International Journal of Biomedical and Pharmaceutical Sciences ©2009 Global Science Books

Acetylcholinesterase Inhibitors of Natural Origin

Melanie-Jayne R. Howes[1*] • Peter J. Houghton[2]

[1] Royal Botanic Gardens, Jodrell Laboratory, Kew, Richmond, Surrey, United Kingdom
[2] Department of Pharmacy, King's College London, Franklin-Wilkins Building, London, United Kingdom
Corresponding author: * **m.howes@kew.org**

ABSTRACT

The endogenous neurotransmitter acetylcholine (ACh), found in vertebrates, stimulates cholinergic (muscarinic and nicotinic) receptors to mediate cholinergic neuronal transmission. ACh has a short half-life, as it is rapidly hydrolysed in the neuronal synaptic cleft by the enzyme acetylcholinesterase (AChE). Modulation of cholinergic function has been recognised as a therapeutic target in some disease states and one approach to achieve this is to prolong the action of ACh through the use of AChE inhibitors. Consequently, AChE inhibitors have been investigated for a number of therapeutic applications including glaucoma, myasthenia gravis, anti-muscarinic poisoning and dementia. Many inhibitors of AChE have been derived from natural sources, with alkaloids generally being the most potent, although other compounds including some terpenoids have also been shown to inhibit AChE. It is particularly interesting that of the four drugs currently licensed in Europe to alleviate cognitive symptoms in Alzheimer's disease, two (galantamine and rivastigmine) are derived from natural sources. Natural products continue to be investigated for anti-AChE activity to identify compounds that may have therapeutic potential, or that provide templates for the development of new drugs, with a particular focus on the alleviation of cognitive disorders. Many plants reputed to enhance cognitive function in a variety of traditional practices of medicine have been investigated to determine any pharmacological basis for their historical uses, and some of the extracts from these plants have shown promising AChE inhibitory effects, although for some plants the active compounds are yet to be elucidated. There are a number of other plants that are not associated with a traditional use related to cognitive disorders but their extracts have also been shown to inhibit AChE. Some of those plant extracts and compounds of natural origin that have shown inhibitory activity against AChE, and their therapeutic relevance, are discussed.

Keywords: acetylcholinesterase, alkaloids, Alzheimer's disease, cholinesterase, galantamine, physostigmine, terpenoids
Abbreviations: ACh, acetylcholine; **AChE**, acetylcholinesterase; **AD**, Alzheimer's disease; **BChE**, butyrylcholinesterase; **ChAT**, choline acetyltransferase; **ChE**, cholinesterase; **CNS**, central nervous system; **MG**, myasthenia gravis; **MAO**, monoamine oxidase; **NMDA**, *N*-methyl-D-aspartate; **PD**, Parkinson's disease; **SERT**, serotonin transporter; **TCM**, traditional Chinese medicine

CONTENTS

INTRODUCTION

The endogenous neurotransmitter acetylcholine (ACh), found in vertebrates, stimulates cholinergic (muscarinic and nicotinic) receptors to mediate cholinergic neuronal transmission. ACh is synthesised presynaptically in neurons from choline and acetyl coenzyme A, an action catalysed by the enzyme choline acetyltransferase (ChAT) (Marshall and Parsons 1987). ACh is stored in synaptic vesicles and is released upon neuronal stimulation into the synaptic cleft (Marshall and Parsons 1987) and so binds to the receptor. However, ACh is rapidly hydrolysed (at the ester bond to

Received: 1 April, 2009. Accepted: 31 August, 2009.

Invited Review

yield choline and acetate) under the action of the enzyme acetylcholinesterase (AChE) resulting in the loss of receptor stimulation and consequently modulates cholinergic neurotransmission. Cholinergic function has been recognised as a therapeutic target in some disease states and one approach to achieve this is to prolong the action of ACh through the use of AChE inhibitors. Consequently, AChE inhibitors have been investigated for a number of therapeutic applications including glaucoma, myasthenia gravis, anti-muscarinic poisoning and dementia.

There are numerous examples of chemically diverse compounds that have shown AChE inhibitory activity, with many of these compounds being of natural origin. These include compounds that are obtained directly from natural sources and those, both semi-synthetic and synthetic, which have chemical structures based on a natural compound. For example, two of the four drugs licensed to alleviate symptoms in dementia (the AChE inhibitors galantamine and rivastigmine) are both derived from natural origin. Galantamine is an alkaloid originally isolated from some Amaryllidaceae species and physostigmine, which is also an alkaloid but from *Physostigma venenosum* Balf. (Leguminosae), was used as a template to develop rivastigmine.

Natural products continue to be a source of compounds that show anti-AChE activity and some have been investigated for their potential for use in disorders that involve cholinergic dysfunction in the underlying pathology. Although AChE inhibitors have a number of therapeutic applications, most studies have focused on their potential to alleviate symptoms in cognitive disorders, particularly dementia. Many plants reputed to influence cognition in various traditional practices of medicine have been investigated to determine any pharmacological basis for their historical uses, with some extracts from these plants showing promising AChE inhibitory effects. Other plant extracts that are not associated with a traditional use related to cognitive disorders have also been shown to inhibit AChE. For some of the plants studied for AChE inhibitory effects the active compounds are unknown, but structural elucidation of many active compounds has been described, with many of these compounds providing templates for the development of new AChE inhibitors. These naturally-derived anti-AChE compounds, and their therapeutic relevance, are discussed.

Acetylcholinesterase (AChE)

AChE is a large glycoprotein and is mainly distributed in neurons, muscles and erythrocytes in vertebrates and it occurs as different molecular forms (Brimijoin 1983; Massoulié *et al*. 1993; Giacobini 2004). AChE is a complex α/β hydrolase-fold type protein with an overall ellipsoid shape containing a deep gorge about 20 Å in depth. At the bottom of this gorge are four main subsites known as the "esteratic site", the "oxyanion hole", the "anionic subsite" and the "acyl pocket" and it is in this region where ACh hydrolysis appears to occur, although the initial binding of ACh is thought to occur at an outer region known as the "peripheral site", located near the active site gorge (Massoulié *et al.* 1993; Houghton *et al.* 2006). Cholinesterases are among the fastest hydrolytic enzymes known (Massoulié *et al*. 1993).

Other functions of AChE include a role as an adhesion protein, a bone matrix protein, and it is associated with neurite outgrowth, apoptosis and the promotion of β-amyloid assembly to form amyloid fibrils, which are characteristically found in the brain tissue of Alzheimer's disease (AD) patients (Zhang 2004; Inestrosa *et al*. 2008; Jiang and Zhang 2008; Johnson *et al*. 2008; Massoulié *et al*. 2008). Another type of cholinesterase (ChE) is butyrylcholinesterase (BChE), which is mainly found in serum and glia, and it hydrolyses toxic esters such as cocaine and it is considered to have a detoxifying action (Giacobini 2004), although its role has not been clearly defined, and no endogenous natural substrate has been identified. Cholinesterases may also play a role in the immune responses, which has been suggested to occur in myasthenia gravis (MG), and they have been implicated in the pathogenesis of AD and cancer (Massoulié *et al*. 2008; Muñoz-Torrero 2008).

Inhibitors of AChE: Applications

Since AChE inhibitors can prolong the half-life and therefore availability of ACh in the neuronal synaptic cleft, they have been investigated for a number of therapeutic applications for disorders associated with cholinergic dysfunction. Memory loss in dementia is associated with a deficit of ACh, which influences cognitive function, and a cholinergic deficit has been correlated with the severity of AD, the most common type of dementia and estimated to affect 27 million people worldwide in 2006 (Perry *et al*. 1978; Perry 1986; Giacobini 1990; Grossman *et al*. 2006; Barten and Albright 2008). AD is a progressive, neurodegenerative disease that mainly affects the elderly population and the symptoms associated with cognitive dysfunction include loss of memory and recognition skills (Förstl *et al*. 1995; Desgranges *et al*. 1998; Grossman *et al*. 2006). The pathological features of AD include senile plaques (amyloid depositions) and neurofibrillary tangles (tau), oxidative and inflammatory processes and neurotransmitter disturbances in the central nervous system (CNS) (Cavalli *et al*. 2008), although the pathophysiology of AD is not completely understood. Dementia can also occur in patients with a variety of other diseases including Huntington's disease, Parkinson's disease (PD), Lewy body disease, Pick's disease and vascular disease (Berman and Greenamyre 2006; Grossman *et al*. 2006). Although PD involves a progressive loss of dopaminergic neurons and is typically characterised by tremor, dyskinesia and bradykinesia, approximately 24–31% of PD patients develop dementia (Dodel *et al*. 2008).

It was therefore rational that inhibitors of AChE were explored for their potential to modulate the cholinergic system and provide symptomatic relief in dementia. In recent years some AChE inhibitors (donepezil (Aricept®), rivastigmine (Exelon®) and galantamine (Reminyl®)) have been licensed for the symptomatic relief of dementia in AD or PD. Although modulation of cholinergic neurotransmission is the principal therapeutic action of AChE inhibitors, some AChE inhibitors are reported to interfere with β-amyloid metabolism and thus could play a role in the prevention of senile plaque formation in AD (Castro and Martinez 2006; Pákáski and Kálmán 2008) and some have been associated with neuroprotective effects (Muñoz-Torrero 2008; Sugimoto 2008). It is also hypothesised that AChE inhibitors act by improving cerebral blood flow, an effect assumed to enhance cognition, since basal forebrain cholinergic neurons have projections to cerebral blood vessels and ACh is a vasodilator (Claassen and Jansen 2006). However, further research is required to substantiate this cholinergic-vascular hypothesis, particularly as other studies suggest that AChE inhibitors may decrease α7-nicotinic ACh receptor-mediated vasodilation in the cerebral circulation (Mozayan *et al*. 2006).

MG is a chronic autoimmune disorder that affects neuromuscular transmission, causing skeletal muscle weakness, and it is estimated that approximately 80–85% of patients show autoimmunity against postsynaptic nicotinic receptors (Maggi *et al*. 2008). In MG, anti-ChE drugs increase the availability of ACh and therefore enhance neuromuscular transmission in voluntary and involuntary muscles. This provides temporary symptomatic relief, but excessively high doses may impair neuromuscular transmission and cause a depolarising block leading to 'cholinergic crisis' (Mehndiratta *et al*. 2008). AChE inhibitors that have been used to treat symptoms in MG include neostigmine, pyridostigmine and distigmine. In anaesthesia, AChE inhibitors such as neostigmine are used to reverse the effects of the non-depolarising (competitive) neuromuscular blocking drugs.

Glaucoma is an ocular disorder, characterised by the loss of the visual field associated with damage to the optic

nerve, usually due to raised intra-ocular pressure. Drugs that can reduce the intra-ocular pressure are therefore used in the treatment of glaucoma. The AChE inhibitor physostigmine has been used alone and in combination with other miotic drugs such as pilocarpine to cause contraction of the ciliary muscle, which opens up the drainage channels to decrease intra-ocular pressure in glaucoma, although physostigmine is rarely used now for this purpose due to contact sensitivity reactions (Neal 1992). Some drugs (e.g. some anti-histamines, atropine) or plants (e.g. *Atropa belladonna* L. (deadly nightshade), *Datura stramonium* L. (jimsonweed, thorn apple, locoweed) (Solanaceae)) can induce anti-cholinergic effects when administered at clinical or toxic doses. AChE inhibitors such as physostigmine have been used to reverse these toxic effects, although they are not used routinely for this purpose.

