.org/10.2147/DDDT.S86463.

Desai, A.K., Grossberg, G.T., 2005. Rivastigmine for Alzheimer's disease. Expert Rev. Neurother. 5 (5), 563–580. https://doi.org/10.1586/14737175.5.5.563.

Dias Viegas, F.P., de Freitas Silva, M., Divino da Rocha, M., Castelli, M.R., Riquiel, M.M., Machado, R.P., Vaz, S.M., Simões de Lima, L.M., Mancini, K.C., Marques de Oliveira, P.C., Morais, É.P., Gontijo, V.S., da Silva, F.M.R., D'Alincourt da Fonseca Peçanha, D., Castro, N.G., Neves, G.A., Giusti-Paiva, A., Vilela, F.C., Orlandi, L., Camps, I., Veloso, M.P., Leomil Coelho, L.F., Ionta, M., Ferreira-Silva, G.Á., Pereira, R.M., Dardenne, L.E., Guedes, I.A., de Oliveira Carneiro Junior, W., Quaglio Bellozi P, M., Pinheiro de Oliveira, A.C., Ferreira, F.F., Pruccoli, L., Tarozzi, A., Viegas Jr., C., 2018. Design, synthesis and pharmacological evaluation of N-benzyl-piperidinyl-aryl-acylhydrazone derivatives as donepezil hybrids: discovery of novel multi-target anti-alzheimer prototype drug candidates. Eur. J. Med. Chem. 147, 48–65. https://doi.org/10.1016/j.ejmech.2018.01.066.

Dimitrova, D.S., Getova-Spassova, D.P., 2006. Effects of galantamine and donepezil on active and passive avoidance tests in rats with induced hypoxia. J. Pharmacol. Sci. 101, 199–204. https://doi.org/10.1254/jphs.FPE05006X.

Eldufani, J., Blaise, G., 2019. The role of acetylcholinesterase inhibitors such as neostigmine and rivastigmine on chronic pain and cognitive function in aging: a review of recent clinical applications. Alzheimer's Dementia 5, 175–183. https://doi.org/10.1016/j.trci.2019.03.004.

English, C., 2012. Donepezil 23 mg: is it more advantageous compared to the original? Ment. Health Clin. 1 (11), 272–273. https://doi.org/10.9740/mhc.n106739.

Enz, A., Amstutz, R., Boddeke, H., Gmelin, G., Malanoski, J., 1993. Brain selective inhibition of acetylcholinesterase: a novel approach to therapy for Alzheimer's disease. Brain Res. 98, 431–438. https://doi.org/10.1016/S0079-6123(08)62429-2.

Enz, A., Gentsch, C., 2004. Co-administration of memantine has not effect on the in vitro or ex vivo determined acetylcholinesterase inhibition of rivastigmine in the rat brain. Neurofarmacology 38, 408–413.

Fancellu, G., Chand, K., Tomás, D., Orlandini, E., Piemontese, L., Silva, D.F., Cardoso, S. M., Chaves, S., Santos, M.A., 2020. Novel tacrine-benzofuran hybrids as potential multi-target drug candidates for the treatment of Alzheimer's Disease. J. Enzym. Inhib. Med. Chem. 35 (1), 211–226. https://doi.org/10.1080/14756366.2019.1689237.

Farlow, M.R., 2003. Clinical pharmacokinetics of galantamine. Clin. Pharmacokinet. 42 (15), 1383–1392. http://doi-org-443.webvpn.fjmu.edu.cn/10.2165/00003088-200342150-00005.

Farlow, M.R., Grossberg, G.T., Sadowsky, C.H., Meng, X., Somogyi, M.A., 2013. 24-week, randomized, controlled trial of rivastigmine patch 13.3 mg/24 h versus 4.6 mg/24 h in severe Alzheimer's dementia. CNS Neurosci. Ther. 19 (10) https://doi.org/10.1111/cns.12158, 745-552.

Furukawa-Hibi, Y., Alkam, T., Nitta, A., Matsuyama, A., Mizoguchi, H., Suzuki, K., Moussaoui, S., Yu, Q.S., Greige, N.H., Nagai, T., Yamada, K., 2011. Butyrylcholinesterase inhibitors ameliorate cognitive dysfunction induced by amyloid-β peptide in mice. Behav. Brain Res. 225 (1), 222–229. https://doi.org/10.1016/j.bbr.2011.07.035.

Giacobini, E., 1998. Cholinesterase inhibitors for Alzheimer's disease: from tacrine to future applications. Neurochem. Int. 32, 413–419. https://doi.org/10.1016/S0197-0186(97)00124-1.

