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Status of Acetylcholinesterase and Butyrylcholinesterase in Alzheimer's Disease and Type 2 Diabetes Mellitus

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Abstract

Both Alzheimer's disease (AD) and Type 2 diabetes mellitus (T2DM) share the presence of systemic and neuro-inflammation, enhanced production and accumulation of β -amyloid peptide and abnormal levels of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Altered levels of AChE and BuChE both in AD as well as in T2DM imply that those two enzymes may be playing a pivotal role in the pathogenesis of the two disorders. AD and T2DM are both characterized by elevated levels of AChE and BuChE in the plasma. On the other hand, in AD the brain levels of AChE go down while those of BuChE go up, resulting in deregulation in balance between AChE and BuChE. This imbalance and change in the AChE/BuChE ratio causes cholinergic deficit in the brain, i.e. deficiency in the brain neurotransmitter acetylcholine. With better understanding of the inter-relationship of AChE and BuChE levels in normality as well as abnormality, AD and T2DM can be effectively treated. Thus, general cholinesterase inhibitors that inhibit both AChE and BuChE as well as highly selective BuChE inhibitors may have potential therapeutic benefits in the treatment of AD and other related dementias.

Keywords

Acetylcholinesterase; Alzheimer's disease; butyrylcholinesterase; cholinergic deficit; cholinesterase inhibitors; type 2 diabetes mellitus

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

INTRODUCTION

The enzyme acetylcholinesterase (AChE) (EC 3.1.1.7) is a specific esterase that belongs to the carboxylesterase family of enzymes. It predominantly hydrolyzes the neurotransmitter acetylcholine (ACh). AChE is found in high concentrations mainly in the red blood cells as well as in the brain at neuromuscular junctions and cholinergic brain synapses. On the other hand, the enzyme butyrylcholin-esterase (BuChE) (also known as “pseudo” cholinesterase; EC 3.1.1.8) is a non-specific type of cholinesterase enzyme that hydrolyzes different types of choline esters and it ubiquitously exists throughout the body, most importantly, in the human liver, blood serum, pancreas and the central nervous system. In the brain, BuChE is primarily associated with glial cells and endothelial cells [1, 2].

These two enzymes share 65% of structural homology. In normality, BuChE plays a supportive role in the brain and accounts for about 10% cholinesterase activity in the temporal cortex. Just like AChE, the enzyme BuChE is involved in the hydrolysis of the neurotransmitter ACh and, hence, it has been proposed as a viable therapeutic target in AD, a disorder which is characterized by cholinergic deficit as it will be discussed later. AChE and BuChE have different K_m (Michaelis Menten Constant) values and, hence, different kinetic responses to the concentrations of ACh. At low concentrations of ACh, AChE is highly efficient but BuChE is much less efficient. However, at higher ACh concentrations, AChE becomes substrate inhibited while BuChE becomes highly efficient in the hydrolysis of ACh [3].

Owing to its low expression in the normal mammalian brain as compared with AChE, the importance of BuChE in human brain was underestimated at first [4]. However, later studies confirmed the importance of BuChE within the nervous system, particularly regarding its pivotal role in coregulating the levels of ACh in the brain in neurodegenerative disorders such as AD [5]. In AD, while the levels of AChE go down by as much as 85% in certain brain areas, BuChE levels (specifically the G1 form) go up as the disease progresses [6, 7]. Consequently, the ratio of BuChE to AChE changes from 0.2 up to as much as 11 in cortical areas of the brain suffering from AD [8]. When the ratio of AChE to BuChE alters with AD progression, this changes the auxiliary role that BuChE plays in normality and, instead, makes BuChE an enzyme of increasing functional importance [9].

Low-grade systemic inflammation has been shown to be a pathological link between AD and T2DM. Various studies have demonstrated that inflammatory pathways play an important role linking T2DM and neurodegenerative diseases such as AD [10–13]. At the same time, it has been noted that altered plasma and tissue levels of AChE and BuChE may also serve as markers for the existence of low-grade systemic inflammation in disorders such as T2DM and AD [14]. In fact, it has been proposed that activities of AChE and BuChE are elevated in diseases such as T2DM and AD involving low-grade systemic inflammation even when plasma, cerebrospinal fluid and tissue concentrations of C-reactive protein, interleukin-6, tumor necrosis factor (TNF- α) and other markers of inflammation are not noticeably higher [15]. *In vitro* studies have demonstrated that selective BuChE inhibition results in lowering the production of pro-inflammatory cytokines such as interleukin-1b and TNF- α in peripheral blood mononuclear cell cultures [16]. Acetylcholine is anti-inflammatory in

nature. The acetylcholine receptor modulates interactions between the immune system and the nervous system. Abnormally elevated levels of AChE and BuChE result in reduced level of ACh, thereby causing reduction or absence of anti-inflammatory actions exerted by ACh on the body. Hence, higher levels of AChE and BuChE in systemic circulation result in inflammation.

