RESEARCH ARTICLE

Acetylcholinesterase Inhibitors for Alzheimer's disease: Past, Present, and Potential Future

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Abstract

Alzheimer's Diseases (AD) is a neurodegenerative disorder characterized by progressive neuronal loss leading to cognitive decline. Although there is yet to be a cure nor a way to reverse the neuronal damage, there are current treatments to amend some of the cognitive symptoms associated with AD. Acetylcholinesterase inhibitors (AChEi) are the primary agents of choice and have had profound implications throughout the past decades. AChEi such as donepezil, rivastigmine, and galantamine mediates and increases cholinergic activities in the central nervous system (CNS), and have been shown to improve and preserve cognition in AD patients. Beyond the current drugs on the market, investigational discoveries continue to explore the potential of safer and more efficacious AChEi agents for the treatment of AD. There have been quite a few challenges, given the high failure rates. Yet, these very trials and studies have been a fundamental step towards better understanding the treatments of AD and have provided some insight on the potential to surpass what is currently available.

Keywords: Alzheimer's Diseases; acetylcholinesterase inhibitors; approved drugs

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder typically characterized by gradual cognitive decline, progressive memory loss, and central nervous system (CNS) neuronal dysfunction.[1] AD is the most common form of dementia with an estimate of over 5 million individuals being afflicted in the United States.[2,3] These numbers are projected to grow, with the geriatric population being most at risk for developing AD.[4] It has been estimated ~48% of individuals within geriatric long-term care have AD or dementia.[5] The overall economic cost of AD is quite substantial, with an estimated of over $200 billion annually.[4] To date, there is no cure for AD. Nothing has been approved to reverse the CNS neuronal loss nor halt the progression in cognitive decline for AD. Although the definitive cause for AD is yet to be determined, there is some understanding of the hallmarks and certain mechanisms seen within AD. First, the pathophysiology of AD displays neuritic plaques associated with extracellular accumulation of amyloid beta.[1] Additionally, hyperphosphorylation of tau proteins are associated with intracellular neuro-fibular tangles.[6] There has yet to be a clear consensus on which proceeds which, however, it is likely that both mechanisms may play a role in the neural degeneration and neurotransmissions abnormality seen with AD. Regardless of the route, the neurotransmitter abnormalities lead to the lowering of acetylcholine (ACh) concentation in the CNS, and perhaps to a lesser extent, an increase in glutamate activity. These are thought to be associated with progressive cognitive decline or contributing factors to the progression of AD.

The primary therapeutic agents used for AD are acetylcholinesterase inhibitors (AChEi).[7] These agents are used to mediate and increase CNS cholinergic transmissions with the goal to address some of the cognitive symptoms and preserve an extent of the patient's activities of daily living (e.g. walking, grooming, bathing, toileting, dressing, managing medication, etc.).[8] By no means is addressing AD with AChEi curative and arguably the clinical improvement with these medications are modest. However, it has been shown to slow the progression of AD cognitive decline.[9-12]

Since AChEi are the primary target available for the treatment of AD, it is important to understand the past, present, and potential future of this target. Hence the primary focus of this review is to describe the overall mechanism of AChEi and ACh, previous studies of AChEi agents, and the potential AChEi agents being investigated for the treatment of AD, including some natural products.

2. ACh and AChEi mechanisms and role in AD with clinical implications/target

With AD patients there is a clear neurochemical imbalance. The most significant being the decrease in ACh neurotransmission.[6] ACh is a neurotransmitter responsible for many roles pertinent to normal physiological function in the peripheral and central nervous systems. In the peripheral nervous system, ACh is vital in parasympathetic and somatic functions inducing gastric secretions, muscle contractions, and the lowering of heart rate when conserving energy. In the CNS, ACh plays a critical role in cognition and memory.[13] Known agents with anticholinergic activity used in the treatment of urinary incontinence or symptomatic bradycardia etc. (e.g. oxybutynin and atropine respectively) with a strong affinity to CNS receptor have been shown to effects cognitive impairment (confusion, delirium, etc.).[14,15] There are claims that these cognitive impairment adverse drug reaction

caused by the anticholinergic are somewhat striking to the state of cognitive decline seen in dementia of AD, albeit transient.[16,17] Furthermore, these agents can potentially worsen the cognitive decline in AD patients.[18] Thus, it would be logical to see that targeting ACh transmissions may have some significance or implications on the cognitive symptoms of AD. In practice, to mediate ACh neurotransmissions for AD, agents that target acetylcholinesterase (AChE) are utilized.[9-12]

Acetylcholinesterase (AChE) catalyzes the breakdown of acetylcholine. Due to this, the inhibition of AChE has been widely investigated for AD as a means to increase the AC in the synapse. Normally, the most prominent isoform of AChE seen in CNS regions thought to be significant in memory processing (e.g. hippocampus, cerebral cortex, forebrain nuclei region, etc.) is G4 (tetrameric).[19] The primary sites of interest for the development of drugs are the peripheral binding site that interacts with the cationic portion of ACh and the active site for the ester hydrolysis.[24] This is critical for drug design because certain agents target this mechanism by acylating AChE with a more hydrolysis resistant functional groups (e.g. carbamoyl moiety). By doing this, it will impede regeneration, thus prolonging the AChE in its inactive state

A limitation AChEi is that there must be the presence of intact cholinergic neurons in order for the clinically beneficial effects to be seen. In certain cases, AChEi are discontinued as patients receive minimal or no clinically beneficial effects due to the severity of their advanced stage AD.[8,21-22] Some agents require high doses to ensure enough can cross the BBB, however, the adverse drug reactions (ADR) and toxicity must also be considered. Due to the extensive role of ACh peripherally and centrally, concern of adverse drug reactions are warranted. With the current AChEi, it is not uncommon for patients to experience nausea, vomiting, diarrhea, headaches, and/or insomnia which can lead to non-adherence or discontinuation.[8-12] Fortunately, some of the ADR can be addressed by measures such as switching dosage forms and taking the AChEi at specific times of the day.[23] Furthermore, with the increased cholinergic activity, clinical implications need to be considered. For example, caution is noted when AChEi are taken with drugs that slow down heart rate (calcium channel blockers, beta blockers, etc.), increase gastric secretions due to increasing GIT bleed risks (NSAIDs), or anticholinergics used for conditions such as urinary incontinence.[24]

3. AChEi that developed into the market for AD

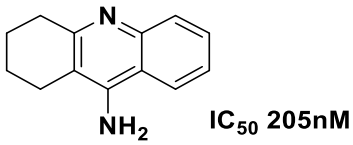
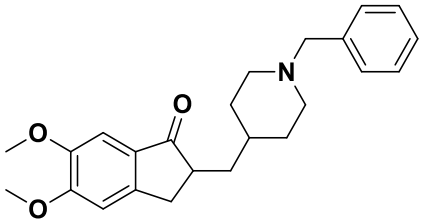