Gracon, S.I., Knapp, M.J., Berghoff, W.G., Pierce, M., DeJong, R., Lobbestael, S.J., Symons, J., Dombey, S.L., Luscombe, F.A., Kraemer, D., 1998. Safety of tacrine: clinical trials, treatment IND, and postmarketing experience. Alzheimer Dis. Assoc. Disord. 12 (2), 93–101. https://doi.org/10.1097/00002093-199806000-00007.

Greig, S.L., 2015. Memantine ER/donepezil: a review in alzheimer's disease. CNS Drugs 29, 963–970. https://doi.org/10.1007/s40263-015-0287-2.

Grossberg, G.T., 2003. Cholinesterase inhibitors for the treatment of Alzheimer's disease: getting on and staying on. Curr. Ther. Res. Clin. Exp. 64, 216–235. https://doi.org/10.1016/S0011-393X(03)00059-6.

Grossberg, G.T., Desai, A.K., 2003. Management of Alzheimer's disease. J. Gerontol. Med. Sci. 58 (4), 331–353. https://doi.org/10.1093/gerona/58.4.M331.

Grossberg, G.T., Manes, F., Allegri, R.F., Gutierrez-Robledo, L.M., Gloger, S., Xie, L., 2013. The safety, tolerability, and efficacy of once-daily memantine (28 mg): a multinational, randomized, double-blind, placebo-controlled trial in patients with moderateto-severe Alzheimer's disease taking cholinesterase inhibitors. CNS Drugs 27, 469–478. https://doi.org/10.1007/s40263-013-0077-7.

Heilbronn, E., 1961. Inhibition of cholinesterase by tetrahydroaminacrin. A. Chem. Snan 15, 1386–1390. https://doi.org/10.3891/acta.chem.scand.15-1386.

Hernandez, C.M., Gearhart, D.A., Parikh, V., Hohnadel, E.J., Davis, L.W., Middlemore, M. L., Warsi, S.P., Waller, J.L., Terry, A.V.J., 2006. Comparison of Galantamine and Donepezil for effects on nerve growth factor, cholinergic markers, and memory performance in aged rats. J. Pharmacol. Exp. Therapeut. 316, 679–694. https://doi.org/10.1124/jpet.105.093047.

Howard, R., McShane, R., Lindesay, J., Ritchie, C., Baldwin, A., Barber, R., Burns, A., Dening, T., Findlay, D., Holmes, C., Jones, R., Jones, R., McKeith, I., Macharouthu, A., O'Brien, J., Sheehan, B., Juszczak, E., Katona, C., Hills, R., Knapp, M., Ballard, C., Brown, R.G., Banerjee, S., Adams, J., Johnson, T., Bentham, P., Phillips, P.P., 2015. Nursing home placement in the Donepezil and Memantine in Moderate to Severe Alzheimer's Disease (DOMINO-AD) trial: secondary and post-hoc analyses. Lancet Neurol. 14 (12), 1171-1181. https://doi.org/10.1016/S1474-4422(15)00258-6.

Ismaili, L., Refouvelet, B., Benchekroun, M., Brogi, S., Brindisi, M., Gemma, S., Campiani, G., Filipic, S., Agbaba, D., Esteban, G., Unzeta, M., Nikolic, K., Butini, S., Marco-Contelles, J., 2017. Multitarget compounds bearing tacrine- and donepezil-like structural and functional motifs for the potential treatment of Alzheimer's disease. Prog. Neurobiol. 151, 4–34. https://doi.org/10.1016/j.pneurobio.2015.12.003.

Iwasaki, K., Egashira, N., Hatip-Al-Khatib, I., Akiyoshi, Y., Arai, T., Takagaki, Y., Watanabe, T., Mishima, K., Fujiwara, M., 2006. Cerebral ischemia combined with β-amyloid impairs spatial memory in the eight-arm radial maze task in rats. Brain Res. 1097 (1), 216–223. https://doi.org/10.1016/j.brainres.2006.04.073.

Jacobson, S.A., Sabbagh, M.N., 2008. Donepezil: potential neuroprotective and disease-modifying effects. Expet Opin. Drug Metabol. Toxicol. 4, 1363–1369. https://doi.org/10.1517/17425255.4.10.1363.

Jann, M.W., 2000. Rivastigmine, a new-generation cholinesterase inhibitor for the treatment of Alzheimer's disease. Pharmacotherapy 20, 1–12. https://doi.org/10.1592/phco.20.1.1.34664.