AChE inhibitors have also been investigated for their efficacy in some other disorders that involve cholinergic dysfunction. Although disruption of the cholinergic system is considered one of the mechanisms that occur in delirium, there is no convincing evidence to date that AChE inhibitors are an effective treatment and further studies to determine any potential therapeutic role are needed (Bourne *et al*. 2008; Overshott *et al*. 2008). AChE also degrades ACh in the insect synapse and inhibition of AChE is the primary target for some insecticides, particularly the organophosphates and the carbamates, which are potent AChE inhibitors (Hassall 1990; Denholm *et al*. 1999; Jett 2008).

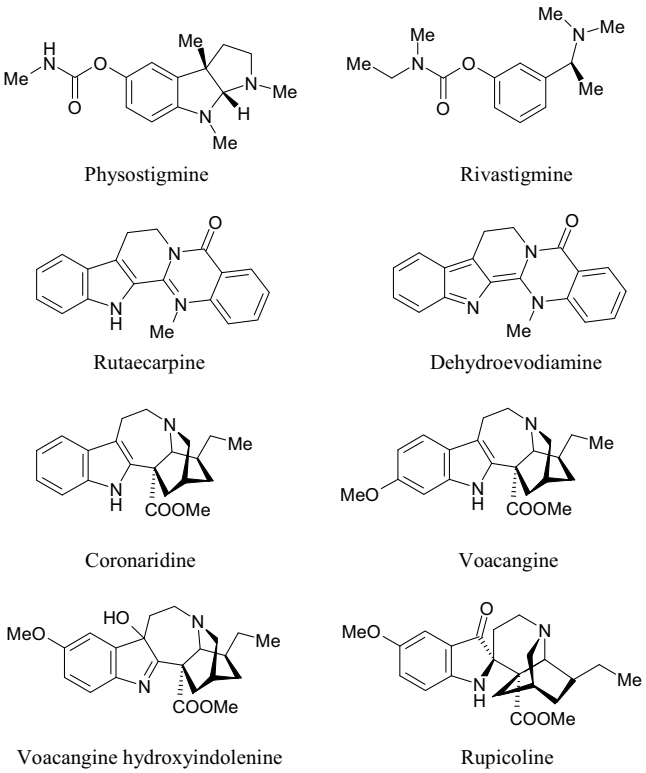


Fig. 1 Indole alkaloid derivatives that inhibit AChE.

ALKALOIDS AS INHIBITORS OF ACHE

Indole alkaloids

Physostigma venenosum Balf. (Leguminosae) seeds, commonly known as calabar beans, were traditionally used in Africa for ritual deaths associated with the funeral of a chief and as an ordeal poison, believed to establish the guilt or innocence of persons accused of a crime; rapid death indicated that the suspect was guilty, perhaps due to nervous sipping by guilty suspects enabling greater absorption of the toxic compounds (Houghton *et al*. 2006). The adverse effects of the calabar bean (also known as the ordeal bean or chopnut) include bradycardia, muscle cramps, salivation, nausea, respiratory failure and CNS effects, actions associated with cholinergic stimulation. It is now known that an alkaloid with a pyrroloindole skeleton, physostigmine (**Fig. 1**), from *P. venenosum* seeds is a potent, short-acting and reversible inhibitor of AChE (Julien and Pikl 1935; Kamal *et al*. 2000) and can explain the toxic effects.

It is the carbamate in the physostigmine structure that is responsible for the ChE inhibition, since hydrolysis of the ester bond produces the inactive eseroline (Houghton *et al*. 2006). It is the carbonyl group of physostigmine that interacts with the OH of a serine in AChE to form an ester with the urethane part of the molecule, consequently interfering with AChE activity (Houghton *et al*. 2006). Physostigmine, also known as eserine, inhibits both G_1 and G_4 AChE forms, the main AChE isoenzymes present in mammalian CNS (Ogane *et al*. 1992). In addition, physostigmine inhibits BChE with similar potency, an enzyme that has been implicated in the pathology of AD (Greig *et al*. 2001). Since it is an inhibitor of ChE, physostigmine has been investigated for therapeutic potential in some cognitive disorders. Studies show physostigmine to protect mice against cognitive impairment caused by oxygen deficit and to improve learning, and to antagonise scopolamine-induced impairment of cognitive function in rats (Yoshida and Suzuki 1993). In human studies, physostigmine produced significant cognitive benefits in both normal and AD patients (Sitaram *et al*. 1978; Shu 1998). As physostigmine can penetrate the blood-brain barrier, it has been used to reverse the CNS effects of poisoning with anti-muscarinic agents, but is not primarily used for this purpose now.

Attempts have been made to develop analogues of physostigmine to optimise the potency of AChE inhibition and modify the pharmacokinetic profile, particularly to identify compounds which have a longer half-life than physostigmine and which are sufficiently lipophilic to enable the blood-brain barrier to be crossed and so target the CNS. Structure-activity relationship studies have enabled a series of carbamate derivatives to be developed that potently and selectively inhibit AChE (Tumiatti *et al*. 2008). Substitution of the carbamoylmethyl group in position 5 of the side chain of physostigmine with a carbamoylheptyl group yielded a compound, eptastigmine (heptyl-physostigmine tartrate) that inhibits both AChE and BChE (Braida and Sala 2001). Although eptastigmine could improve cognition in AD patients, haematologic adverse effects prevented further clinical investigations (Braida and Sala 2001). Cymserine is a synthetic analogue of physostigmine and it potently and concentration-dependently binds with human BChE (Kamal *et al*. 2006a), and bisnorcymserine (*N*-demethylated cymserine) potently inhibits BChE *in vitro* (Kamal *et al*. 2006b), although the therapeutic potential of these compounds requires further study.

Phenserine is a third-generation derivative of physostigmine that inhibits both AChE and β-amyloid precursor protein (Butler 2008). (-)-Phenserine is less toxic than physostigmine and the synthetic AChE inhibitor tacrine, and it enhanced cognition *in vivo* (Loizzo *et al*. 2008). A small-scale clinical study in 20 AD patients (30 mg/day) showed phenserine to improve cognition (Kadir *et al*. 2008) however, phase III trials did not show this drug to differ to the effects of the placebo in AD patients; the (+)-enantiomer (Posiphen™) has also been investigated in phase I trials but additional trials are not planned (Butler 2008). A related compound is tolserine, which only differs in structure from phenserine by methyl substitution in the C2' position, and it has improved selectivity for AChE compared to BChE (Loizzo *et al*. 2008). Various other methyl, dimethyl and trimethyl substituted derivatives of physostigmine and phenserine have also been investigated for their ChE inhibitory potency (Loizzo *et al*. 2008), although none of these compounds appears to have been considered for clinical studies. Xanthostigmine is also based on the structure of physostigmine and is a potent inhibitor of AChE; derivatives of this compound have been developed that not only inhibit the

catalytic activity of AChE, but also inhibit AChE-induced β-amyloid aggregation (Cavalli *et al*. 2008). The carbamate type reversible AChE inhibitor rivastigmine (**Fig. 1**) is also derived from physostigmine. Rivastigmine inhibits AChE in the cortex and hippocampus and preferentially inhibits the G_1 form of AChE (Polinsky 1998); it also potently inhibits both AChE and BChE in Alzheimer's plaques and tangles (Eskander *et al*. 2005). In AD patients it improved cognition, an effect correlated with the level of AChE inhibition (Agid *et al*. 1998; Cutler *et al*. 1999; Kumar *et al*. 1999; Grossberg and Desai 2001). Since there has been clinical evidence of efficacy and appropriate safety, rivastigmine is now licensed for use in Europe for the symptomatic treatment of mild to moderate dementia in AD or PD.

Since other neurotransmitter disturbances in addition to the cholinergic changes can also occur in AD, some compounds have been developed that combine the propargylamine pharmacophore of the monoamine oxidase (MAO) inhibitor selegiline with physostigmine, with the aim of producing compounds with both AChE and MAO inhibitory activities. One compound based on this type of structure (an imino 1,2,3,4-tetrahydrocyclopent[*b*]indole carbamate) inhibited both AChE and MAO-A, but was not developed further due to poor oral bioavailability and brain penetration when administered *in vivo* (Fink *et al*. 1996). Other compounds synthesised include structural features of rivastigmine and fluoxetine, a serotonin transporter (SERT) inhibitor, with the aim of providing compounds with cognitive and anti-depressant actions. One of these compounds, (*S*)-RS-A259, has shown some promise since it could inhibit AChE and SERT *in vitro* and *in vivo* (Cavalli *et al*. 2008).

Other physostigmine analogues in clinical use include neostigmine, pyridostigmine and distigmine. Unlike physostigmine (a tertiary amine) these compounds contain a quaternary N within their structures, thus restricting their ability to cross the blood-brain barrier, so are not considered as useful candidates for treatment of cholinergic disorders of the CNS. They are however, licensed drugs for the treatment of symptoms in MG, and distigmine is also used for urinary retention and neurogenic bladder.

Rutaecarpine and dehydroevodiamine (**Fig. 1**) are indole alkaloids from the plant described as *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae) in the Pharmacopoeia of the People's Republic of China (2005), which is used in traditional Chinese medicine (TCM) for its reputed cardiotonic, restorative and analgesic effects (Chinese Pharmacopoeia Commission 2005). Both dehydroevodiamine and an extract from *E. rutaecarpa* inhibit AChE *in vitro*, and reverse scopolamine-induced memory impairment in rats (Park *et al*. 1996). Attempts to exploit the anti-ChE properties of this plant have included studies to modify the rutaecarpine and dehydroevodiamine chemical structures to develop new compounds with structural components of these alkaloids with those of the synthetic AChE inhibitor tacrine. The synthetic analogues developed, (8*Z*)-5,6-dihydro-8*H*-isoquino[1,2-*b*]quinazolin-8-imine, 5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazoline, 5,7,8,13-tetrahydroindolo[2′,3′:3,4]pyrido[2,1-*b*]quinazoline and *N*-(2-phenylethyl)-*N*-[(12*Z*)-7,8,9,10-tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-ylidene]amine, inhibited AChE and BChE *in vitro*, with the latter two compounds showing greater affinity for BChE (Decker 2005). In contrast, the dibenzo-analogue of dehydroevodiamine (13-methyl-5,8-dihydro-6*H*-isoquino[1,2-*b*]quinazolin-13-ium chloride) showed greater selectivity for AChE, compared to BChE (Decker 2005).

Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult. (Apocynaceae) root is used traditionally in Thai medicine for its reputed rejuvenating and neurotonic effects (Ingkaninan *et al*. 2003). A methanolic extract of the root showed the most potent inhibition of AChE *in vitro* of 32 different Thai plants studied, and this effect was suggested to be due to the alkaloids present in the root (Ingkaninan *et al*. 2003). Subsequent studies revealed that the root and stem extracts from *T. divaricata* inhibited AChE (99.7 and 94.7% inhibition, respectively) more potently than leaf and flower extracts (41.8 and 51.8% inhibition, respectively) at 0.1 mg mL^{-1} *in vitro* (Ingkaninan *et al*. 2006a). Of four bisindole alkaloids isolated from the root, only 19,20-dihydrotabernamine and 19,20-dihydroervahanine inhibited AChE more potently than galantamine *in vitro*; conodurine and tabernaelegantine A were inactive (Ingkaninan *et al*. 2006a). An ethanol extract of the roots also inhibited cortical AChE activity *in vivo* (Chattipakorn *et al*. 2007), but the individual alkaloids from this plant have yet to be investigated for their effects *in vivo*. Studies on a different species, *T. australis* Müll. Arg. (synonym: *T. catharinensis* A.DC.), showed the indole alkaloids coronaridine, voacangine, voacangine hydroxyindolenine and rupicoline (**Fig. 1**) isolated from the stalks to inhibit AChE (Andrade *et al*. 2005). Indole alkaloids from *T. laeta* Mart. stalks show inhibitory activity against AChE (coronaridine and 19-*epi*-isovoacristine), BChE (heyneanine and N_b-methylvoachalotine), or both (conodurine) (Vieira *et al*. 2008).