Fig. 1. Structure of tacrine

Tacrine (Figure 1) was the first AChEi to be FDA approved for the treatment of AD cognitive symptoms in 1993 under the brand name Cognex.[25] Tacrine is an aminoacridine derivative, specifically 1,2,3,4-tetrahydro-9-acridinamine.[26,27] Although it was able to cross the BBB, there were extensive peripheral adverse effects such as nausea, vomiting, and diarrhea.[28,29] Tacrine interacts at the AChE binding site via pi-stacking. Tacrine had an IC_{50} of 205nM (Figure 1) towards the AChE receptors.[30] There were some limitations presented with tacrine, such as concerns with its hepatotoxicity profile and the dosing frequency due to low oral bioavailability.[26,27] Due to concerns of hepatotoxicity, patients taking tacrine required liver monitoring,

specifically looking at hepatocellular damage and elevated liver enzymes. Recommendations proposed were to either lower the dose or stop the medication when transaminase levels were 3-5+ times above the upper limits.[28] Tacrine was given at 10-40mg every 6 hours by oral route without food.[27,28] This four times a day dosing frequency was inconvenient to many patients and was not conducive to medication adherence. Oral bioavailability of tacrine was quite low due to extensive first pass metabolism by CYP1A2. The elimination half-life of tacrine was ~1.5-5 hours with ~5-30% of tacrine entering the bloodstream, however, this was lowered when taken with food.[28,29] Tacrine was discontinued from the US market in 2013 with the introduction of safer alternatives.[26]

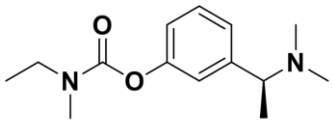


IC50 ~11.6nM

Donepezil

Fig. 2. Structure of donepezil

Donepezil (Figure 2) was the second approved AChEi by the FDA for the treatment of AD cognitive symptoms in 1996 under the brand name Aricept.[11] Donepezil is able to cross the BBB and has a strong affinity to AChE (IC_{50} value of 11.6 nM) in the CNS and to a lesser extent butylcholinesterase in the periphery.[30-32] Although there are concerns with donepezil, it is one of the most prescribed treatments for AD to date.[33] Concerns include considerations for QT-prolongations and higher cholinergic ADR profile due to long half-life and high receptor affinity.[11,34] With the QT-prolongation profile, risk-benefits need to be weighed and caution must be considered with patients at risk of arrhythmias. Furthermore, as with all AChEi, considerations of bradycardia should also be noted.[30] Other cholinergic ADR, which patients commonly complain about are nausea, vomiting, headaches, and insomnia.[11] However, the adverse effect issues can be addressed by adjusting the medication dose schedule.[23] The extended elimination half-life allows donepezil to be dosed at a once daily frequency 15-23mg which allows for improved medication adherence with AD patients.[11] Donepezil has an oral bioavailability of ~100% and the absorption is minimally affected by food intake. In terms of distribution, donepezil is ~96% plasma bounded; more so to albumin than to α-glycoprotein.[11] Donepezil is primarily metabolized by CYP3A4 and CYP2D6. Unchanged donepezil and its metabolites are excreted into the urine with an elimination half-life of ~70 hours.[11] In 2014, Namzaric was FDA approved for the treatment of AD as a combination of products containing donepezil and memantine extended release (NMDA antagonist).[12]



IC50= 4.3μM

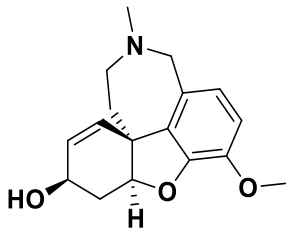
Rivastigmine

Fig. 3. Structure of rivastigmine.

Rivastigmine (Figure 3) is another AChEi approved by the FDA for the treatment of AD cognitive symptoms in 2000 under the brand name Exelon.[9] Though there were concerns related to cholinergic gastrointestinal tract (GIT) adverse effects (nausea, vomiting, diarrhea, etc.), there are minimal to no concerns with hepatotoxicity.[35,36] Interestingly, the GIT ADR issue seems to have been addressed with the approval of the transdermal dosage

formulation in 2007.[24,35] In addition, for the oral dosage form of rivastigmine, starting low and slow titration can help in GIT ADR tolerability. Rivastigmine is administered orally at 1.5-6mg twice daily with food.[36] Rivastigmine transdermal is administered at 4.6-13.3mg once daily while rotating sites.[9] There is a greater extent of first past metabolism with the oral formulation, as only ~36% makes it to the site of action and it is also recommended to take rivastigmine with food. Although food can delay absorption, there is an increase in AUC and increased medication tolerability by decreasing ADR. Rivastigmine is weakly bound to plasma protein with ~40% of it distributed by albumin. Rivastigmine is metabolized by the cholinesterase in plasma and at the site of action. The EXPRESS (Exelon in Parkinson's disease dementia study) trial in 2006 granted rivastigmine FDA approval for AD and dementia(s) associated with Parkinson's Disease.[37] The EXPRESS trial was a randomized, double blind, intervention testing rivastigmine against placebo for Parkison's associated dementia, utilizing the AD assessment scale and the cooperative study-clinical global impression of change scores as a measure for primary outcomes of efficacy.[37] The EXPRESS trial demonstrated moderate, albeit clinically significant results with cognitive improvements favoring rivastigmine over placebo.

Rivastigmine is able to cross the BBB and has a high affinity to the G1 isoform in the CNS. Studies have shown the IC_{50} of rivastigmine to AChE is ~4.3nM.[31] Rivastigmine binds to the site of esterase hydrolysis in AChE and butylcholinesterase. Upon reaction, it leaves behind a carbamate moiety attached to the AChE which requires significant energy to hydrolyze and reactivate/regenerate the enzyme.[38] The regeneration hydrolysis takes ~10 hours and thus is often referred to as pseudo-irreversible inhibition.[39] Of note, rivastigmine was initially researched as a semi-synthetic derivative of physostigmine which is another carbamates AChEi that was once believed to have potential in the treatment of AD.



IC50= 410 nM

Galantamine

Fig. 4. Structure of galantamine with IC_{50} value.