Jarrott, B., 2017. Tacrine: in vivo veritas. Pharmacol. Res. 116, 29-31. https://doi.org/10.1016/j.phrs.2016.12.033.

Jiang, D., Yang, X., Li, M., Wang, Y., Wang, Y., 2015. Efficacy and safety of galantamine treatment for patients with Alzheimer's disease: a meta-analysis of randomized controlled trials. J. Neural. Transm. 122, 1157–1166. https://doi.org/10.1007/s00702-014-1358-0.

Joe, E., Ringman, J.M., 2019. Cognitive symptoms of Alzheimer's disease: clinical management and prevention. BMJ 367, l6217. https://doi.org/10.1136/bmj.l6217.

Johnson, J.A., Johnson, D.A., Kraft, A.D., Calkins, M.J., Jakel, R.J., Vargas, M.R., Chen, P.C., 2008. The Nrf2-ARE pathway: an indicator and modulator of oxidative stress in neurodegeneration. Ann. N. Y. Acad. Sci. 1147, 61–69. https://doi.org/10.1196/annals.1427.036.

Karl, T., Garner, B., Cheng, D., 2017. The therapeutic potential of the phytocannabinoid cannabidiol for Alzheimer's disease. Behav. Pharmacol. 28, 142–160. https://doi.org/10.1097/FBP.0000000000000247.

Kavanagh, S., Gaudig, M., Van Baelen, B., Adami, M., Delgado, A., Guzman, C., Jedenius, E., Schäuble, B., 2011. Galantamine and behavior in Alzheimer disease: analysis of four trials. Acta Neurol. Scand. 124, 302–308. https://doi.org/10.1111/j.1600-0404.2011.01525.x.

Kim, S.H., Kandiah, N., Hsu, J.L., Suthisisang, C., Udommongkol, C., Dash, A., 2017. Beyond symptomatic effects: potential of donepezil as a neuroprotective agent and disease modifier in Alzheimer's disease. Br. J. Pharmacol. 174, 4224–4232. https://doi.org/10.1111/bph.14030.

Kishi, T., Matsunaga, S., Oya, K., Ikuta, T., Iwata, N., 2015. Protection against brain atrophy by anti-dementia medication in mild cognitive impairment and Alzheimer's disease: meta-analysis of longitudinal randomized placebo-controlled trials. Int. J. Neuropsychopharmacol. 18, 1–7. https://doi.org/10.1093/ijnp/pyv070.

Kita, Y., Ago, Y., Higashino, K., Asada, K., Takano, E., Takuma, K., Matsuda, T., 2014. Galantamine promotes adult hippocampal neurogenesis via M1 muscarinic and α7 nicotinic receptors in mice. Int. J. Neuropsychopharmacol. 17 (12), 1957–1968. https://doi.org/10.1017/S1461145714000613.

Knight, R., Khondoker, M., Magill, N., Stewart, R., Landau, S., 2018. A systematic review and meta-analysis of the effectiveness of acetylcholinesterase inhibitors and memantine in treating the cognitive symptoms of dementia. Dement. Geriatr. Cognit. Disord. 45, 131–151. https://doi.org/10.1159/000486546.

Korabecny, J., Zemeka, F., Soukup, O., Spilovska, K., Musilek, K., Junb, D., Nepovimova, E., Kuc, K., 2014. Pharmacotherapy of Alzheimer's Disease: Current State and Future Perspectives Drug Design and Discovery in Alzheimer's Disease, pp. 3–39. https://doi.org/10.1016/B978-0-12-803959-5.50001-5.

Kozurkova, M., Hamulakova, S., Gazova, Z., Paulikova, H., Kristian, P., 2011. Neuroactive multifunctional tacrine congeners with cholinesterase, anti-amyloid aggregation and neuroprotective properties. Pharmaceuticals 4 (2), 382–418. https://doi.org/10.3390/ph4020382.

Lane, R.M., Potkin, S.G., Enz, A., 2006. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. Int. J. Neuropsychopharmacol. 9 (1), 101–124. https://doi.org/10.1017/S1461145705005833.

Li, H., Liu, Y., Tian, D., Tian, L., Ju, X., Qi, L., Wang, Y., Liang, C., 2020. Overview of cannabidiol (CBD) and its analogues: Structures, biological activities, and neuroprotective mechanisms in epilepsy and Alzheimer's disease. Eur. J. Med. Chem. 192, 112–163. https://doi.org/10.1016/j.ejmech.2020.112163.

Li, J., Wu, H.M., Zhou, R.L., Liu, G.J., Dong, B.R., 2008. Huperzine A for Alzheimer's disease. Cochrane Database Syst. Rev. (2), CD005592. https://doi.org/10.1002/14651858.CD005592.pub2.