ROLE OF AChE AND BuChE IN THE PATHOGENESIS OF AD

Anatomical findings from the brains of AD patients have revealed damage to the nuclei containing cholinergic neurons suggesting the role of cholinergic system in AD. Earlier studies have demonstrated that nucleus basalis and hippocampus are affected in AD patients with severe deficits occurring especially in the nucleus basalis [17]. Loss of cholinergic activity in areas of the brain responsible for higher mental functions contributes to the pathogenesis of AD. Extracellular senile plaques, intracellular neurofibrillary tangles and localized A β formation and accumulation are the hallmarks of AD. Senile plaques found deposited in the brains of AD sufferers contain extracellular deposits of human beta-amyloid (A β) peptide [18, 19]. It has been reported that a region near the C-terminus of human AChE is weakly homologous to the N-terminus of Alzheimer's disease A β peptide [20]. This further suggests that AChE may be involved in the formation of amyloid fibrils in senile plaques and the toxicity associated with such deposits.

Evidence from *in vitro* studies using electron microscopy and Congo Red binding suggests that AChE strongly stimulates A β peptide fibrils assembly [21]. The elongated unbranched fibrils of A β that bind to Congo Red are comprised of AChE and apolipoprotein E besides other things. In addition, physical, biochemical and morphological data provide evidence that AChE accelerates the assembly of A β peptides to form amyloid-AChE complex, strongly suggesting the role of human AChE in accelerating A β fibrillogenesis [22]. Those A β -AChE complexes were found to be more neurotoxic than A β aggregates alone in primary cultured chick retina neurons and PC12 cells [23]. Likewise, in an *in vivo* study monitoring and comparing the neurotoxicity of A β fibrils alone and A β -AChE complexes, it has been shown that when human A β peptide fibrils formed a complex with AChE to generate A β -AChE, the resulting A β -AChE complexes were bigger in size than those observed in matched controls injected with the same amount of A β peptide alone. Also, A β -AChE complexes proved more toxic than A β peptide fibrils alone. Most importantly, the hippocampal injection of A β -AChE complexes to rats resulted in the formation of Alzheimer-like lesions in the rat brain. In fact, A β -AChE injected animals showed stronger neuronal cell loss in comparison with A β -injected animals. This suggests that AChE is associated with some of the neurodegenerative changes seen in AD brains by promoting amyloid formation, astrocytosis and neural cell loss caused by A β -AChE complex formation [24].

The important role of AChE in AD pathogenesis is further supported by recent studies conducted on double-transgenic mice that overexpress both human AChE and amyloid precursor protein (APP). Those doubly-transgenic mice are produced when Tg2576 mice (which express human APP and show amyloid plaque deposits in their brains at age 6 month) are crossed with transgenic mice expressing human AChE. The resulting hybrids

express both transgenes in their brains. β -amyloid plaques are formed 30–50% sooner in double-APP-AChE transgenic mice as compared to those mice that overexpress APP alone. Plaque numbers were higher and more abundant in doubly transgenic animals at 9 and 12 months and so was the total amyloid content in the brain as assessed by quantitative immunoassay *via* enzyme-linked immunosorbent assay. These findings provide further evidence that AChE promotes β -amyloid plaques in cerebral cortex and may play a pivotal role in the pathogenesis of AD [25].

The association of BuChE to AD has been studied in different ethnic groups. It has been shown that BuChE may promote the risk of AD either alone or in synergic association of butyrylcholinesterase-K variant and apolipoprotein epsilon 4 allele [26, 27]. It has been found that BuChE-K variant and its corresponding apolipoprotein allele act synergistically to increase the risk of late-onset AD, especially in age groups less than 75 years old [28]. Furthermore, abnormally high levels of BuChE in the brains of AD sufferers contribute to the pathogenesis of AD. Comparing the brains of AD patients versus healthy subjects, it has been observed that BuChE correlates positively with the formation of neocortical amyloid-rich neuritic plaques and neurofibrillary tangles [29].