Galantamine (Figure 4) was FDA approved for the treatment of AD cognitive symptoms in 2001, formerly under the brand name Reminyl, now Razadyne.[10] Galantamine is a natural alkaloid derived from *Galanthus Nivalis*.[40-42] There is minimal to no concerns of hepatotoxicity, however, galantamine has renal dosing considerations.[40] Patients with a renal clearance of 9-59ml/min are limited to ≤16mg/day, while patients with severe impairment w/ CrCl <9 are recommended to not take galantamine.[10] There is limited information on galantamine and its metabolites interaction with dialysis (hemodialysis and peritoneal). Similarly, with other AChEi, there have been patient complaints of GIT ADR (nausea, vomiting, and diarrhea) though taking galantamine with a meal can help with drug tolerability.[10] Furthermore, starting with a low and slow titration can assist in GIT ADR tolerability. Galantamine has an immediate release (IR) formulation and an extended release (ER) formulation. Galantamine IR is given at 4-12mg twice daily orally with food (preferably with breakfast and an evening meal).[10] The bioavailability of galantamine IR formulation

is ~90%. When taken with a meal there is a delay in absorption but an increase in AUC and an increase in medication tolerability. Galantamine has a low plasma protein binding affinity with ~18% bound. Furthermore, galantamine is extensively metabolized in the liver primarily through CYP2D6 and to a lesser degree CYP3A4.[10] Approximately 95% is excreted in the urine, composed of unchanged galantamine and its various metabolites. Furthermore, there is a definitive increase in the AUC for renally impaired patients. Concentrations of galantamine are higher in AD patients compared to healthy young subjects; though this may be due to some of the risk factors/comorbidity associated with AD and advanced age. Furthermore, women seem to have a lower clearance compared to men.[10] Galantamine has an elimination half-life of ~7 hours.

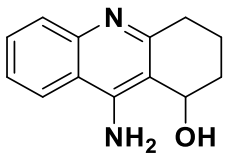
Since galantamine is a tertiary amine oppose to a quaternary ammonium salt, it is able to cross the BBB and bind to the active site of AChE.[42] In addition to inhibiting AChE, galantamine also interacts with nicotinic cholinergic receptors through allosteric binding.[43,44] There is limited data on whether or not modulation of nicotinic receptors has any clinical significance with AD, however, there are studies that are currently investigating this mechanism's role in the treatment of AD[43]. Galantamine has an IC_{50} to AChE of 410nM.[31] There have been investigations in other compounds modeled after galantamine for the treatment of AD such as the novel galantamine–camphene hybrid with promising IC_{50} data.

Table 1: AChEi marketed for the treatment of AD

AChEi currently used for AD treatment	IC_{50} to AChE	Trial and Testing	Reference/Citation
Donepezil	11.6 nM	FDA-Approved	11, 31-34
Rivastigmine	4.3 μM	FDA-Approved	9, 31, 35-39
Galantamine	410 nM	FDA Approved	10, 31, 40
Tacrine	11 μM	FDA-Approved; Taken off market as of 2013	25-31

4. Investigational AChEi that has yet to reach the market for AD

4.1 Tacrine derivatives (acridines)



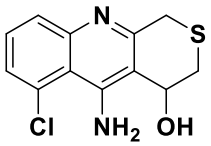
IC50= 3.27 μM

Velnacrine

Fig. 5. Structure of Velnacrine.

Velnacrine (Figure 5) is a known active metabolite derived from tacrine, more specifically 1-hydroxy-tacrine.[45,46] Previously there were interest in investigating velnacrine as a potential AChEi used in the treatment of AD with the ideas of addressing the hepatotoxicity ADR profile seen with tacrine.[45] Velnacrine has an IC_{50} to AChE of 3.27uM.[47-49] Similar to tacrine, velnacrine was theorized to act on the AChE active site via aromatic interactions.[47] Unfortunately, results were not promising

from a velnacrine double blind, randomize, clinical trial against placebo in the 1990s.[45,46] In one such trial, 236 individuals were administered velnacrine at 10-75mg three times daily or placebo in the same frequency, from week 1-7.[45] With a wash out period that followed thereafter, individuals were assigned to the dose they best responded to in weeks 10-15. 119 patients dropped out of the trial before the wash out period due to various reasons; with lack of efficacy and adverse reaction making up the majority. Furthermore, 26 individuals that moved onto week 10-15 dropped out due to adverse drug reactions.[46] The ADR profile of velnacrine included elevated liver enzymes, nausea, vomiting, diarrhea, and rash.[45,46] Nonetheless, there is still significant interest with the acridines analogs.[45,50]

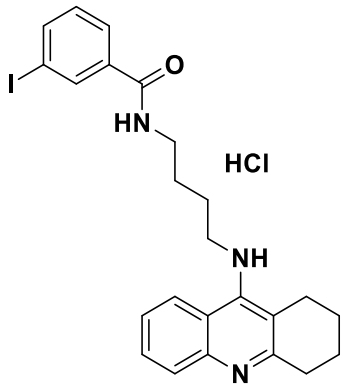


IC50= 0.32 μM

Compound 5eg

Fig. 6. Structure of velnacrine thiaanalogue Compound 5eg.[47]

Various velnacrine thiaanalogues were investigated as potential agents for AD treatment.[47] Modifications of velnacrine's cyclohexyl ring and aromatic ring were performed. Each compound underwent in vitro testing for AChE inhibition with human red blood cells, in addition to acute toxicity testing on mice. Depending on the compound, IC_{50} ranged from 0.32uM - >100uM to AChE.[47] The chlorinated compound 5eg (Figure 6) presented with the best IC_{50} of 0.32uM, though many other chlorinated compounds were deemed inactive.[47]

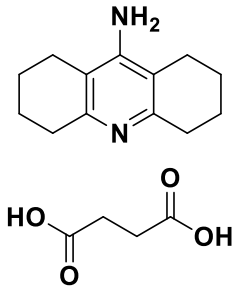


IC50= 31.2 nM

Compound 3f

Fig. 7. Structure of THA-IBA compound 3f.[49]

More recently, investigations on tetrahydroacridine derivatives with iodobenzoic acid (THA-IBA) showed some promising results with claims of a multifunctional mechanism against AD.[49] The primary mechanism of interest was the inhibition of AChE, however, it was also investigated for beta-amyloid aggregation inhibition.[49] With acridine derivatives, there are inherent concerns regarding hepatotoxicity and bioavailability requiring frequent doses. The researchers addressed the hepatotoxicity issue with THA-IBA by substituting the free amine group that was theorized to be responsible for the liver toxicity.[49] Certain THA-IBA compounds have IC_{50} values more potent than tacrine. Notable, a compound referred to as 3f tested with an IC_{50} of 31.2nM (Figure 7).[49] The proposed mechanism of action on AChE inhibition was similar to that of tacrine's aromatic interactions with binding sites of AChE. The novel THA-IBA design has potential as a candidate for AD treatment.[49] Theoretically, if the issues with hepatotoxicity and bioavailability were to be solved, this would be a revolutionary step for the acridine derivatives for use as an AChEi in the treatment of AD.