Lin, H., Li, Q., Gu, K., Zhu, J., Jiang, X., Chen, Y., Sun, H., 2017. Therapeutic agents in Alzheimer's disease through a multitarge directed ligands strategy: recent progress based on tacrine core. Curr. Top. Med. Chem. 17 (27), 3000–3016. https://doi.org/10.2174/1568026617666170717114944.

Liu, Y., Zhang, Y., Zheng, X., Fang, T., Yang, X., Luo, X., Guo, A., Newell, K.A., Huang, X. F., Yu, Y., 2018. Galantamine improves cognition, hippocampal inflammation, and synaptic plasticity impairments induced by lipopolysaccharide in mice. J. Neuroinflammation 15 (1), 112. https://doi.org/10.1186/s12974-018-1141-5.

Madav, Y., Wairkar, S., Prabhakar, B., 2019. Recent therapeutic strategies targeting beta amyloid and tauopathies in Alzheimer's disease. Brain Res. Bull. 146, 171–184. https://doi.org/10.1016/j.brainresbull.2019.01.004.

Maelicke, A., 2000. Allosteric modulation of nicotinic receptors as a treatment strategy for Alzheimer's disease. Dement. Geriatr. Cognit. Disord. 11 (Suppl. 1), 11–18. https://doi.org/10.1159/000051227.

Maelicke, A., Hoeffle-Maas, A., Ludwig, J., Maus, A., Samochock, M., Jordis, U., Koepke, A.K.E., 2010. Memogain is a galantamine pro-drug having dramatically reduced adverse effects and enhanced efficacy. J. Mol. Neurosci. 40 (1–2), 135–137. https://doi.org/10.1007/s12031-009-9269-5.

Márquez, F., Yassa, M.A., 2019. Neuroimaging biomarkers for alzheimer's disease. Mol. Neurodegener. 14 (1), 21. https://doi.org/10.1186/s13024-019-0325-5, 2019 Jun 7.

Meng, F.C., Mao, F., Shan, W.J., Qin, F., Huang, L., Li, X.S., 2012. Design, synthesis, and evaluation of indanone derivatives as acetylcholinesterase inhibitors and metal-chelating agents. Bioorg. Med. Chem. Lett 22 (13), 4462–4466. https://doi.org/10.1016/j.bmcl.2012.04.029.

Mercier, F., Lefevre, G., Huang, H.L., 2007. Rivastigmine exposure provided by a transdermal patch versus capsules. Curr. Med. Res. Opin. 23, 3199–3204. https://doi.org/10.1185/030079908X253438.

Mesulam, M., Guillozet, A., Shaw, P., Quinn, B., 2002. Widely spread butyrylcholinesterase can hydrolyze acetylcholine in the normal and Alzheimer brain. Neurobiol. Dis. 9, 88–93. https://doi.org/10.1006/nbdi.2001.0462.

Mesulam, M.M., Geula, C., 1991. Acetylcholinesterase-rich neurons of the human cerebral cortex: cytoarchitectonic and ontogenetic patterns of distribution. J. Comp. Neurol. 306 (2), 193–220. https://doi.org/10.1002/cne.903060202.

Meunier, J., Ieni, J., Maurice, T., 2006. The anti-amnesic and neuroprotective effects of donepezil against amyloid β25-35 peptide-induced toxicity in mice involve an interaction with the σ1receptor. Br. J. Pharmacol. 149, 998–1012. https://doi.org/10.1038/sj.bjp.0706927.

Mishra, D.N., 2019. Nose to brain delivery of galantamine loaded nanoparticles: in-vivo pharmacodynamic and biochemical study in mice. Curr. Drug Deliv. 16 (1), 51–58. https://doi.org/10.2174/1567201815666181004094707.

Mohammad, D., Chan, P., Bradley, J., 2017. Acetylcholinesterase inhibitors for treating dementia symptoms- a safety evaluation. Expet Opin. Drug Saf. 16 (9), 1009–1019. https://doi.org/10.1080/14740338.2017.1351540.

Mohapel, P., Leanza, G., Kokaia, M., Lindvall, O., 2005. Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning. Neurobiol. Aging 26, 939–946. https://doi.org/10.1016/j.neurobiolaging.2004.07.015.

Multum, C., 2019. Donepezil Medically Reviewed by Drugs com https://www.drugs.com/mtm/donepezil.html.