With the progression of AD, the G1 form of BuChE increasingly accumulates in plaques and tangles. Levels of the G1 form of BuChE have been shown to correlate positively with plaque density and pathogenicity [30]. BuChE begins to be linked to amyloid plaques at around the same time when A β deposits start to acquire a compact beta-pleated sheet conformation. This is supported by studies on the brains of aged, demented and age-matched nondemented individuals in which the total cortical area covered by plaque-like A β and BuChE deposits was measured at two regions of the temporal cortex. The proportional plaque area displaying BuChE-reactivity was fivefold to six fold higher in the brains of demented subjects than in the nondemented individuals [31]. It has been suggested that BuChE could be making a noticeable contribution in the transformation of A β from an initially benign form to an ultimately malignant form of amyloid plaques causing brain tissue degeneration and clinical dementia in disorders such as AD [32, 33].

ROLE OF AChE AND BuChE IN THE PATHOGENESIS OF T2DM

Higher levels of AChE as well as BuChE are observed in T2DM. AChE activity has been found higher by an order of magnitude in islets of Langerhans than in the exocrine tissue from rat pancreas and this difference in activity was observed in healthy, control rats as well as rats made diabetic with streptozotocin. In addition, AChE activity in islets from diabetic rats was about 30–40% higher than in islets from control rats [34]. Likewise, when the activity of serum BuChE was investigated in diabetic versus normal controls, it was found that BuChE activity was significantly elevated in both type 1 diabetes (8.10 ± 3.35 units/mL) and type 2 diabetes (7.22 ± 1.95 units/mL) patients compared with the control subjects (4.23 ± 1.89 units/mL) ($P < 0.001$). It was also found in this study that serum activity of BuChE was correlated with insulin sensitivity particularly in T2DM patients [35].

Studies have shown that BuChE may be indirectly contributing to the pathogenesis of T2DM by causing insulin resistance [36]. In fact, a locus on chromosome 3q27, which is near the

chromosomal locus of BuChE (3q26), has been linked to T2DM in a French population in one study [37]. Furthermore, BuChE-K variant on chromosome 3q was found to be associated with T2DM in white Caucasian subjects. Hence, the association of the BuChE gene (3q26) with T2DM may possibly be related to an identified susceptibility on chromosome 3q27 but independent of islet function [38]. When different risk factors were studied that are independently correlated with BuChE activity for coronary artery disease, it was found that BuChE activity was positively correlated with lipoprotein synthesis, hypertension and diabetes [39].

In a study conducted on 1,097 subjects from the Newfoundland adult population, evidence was found for the involvement of elevated levels of serum BuChE in the pathophysiological processes causing T2DM symptoms such as serum triglyceride levels, abdominal obesity, fasting blood glucose levels, and fasting insulin levels [40]. BuChE may be playing a role in causing T2DM through amyloid fibrils formation. It has been shown that the presence of amylin in pancreatic islet cells of T2DM patients causes apoptosis of beta cells by nitric oxide inactivation, lipid peroxidation and formation of excessive superoxide radicals (Fig. 1) [41].

TARGETING AChE AND BuChE TO MANAGE AD AND T2DM

Clinical studies have shown that plasma levels of both AChE and BuChE are significantly higher in AD patients as compared with normal, healthy persons. Moreover, levels of β -amyloid are on average about 2-times higher in AD patients compared to normal, healthy subjects [42, 43]. Likewise, the activities of both AChE and BuChE are found to be higher in diabetic patients versus normal controls [44, 45]. This means that abnormal plasma levels of AChE and BuChE may serve as markers to predict the development of T2DM and AD in addition to serving as therapeutic targets [46]. With better understanding of the inter-relationship of AChE and BuChE levels in normality as well as abnormality, AD and T2DM can be managed.