Octahydroaminoacridine succinate

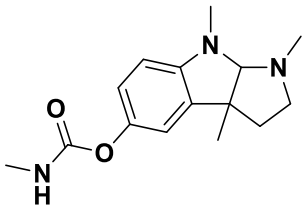
Fig. 8. Structure of octohydroaminoacridine succinate. IC_{50} was unspecified.

In terms of ongoing clinical trials for the acridine AChEi, an agent of note is octohydroaminoacridine succinate (Figure 8). Having completed a Phase 2 clinical trial in China in 2011,[50] Shanghai Mental Health Center has been recruiting for a double blind, randomized, parallel assignment intervention with octohydroaminoacridine succinate against donepezil and placebo for mild to moderate AD. This trial is estimated to be completed prior to the Spring of 2021 (Clinical Trial Identifier: NCT03283059).

Table 2: Tacrine derivatives (acridine) with AChEi properties being researched in AD treatment

AChEi that has been or is being researched in AD treatment	IC_{50} to AChE	Trial and Testing	Reference/Citations
Velnacrine	~3.27μM	Clinical Trials 1990s	45-47
Velnacrine thiaanalogues	0.32μM (Compound 5eg)	Animal studies, In vitro and in vivo testing	47
Tetrahydroacridine derivatives with iodobenzoic acid moiety	31.2nM (Compound 3f)	In vitro and in vivo testing. Computer modeling.	49
Octohydroaminoacride succinate	[Not specified]	Clinical Trial; Recruiting for Phase 3 Clinical trial	50 Clinical Trial ID: (NCT03283059)

4.2 Carbamates derivatives



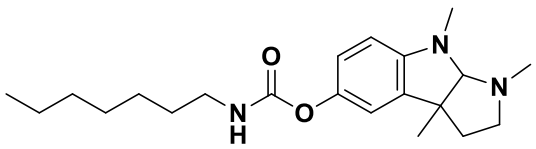
IC50= ~0.15 μM

Physostigmine

Fig. 9. Structure of physostigmine.

Physostigmine (Figure 9) is a carbamate AChEi derived from the Calabar bean and it is used for a variety of indications such as glaucoma and reversal of cholinergic toxicity.[51,52] Physostigmine has an IC_{50} of 0.15uM towards AChE.[53] Physostigmine completed a Phase 3 clinical trial in 1996 under the trade name Synapton. The formulations which physostigmine tested were through a controlled release oral and transdermal application, though incidences of

adverse drug reactions for both were still significantly high.[51,53]

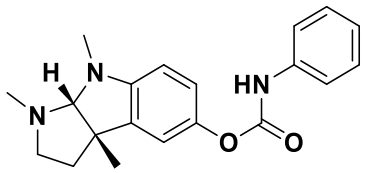


IC50= ~0.11 μM

Epistigmine

Fig. 10. Structure of epistigmine.

Epistigmine (Figure 10) is a physostigmine derivative modified with a heptyl chain. Epistigmine has an IC_{50} of 0.11uM and was investigated in an AD clinical trial under the name MF201.[54,55] Epistigmine was administered at 15-20mg three times daily. In terms of efficacy, epistigmine performed well against placebo,[54] however, trials were suspended indefinitely as of 1999 due to the emergence of hematological adverse events resulting in granulocytopenia in participants.[55]

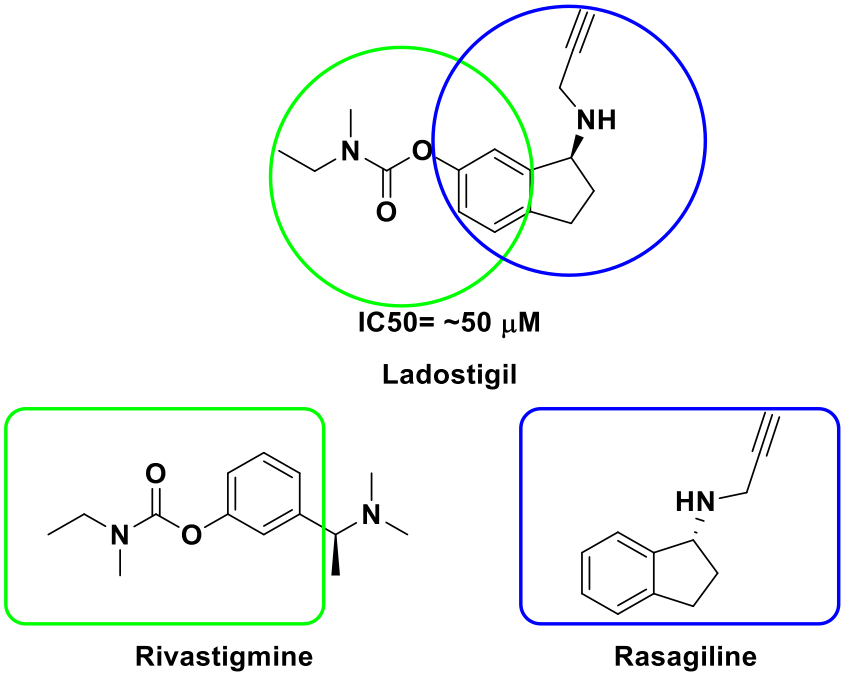


IC50= ~22 nM

Phenserine

Fig. 11. Structure of phenserine.

Phenserine (Figure 11) is another physostigmine derivative investigated for the treatment of AD. Phenserine has an IC_{50} of ~22nM to AChE[56]. Unfortunately, Axonyx biopharmaceutical stopped two Phase 3 clinical trials in 2005 due to failure to improve cognition.[56] The (+) enantiomer of phenserine is currently being investigated in continuing trials under the name of Posiphen-Discover Study (NCT02925650). The trial started in 2017 with studies estimated to conclude in December of 2020. However, it was recently noted that Posiphen does not appear to have as high of an inhibitory activity to AChE as compared to phenserine.[57]



IC50= ~50 μM

Ladostigil

Rivastigmine

Rasagiline

Fig. 12. Structure of ladostigil with rivastigmine and rasgilline to emphasize pharmacophores.[58]