Nordberg, A., Ballard, C., Bullock, R., Darreh-Shori, T., Somogyi, M., 2013. A review of butyrylcholinesterase as a therapeutic target in the treatment of Alzheimer's disease. Prim. Care Companion CNS Disord. 15 (2) https://doi.org/10.4088/PCC.12r01412.

Ogura, H., Kosasa, T., Kuriya, Y., Yamanishi, Y., 2000. Comparison of inhibitory activities of donepezil and other cholinesterase inhibitors on acetylcholinesterase and butyrylcholinesterase in vitro. Methods Find Exp. Clin. Pharmacol. 22 (8), 609–613. https://doi.org/10.1358/mf.2000.22.8.701373.

Olin, J., Schneide, r L., 2006. Galantamine for Alzheimer's disease and mild cognitive impairment. Cochrane Database Syst. Rev. 1 (1) https://doi.org/10.1002/14651858.CD001747.pub3.

Onor, M.L., Trevisiol, M., Aguglia, E., 2007. Rivastigmine in the treatment of Alzheimer's disease: an update. Clin. Interv. Aging 2 (1), 17–32. https://doi.org/10.2147/ciia.2007.2.1.17.

Pang, Y.P., Brimijoin, S., 1997. Tha Analogs Useful as Cholinesterase inhibitors. Mayo Foundation For Medical Edu-cation And Research, USA. Patent WO1997021681.

Parnetti, L., Chiasserini, D., Andreasson, U., Ohlson, M., Hüls, C., Zetterberg, H., Minthon, L., Wallin, A.K., Andreasen, N., Talesa, V.N., Blennow, K., 2011. Changes in CSF acetyl and butyrylcholinesterase activity after long-term treatment with AChE inhibitors in Alzheimer's disease. Acta Neurol. Scand. 124 (2), 122–129. https://doi.org/10.1111/j.1600-0404.2010.01435.

Piamonte, B.L.C., Espiritu, A.I., Anlacan, V.M.M., 2020. Effects of citicoline as an adjunct treatment for alzheimer's disease: a systematic review. J Alzheimers Dis. 76 (2), 725–732. https://doi.org/10.3233/JAD-200378.

Perry, E., Martin-Ruiz, C., Lee, M., Griffiths, M., Johnson, M., Piggott, M., Haroutunian, V., Buxbaum, J.D., Nāsland, J., Davis, K., Gotti, C., Clementi, F., Tzartos, S., Cohen, O., Soreq, H., Jaros, E., Perry, R., Ballard, C., McKeith, I., Court, J., 2000. Nicotinic receptor subtypes in human brain ageing. Alzheimer and Lewy body diseases. Eur. J. Pharmacol. 393, 215–222. https://doi.org/10.1016/S0014-2999(00)00064-9.

Perry, E., McKeith, I., Ballard, C., 2003. Butyrylcholinesterase and progression of cognitive deficits in dementia with Lewy bodies. Neurology 60, 1852–1853. https://doi.org/10.1212/01.WNL.0000068336.84399.9E.

Piazzi, L., Rampa, A., Bisi, A., Gobbi, S., Belluti, F., Cavalli, A., Bartolini, M., Andrisano, V., Valenti, P., Recanatini, M., 2003. 3-(4-{[benzyl(methyl)amino] methyl}phenyl)-6,7-dimethoxy-2H-2-chromenone(AP2238) inhibits both acetylcholinesterase and acetylcholinesterase-induced beta-amyloid aggregation: a dual function lead for Alzheimer's disease therapy. J. Med. Chem. 46, 2279–2282. https://doi.org/10.1021/jm0340602.

Pini, L., Pievani, M., Bocchetta, M., et al., 2016. Brain atrophy in Alzheimer's Disease and aging. Ageing Res. Rev. 30, 25-48. https://doi.org/10.1016/j.arr.2016.01.002.

Potkin, S.G., Anand, R., Fleming, K., Alva, G., Keator, D., Carreon, D., Messina, J., Wu, J. C., Hartman, R., Fallon, J.H., 2001. Brain metabolic and clinical effects of rivastigmine in Alzheimer's disease. Int. J. Neuropsychopharmacol. 4 (3), 223–230. https://doi.org/10.1017/S1461145701002528.

Raskind, M.A., Peskind, E.R., Wessel, T., Yuan, W., 2000. Galantamine in AD: a 6-month randomized, placebo-controlled trial with a 6-month extension. Neurology 54 (12), 2261–2268. https://doi.org/10.1212/WNL.54.12.2261.

Reid, R.T., Sabbagh, M.N., 2008. Effects of cholinesterase inhibitors on rat nicotinic receptor levels in vivo and in vitro. J. Neural. Transm. 115 (10), 1437–1444. https://doi.org/10.1007/s00702-008-0107-7.