By effectively managing the treatment of T2DM with drugs that reduce peripheral inflammation and also act as cholinesterase inhibitors, we may be able to manage and delay the onset or progression of AD as the two disorders are related [47, 48]. In this regards, one promising candidate is the Chinese herbal extract “SK0506” which is an inhibitor of BuChE. SK0506 has been used as anti-diabetic testing agent in animal studies. In one study, SK0506 treatment resulted in lowering of BuChE activity in adipose tissues and skeletal muscle of rats and also significantly lowered the levels of hepatic and serum triglycerides and inflammatory markers (interleukin-6 and TNF- α) in rats fed with high fat diet [49]. Likewise, in a separate study on rodent model of the metabolic syndrome induced by high-fat fed diet, SK0506 was found to significantly enhance glycogen synthesis and glucose uptake in adipose tissues during hyperinsulinemic clamp [50].

Similarly, α -lipoic acid and its reduced form, dihydrolipoic acid are multifunctional antioxidants and have been shown to have anti-diabetic properties [51]. α -lipoic acid is also used as a dietary supplement. Both α -lipoic acid and dihydrolipoic acid have been shown to prevent glycation reactions by binding to proteins such as albumin *via* hydrophobic binding

[52]. Recently, a group of researchers reported a series of hybrid molecules between α -lipoic acid and the acetylated or methylated polyphenol compounds synthesized and tested for *in vitro* AChE and BuChE activity. The half maximal inhibitory concentration (IC_{50}) values of all hybrid molecules for BuChE inhibition were lower than those for the parent compounds. However, one of the hybrid compounds, α -lipoic acid-acetyl protected caffeic acid, was reported to be a potent BuChE inhibitor with IC_{50} of $0.5 \pm 0.2 \mu M$ and more than 800 fold selectivity for BuChE over AChE [53]. There is a need to conduct randomized clinical trials to confirm the potential of cholinesterase inhibitors such as SK0506, α -lipoic acid and its derivatives in treating T2DM.

As of now, cholinesterase inhibitors have been proven to be the most effective in the treatment of AD owing to their ability to affect the cognitive and functional domains of AD [54]. There is experimental evidence indicating that general cholinesterase inhibitors targeting AChE and BuChE as well as highly selective BuChE inhibitors have potential therapeutic benefits in the treatment of AD and other related dementias. Cholinesterase inhibitors enhance cholinergic activity by inhibiting enzymes (AChE and/or BuChE) that hydrolyze ACh following synaptic release and, hence, prolong the activity of ACh molecules [55, 56]. Patients treated with cholinesterase inhibitors show amelioration of AD-related behavioral disturbances [57, 58].

Physostigmine, a plant alkaloid, was the earliest drug among the AChE inhibitors to be used in the treatment of AD [59, 60]. Despite its ability to cross the blood-brain barrier, the clinical use of physostigmine was halted due to peripheral side effects, insufficient evidence of efficacy in the clinical studies and fast clearance from the body [61]. Consequently, synthetic analogues of physostigmine were synthesized such as quilostigmine, neostigmine and phenserine. Kinetic studies of human erythrocyte AChE inhibition by phenserine indicate that this compound inhibits the activity of human erythrocyte AChE in a concentration-dependent fashion with IC_{50} of 45.3 nM [62]. Further, investigations had led to the development of another novel experimental anti-AD therapeutic agent tolserine, a structural analog of phenserine. With an IC_{50} value of 8.13 nM, tolserine proved to be more potent inhibitor of human AChE compared to its structural analogues phenserine and physostigmine [63].

Currently, the most common drugs used to treat AD across the world are rivastigmine, galantamine, memantine and donepezil. With the exception of memantine, all of these drugs are AChE inhibitors [64, 65]. Rivastigmine acts as a dual reversible inhibitor of both AChE and BuChE. Clinical administration of rivastigmine has been shown to provide long-term symptomatic treatment to AD patients with dementia [66]. Donepezil, on the other hand, is a selective inhibitor of AChE. In preclinical studies on AChE-deficient mice (AChE $^{-/-}$ mice), rivastigmine dosing has been shown to enhance the levels of extracellular ACh by inhibiting BuChE whereas infusion of donepezil did not affect hippocampal ACh levels, although donepezil increased ACh levels by more than twofold in wild-type mice [67]. Donepezil is 1000 fold more selective for AChE over BuChE and improves working memory and long-term memory in animal studies. Clinical studies have shown that donepezil produced dose-related improvements in AD patients with statistically significant effects [68].