Ladostigil (Figure 12) structure resembles that of a hybrid between rivastigmine (Figure 12) and rasagiline (Figure 12). It was noted that due to ladostigil sharing both pharmacophores, the mechanism was theorized to possess carbamate meditated inhibition of AChEi, as seen with rivastigmine, as well as the monoamine oxidase B inhibition, seen with rasagiline.[58] Ladostigil has an IC_{50} of 50 uM towards AChE and 37.1 uM towards monoamine oxidase B.[58] Although initially investigated for the treatment of AD, Avraham Pharma changed ladostigil's indication towards treating the progression of mild cognitive impairments instead. This change was due to failures to reach efficacy endpoint during the initial Phase 2 clinical trial that ended in 2012 (NCT01354691). But other homoisoflavonoid derivatives modeled after ladostigil continue to be investigated for the treatment of AD.[58]

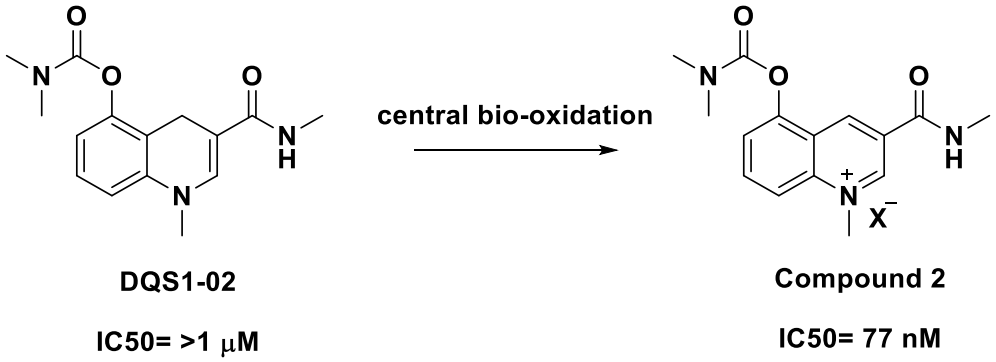


Fig. 13. Produg, DQS1-02 bio-oxidation to active metabolite.[59]

Dihydroquinoline carbamate (DQS1-02) (Figure 13) is a prodrug of its quinolinium form. As a prodrug, DQS1-02 has an IC_{50} value of >1uM towards AChE.[59] However, once it is bioactivated to Compound 2 it has an IC_{50} value of 77 nM (Figure 13). Pharmacokinetic and toxicity studies were also performed on DQS1-02 with positive results indicating that there would not be any conflict of note for development.[59] Overall, DQS1-02 seems to be a promising agent currently under development for AD treatment.[59]

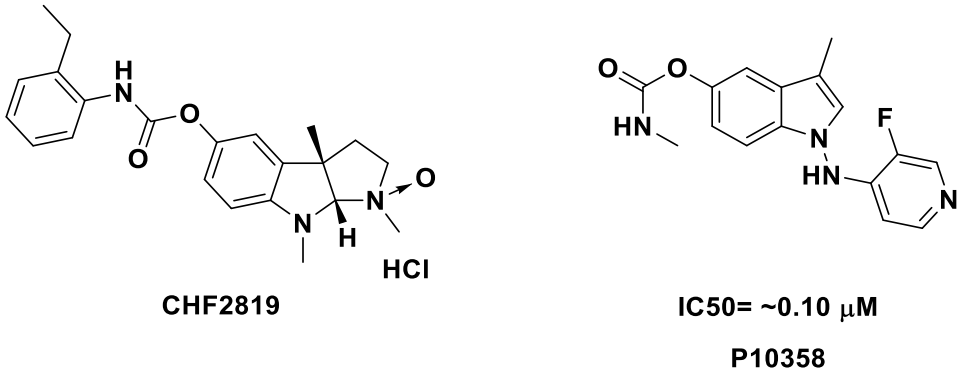


Fig. 14. Structure of CHF2819 and P10358.[60-62]

Other carbamates investigated for AD treatment were CHF2819 (Figure 14) and P10358 (Figure 14).[60-62] There were claims of CHF2819 being 2.6x less potent then physostigmine however the IC_{50} values were not specified clearly aside from being in the uM levels.[61] P10358 on the other hand was indicated to have an IC_{50} value of 0.10uM towards AChE.[60] P10358 was tested on mice with promising safety profiles with a wide therapeutic index, though there are limited clinical data.[60]

Table 3: **Carbamate derivatives with AChEi properties being researched in AD treatment**

AChEi that has been or is being researched in AD treatment	IC_{50} to AChE	Trial and Testing	Reference/Citations
Physostigmine	0.15μM	Clinical Trial; Phase 3 in 1996	51-53
Epistigmine	0.11uM	Clinical Trial; Tests halted due to hematologic ADR	54-55
Phenserine	22nM	Clinical Trial; 2 Phase 3 Halted due to efficacy	56
Posiphen [(+) Phenserine enantiomer)]	[Not specified aside from no binding affinity to AChE]	Clinical Trial; ongoing DISCOVER study	57 Clinical Trial ID: (NCT02925650)
Ladostigil	1.05μM	Clinical Trial; Indication change due to efficacy (failure to meet primary endpoint)	58 Clinical Trial ID: (NCT01354691)
Dihydroquinoline carbamate (DQS1-02)	77nM (Bioactivated)	In vitro, in vivo testing, computer modeling	59
CHF2819	[Not specified; other than 2.6x less potent than physostigmine in uM level]	Animal study, in vitro and in vivo testing	61
P10358	0.10μM	Animal study, in vitro and in vivo testing	60

4.3 Galantamine derivatives

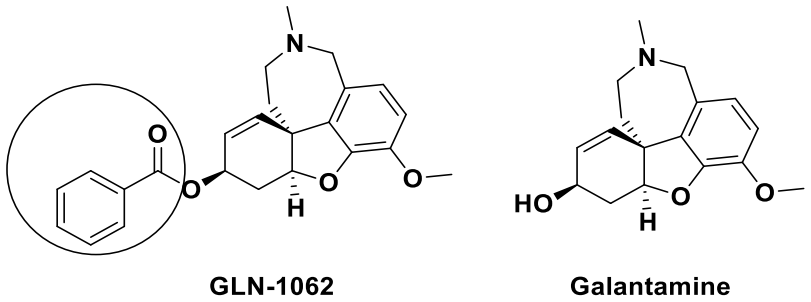


Fig. 15. Structure of GLN-1062 prodrug in contrast to galantamine

GLN-1062 (Figure 15) is the ester prodrug of galantamine, investigated for the treatment of AD.[63] The intention of the addition of a benzoate group was to decrease peripheral ADR and increase lipophilicity for BBB penetration.[63] There were clinical trials in the 2000s with an intranasal formulation dosed at 6mg/kg BID which seemed to have been cleared without issue.[63] However, there has not been any listed clinical trials since late 2014. However, a recent announcement was made by Alpha Cognition Inc. in the spring of 2020 regarding the development of GLN-1062, now renamed to Alpha-1062, for potential regulatory approval in Japan and USA by ~2023.