Rinner, U., Dankcand, C., Hudlickyd, T., 2017. GALANTHAMINE (chapter 11). http://dx.medra.org/10.17374/targets.2017.20.283.

Rizzo, S., Bartolini, M., Ceccarini, L., Piazzi, L., Gobb, i S., Cavalli, A., Recanatini, M., Andrisano, V., Rampa, A., 2010. Targeting Alzheimer's disease:Novel indanone hybrids bearing a pharmacophoric fragment of AP2238. Bioorg. Med. Chem. 18 (5), 1749–1760. https://doi.org/10.1016/j.bmc.2010.01.071.

Rockwood, K., Fay, S., Gorman, M., 2010. The ADAS-cog and clinically meaningful change in the VISTA clinical trial of galantamine for Alzheimer's disease. Int. J. Geriatr. Psychiatr. 25 (2), 191–201. https://doi.org/10.1002/gps.2319.

Rong, C., Lee, Y., Carmona, N.E., Cha, D.S., Ragguett, R.M., Rosenblat, J.D., Mansur, R. B., Ho, R.C., McIntyre, R.S., 2017. Cannabidiol in medical marijuana: research vistas and potential opportunities. Pharmacol. Res. 121, 213–218. https://doi.org/10.1016/j.phrs.2017.05.005.

Rosenblatt, A., Gao, J., Mackell, J., Richardson, S., 2010. Efficacy and safety of donepezil in patients with Alzheimer's disease in assisted living facilities. Am. J. Alzheimer's Dis. Other Dementias 25, 483–489. https://doi.org/10.1177/1533317510372923.

Rosler, M., Anand, R., Cicin-Sain, A., Gauthier, S., Agid, Y., Dal-Bianco, P., Stähelin, H.B., Hartman, R., Gharabawi, M., 1999. Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. BMJ 318 (7184), 633–638. https://doi.org/10.1136/bmj.318.7184.633.

Roy, K.K., Tota, S.K., Shukla, R., Nath, C., Saxena, A.K., 2015. Substituted 1, 2, 3, 4-Tetrahydroquinolin-7-Yl Carbamates Their Preparation and Use There of as Acetylcholinesterase (AChE) Inhibitors for the Treatment of Alzheimer's and Other Neurodegenerative Disease. US8946261B2.

Russo, E., Guy, G.W., 2006. A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol Med. Hypotheses 66, 234–246. https://doi.org/10.1016/j.mehy.2005.08.026.

Samadi, A., de la Fuente Revenga, M., Pérez, C., Iriepa, I., Moraleda, I., Rodríguez-Franco, M.I., Marco-Contelles, J., 2013. Synthesis, pharmacological assessment, and molecular modeling of 6-chloropyridonepezils: new dual AChE inhibitors as potential drugs for the treatment of Alzheimer's disease. Eur. J. Med. Chem. 67, 64–74. https://doi.org/10.1016/j.ejmech.2013.06.021.

Samadi, A., Estrada, M., Pérez, C., Rodríguez-Franco, M.I., Iriepa, I., Moraleda, I., Chioua, M., Marco-Contelles, J., 2012. Pyridonepezils, new dual AChE inhibitors as potential drugs for the treatment of Alzheimer's disease: synthesis, biological assessment, and molecular modeling. Eur. J. Med. Chem. 57, 296–301. https://doi.org/10.1016/j.ejmech.2012.09.030.

Sameem, B., Saeedi, M., Mahdavi, M., Shafiee, A., 2017. A review on tacrine-based scaffolds as multi-target drugs (MTDLs) for Alzheimer's disease. Eur. J. Med. Chem. 128, 332–345. https://doi.org/10.1016/j.ejmech.2016.10.060.

Saxena, M., Dubey, R., 2019. Target enzyme in Alzheimer's disease: acetylcholinesterase inhibitors. Curr. Top. Med. Chem. 19 (4), 264–275. https://doi.org/10.2174/1568026619666190128125912.

Scott, L.J., Goa, K.L., 2000. Galantamine: a review of its use in Alzheimer's disease. Drugs 60 (5), 1095–1122. https://doi.org/10.2165/00003495-200060050-00008.

Seltzer, B., 2010. Galantamine-ER for the treatment of mild-to-moderate Alzheimer's disease. Clin. Interv. Aging 5, 1–6. https://doi.org/10.2147/CIA.S4819.