An aqueous extract of *Desmodium gangeticum* DC. (Leguminosae) inhibited AChE in the CNS and produced anti-amnesic effects *in vivo* (Joshi and Parle 2006), suggesting that compounds from this plant may modulate cholinergic function. Investigations on the compounds responsible for the observations *in vivo* show indole alkaloids from both *D. gangeticum* and *D. pulchellum* Benth. to inhibit AChE, with the most potent being legucin A (Houghton *et al*. 2006). Other indole alkaloids that inhibit AChE are the glucoalkaloids turbinatine and desoxycordifoline from the bark and leaves of *Chimarrhis turbinata* DC. (Rubiaceae), which produce moderate inhibition *in vitro* (Cardoso *et al*. 2004).

Isoquinoline and quinoline alkaloids

The alkaloid galantamine (also known as galanthamine) (**Fig. 2**) was originally isolated in the mid twentieth century from *Galanthus woronowii* Losinsk. (Amaryllidaceae), commonly known as the 'snowdrop' but has now also been isolated from some species of *Narcissus* (Amaryllidaceae) and *Leucojum aestivum* L. (Amaryllidaceae) (Heinrich and Teoh 2004). The AChE inhibitory activity of galantamine is well-documented. It binds at the base of the active site gorge of AChE (*Torpedo californica* AChE) and interacts with both the choline-binding site and the acyl binding pocket, and the tertiary amine is suggested to form an H-bond (via the *N*-methyl group) near the top of the gorge (to Asp-72) (Greenblatt *et al*. 1999). Galantamine is more selective for AChE than BChE, and is well-absorbed from the gastrointestinal tract (Bickel *et al*. 1991; Fulton and Benfield 1996). A number of clinical, multi-centre, randomised, controlled trials, show galantamine to be well-tolerated and to significantly improve cognitive function when administered to AD patients (Wilcock *et al*. 2000; Wilkinson and Murray 2001) and it is a licensed drug for treating symptoms of mild to moderate dementia in AD. Some studies suggest that galantamine may also be of some therapeutic value in vascular Lewy body dementia (Small *et al*. 2003; Edwards *et al*. 2007) and in schizophrenia, since it improved memory and attention (Schubert *et al*. 2006). Synthetic galantamine derivatives have been developed including quaternary ammonium derivatives, esters and carbamates of 6-*O*-demethylgalantamine (Loizzo *et al*. 2008). Structure-activity relationship studies revealed that some heterodimeric alkylene linked bis-galantamine derivatives were more potent than galantamine in the inhibition of AChE, and that the length of the alkylene link and the presence of an iminium function were important for inhibitory potency (Loizzo *et al*. 2008).

Other alkaloids from species of *Narcissus* have been investigated for their anti-ChE activity. In one study, 26 extracts from different species of *Narcissus* and 23 Amaryllidaceae alkaloids were tested, but only seven of these alkaloids, either galantamine or lycorine (**Fig. 2**) structural types, inhibited AChE (López *et al*. 2002). This study showed 11-hydroxygalantamine (**Fig. 2**) to have similar in-

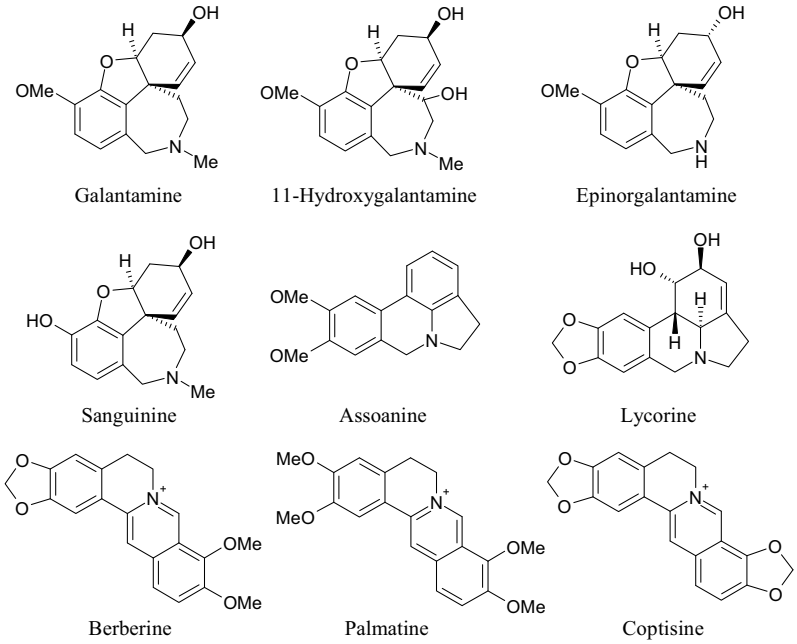


Fig. 2 Isoquinoline alkaloid derivatives that inhibit AChE.

hibitory potency to galantamine, epinorgalantamine (**Fig. 2**) was less potent and sanguinine (**Fig. 2**) was even more potent than galantamine, an effect attributed to the additional hydroxyl group in this structure, for interaction with AChE (López *et al*. 2002). Assoanine (**Fig. 2**) was the most active AChE inhibitor of the lycorine-type alkaloids, but it was still four-fold less potent than galantamine (López *et al*. 2002). When 23 Amaryllidaceae alkaloids were tested for AChE inhibition in a separate study, the lycorine-type alkaloids showed greater activity than the crinine- and tazettine-type alkaloids, with 1-*O*-acetyllycorine being two-fold more potent than galantamine (Elgorashi *et al*. 2004). Other studies show the alkaloid ungiminorine from the *Narcissus* cultivar 'Sir Winston Churchill' to be a relatively weak AChE inhibitor (Ingkaninan *et al*. 2000), but ungeremine, isolated from *Nerine bowdenii* W.Watson (Amaryllidaceae) and from species of *Galanthus* and *Narcissus* showed stronger AChE inhibition than galantamine (Labraña *et al*. 2002; Rhee *et al*. 2004; Berkov *et al*. 2007).

An investigation of the bulb extracts from *Crinum jagus* (J.Thomps.) Dandy and *C. glaucum* A.Chev. (Alliaceae), plants used traditionally in Nigeria for treating memory loss, showed the bulb extracts and some alkaloids isolated from *C. glaucum* (hamayne and lycorine) to inhibit AChE, but less potently than physostigmine (Houghton *et al*. 2004). There are also several isoquinoline alkaloids from the corms of *Colchicum speciosum* Steven (Colchicaceae) that are reversible inhibitors of both AChE and BChE *in vitro* (Rozengart *et al*. 2006).

Benzylisoquinoline alkaloids from various plant families, including members of the Berberidaceae, Papaveraceae, Ranunculaceae and Menispermaceae have been investigated for ChE inhibition. Of these compounds berberine, palmatine and sanguinarine are reported to inhibit AChE and BChE, with the latter considered the most potent inhibitor (Schmeller *et al*. 1997). However, a later study showed berberine, palmatine and coptisine (**Fig. 2**), which occur in *Coptis chinensis* Franch. (Ranunculaceae) (Howes and Houghton 2003; Howes *et al*. 2003), to potently inhibit AChE but not BChE (Shigeta *et al*. 2002), an indication that the inhibitory potency of some compounds appears to vary between different studies. Although, a further study confirmed the AChE inhibitory potency when of four alkaloids isolated from the aerial parts of the TCM plant *Corydalis speciosa* Maxim. (Papaveraceae), berberine and palmatine were the most potent, with corynoxidine and protopine being less active (Kim *et al*. 2004a). Other alkaloids from *Corydalis* species have been tested for their effects on AChE and those most active include epiberberine, pseudodehydrocorydaline, pseudocoptisine and pseudoberberine (Kim 2002; Hung *et al*. 2008a). The anti-ChE activities of pseudoberberine and pseudocoptisine were considered to explain the ability of these alkaloids to alleviate scopolamine-induced memory impairment in rodents (Hung *et al*. 2008a and 2008b). In another study, berberine and sanguinarine were more potent AChE inhibitors than chelidonine, alkaloids that occur in numerous plants including *Chelidonium majus* L. (Papaveraceae) (Kuznetsova *et al*. 2002). The related compounds 8-hydroxydihydrosanguinarine and 8-hydroxydihydrochelerythrine, also from *Chelidonium majus*, show selective inhibition of AChE compared with BChE (Cho *et al*. 2006). (±)-Meptazinol is an opioid analgesic (Tumiatti *et al*. 2008), with a structure based on the natural alkaloid morphine, from *Papaver somniferum* L. (Papaveraceae). Although the (*R*)-(+)-enantiomer of meptazinol is a more potent analgesic than the (*S*)-(-)-enantiomer, the latter shows more potent anti-AChE activity (Tumiatti *et al*. 2008).

Other alkaloids in this class have been isolated from *Stephania* species (Menispermaceae). *S. suberosa* L.L.Forman is reputed to be rejuvenating and to show neurotonic effects in Thai medicine (Ingkaninan *et al*. 2003). A methanolic extract of the root potently inhibits AChE *in vitro*, an effect suggested to be due to the alkaloids present (Ingkaninan *et al*. 2003). Studies on the compounds from a different species, *S. venosa* Spreng. (also used in traditional Thai medicine) showed the alkaloids stepharanine, cyclanoline and *N*-methyl stepholidine to inhibit AChE more potently than stepholidine and corydalmine (Ingkaninan *et al*. 2006b). Thus, it was concluded that the positive charge at the nitrogen of the tetrahydroisoquinoline portion, steric substitution at the nitrogen, planarity of the molecule or substitutions at C-2, -3, -9 and -10 can modify the AChE inhibitory effects of protoberberine alkaloids (Ingkaninan *et al*. 2006b). Stephaoxocanidine, also from *Stephania* species, has been used as a template to synthesise analogues that inhibit AChE *in vitro* and one such compound, 5,6-dimethoxy-7*H*-8-oxa-1-aza-phenalen-9-one, was as active as a galantamine-enriched *Narcissus* extract (Bianchi *et al*. 2005). The bisbenzylisoquinoline alkaloids fangchinoline, atherospermoline and fenfangjine E from *S. tetrandra* S.Moore root and five bisbenzylisoquinoline alkaloids from *Cocculus pendulus* Diels (Menispermaceae) also inhibit AChE (Houghton *et al*. 2006; Markmee *et al*. 2006).

Lindoldhamine is a bisbenzylisoquinoline alkaloid from *Triclisia sacleuxii* Diels (Menispermaceae) leaves and it inhibits AChE *in vitro*, but less potently than galantamine (Murebwayire *et al*. 2009).

Some quinoline alkaloids from the aerial parts of *Skimmia laureola* (DC.) Dcne. (Rutaceae) include methyl isoplatydesmine, 3-hydroxy,2,2,6-trimethyl-3,4,5,6-tetrahydro-2*H*-pyrano[3,2-*c*] quinoline 5-one and ribalinine, which are linear mixed type AChE inhibitors, and the latter two compounds also show this type of inhibition against BChE; methyl isoplatydesmine was identified as a non-competitive inhibitor of BChE (Rahman *et al*. 2006).