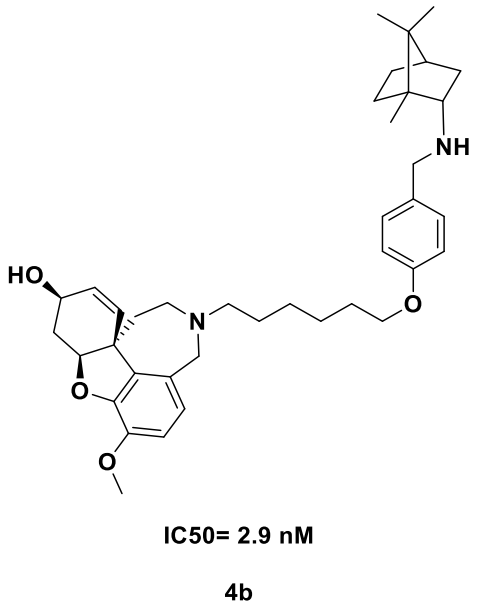
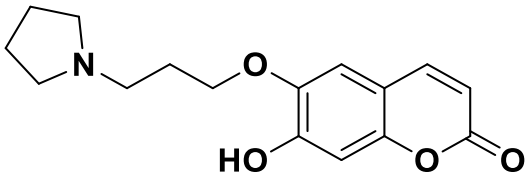


Fig. 16. Structure of Galantamine–camphane hybrid.[64]

Galantamine–camphane hybrids were investigated for the treatment of AD. Researchers were interested in increasing the potency of galantamine to AChE and were successful with various compounds.[64] These hybrids boasted a highly potent IC_{50} of 0.0029uM-0.77uM to AChE. The compound most potent was referred to as 4b (Figure 16), with an IC_{50} value of 2.9nM to AChE.[64] Its oral bioavailability is ~90% and the hybrids were claimed to have good penetration of the BBB.[64]



IC50= ~2.87 μM

Compound 3f

Fig. 17. Structure of AASC derivative Compound 3f.[65]

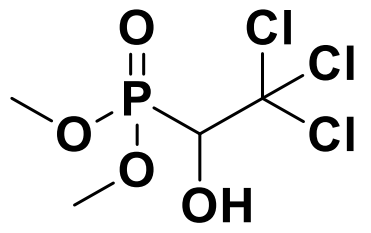
Aminoalkyl-substituted coumarin (AASC) derivatives were also investigated as AChEi's. This is a modified natural product compound which is loosely based on galantamine's pharmacophore.[65] The IC_{50} values ranged from 2.87 to 26.98uM towards AChE with the greatest inhibition potential coming from Compound 3f (Figure 17).[65] Other coumarin derivatives have been investigated as natural product analogs as AChEi for the treatment of AD.[66] Furthermore, 3-benzofuranone has also been recently explored in the treatment of AD with other studies showing similarly potent ranges of IC_{50} values.[66]

Table 4: **Galantamine derivatives with AChEi properties being researched in AD treatment**

AChEi that has been or is being researched in AD treatment	IC_{50} to AChE	Trial and Testing	Reference/Citations
GLN-1062 or Alpha-1062 (galantamine prodrug)	[Not specified]	Clinical Trial	63
Galantamine-camphane hybrid	2.9nM	In vitro, in vivo testing computer modeling	64
Aminoalkyl-substituted coumarin modeled after galantamine	2.87μM (Compound 3f)	In vitro, in vivo testing computer modeling	65

4.4 Novel Models

4.41 Organophosphates



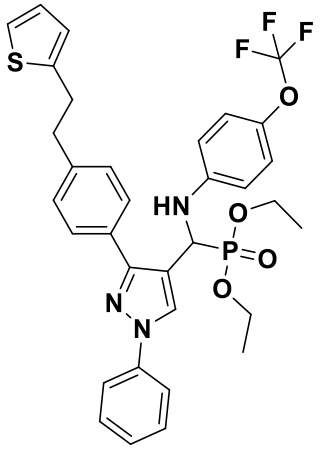
IC50= ~36.7 μM

Metrifonate

Fig. 18. Structure of metrifonate

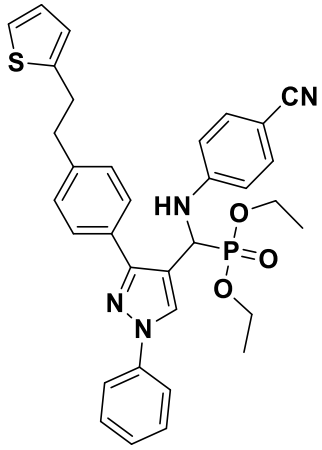
4.42 Pyrazole

Metrifonate (Figure 18) is an organophosphorus irreversible inhibitor of AChE with an IC_{50} value of 36.7uM.[67] Historically metrifonate has been used in the treatment of parasitic flatworm infections, referred to as schistosomiasis.[68] Metrifonate underwent clinical trials in 1999 for AD and was deemed efficacious.[67] However, metrifonate presented with several issues regarding ADR. Approximately 60% of the participants experienced ADR pertaining to nausea, vomiting, headaches, diarrhea, and malaise.[67,68] Some patients experienced respiratory paralysis and neuromuscular complications.[67] Subsequently, the FDA did not approve metrifonate for the treatment of AD.[68] To date, the use of metrifonate is restricted for use as an agricultural insecticide.



IC50= 55 nM

Compound 4ah



IC50= 17 nM

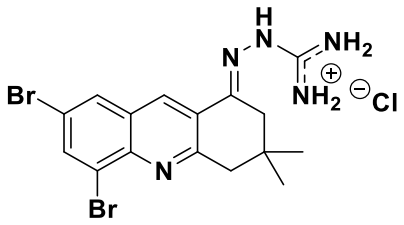
Compound 4bh

Fig. 19. Structure of Compounds 4ah and 4bh.[69]

Recently, a novel AChEi pyrazole bearing α-aminophosphonate derivatives was explored as a potential AD treatment.[69] The IC_{50} value were very promising for a few of these derivatives. Compounds referred to as 4ah and 4bh displayed IC_{50} values 0.055uM and 0.017uM (Figure 19), respectively.[69] The mechanism of action was explored through computer modeling indicated a pi-stacking interaction being the primary mode of inhibition.[69] Although clinical data has yet to

be reported, these novel compounds may have potential as an AChEi in the AD market.