Galantamine is another AChE inhibitor drug commonly used to treat AD. A tertiary alkaloid extracted from several species of Amaryllidaceae, galantamine acts as a non-competitive inhibitor of AChE with half-life of about 10 hours and demonstrates only limited affinity for BuChE [69, 70]. Results from large randomized, double-blind, placebo-controlled trials of up to 6 months duration have demonstrated that galantamine significantly improved cognitive and global function and ability to perform every day activities in AD patients relative to placebo and baseline [71].

Berberine is a natural isoquinoline alkaloid that holds promise as a potential anti-AD candidate. It acts as a dual AChE/BuChE inhibitor [72]. Berberine can inhibit AChE with an IC₅₀ of 0.44 μ M and it can inhibit BuChE with an IC₅₀ of 4.44 μ M [73]. Berberine also causes reduction in A β levels. Asai *et al.* has reported that berberine can alter APP processing and, hence, cause reduction of A β levels in human neuroglioma H4 cells that stably express Swedish-type of APP at the range of berberine concentration without cellular toxicity [74].

In disorders such as AD, AChE activity goes down to only 33–45% of normal values as the disease progresses while the activity of BuChE goes up by as much as 40–90% in certain brain areas [75, 76]. This drastic alteration of AChE to BuChE ratio causes a change in the normally supportive role of BuChE in hydrolyzing excess ACh only. This implies that BuChE inhibition may serve as an appropriate therapeutic target to treat AD. Furthermore, when transgenic mice with overexpressed human mutant APP were treated with selective and potent BuChE inhibitors, it resulted in lowering of A β peptide brain levels than in control mice and reduced intra- and extracellular β -amyloid precursor protein. Selective BuChE inhibition in rat brain slices resulted in improved long-term potentiation. Furthermore, those potent and brain-targeted BuChE inhibitors also resulted in improved cognitive performance in animal studies [77].

Various selective BuChE inhibitors have been tested as potential therapeutic anti-AD candidates. Notable among those compounds are the two structural analogs of cymserine, namely, bisnorcymserine and phenethylcymserine, which have proven to be 15, 110 and 5000-fold more selective for BuChE than AChE respectively [78–82]. It has been demonstrated in animal studies that these two cymserine-derivatives, i.e. phenethylcymserine and bisnorcymserine, are nontoxic and they improve learning in elderly rats in a 14-unit T maze compared with untreated controls [83]. Preclinical studies conducted on animals with cholinergic forebrain lesions have demonstrated that phenethylcymserine and bisnorcymserine cause *in vivo* reduction in the levels of APP found in cerebrospinal fluid [84]. Dosing of both of these BuChE inhibitors results in reduced intracellular and extracellular levels of APP in a dose and time dependent manner [85–87]. Likewise, MF-8622 is a BuChE-specific carbamate inhibitor that holds promise in the treatment of AD. Animal studies have reported a 15-fold increase in the extracellular concentration of ACh in rat brains injected intracortically with MF-8622 [88, 89].

Ethopropazine is another drug that inhibits BuChE selectively and it has been shown clinically to cause reduction in the formation of β -amyloid in patients suffering from AD [90, 91]. Two other noteworthy anti-AD compounds that show weak BuChE inhibition are

HLA20 and M30D, even though they also exhibit strong AChE inhibition *in vitro*. HLA20 and M30D were designed by merging three FDA-approved drugs rasagline, rivastigmine and donepezil or tacrine. Both of these compounds have demonstrated the ability to modulate APP regulation and β -amyloid reduction (Table 1) [92].

Recently, various thienothiazines have also been explored as anti-AD agents due to their potent BuChE inhibitory activities and also due to the fact that this class of compounds share common structural features with phenothiazines, which are well-known potent BuChE inhibitors. After screening 45 thienothiazines for their BuChE inhibitory activity, Karlsson *et al.* have reported a compound, 3f, which is so far the most active against human BuChE with an IC₅₀ of 0.51 μ M [93]. The bronchodilating compounds metaproterenol and isoproterenol as well as their racemic bisdimethylcarbamate derivatives are another class of compounds that work by selectively inhibiting BuChE five times stronger than AChE [94].