Quinolizidine alkaloids

A TCM remedy prepared from *Huperzia serrata* (Thunb.) Trevis. (Lycopodiaceae) is reputed to alleviate memory loss (Howes and Houghton 2003). Alkaloids isolated from *H. serrata* include huperzines A and B (**Fig. 3**), which are related to the quinolizidine alkaloids. A number of studies *in vivo* have shown huperzine A to improve cognition (Lu *et al*. 1988; Wang *et al*. 2000; Zhou *et al*. 2001). The main mode of action to explain the cognitive benefits of huperzine A is reversible inhibition of AChE, which has been shown both *in vitro* and *in vivo* (Laganière *et al*. 1991; McKinney *et al*. 1991; Ashani *et al*. 1992), with the natural (-)-isomer being a more potent inhibitor of AChE than the (+)-isomer (Zhang *et al*. 2000). Huperzine A also shows greater selectivity for AChE than BChE, which may provide some explanation why it was less toxic than the synthetic AChE inhibitors donepezil and tacrine, and it significantly improved memory and behaviour in AD patients in a multicentre, double-blind trial (Small *et al*. 1997; Shu 1998). In phase IV clinical trials, huperzine A improved memory in elderly, AD and vascular dementia patients, with few adverse effects (Wang *et al*. 2006). Pharmacokinetic studies have indicated that huperzine A is rapidly absorbed, widely distributed in the body and is eliminated at a moderate rate (Wang *et al*. 2006). In 1996, the drug 'Shuangyiping', a tablet formulation of huperzine A, was developed and used for symptomatic treatment of AD in China; huperzine A is also marketed in the USA as a dietary supplement as powdered *H. serrata* for memory impairment (Ma *et al*. 2007). Huperzine B is a less potent and selective AChE inhibitor than huperzine A (He *et al*. 2007).

A series of structural analogues of huperzines A and B have been developed with the aim of identifying more potent AChE inhibitors that could have therapeutic potential. One study investigated 5-substituted huperzine A analogues, with the C-5 amino group in huperzine A substituted with either a hydroxyl group, a fluoro group or an acetoxyl group (Zhou and Zhu 2000). However, none of these 5-substituted analogues were as potent as huperzine A in the inhibition of AChE *in vitro*, indicating that the C-5 amino group is important for the inhibitory potency, perhaps because it forms a quaternary ammonium under physiological conditions to imitate ACh (Zhou and Zhu 2000). The significance of the C-5 amino group is also demonstrated by a study in which the synthetic (*E*)- and (*Z*)-5-desamino huperzine A derivatives were more potent than the 5-fluoro and 5-hydroxyl derivatives, but were not as potent as huperzine A in the inhibition of AChE *in vitro* (Zhou and Zhu 2000; Högenauer *et al*. 2001). Other huperzine A derivatives include (±)-14-fluorohuperzine A, synthesized with the aim of enhancing the H-bond between the C-14 methyl of huperzine A and the backbone carbonyl of His440 on AChE. However, this was unsuccessful as the 14-fluorohuperzine A racemate inhibited AChE *in vitro* 62-times less potently than huperzine A (Zeng *et al*. 1998). Other fluorinated analogues of huperzine A have been developed ((±)-12,12,12-trifluorohuperzine A, (±)-14,14,14-trifluorohuperzine A, (±)-12,12,12,14,14,14-hexafluorohuperzine A and (±)-12-fluorohuperzine A) but disappointingly were less potent AChE inhibitors than huperzine A (Kaneko *et al*. 1996 and 1997). Another synthetic derivative (-)-dimethylhuperzine A (DMHA) showed comparable AChE inhibitory activity to (-)-huperzine A, but the enantiomer (+)-DMHA was inactive (Rajendran *et al*. 2000).

Pharmacomodulation of huperzine A and the synthetic AChE inhibitor tacrine has provided compounds that include a combination of the carbobicyclic substructure of huperzine A and the 4-aminoquinoline substructure of tacrine (Badia *et al*. 1998; Carlier *et al*. 1999). Of these compounds, *rac*-(*E*)-12-amino-13-ethylidene-6,7,10,11-tetrahydro-9-methyl-7-11-methanocycloocta[*b*]quinoline hydrochloride, was shown to be less potent than tacrine in the inhibition of AChE, but it was more active than the (*Z*)-stereoisomer (Badia *et al*. 1998). Other synthetic derivatives which lack the ethylidene substituent are *rac*-12-amino-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*] quinoline hydrochloride and *rac*-12-amino-9-ethyl-6,7,10, 11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride, which promisingly were more potent AChE inhibitors than tacrine (Badia *et al*. 1998).

Some other synthetic compounds related to huperzine A are the huprines X, Y and Z (**Fig. 3**). Huprine X ((-)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride) showed affinity for AChE that was 180-times that of huperzine A, 1200-times that of tacrine and 40-times that of donepezil (Camps *et al*. 2000) and it binds to AChE in a similar way to huperzine A and tacrine at the acylation site in the active site gorge, and it interferes with the binding of peripheral site ligands (Camps *et al*. 2000). Both (±)-huprine X and (±)-huprine Y ((±)-12-amino-3-chloro-9-methyl-6,7,10,11-tetrahydro-7, 11-methanocycloocta[*b*]quinoline hydrochloride) increased the level of ACh in the synaptic cleft more effectively than tacrine (Ros *et al*. 2001). Both (±)-huprines Y and Z, which

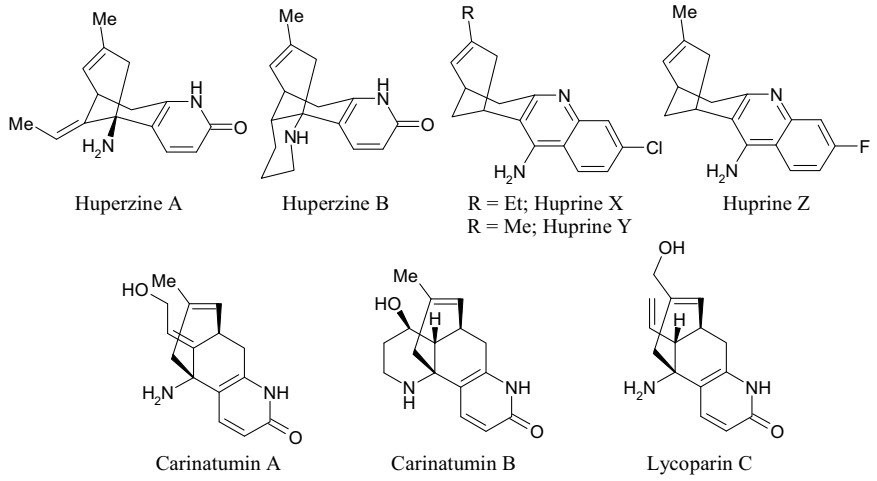


Fig. 3 Quinolizidine alkaloid derivatives that inhibit AChE.

differ in structure by the halogen at position 3 (chlorine and fluorine, respectively), are more potent inhibitors of AChE than BChE and could inhibit brain AChE (*ex vivo*), but (±)-huprine Y was approximately 5-times more potent than (±)-huprine Z (Alcalá *et al*. 2003). Other synthetic huperzine A hybrids feature structural properties of E2020 (donepezil). These synthetic hybrids were developed to promote an interaction between the 5,6,7,8-tetrahydroquinolinone of huperzine A and the active site of AChE, and an interaction between the benzyl piperidine of E2020 and the peripheral binding site of AChE, however these hybrids were not as potent as E2020 in the inhibition of AChE *in vitro* (Zeng *et al*. 1999). A pro-drug of huperzine, Debio 9902 (ZT-1), was shown to be safe and effective when administered once daily to AD patients in a phase IIa clinical trial and further trials are now in progress (Butler 2008).

Some analogues of huperzine B have also been synthesised but although some of these analogues are reported to be up to 4-fold more potent than huperzine B, they were not as potent as huperzine A in the inhibition of AChE (Rajendran *et al*. 2002). One dimer derivative of huperzine B not only potently inhibits AChE, but is also neuroprotective against β-amyloid and it could improve scopolamine-induced spatial performance deficits in rodents (He *et al*. 2007; Muñoz-Torrero 2008), suggesting this compound may have potential to alleviate memory loss and protect against neurodegeneration in cognitive disorders, although it does not appear to have been investigated further.

Carinatumins A, B (**Fig. 3**) and C, which are structurally related to huperzines A and B, have been isolated from the club moss *Lycopodium carinatum* Poir. (Lycopodiaceae); only carinatumins A and B were potent inhibitors of AChE *in vitro* (IC_{50} 4.6 and 7.0 μM, respectively), but they were still not as potent as huperzine A (IC_{50} 0.8 μM) in the inhibition of the enzyme (Choo *et al*. 2007). Also structurally related to the huperzines are the lycoparins A, B and C from *L. casuarinoides*, but only lycoparin C (**Fig. 3**) (which lacks the carboxylic acid at C-15 and the *N*-methyl groups that occur in lycoparins A and B), showed inhibitory activity against AChE *in vitro* (Hirasawa *et al*. 2008). From *L. annotinum* L., different alkaloids that also inhibit AChE have been isolated, including annotine (Halldorsdottir and Olafsdottir 2006).

Piperidine alkaloids

The piperidine alkaloids (-)-spectaline and the (-)-3-*O*-acetyl derivative from *Cassia spectabilis* DC. (Leguminosae) flowers contain structural features similar to that of ACh, thus were selected as templates to design compounds with AChE inhibitory potential. Two of these compounds ((2*R*,3*R*,6*S*)-2-methyl-6-(13-oxotetradecyl)-piperidin-3-yl acetate hydrochloride (LASSBio-767) (**Fig. 4**) and *tert*-butyl (2*R*,3*R*,6*S*)-2-methyl-6-(13-oxotetradecyl)-piperidin-3-yl carbonate hydrochloride (LASSBio-822)) inhibited rat brain AChE more selectively than BChE, and also reversed scopolamine-induced amnesia in mice without producing toxic effects (Viegas *et al*. 2005; Castro *et al*. 2008). Further investigations suggested these compounds non-competitively inhibit AChE and interact with the peripheral anionic site rather than the catalytic triad (Castro *et al*. 2008). Consequently, the piperidine alkaloids isolated from *C. spectabilis* and the semi-synthetic derivatives including LASSBio-767 have been patented as AChE inhibitors with potential to treat pathologies affecting the cholinergic system (Tumiatti *et al*. 2008).

Extracts from the whole plant of *Haloxylon salicornicum* Bunge ex Boiss. (Chenopodiaceae) have yielded the piperidine alkaloids haloxylines A and B (**Fig. 4**), which inhibit both AChE (IC_{50} 25.3 and 20.2 μM, respectively) and BChE (IC_{50} 19.0 and 14.7 μM, respectively) *in vitro*, but neither compound was as active as galantamine (IC_{50} 0.5 and 8.5 μM, against AChE and BChE, respectively) (Ferheen *et al*. 2005). Juliflorine (**Fig. 4**), from the leaves of *Prosopis juliflora* DC. (Leguminosae), is an alkaloid consisting of two piperidine rings connected with a dihydroindilizine moiety and it inhibits both AChE and BChE *in vitro* (IC_{50} 0.42 and 0.12 μM, respectively) (Choudhary *et al*. 2005a). Molecular docking studies indicate that the more hydrophobic dihydroindilizine moiety penetrates deep into the aromatic gorge of AChE whilst the piperidine rings remain at the top of the gorge, enabling interactions with the peripheral anionic and quaternary ammonium binding sites, but it was not shown to extend to the catalytic triad (Choudhary *et al*. 2005a). Cryptadines A and B, which each consist of a piperidine ring and two octahydroquinoline rings, have been isolated from the club moss *Lycopodium cryptomerinum* and inhibit AChE *in vitro*, with the latter being the most potent inhibitor (Koyama *et al*. 2007). A related compound lycoperine A, from *L. hamiltonii*, also inhibits AChE (Hirasawa *et al*. 2006).