4.43 Hyrdrazlones

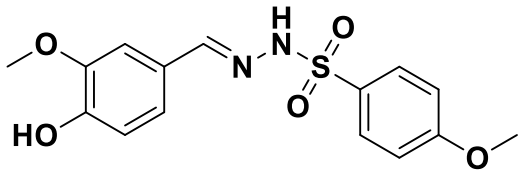


IC50= ~1.97 μM

Compound 2

Fig. 20. Structure of guanylhydrazones Compound 2.[70]

Guanylhydrazones derived agents have been investigated to inhibit AChE with a proposed interaction of its aromatic residue(s) at the peripheral binding site in addition to the histidine residue of the active ester hydrolysis site.[70] The guanylhydrazones derivatives were modeled after tacrine to better facilitate these bindings. The IC_{50} value of the guanylhydrazones ranged from 1.97 to 7.77uM towards AChE with Compound 2 being deemed the most potent (Figure 20).[70] Studies were primarily performed in vitro, in vivo, and through computer modeling with limited clinical data.



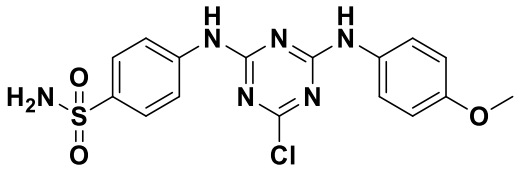
IC50= ~0.64 μM

Compound 6d

Fig. 21. Structure of sulfonylhydrazones Compound 6d.[71]

Sulfonylhydrazones derived agents have been investigated as a potential treatment for AD. For certain sulfonylhydrazones the IC_{50} values ranged from 0.64 to 51.09uM towards AChE.[71] The mechanism proposed, via computer simulation, was the sulfonylhydrazones moiety interacting with both AChE's catalytic active site of ester hydrolysis and the peripheral aromatic binding sites.[71] There have been some promising claims made for a sulfonylhydrazones compound referred to as 6d (Figure 21). Some of these claims include 6d having good oral absorption and BBB penetration.[71] If these hold true clinically, the novel hydrazones have potential as an AChEi in the market for the treatment of AD.

4.44 Triazine with Sulfonamide



AChE Assay: ~96% inhibition value of 200 μM

Compound 2b

Fig. 22. Structure of Sulfonamide substituted triazine, Compound 2b.[72]

Sulfonamides substituted triazine have been explored for the as an AChE targeted towards AD[72]. A compound referred to as 2b appears to be the most potent with the study's AChE inhibition assay method.[72] This AChE inhibition assay method was unique in contrast to the other agents explored as it was calculated based off of a percentage inhibition at 200uM (Figure 22).

4.45 Dimethylbenzimidazolinone

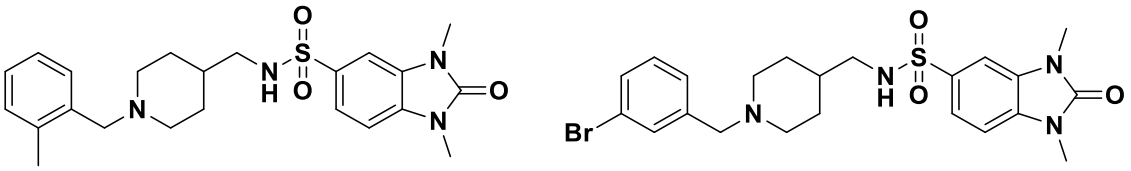


Fig. 23. Structure of Benzylpiperidine derrivatives modified from 15b and 15j.[73]

Benzylpiperidine linked dimethylbenzimidazolinone derivatives are similar to donepezil and were theorized to have a comparative mechanism in interacting with tryptophan at the binding sites of ACh through pi-stacking.[73] The IC_{50} value of these agents towards AChE ranges from 0.39 to 38uM.[73] The most potent of these compounds being referred to as Compounds 15b and 15j (Figure 23).[73] Though no clinical testing has been performed yet, these compounds may have the potential of becoming an agent for the treatment of AD

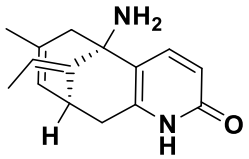
.

Table 5: Novel models and compounds with AChEi properties being researched in AD treatment

AChEi that has been or is being researched in AD treatment	IC_{50} to AChE	Trial and Testing	Reference/Citations
Metrifonate	36.7μM	Clinical Trial: Clinical trial in 1999 Not approved; Test halted due to long term ADR/toxicity	67-68
Pyrazole bearing α-aminophosphonate derivatives	55nM (Compound 4ah) 17nM (Compound 4bh)	In vitro, in vivo testing computer modeling	69
Gaunylhydrazone	1.97uM (Compound 2)	In vitro, in vivo testing computer modeling	70
Sulfonylhydrazone	0.64uM (Compound 6d)	In vitro, in vivo testing computer modeling	71

AChEi that has been or is being researched in AD treatment	IC_{50} to AChE	Trial and Testing	Reference/Citations
Triazine with sulfonamide	[96% of a 200uM AChE inhibition Assay] (Compound 2b)	In vitro, in vivo testing computer modeling	72
Dimethyl-benzimidazolinone	0.39uM (Compound 15b and Compound 15j)	In vitro, in vivo testing computer modeling	73

4.5. Natural products

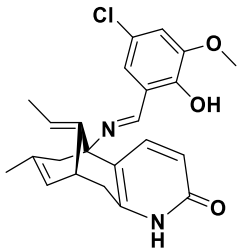


IC50= ~82 nM

Huperzine A

Fig. 24. Structure of huperzine A

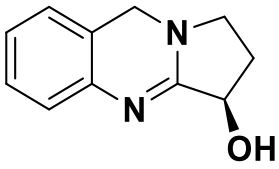
Natural products and their derivatives overall has been long investigated and may present potential compound(s) that can be developed for AD treatment.[74-81]. For example, huperzine A (Figure 24) is a natural alkaloid derived from *Huperzia Serrata* with known activity for AChE inhibition.[74] As such, huperzine A has been widely investigated in the treatment for AD. The IC_{50} value of huperzine A is shown to be 82nM towards AChE.[74] There were several clinical trials conducted in the USA, China, and Europe. In 2005, Neuro-Hitech pharmaceutical conducted a Phase 2 clinical trial for oral huperzine A, but it did not continue onto Phase 3 due to failure to meet efficacy endpoint.[75] In China, huperzine A is an approved agent for the treatment of AD. Additionally, there is an ongoing trial lead by Shanghai Mental Health Center started in 2011, however, informing regarding trial status is currently unknown (NCT01282619). With the given information, it is inconclusive to determine huperzine A's potential future in the treatment of AD.