CONCLUSION

To sum up, numerous *in vitro* and *in vivo* animal and human studies have demonstrated that activities and abnormal levels of AChE and BuChE appear to be closely associated with the pathogenesis of T2DM and AD. Both AChE and BuChE seem to play a crucial role in the pathogenesis of AD and T2DM and this class of esterase enzymes could possibly be an underlying connection responsible for the coexistence of these two disorders [95]. Currently, cholinesterase inhibitors are the treatment of choice for AD albeit the fact that they only provide symptomatic relief. Of course, cholinesterase inhibitors, especially AChE inhibitors, have potential unwanted side effects on the brain as well as the skeletal muscles and digestive system. In terms of the future of targeting AChE and BuChE inhibitors for the treatment of AD and T2DM, research should be geared towards designing such selective cholinesterase inhibitors that can potentially treat both AD and T2DM at the same time. Furthermore, there exists a need to design safe and efficacious cholinesterase inhibitors with a favorable pharmacological profile that would rapidly cross the blood-brain barrier and disappear quickly from the body with minimal or no side-effects on the body.

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LIST OF ABBREVIATIONS

ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
APP	Amyloid precursor protein
Aβ	Beta-amyloid

BuChE	Butyrylcholinesterase
IC₅₀	Half maximal inhibitory concentration
T2DM	Type 2 diabetes mellitus
TNF-α	Tumor necrosis factor

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Fig. 1.
Cholinesterase inhibitors in Alzheimer's disease (AD) and Type 2 Diabetes mellitus (T2DM).

Table 1

Summary of natural and synthesized cholinesterase inhibitors.

Category	Compound/Drug Name	Type (Natural or Synthesized)	Mechanism of Action	Reference(s)
AChE inhibitors	Phenserine	Synthetic analogue of a physostigmine (plant alkaloid)	Inhibits the activity of human erythrocyte AChE in a concentration-dependent fashion with nanomolar IC(50)	[62]
	Tolserine	Synthetic analogue of a physostigmine	More potent inhibitor of human AChE than its structural analogue phenserine	[63]
	Berberine	Plant alkaloid	Dual inhibitor of AChE and BuChE; it can alter APP processing and cause reduction of β -amyloid levels in human neuroglioma H4 cells that stably express Swedish-type of APP	[72–74]
	Rivastigmine	Miotine derivative of physostigmine	Dual inhibitor of AChE and BuChE; Clinical administration shown to provide long-term symptomatic treatment to AD patients with dementia	[66]
	Donepezil	Synthetic novel drug	1000 fold more selective for AChE over BuChE; improves long-term memory in animal studies; produced dose-related improvements in AD patients with statistically significant effects	[68]
	Galantamine	Tertiary alkaloid extracted from botanical sources	Non-competitive, selective inhibitor of AChE; significantly improved cognitive and global function and ability to perform every day activities in AD patients	[71]
BuChE inhibitors	Bisnorcymserine & Phenethylcymserine	Cymserine analogs	Dosing resulted in reduced intracellular and extracellular levels of APP; nontoxic; improved cognitive performance (maze navigation) in aged rats	[78–87]
	MF-8622	Synthetic BuChE-specific carbamate	Animal studies reported a 15-fold increase in the extracellular concentration of ACh in rat brains injected	[88, 89]

Category	Compound/Drug Name	Type (Natural or Synthesized)	Mechanism of Action	Reference(s)
			intracortically with MF-8622	
	Ethopropazine	Synthetic novel drug	Shown clinically to cause reduction in the formation of β -amyloid in patients suffering from AD	[90, 91]
	HLA20 & M30D	Designed by merging three FDA-approved drugs rasagline, rivastagmine and donepezil or tacrine	Shown weak BuChE inhibition; demonstrated the ability to modulate APP regulation and β -amyloid reduction	[92]
	3f	Synthetic derivative of thienothiazine	Highly active against the human BuChE (<i>in vitro</i>) with an IC(50) of 0.51 microM	[93]
	Metaproterenol, Isoproterenol and their racemic Bisdimethylcarbamate derivatives	Synthetic novel drugs used as bronchodilators	This class of compounds works by selectively inhibiting BuChE five times stronger than AChE	[94]

List of Abbreviations: Acetylcholine (ACh); Acetylcholinesterase (AChE); Alzheimer's Disease (AD); Amyloid Precursor Protein (APP); Butyrylcholinesterase (BuChE); Half Maximal Inhibitory Concentration (IC(50)); Type 2 Diabetes Mellitus (T2DM).