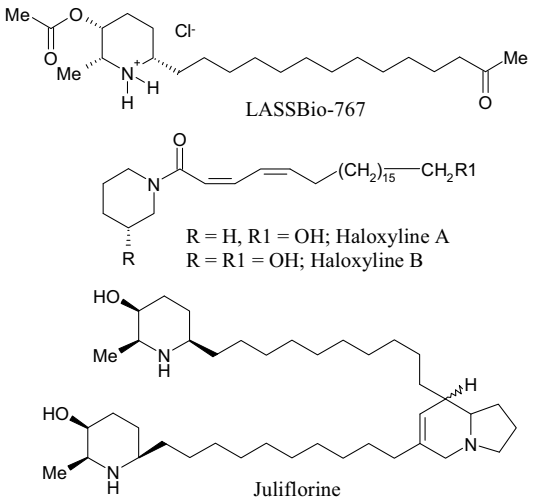
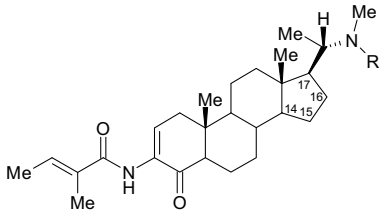


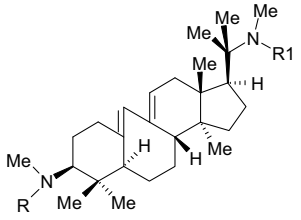
Fig. 4 Piperidine alkaloid derivatives that inhibit AChE.

Steroidal and terpenoid alkaloids

A number of steroidal alkaloids from *Sarcococca* and *Buxus* species (Buxaceae) have shown anti-ChE activities (Devkota *et al*. 2008). These include epoxynepapakistamine A, funtumafrine C and *N*-methylfuntumine from the leaves of *S. coriacea* Sweet, which showed greater inhibitory selectivity against BChE than AChE *in vitro*, but none were as active as physostigmine (Kalauni *et al*. 2002). It has been suggested that the anti-ChE steroidal alkaloids from *Sarcococca* species show greater selectivity for BChE compared to AChE due to the wider aromatic gorge in BChE, which could accommodate these bulky steroid structures more efficiently than AChE (Khalid *et al*. 2004a). Some steroidal alkaloids from the whole plant of *S. saligna* Müll.Arg. also appear to show greater selectivity for BChE than AChE (Atta-ur-Raman *et al*. 2004; Khalid *et al*. 2004b). In one study, none of the seven alkaloids from *S. saligna* tested were as active as galantamine in the inhibition of AChE, but three of these alkaloids, 16-dehydrosarcorine, sarcovagenine C and salignarine C, were more potent than galantamine in the inhibition of BChE (Atta-ur-Raman *et al*. 2004). Other relatively weak anti-ChE steroidal alkaloids from this species include isosarcodine, sarcorine, sarcodine, sarcocine and alkaloid C (Khalid *et al*. 2004b; Gilani *et al*. 2005). The nitrogen substituents at C-3 and C-20 (protonated at physiological pH) were associated with the inhibitory potency of these alkaloids. The presence of electron-withdrawing substituents is considered important for AChE inhibition when positioned at C-3 rather than C-20, as isosarcodine, which has acetamide substitution at C-3, was a more potent inhibitor of AChE (IC_{50} 10.3 μM) than sarcodine (IC_{50} 49.8 μM), which has acetamide substitution at C-20 (Khalid *et al*. 2004b). Molecular docking studies suggest ring A of the steroidal skeleton and the C-3 substituents of these com-



R = H, $\triangle^{14,15}$; Hookerianamide F
R = CH_3, $\triangle^{16,17}$; Sarcovagenine C
R = CH_3; Sarcovagine D



R = H, R1 = CH_3; Buxamine B
R = CH_3, R1 = H; Buxamine C

Fig. 5 Steroidal alkaloid derivatives that inhibit AChE.

pounds are positioned at the bottom of the aromatic gorge of AChE, while C-20 substituents remain outside the gorge (Khalid *et al*. 2004b). Other structure-activity relationship studies on the anti-AChE steroidal alkaloids from *S. saligna* consider the A-ring of the steroid nucleus as important for inhibitory activity (Zaheer-ul-Haq *et al*. 2003).

Steroidal alkaloids from the whole plant of *S. hookeriana* Baill. were inhibitory against AChE and BChE activity *in vitro*, but were not as active as galantamine in the inhibition of AChE; their inhibitory potencies against AChE were hookerianamide H > dictyophlebine > sarcovagine C > N_a-methylepipachysamine D > hookerianamide I (Devkota *et al*. 2007). In another study, nine steroidal alkaloids from the same species inhibited AChE and BChE *in vitro*, but even the three most potent inhibitors of AChE, hookerianamide F, sarcovagenine C and sarcovagine D (**Fig. 5**) (IC_{50} 1.6, 1.5, 2.2 μM, respectively) were less active than galantamine (IC_{50} 0.5 μM) (Choudhary *et al*. 2005b). The structural features of these alkaloids that were associated with inhibitory activity were the tigloylamino group at position C-3 and the carbonyl moiety in ring A (Choudhary *et al*. 2005b).

Buxamines B and C (**Fig. 5**) (from *Buxus papillosa* C.K.Schneid. and *B. hyrcana* Pojark. (Buxaceae)) concentration-dependently and non-competitively inhibit AChE (IC_{50} 74 μM and 7.5 μM, respectively) (Khalid *et al*. 2005). The substitutions at C-3 and C-20 are the only structural differences between these two compounds and it is proposed that the amino substituents at C-20, and C-3 are particularly important for the anti-ChE effects. Docking studies suggest that buxamine C is a more potent inhibitor because it penetrates deeper into the AChE gorge than buxamine B and the positioning of the C-3 tertiary amino group resembles the quarternary ammonium group of ACh or decamethonium better than the secondary amino group of buxamine B (Khalid *et al*. 2005). The alkaloids buxakashmiramine, cycloprotobuxine C, cyclovirobuxeine A, cyclomicrophylline A, buxakarachiamine and buxahejramine from *B. papillosa* leaves inhibit BChE more selectively than AChE, except for the latter two compounds which were only tested for AChE inhibition (Atta-ur-Raman *et al*. 2001). Buxakashmiramine (IC_{50} 25.4 μM) was the most active AChE inhibitor in this study but it was not as potent as physostigmine (IC_{50} 0.04 μM) (Atta-ur-Raman *et al*. 2001). Other steroidal alkaloids from *B. hyrcana* that inhibit AChE activity include (+)-benzoylbuxidienine, (+)-buxapapillinine, (+)-buxaquamarine, (+)-irehine, (+)-O^6-buxafurandiene and (+)-7-deoxy-O^6-buxafurandiene; the latter two compounds were the most potent of those tested, possibly due to the tetrahydrofuran ring in their structures (Babar *et al*. 2006). Steroidal alkaloid derivatives synthesised from *N*-3-isobutyrylcycloxobuxidine F, a compound isolated from *B. balearica* Lam., are recorded in a patent as AChE inhibitors (Tumiatti *et al*. 2008). These semi-synthetic alkaloids are suggested to bind simultaneously to both the catalytic and peripheral sites of AChE (Sauvaître *et al*. 2007).

Impericine, forticine, delavine, persicanidine A and imperialine, steroidal alkaloids from the bulbs of *Fritillaria imperialis* L. (Liliaceae), show both anti-AChE and anti-BChE activities *in vitro*, but were less active than physostigmine (Atta-ur-Raman *et al*. 2002). Several steroidal alkaloids isolated from other *Fritillaria* species have been evaluated for their ability to inhibit both AChE and BChE *in vitro*. *N*-Demethylpuqietinone, hupeheninoside (from *F. monantha* Migo; synonyms: *F. puqiensis* G.D.Yu & G.Y.Chen and *F. hupehensis* P.K.Hsiao & K.C.Hsia), ebeiedinone (from *F. ebeiensis* var. *purpurea* G.D.Yu & P.Li), yibeinoside A (from *F. pallidiflora* Schrenk) and chuanbeinone (from *F. delavayi* Franch.) inhibited both AChE and BChE, with *N*-demethylpuqietinone showing greater selectivity for AChE and hupeheninoside and chuanbeinone were more selective for BChE (Lin *et al*. 2006).

Steroidal glycoalkaloids occur in some members of the Solanaceae and may act as defense compounds, protecting the plants that contain them from pests and diseases. The toxicity of the green parts of *Solanum* species, such as the potato, is mainly attributed to these toxic alkaloids, and this has implications for food safety. Solanine and chaconine, steroidal glycoalkaloids based on the aglycone solanidine, occur in *Solanum* species and have a moderately strong anti-AChE effect, which might explain why consumption of plant material containing high amounts of these compounds produces gastrointestinal and CNS disturbances (Roddick *et al*. 2001; Houghton *et al*. 2006). Some other alkaloids also inhibit AChE but have not been considered for further study as potential drug candidates due to their relatively weak ChE inhibitory activities, compared to other more promising naturally derived compounds. These include a stigmastane type steroidal alkaloid, 4-acetoxy-plakinamine B, isolated from the sponge *Corticum* sp., which is a mixed-competitive inhibitor of AChE, but was approximately six-fold less active than galantamine (Langjae *et al*. 2007). *Aconitum falconeri* Stapf (Ranunculaceae) has been used traditionally in India as a sedative and to treat neuralgia and two diterpenoid alkaloids, faleoconitine and pseudaconitine, from the roots are moderate inhibitors of AChE (Atta-ur-Rahman *et al*. 2000). From another species, *A. heterophyllum* Wall., the diterpenoid alkaloids heterophyllinins A and B from the roots were more selective (13-fold) for inhibition of BChE compared to AChE (Nisar *et al*. 2009).

Other alkaloids

Menispermum dauricum DC. (Menispermaceae), a plant used in TCM for some inflammatory disorders including rheumatic pain (Chinese Pharmacopoeia Commission 2005), is a source of some oxoisoaporphine alkaloids. It is suggested that the 1-azabenzanthrone moiety in the chemical structures of these alkaloids can bind to the peripheral anionic site of AChE, thus inhibiting its activity (Tumiatti *et al*. 2008). It is therefore not surprising that semi-synthetic derivatives of these alkaloids show anti-AChE activity and some are being investigated further for potential use as AD treatment (Tumiatti *et al*. 2008).

Tapsine is a particularly interesting protoalkaloid from *Magnolia* x *soulangiana* Soul.-Bod. (Magnoliaceae) leaves, as it produced long-acting and concentration-dependent inhibition of AChE (IC_{50} 0.3 μM), and was more active than galantamine (IC_{50} 3.2 μM); molecular docking studies suggest tapsine binds closely to the catalytic triad in AChE via π-stacked interactions between the planar aromatic ligand and Trp84 and Phe330 of AChE, anchoring of the

cationic side chain with His444 reaching into the catalytic site and H-bonding with active site water molecules and Ser122 (Rollinger *et al*. 2006). Sinapine, an ester of sinapic acid and choline, is present in a number of plants including *Raphanus sativus* L. (Brassicaceae) and it shows potent AChE inhibition *in vitro* and in brain tissue (Tumiatti *et al*. 2008).

In addition to plants, some marine organisms have been investigated for bioactive compounds that inhibit ChE. One compound from the marine sponge *Petrosia* n. sp., a pentacyclic pyridoacridine alkaloid petrosamine, was six-fold more potent than galantamine in the inhibition of AChE *in vitro*, and molecular docking studies suggest that the quaternary ammonium group of petrosamine is responsible for the major interactions with the amino acid residues of the catalytic triad of the AChE gorge (Nukoolkarn *et al*. 2008). The AChE inhibitory potency of this compound suggests it has potential for therapeutic use, but further studies are required to assess relevant activities and toxicity *in vivo*.