Shen, Y., Sheng, R., Zhang, J., He, Q., Yang, B., Hu, Y., 2008. 2-Phenoxy-indan-1-one derivatives as acetylcholinesterase inhibitors: a study on the importance of modifications at the side chain on the activity. Bioorg. Med. Chem. 16 (16), 7646–7653. https://doi.org/10.1016/j.bmc.2008.07.014.

Shigeta, M., Homma, A., 2001. Donepezil for Alzheimer's disease: pharmacodynamic, pharmacokinetic, and clinical profiles. CNS Drug Rev. 7 (4), 353–368. https://doi.org/10.1111/j.1527-3458.2001.tb00204.x.

Snape, M.F., Misra, A., Murray, T.K., De Souza, R.J., Williams, J.L., Cross, A.J., Green, A. R., 1999. A comparative study in rats of the in vitro and in vivo pharmacology of the acetylcholinesterase inhibitors tacrine, donepezil and NXX-066. Neuropharmacology 38 (1), 181–193. https://doi.org/10.1016/S0028-3908(98)00164-6.

Sola, I., Viayna, E., Gómez, T., Galdeano, C., Cassina, M., Camps, P., Romeo, M., Diomede, L., Salmona, M., Franco-Schaeffer, M., Colantuono, D., Robin, D., Brunner, D., Taub, N., Hutter-Paier, B., Muñoz-Torrero, D., 2015. Multigram synthesis and in vivo efficacy studies of a novel multitarget anti-Alzheimer's compound. Molecules 20 (3), 4492–4515. https://doi.org/10.3390/molecules20034492.

Soukup, O., Jun, D., Zdarova-Karasova, J., Patocka, J., Musilek, K., Korabecny, J., Krusek, J., Kaniakova, M., Sepsova, V., Mandikova, J., Trejtnar, F., Pohanka, M., Drtinova, L., Pavlik, M., Tobin, G., Kuca, K., 2013. A resurrection of 7-MEOTA: a comparison with tacrine. Curr. Alzheimer Res. 10 (8), 893–906. https://doi.org/10.2174/1567205011310080011.

Spowart-Manning, L., van der Staay, F.J., 2005. Spatial discrimination deficits by excitotoxic lesions in the Morris water escape task. Behav. Brain Res. 156 (2), 269–276. https://doi.org/10.1016/j.bbr.2004.05.031.

Sugimoto, H., Ogura, H., Arai, Y., Limura, Y., Yamanishi, Y., 2002. Research and development of donepezil hydrochloride, a new type of acetylcholinesterase inhibitor. Jpn. J. Pharmacol. 89 (1), 7–20. https://doi.org/10.1254/jjp.89.7.

Szeto, J.Y.Y., Lewis, S.J.G., 2016. Current treatment options for Alzheimer's disease and Parkinson's disease dementia. Curr. Neuropharmacol. 14 (4), 326–338. https://doi.org/10.2174/1570159X14666151208112754.

Tariot, P.N., Solomon, P.R., Morris, J.C., Kershaw, P., Lilienfeld, S., Ding, C., 2000. A 5-month, randomized, placebo-controlled trial of galantamine in AD. Neurology 54 (12), 2269–2276. https://doi.org/10.1212/WNL.54.12.2269.

Terra, B.S., da Silva, P.H.C., Tramarin, A., Franco, L.L., da Cunha, E.F.F., Macedo Junior, F., Ramalho, T.C., Bartolini, M., Bolognesi, M.L., de Fatima, A., 2018. Two novel donepezil-lipoic acid hybrids: synthesis, anticholinesterase and antioxidant activities and theoretical studies. J. Braz. Chem. Soc. 29, 738–747. https://doi.org/10.21577/0103-5053.20170196.

Traini, E., Carotenuto, A., Fasanaro, A.M., Amenta, F., 2020. Volume Analysis of brain cognitive areas in alzheimer's disease: interim 3-year results from the ASCOMALVA trial. J Alzheimers Dis. 76 (1), 317–329. https://doi.org/10.3233/JAD-190623.

Tsoi, K.K.F., Hirai, H.W., Chan, J.Y.C., Kwok, T.C.Y., 2016. Time to treatment initiation in people with Alzheimer disease: a meta-analysis of randomized controlled trials. J. Am. Med. Dir. Assoc. 17, 24–30. https://doi.org/10.1016/j.jamda.2015.08.007.