DEBIO-9902/ZT-1

Fig. 25. Structure of DEBIO-9902/ZT-1. IC_{50} was unspecified.[76]

A prodrug formulation of huperzine A under the trade names of ZT-1 and DEBIO-9902 (Figure 25) has made a push for clinical development.[76] A Phase 2 clinical investigation, referred to as the BRAINz trial, was led by Debiopharm International SA (Clinical Trial Identifier: NCT00423228). The distinguishing feature of this trial was the dosage form in which ZT-1 was delivered. They opted for a subcutaneous implant administered ~9-15mg, once monthly. This novel idea would address drug adherence concerns.[76] Additionally, ZT-1 had an oral sustained release formulation that proceeded through trials as well. Unfortunately, both formulation of ZT-1 has been halted and discontinued after the completion of trial in 2008.

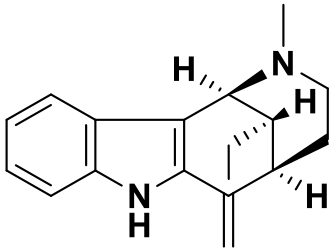


IC50= ~11.24 μM

Vasicine

Fig. 26. Structure of extracted vasicine from *Adhatoda vasica*

The *Adhatoda vasica* plant and its derivatives have been investigated for the treatment of AD. The plant itself and various compounds extracted were tested for AChE inhibition.[77] Most notably, the extracted compound vasicine yielded and IC_{50} values of 11.24uM towards AChE (Figure 26).[77] The mechanism of action towards AChE was predicted to be a combination of aromatic interaction binding and hydrogen bonding with the active site of ester hydrolysis, similar to tacrine and galantamine.[77]

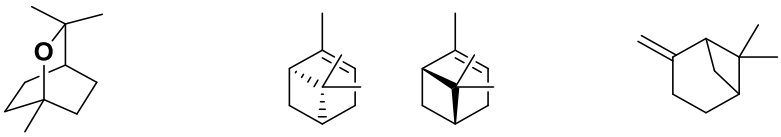


IC50= ~0.45 μM

Uleine

Fig. 27. Structure of uleine from *Himatanthus lancifolius*

Uleine (Figure 27) extracted from *Himatanthus lancifolius* is a natural alkaloid investigated for AD with an IC_{50} value of 0.45uM.[78] Although there were claims that there are other indole alkaloids with AChEi activity, there has not been clear data of their use in the treatment of AD.



IC50= ~0.4-7 mM **IC50= ~0.67 mM** **IC50= ~1.5 mM**

1,8-cineole **(+/-)-a-pinene** **beta-pinene**

Fig. 28. Structures of various extracted compounds from *Salvia lavandulaefolia*

Of note, *Salvia lavandulaefolia* extracts, commonly known as the Spanish sage, was investigated for the treatment of AD. The IC_{50} value of each component (Figure 28) was tested for the inhibition of AChE in vitro via an Ellman's assay.[79] IC_{50} value were quite modest ranging from 0.4 to >5mM[79]. An open-label trial to evaluate the tolerability of 2% sage oil extracts administered at 2.5 grams a day was established.[79] The authors claimed positive clinical outcomes however, the study did not have a placebo control.[79] There is limited data and more information would be needed before any conclusions can be drawn.

Most recently, several other natural products have been discussed for its use against AD such as green tea, ginseng, yuan zhi root, and other plants cultivated within the field of traditional medicine.[80,81] Many of these studies have determined inhibitory activity towards AChE with respective IC_{50} values, while others claim potential with only preliminary evidence.[80,82] Natural products

have continued to inspire new discoveries of treatments throughout history, whether directly or modified.

Table 6: Mentioned natural products investigated as AChEi for treatment of AD

AChEi that has been or is being researched in AD treatment	IC_{50} to AChE	Trial and Testing	Reference/Citations
Hupazine A	~82nM	Studied in Animal. In vivo and in vitro testing. Completed Phase 2 Clinical trial and ongoing Phase 3 Clinical trial	74-75 Clinical trial ID: (NCT01282619)
ZT-1: Pro drug of Hupazine A	Not specified	Completed P1 and P2a clinical trial (BRAINz)	76 Clinical trial ID: (NCT00423228)
Adhatoda Vasica (vasicine extract)	11.24 μM (Vasicine)	In vitro and In vivo testing	77
Uleine from Himatanthus lancifolius	0.45μM (uleine)	In vitro and in vivo Assays (Microplate, colorimetric on TLC	78
Salvia lavandulaefolia (Spanish Sage)	[0.4mM->5 mM] (Extracts component/oil)	Animal studies, in vitro testing, open label clinical test	79

Conclusion

The use of AChEi has been a revolutionary step in the treatment of AD. Throughout the past decades, the few drugs that made it to market have helped the lives of millions. Although the development of new AChEis may seems to stagnate, research continues to push forwards. Due to the nature of AD, clinical failures and discontinuations tend to be from the concerns of efficacy and safety or the lack thereof. Regardless, this illustrates that efficacy and safety are held to the highest of standards in order to ensure our patients and the people of our communities continue to receive the care they deserve. As the population continues to age, the need for treatments to address geriatric therapies such as dementias and AD has never been greater. The collective cost of these geriatric therapies in USA alone is estimated to be over $200 billion annually as of 2020[4,83]. This will continue to climb with the growing geriatric population. From past studies and trials, novel methods and ideas continue forward in the treatment of AD. There is still much room for improvements with the AChEi currently available today and even more so, with the endeavor in developing a safer and more efficacious treatment for AD.

Although a vast array of different AChEi, with a multitude of different structural moieties have been identified, no new drugs have reached the market for this target in decades. The most compelling have excellent IC_{50}'s but to-date have not shown statistically relevant clinical results (Table 7).

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Declaration of Competing Interest: The authors declare no conflict of interest.

Table 7: The most potent compounds investigated as AChEi of each group for the treatment of AD.

AChEi that has been or is being researched in AD treatment	IC_{50} to AChE	Trial and Testing	Reference/Citation
Donepezil	11.6 nM	FDA-Approved	11, 31-34
Tetrahydroacridine derivatives with iodobenzoic acid moiety	31.2nM (Compound 3f)	In vitro and in vivo testing. Computer modeling.	49
Phenserine	22nM	Clinical Trial; 2 Phase 3 Halted due to efficacy	56
Galantamine-camphane hybrid	2.9nM	In vitro, in vivo testing computer modeling	64
Pyrazole bearing α-aminophosphonate derivatives	17nM (Compound 4bh)	In vitro, in vivo testing computer modeling	69
Hupazine A	~82nM	Studied in Animal. In vivo and in vitro testing. Completed Phase 2 Clinical trial and ongoing Phase 3 Clinical trial	74-75 Clinical trial ID: (NCT01282619)

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