Arecoline is a reduced pyridine derivative and is the major alkaloid in *Areca catechu* L. (Arecaceae), commonly known as betel nut (Houghton and Howes 2005). Arecoline improved scopolamine-induced cognitive impairment and passive avoidance performance *in vivo*, indicating a cholinergic action (Carey *et al*. 1992; Riekkinen *et al*. 1993). When administered to AD patients, arecoline enhanced verbal memory (Soncrant *et al*. 1993) and it improved cognitive function and recognition skills (Tariot *et al*. 1988; Raffaele *et al*. 1996). The suggested cholinergic action may be due to arecoline acting as an M_1/M_3 partial agonist (Lim and Kim 2006) or by binding to M_2 muscarinic receptors (Yang *et al*. 2000). However, anti-ChE activity could also explain the cognitive improvements observed with arecoline. To investigate this, an extract and fractions from *A. catechu* were assessed for AChE inhibitory activity *in vitro* and were found to be active, however, when some of the known constituents, including arecoline, arecaidine and catechin, were tested for anti-AChE activity they were inactive (Gilani *et al*. 2004). In contrast, a more recent study shows that arecoline competitively inhibits AChE *in vitro* and in the nervous tissue of the mollusc *Lymnaea acuminata*, suggesting that plants containing arecoline may be useful molluscicides (Jaiswal *et al*. 2008).

MONOTERPENOIDS, SESQUITERPENOIDS AND OTHER ESSENTIAL OIL COMPONENTS AS INHIBITORS OF ACHE

Sage (*Salvia* species (Lamiaceae)) is documented in 16th and 17th century English herbals, to improve the memory (Perry *et al*. 2001). In the 16th century English herbal by Gerard, sage is described to be 'Singularly good for the head and brain and quickenethe the nerves and memory' and Culpeper wrote of sage 50 years later that 'It also heals the memory, warming and quickening the senses', and in the 18th century, Hill suggests that 'Sage will retard that rapid progress of decay that treads upon our heels so fast in latter years of life, will preserve faculty and memory more valuable to the rational mind than life itself' (Perry *et al*. 2001). Studies to investigate if the reputed cognitive effects of sage in traditional medicine could be explained by AChE inhibitory activity have focused on extracts and essential oils from *S. officinalis* L. and *S. lavandulifolia* Vahl. The steam-distilled oils from both of these species and an ethanol extract from *S. officinalis* inhibit AChE *in vitro* (Perry *et al*. 1996) and studies *in vivo* show positive effects of *Salvia* species on cholinergic function and cognition (Perry *et al*. 2002; Eidi *et al*. 2006). When administered orally to rats, *S. lavandulifolia* oil decreased AChE activity in the striatum and the hippocampus, when compared with control rats, suggesting the oil constituents or their metabolites could cross the blood-brain barrier (Perry *et al*. 2002). The main compounds responsible for the anti-AChE activity of the *S. lavandulifolia* oil are considered to be 1,8-cineole and α-pinene (**Fig. 6**), as these cyclic monoterpenoids inhibited

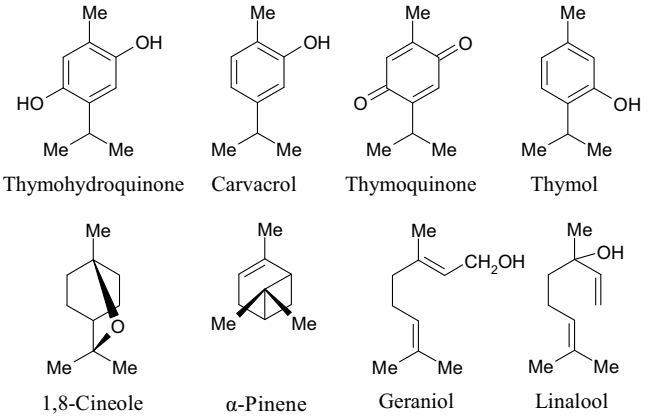


Fig. 6 Monoterpenoids that inhibit AChE.

AChE *in vitro*, although other oil constituents may also have contributed, perhaps synergistically (Perry *et al*. 2000; Savelev *et al*. 2003). Although some interesting activities in relation to AChE and cognition have been observed with *Salvia* species and the essential oil constituents, the monoterpenoids are considerably less potent AChE inhibitors (by a factor of at least 10^3) than the alkaloid inhibitors such as physostigmine (Perry *et al*. 2000).

Studies on the anti-ChE effects of *Narcissus* species (Amaryllidaceae) have focused on the active alkaloids, particularly galantamine and related compounds (López *et al*. 2002; Ingkaninan *et al*. 2003; Elgorashi *et al*. 2004). However, the absolute obtained from *N. poeticus* L. has also been explored and it selectively inhibits BChE more potently than AChE and some of the constituents have been reported as ChE inhibitors (Okello *et al*. 2008). The components of this absolute included the monoterpenoids geranial, neral, linalool and cineole, which reversibly and competitively inhibit electric eel AChE *in vitro* (Ryan and Byrne 1988; Picollo *et al*. 2008), but these were only minor components of the absolute (detected at ≤ 0.4%). Geraniol (detected in the absolute at 0.1%) and linalool (**Fig. 6**) inhibit human erythrocyte AChE, although these monoterpenoids were not as potent as 1,8-cineole (Perry *et al*. 2000). Other known inhibitors of AChE that were minor components in this absolute (detected at ≤ 0.2%) were cymene (Miyazawa and Yamafuji 2006) and the phenylpropanoid eugenol (Picollo *et al*. 2008; Kumar *et al*. 2009). Since these known inhibitors of AChE were only minor components in the *N. poeticus* absolute they may not have contributed significantly to the observed anti-ChE activities, although a synergistic action may provide some explanation. Some studies have investigated potential synergism between essential oil components for AChE inhibition. In one study the sesquiterpenoid caryophyllene oxide was a relatively weak AChE inhibitor alone, but a synergistic inhibition of AChE was observed when it was combined with 1,8-cineole (Savelev *et al*. 2003). The three main compounds identified in the *N. poeticus* absolute were benzyl benzoate, benzyl alcohol and phenylethyl alcohol but the latter two compounds only showed moderate inhibition of AChE (Okello *et al*. 2008) and an earlier study showed benzyl alcohol to inhibit AChE only at very high concentrations (> 150 mM) (Tanaka *et al*. 1984).

Tea tree (*Melaleuca* species (Myrtaceae)) oil is used as an alternative treatment to conventional medicines for head lice (*Pediculus humanus capitis*) infestation and studies have investigated the insecticidal mode of action of this oil. It was found that two of the main monoterpenoid constituents of tea tee oil, terpinen-4-ol and 1,8-cineole, can inhibit AChE *in vitro* (IC_{50} 10.3 and 0.04 mM, respectively) which might explain the insecticidal action (Mills *et al*. 2004). However, although 1,8-cineole was the most effective AChE inhibitor of twenty monoterpenoids investigated, this activity was not correlated with head lice intoxication

suggesting that a different mode of action might explain the pediculicidal activity of monoterpnoids such as 1,8-cineole (Picollo *et al*. 2008). Another insecticidal monoterpenoid that inhibits AChE is pulegone-1,2-epoxide from *Lippia stoechadifolia* (L.) Kunth (Verbenaceae) (Howes *et al*. 2003).

Structure-activity relationships for anti-ChE monoterpenoids have been investigated and studies show some bicyclic monoterpenoids with a pinane skeleton (hydrocarbon compounds such as (-)-α-pinene) to be more potent AChE inhibitors than alcohol or ketone monoterpenoids such as (-)-verbenone and (-)-myrtenol, and for bicyclic monoterpenoids with a carane or pinane skeleton, the position of the C = C double bond was associated with the potency of AChE inhibition (e.g. (+)-3-carene was more active than (+)-2-carene) (Miyazawa and Yamafuji 2005). These *in vitro* studies also revealed that (+)-α-pinene, (-)-α-pinene, (+)-2-carene and (+)-3-carene are uncompetitive AChE inhibitors and (-)-β-pinene, (+)-fenchol, (+)-fenchone and (-)-fenchone are competitive AChE inhibitors (Miyazawa and Yamafuji 2005).

Leontopodium alpinum Cass. (Asteraceae) is used traditionally in alpine folk medicine for various conditions. A root extract from this plant increased extracellular ACh in rat brain and inhibited AChE *in vitro* (Hornick *et al*. 2008). Some sesquiterpenoids isolated from the root also increased extracellular ACh in rat brain, but only silphiperfolene acetate showed weak AChE inhibitory activity *in vitro* (Hornick *et al*. 2008). Sesquiterpenoids of the agarofuran and dihydroagarofuran types from aerial parts of *Maytenus disticha* Urb. and seeds of *M. boaria* Molina (Celastraceae) are also inhibitors of AChE (Céspedes *et al*. 2001; Alarcón *et al*. 2008). Several compounds from the marine organism *Cladiella* have also been tested to identify those responsible for AChE inhibition, but only the sesquiterpenoid cladidiol was a moderate AChE inhibitor (Ata *et al*. 2004).

Acorus calamus L. (Acoraceae) root has been used in Ayurvedic medicine for various indications including memory disorders and is also reported to improve cognition in some *in vivo* studies (Mukherjee *et al*. 2007a). The traditional use of this plant may be explained by an AChE inhibitory action, since hydroalcoholic extracts and the essential oil from this plant inhibited AChE *in vitro*, with the latter being the more potent (Mukherjee *et al*. 2007b). Two of the active AChE inhibitory components from the essential oil were identified as α- and β-asarone (Mukherjee *et al*. 2007b). Other essential oils that inhibit AChE include those from *Melissa officinalis* L. (Houghton *et al*. 2007), *Mentha suaveolens* Ehrh., *Lavandula angustifolia* Mill., *L. pedunculata* (Mill.) Cav. (Lamiaceae) (Ferreira *et al*. 2006), *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) (Siramon *et al*. 2008), *Cymbopogon schoenanthus* Spreng. (Poaceae) (Khadri *et al*. 2008) and *Thymus vulgaris* L. (Lamiaceae) (Loizzo *et al*. 2008). The AChE inhibitory potency of the oil and constituents of *T. vulgaris* is thymohydroquinone > carvacrol > thymoquinone > essential oil > thymol > linalool (monoterpenoid chemical structures are shown in **Fig. 6**) (Loizzo *et al*. 2008). The essential oil from *Angelica sinensis* (Oliv.) Diels (Apiaceae) contains (*Z*)-ligustilide, which is associated with some interesting effects *in vivo*, as it prevents hypoperfused cognitive deficits that may be mediated by cholinergic effects, since it increased ChAT activity and inhibited AChE activity in ischemic brain tissues (Kuang *et al*. 2008).

OTHER TERPENOIDS AND STEROIDAL DERIVATIVES AS INHIBITORS OF ACHE

Diterpenoids

A number of diterpenoids that occur in members of the Lamiaceae have shown AChE inhibitory activities. The first diterpenoids to show AChE inhibitory activity were isolated from an anti-AChE extract from *Salvia miltiorhiza* Bunge root (Ren *et al*. 2004; Houghton *et al*. 2007). *S. miltiorhiza*

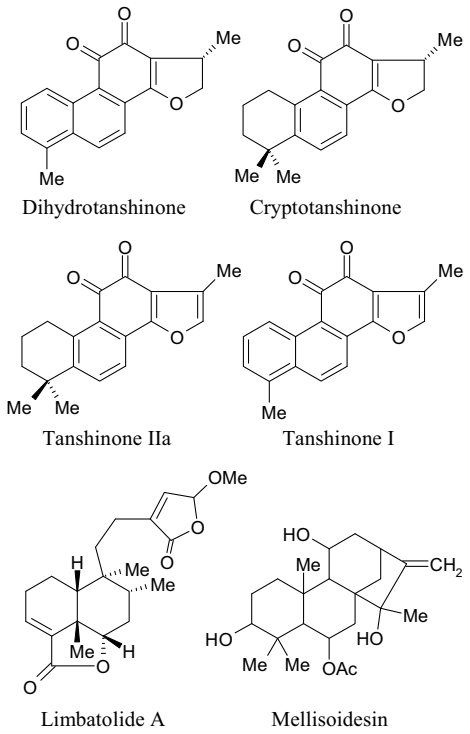


Fig. 7 Diterpenoids that inhibit AChE.