Tufekci, K.U., Bathway, E., Genc, S., Genc, K., 2011. The Nrf2/ARE pathway: a promising target to counteract mitochondrial dysfunction in Parkinson's disease. Parkinsons Dis. 314082. https://doi.org/10.4061/2011/314082.

van Dyck, C.H., Newhouse, P., Falk, W.E., Mattes, J.A., 2000. Extended-release physostigmine in Alzheimer disease: a multicenter, double-blind, 12-week study with dose enrichment. Physostigmine Study Group. Arch. Gen. Psychiatr. 57 (2), 157–164. https://doi.org/10.1001/archpsyc.57.2.157.

Wagstaff, A.J., McTavish, D., 1994. Tacrine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in Alzheimer's disease. Drugs Aging 4 (6), 510–540. https://doi.org/10.2165/00002512-199404060-00006.

Watkins, P.B., Zimmerman, H.J., Knapp, M.J., Gracon, S.I., Lewis, K.W., 1994. Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. J. Am. Med. Assoc. 271 (13), 992–998.

Wilcock, G.K., Lilienfeld, S., Gaens, E., 2000. Efficacy and safety of galantamine in patients with mild to moderate Alzheimer's disease: multicentre randomised controlled trial. Galantamine International-1 Study Group. BMJ 321 (7274), 1445–1449. https://doi.org/10.1136/bmj.321.7274.1445.

Wilkinson, D., Murray, J., 2001. Galantamine: a randomized, double-blind, dose comparison in patients with Alzheimer's disease. Int. J. Geriatr. Psychiatr. 16 (9), 852–857. https://doi.org/10.1002/gps.409.

Wlodek, S.T., Antosiewicz, J., McCammon, J.A., Straatsma, T.P., Gilson, M.K., Briggs, J. M., Humblet, C., Sussman, J.L., 1996. Binding of tacrine and 6-chlorotacrine by acetylcholinesterase. Biopolymers 38 (1), 109–117. https://doi.org/10.1002/(SICI)1097-0282(199601)38:1<109::AID-BIP9>3.0.CO;2-#.

Wright, C.I., Geula, C., Mesulam, M.M., 1993. Neurological cholinesterases in the normal brain and in Alzheimer's disease: relationship to plaques, tangles, and patterns of selective vulnerability. Ann. Neurol. 34 (3), 373–384. https://doi.org/10.1002/ana.410340312.

Wu, M.Y., Esteban, G., Brogi, S., Shionoya, M., Wang, L., Campiani, G., Unzeta, M., Inokuchi, T., Butini, S., Marco-Contelles, J., 2017. Donepezil-like multifunctional agents:Design, synthesis, molecular modeling and biological evaluation. Eur. J. Med. Chem. 121, 864–879. https://doi.org/10.1016/j.ejmech.2015.10.001.

Wu, Z., Zhao, L., Chen, X., Cheng, X., Zhang, Y., 2015. Galantamine attenuates amyloid-β deposition and astrocyte activation in APP/PS1 transgenic mice. Exp. Gerontol. 72, 244–250. https://doi.org/10.1016/j.exger.2015.10.015.

Xu, A.J., Chen, Z., Yanai, K., Huang, Y.W., Wei, E.Q., 2002. Effect of 3-[1-(phenylmethyl)-4-piperidinyl]-1-(2,3,4,5-tetrahydro-1H-1-benzazepin-8-yl)-1-propanone fumarate, a novel acetylcholinesterase inhibitor, on spatial cognitive impairment induced by chronic cerebral hypoperfusion in rats. Neurosci. Lett. 331 (1), 33–36. https://doi.org/10.1016/S0304-3940(02)00830-3.

Yang, G., Wang, Y., Tian, J., Liu, J., 2013. Huperzine A for alzheimer's disease: a systematic review and meta-analysis of randomized clinical trials. PLoS One 8 (9), e74916. https://doi.org/10.1371/journal.pone.0074916.

Yuede, C.M., Dong, H., Csernanskya, J.G., 2007. Anti-dementia drugs and hippocampal-dependent memory in rodents. Behav. Pharmacol. 18 (5–6), 347–363. https://doi.org/10.1097/FBP.0b013e3282da278d.

Zhang, C., Du, Q.Y., Chen, L.D., Wu, W.H., Liao, S.Y., Yu, L.H., Liang, X.T., 2016. Design, synthesis and evaluation of novel tacrine-multialkoxybenzene hybrids as multi-targeted compounds against Alzheimer's disease. Eur. J. Med. Chem. 116, 200–209. https://doi.org/10.1016/j.ejmech.2016.03.077.

Zhou, J., Fu, Y., Tang, X.C., 2001. Huperzine A and donepezil protect rat pheochromocytoma cells against oxygen-glucose deprivation. Neurosci. Lett. 306 (1–2), 53–56. https://doi.org/10.1016/S0304-3940(01)01855-9.