(also known as Chinese sage or 'dan shen') has been used in TCM to stabilise the heart and calm the nerves and to treat circulatory disorders, insomnia and neurasthenia (Tang and Eisenbrand 1992; Huang 1993); the Pharmacopoeia of the People's Republic of China (2005) includes the root as a remedy for fidgets and insomnia, amongst other indications (Chinese Pharmacopoeia Commission 2005). Of the four AChE inhibitory diterpenoids from this plant, the most active were dihydrotanshinone and cryptotanshinone (**Fig. 7**) (Ren *et al*. 2004). These dihydrofurans were more active than the furans, tanshinones I and IIa (**Fig. 7**), indicating that the more flexible dihydrofuran improves the binding affinity to the active site of the enzyme (Ren *et al*. 2004). There is a lack of pharmacokinetic studies regarding these compounds, but tanshinone IIa has poor bioavailability (Hao *et al*. 2006) and penetration of cryptotanshinone across the blood-brain barrier may be limited *in vivo* (Yu *et al*. 2007). However, oral administration of tanshinones I and IIa, cryptotanshinone and 15,16-dihydrotanshinone could reverse scopolamine-induced cognitive impairments in mice, and both cryptotanshinone and 15,16-dihydrotanshinone inhibited AChE activity for 3 – 6 h in an *ex vivo* study (Kim *et al*. 2007a), suggesting that the poor pharmacokinetic profile suggested for these compounds may not limit efficacy *in vivo*. More extensive studies on the pharmacokinetics in relation to efficacy and toxicity for these diterpenoids are needed to assess their therapeutic potential.

Three *cis*-clerodane diterpenoids, limbatolides A (**Fig. 7**), B and C, isolated from the roots of *Otostegia limbata* (Benth.) Boiss. concentration-dependently inhibit both AChE and BChE, with the strongest inhibitor of AChE being limbatolide A (IC_{50} 38.5 μM), possibly due to the lactone ring at position C-4/C-6, which is absent in limbatolides B and C (IC_{50} 47.2 and 103.7 μM, respectively), and substitution of the methoxy group at C-15 with an H-atom could also reduce activity, as the least potent of these diterpenoids was limbatolide C (Ahmad *et al*. 2005). Melissoidesin (**Fig. 7**) is an *ent*-kaurane diterpenoid from the leaves of *Isodon wightii* (Benth.) H.Hara and it inhibits AChE *in vitro*, but not as potently as physostigmine (IC_{50} 215 and 143 μg mL^{-1}, respectively) (Thirugnanasampandan *et al*. 2008). Other diterpenoids from members of the Lamiaceae include four obtained from the whole plant of *Ajuga bracteosa* Wall. ex Benth. that inhibit AChE (IC_{50} 14.0–

35.2 μM) and BChE (IC_{50} 10.0–19.0 μM) in a concentration-dependent manner (Riaz *et al*. 2007) and (16*S*)-coleon E, which inhibits AChE by 61% at 1 mg mL^{-1} and may partly explain the anti-AChE activity of *Plectranthus barbatus* Andrews leaf extract (Falé *et al*. 2009).

The fruit of *Detarium microcarpum* Harms (Leguminosae) is used in African traditional medicine for a variety of disorders and for magical treatments. Some clerodane diterpenoids (3,4-epoxyclerodan-(13*E*)-en-15-oic acid, 5α, 8α-(2-oxokolavenic acid), 3,4-dihydroxyclerodan-(13*Z*)-en-15-oic acid and 2-oxokolavenic acid) from the fruit inhibit AChE (Cavin *et al*. 2006). The most active of these diterpenoids was 5α,8α-(2-oxokolavenic acid) but it was still 10-fold less potent than galantamine (Cavin *et al*. 2006). From the leaves of *Hypoestes serpens* R.Br. (Acanthaceae) two isoprimarane diterpenoids showed AChE inhibitory activity in a TLC bioautographic assay, but quantities required for inhibition were 50- and 20-fold higher for 7β-hydroxyisopimara-8,15-dien-14-one and 14α-hydroxyisopimara-7,15-dien-1-one respectively, than for galantamine (Rasoamiaranjanahary *et al*. 2003).

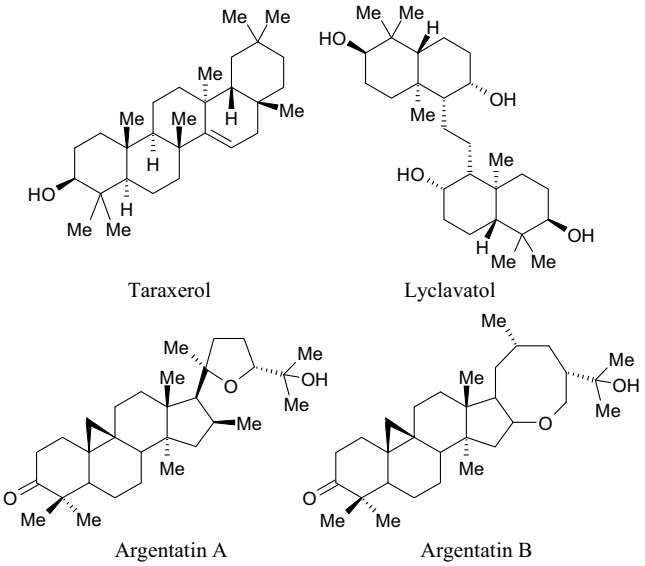


Fig. 8 Triterpenoids that inhibit AChE.

Triterpenoids and steroidal derivatives

Some studies on *Polygala tenuifolia* Willd. (Polygalaceae) root extract may explain the use of this plant in TCM for CNS effects (Howes and Houghton 2003). Root extracts reversed scopolamine-induced cognitive impairment and to some extent, improved memory and behavioural disorders induced by CNS lesions in rodents, they showed a neuroprotective action against glutamate and amyloid precursor protein and dose-dependently inhibited AChE activity *in vitro* (Park *et al*. 2002; Chen *et al*. 2004; Lee *et al*. 2004a), indicating a cholinergic effect in addition to other modes of action that may be relevant to alleviate cognitive disorders. Tenuifolin, a triterpenoid glycoside from the roots of this plant, improved learning and memory in aged mice, an effect associated with decreasing AChE in the hippocampus of treated mice (Zhang *et al*. 2008). Thus, tenuifolin may explain the reputed and observed biological activities of *P. tenuifolia*, but other compounds that may be responsible for enhancing cognition require investigation.

The Indian medicinal plant *Clitoria ternatea* L. (Leguminosae) has a reputation for promoting intellect (Warrier *et al*. 1995; Misra 1998) and some pharmacological studies offer some explanation for the traditional uses. Alcoholic extracts from both aerial parts and roots attenuated memory deficits in rats, with the latter being the most active (Taranalli and Cheeramkuzhy 2000). Although the cognitive effects of extracts were not directly correlated with AChE inhibition (Taranalli and Cheeramkuzhy 2000), a triterpenoid from *C. ternatea*, taraxerol (**Fig. 8**), inhibits AChE both *in vitro* and in the brain of rodents *in vivo*, but was not as potent as physostigmine (Lee *et al*. 2004b; Kumar *et al*. 2007).

Most of the anti-ChE compounds from *Lycopodium* species (Lycopodiaceae) are alkaloids (Halldorsdottir and Olafsdottir 2006; Choo *et al*. 2007; Hirasawa *et al*. 2008). However, one study identified the triterpenoid α-onocerin from *L. clavatum* L. as a moderately potent inhibitor of AChE (Orhan *et al*. 2003), although a subsequent study did not show this compound to inhibit AChE (Rollinger *et al*. 2005). *Ononis spinosa* L. (Leguminosae) is also a source of α-onocerin, but the anti-AChE triterpenoid isolated from this plant was identified as the tetracyclic lyclavatol (**Fig. 8**), although AChE inhibition was weak (Rollinger *et al*. 2005). Weak AChE inhibitory activity was also observed with the triterpenoid cycloart-23-ene-3,25-diol, from the leaves of *Trichilia dregeana* Harv. & Sond. (Meliaceae) (Eldeen *et al*. 2007). A more promising group of triterpenoids are some jujubogenin glycosides that have been used as templates to design semi-synthetic derivatives that inhibit AChE, which are described in a patent for this purpose (Tumiatti *et al*. 2008). Other triterpenoids reported to inhibit AChE include ursolic acid, found in many species including *Origanum majorana* L. (Lamiaceae), and argentatins A and B (**Fig. 8**) from *Parthenium argentatum* A.Gray (Asteraceae) (Houghton *et al*. 2006).

The use of the Ayurvedic remedy *Withania somnifera* (L.) Dunal (Solanaceae) root, also known as 'ashwagandha' in Sanskrit, dates back almost 4000 years and it is classed among the 'Rasayanas', the rejuvenative tonics (Howes and Houghton 2003; Howes *et al*. 2003). Steroidal derivatives isolated from the root display a number of interesting activities relating to cognitive function (Ghosal *et al*. 1989; Bhattacharya *et al*. 1995). These include the sitoindosides IX and X which augment learning acquisition and memory in young and old rats (Ghosal *et al*. 1989), an action associated with modulation of cholinergic function. When administered to rodents, the sitoindosides VII–X and withaferin A both enhanced and decreased AChE activity in different brain regions, in addition to increasing muscarinic receptor binding (Schliebs *et al*. 1997). AChE inhibition was shown *in vitro* with root extracts (Houghton *et al*. 2007; Vinutha *et al*. 2007) and some withanolides inhibit AChE and BChE; withaferin A, 2,3-dihydrowithaferin A and 5β,6β-epoxy-4β-hydroxy-1-oxowitha-2,14,24-trienolide were more active against AChE activity (Choudhary *et al*. 2004 and 2005c).

Haloxylon recurvum Bunge ex Boiss. (Chenopodiaceae) has been used traditionally in Pakistan for a variety of neural disorders and several alkylated sterols extracted from the whole plant concentration-dependently inhibit AChE and BChE (Ahmed *et al*. 2006). Of these sterols, the most potent inhibitors of AChE were haloxysterols B and C (IC_{50} 0.9 and 1.0 μM, respectively) but were not quite as active as galantamine (IC_{50} 0.5 μM) (Ahmed *et al*. 2006). Cynatroside B was the most potent AChE inhibitor (dose-dependent, reversible and non-competitive inhibition) *in vitro* of several pregnane glycosides obtained from roots of *Cynanchum atratum* Bunge (Asclepiadaceae), which may explain why this compound could ameliorate memory impairment *in vivo* (Lee *et al*. 2005).

MEROTERPENOIDS AND MYCOTOXINS AS INHIBITORS OF ACHE

Arisugacins A and B (**Fig. 9**) from *Penicillium* species are selective inhibitors of AChE, but not BChE and are 200-fold more potent inhbitors of AChE than tacrine *in vitro* (Ōmura *et al*. 1995; Otoguro *et al*. 1997). Binding of arisugacin A to AChE is suggested to be due to electron-donating and electron-withdrawing interactions, in particular, electron density from the dimethoxy group coupled with the electron-withdrawing 2-pyrone ring, a moiety considered crucial for anti-AChE potency (McGlacken and Fairlamb 2005). Terri-

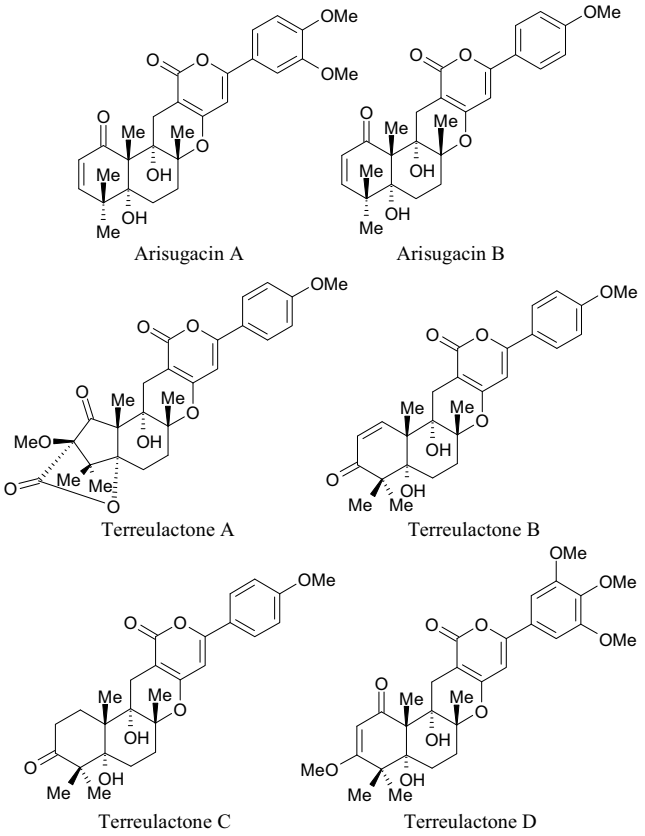


Fig. 9 Meroterpenoids that inhibit AChE.

trems B and C from *Penicillium* species also inhibit AChE and are structurally related to the arisugacins, each consisting of naphtha[2,1-*b*]pyrano[3,4-*e*]pyran and benzene (Shiomi *et al*. 1999). Isoterreulactone A, which is biogenetically related to the arisugacin meroterpenoids, and terreulactones A–D (**Fig. 9**) are meroterpenoids from *Aspergillus terreus* and they dose-dependently and selectively inhibit AChE *in vitro* (Yoo *et al*. 2005). The meroterpenoids colletochlorin B, colletorin B, ilicicolins C, E, and F, as well as the phytotoxin α, β-dehydrocurvularin, from liquid cultures of the phytopathogenic fungus *Nectria galligena* (Hypocreaceae), moderately inhibit AChE, with the most active being colletochlorin B and ilicicolins C and E (Gutiérrez *et al*. 2005). Although demonstrating some promising AChE inhibitory effects, none of these fungal metabolites appear to have been assessed or pursued for clinical use.

Aflatoxin B_1 is a mycotoxin produced by *Aspergillus flavus* which non-competitively and dose-dependently inhibits mouse brain AChE, the G_1 and G_4 molecular isoforms in particular (Cometa *et al*. 2005). However, this toxin is associated with carcinogenic, mutagenic, hepatotoxic and cholinergic effects, the latter possibly related to the anti-ChE activity (Cometa *et al*. 2005), and is therefore not a potential therapeutic compound without structural modifications to reduce the risk of toxic effects.

SHIKIMATE-DERIVED COMPOUNDS AS INHIBITORS OF ACHE

In flowering plants the shikimic acid pathway is a biosynthetic route for the production of compounds that are usually phenolic and derived from one or more phenylpropanoid units, which may be combined with compounds produced from other metabolic pathways (Houghton *et al*. 2006). Shikimic acid derived compounds include some flavonoids, coumarins and tannins.

Flavonoids, xanthones and anthraquinone derivatives

Compared to alkaloids, a relatively low number of flavonoids have been reported to inhibit AChE. Flavonoids from *Buddleja davidii* Franch. (Buddlejaceae) leaves have been identified as AChE inhibitors and the structure-activity relationships of these and other flavonoids were investtigated. Of 17 flavonoids tested for AChE inhibition, only linarin and tilianin (**Fig. 10**) were active and it was concluded that important structural features of flavonoids for AChE inhibition are the presence of a 4'-*O*Me group and a 7-*O*-sugar, and the length of the interglycosidic links of the sugar chain (Fan *et al*. 2008). A separate study confirmed that linarin (acacetin-7-*O*-β-D-rutinoside) from the flower extract of *Mentha arvensis* L. (Lamiaceae) selectively and dose-dependently inhibits AChE (Oinonen *et al*. 2006). Although none of the flavonoid aglycones tested, including the flavanone naringenin (**Fig. 10**), inhibited AChE in the study by Fan *et al*. (2008), a separate study showed naringenin (from *Citrus junos* Siebold ex Tanaka (Rutaceae)) to dose-dependently inhibit AChE *in vitro*, which might explain why this flavanone could ameliorate scopolamine-induced amnesia in rodents (Heo *et al*. 2004). Quercetin (**Fig. 10**), quercitrin, 3-methoxy quercetin and tiliroside, flavonols from *Agrimonia pilosa* Ledeb. (Rosaceae), inhibit AChE but were not as active as tacrine and berberine; however, tiliroside and quercetin were almost two-fold more active than the alkaloid dehydroevodiamine in this study (Jung and Park 2007). The flavone luteolin (**Fig. 10**) also moderately inhibits AChE (IC_{50} > 0.1 mM) (Choi *et al*. 2008). Scutellarein 4'-methyl ether 7-*O*-glucuronide inhibits AChE by 50.5% at 1 mg mL^{-1} and was considered to contribute to the anti-AChE activity of *Plectranthus barbatus* Andrews (Lamiaceae) leaf extract (Falé *et al*. 2009). Licorice is the root of *Glycyrrhiza* species (Leguminosae) and it is widely used in TCM and reported to enhance memory (Cui *et al*. 2008). The isoflavan glabridin from the roots antagonised scopolamine-induced amnesia in mice, an effect that could be explained by it reducing brain ChE activity (Cui *et al*. 2008).

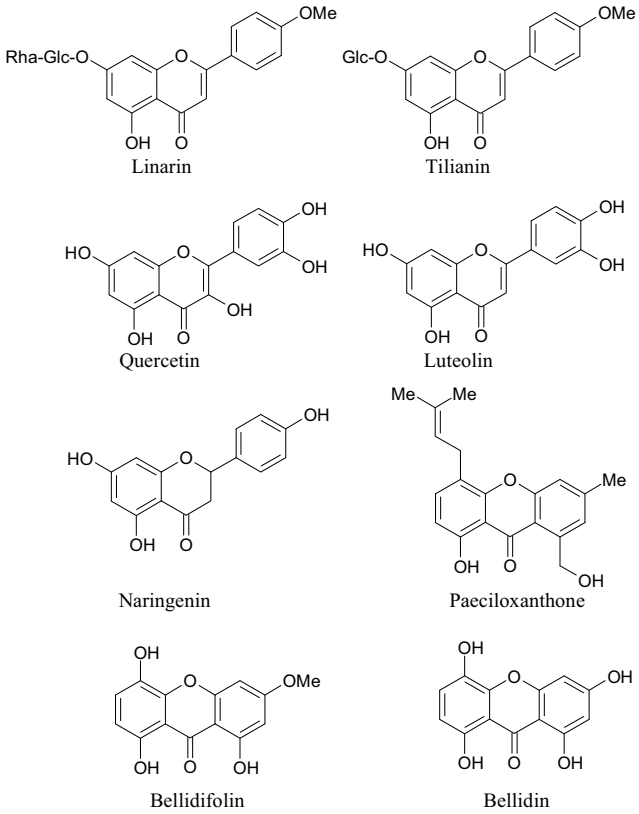


Fig. 10 Flavonoids and xanthones that inhibit AChE.

Some phytoestrogens are associated with cognitive improvements in both animal and clinical studies, and are suggested to protect against AD (Pan *et al*. 2000; File *et al*. 2001; Kim *et al*. 2001), although the mode(s) of action to explain these observations have not been elucidated. However, it is interesting to consider that a diet of soy isoflavones for 16 weeks in aged male rats produced AChE inhibition in the cortex, basal forebrain and hippocampus (Lee *et al*. 2004c), suggesting that anti-ChE activity might be one mechanism to explain why some isoflavones show cognitive benefits.

In general, of the various flavonoids that have been assessed for AChE inhibitory effects, none appear to have stimulated a particular interest for clinical development. However, there are some xanthones that show more promising ChE inhibitory effects that could be subjected to more extensive tests to assess any relevance for clinical use. *Gentiana campestris* L. (Gentianaceae) leaf extract and some of the component xanthones, bellidin, bellidifolin (**Fig. 10**), bellidin 8-*O*-β-glucopyranoside (norswertianolin), and bellidifolin 8-*O*-β-glucopyranoside (swertianolin), inhibit AChE and interestingly, bellidifolin showed similar inhibitory potency to galantamine (Urbain *et al*. 2004). For these xanthones, inhibitory activity was enhanced when a glucopyranosyl was absent and a methoxy group was present in position C-3 (Urbain *et al*. 2004). When 45 non-alkaloidal natural compounds were screened for AChE inhibitory activity six of the seven active compounds were xanthones. The most potent of these had an additional cyclic component and a hydrophobic side-chain which was considered important for inhibitory activity as the other active xanthones lacked these structural components (Brühlmann *et al*. 2004). *Garcinia* species (Clusiaceae) are a source of isoprenylated xanthones and some of the xanthones from the wood of *G. polyantha* Oliv. inhibit BChE (1,3,5-trihydroxyxanthone, 1,5-dihydroxyxanthone, 1,6-dihydroxy-5-methoxyxanthone; IC_{50} 93.0, 2.5, 74.4 μM, respectively) and polyanxanthone B inhibits both BChE (IC_{50} 25.5 μM) and AChE (IC_{50} 46.3 μM), but none of these particular xanthones were as active as galantamine (Louh *et al*. 2008). A xanthone, paeciloxanthone (**Fig. 10**), isolated from the marine fungus *Paecilomyces* sp. also inhibits AChE *in vitro* (IC_{50} 2.25 μg mL^{-1}) (Wen *et al*. 2008).

Anthrone derivatives from *Psorospermum glaberrimum* Hochr. (Clusiaceae) stem bark have been assessed for their ability to inhibit AChE and BChE, but showed more potent inhibition of BChE compared to AChE, with the most potent AChE inhibitor identified as bianthrone 1a (IC_{50} 63.0 μM), which was 126-fold less potent than galantamine (Ndjakou Lenta *et al*. 2008). The anthraquinone derivative aloe-emodin is also a relatively weak inhibitor of AChE (57 % inhibition at 1 mg mL^{-1}) (Orhan *et al*. 2008a).

Coumarins and furanocoumarins

Bergapten, scopoletin (**Fig. 11**), 4-methylumbelliferone and a furanocoumarin mixture from *Heracleum crenatifolium* Boiss. (Apiaceae) inhibited AChE (> 50% at 1 mg mL^{-1}), while the latter three, in addition to umbelliferone and 8-methoxypsoralen, also inhibited BChE (> 50% at 1 mg mL^{-1}) (Orhan *et al*. 2008a). Using pharmacophore modelling in a virtual screen study, the coumarins scopoletin and its glucoside scopolin (**Fig. 11**) were identified as potential anti-ChE compounds so were isolated from *Scopolia carniolica* Jacq. (Solanaceae) rhizomes to investigate the predicted effects on AChE. Both coumarins dose-dependently inhibited AChE only moderately, but significantly they increased extracellular ACh concentrations in rat brain when tested *in vivo* (Rollinger *et al*. 2004). In another study scopoletin, obtained from the twigs of *Vaccinium oldhami* Miq. (Ericaceae), was shown to inhibit AChE dose-dependently and non-competitively *in vitro* (Lee *et al*. 2004b). The AChE inhibitory potency of simple coumarins such as umbelliferone and scopoletin is considered to be moderate to low, and substitutions in position C-7 of the coumarin nucleus by

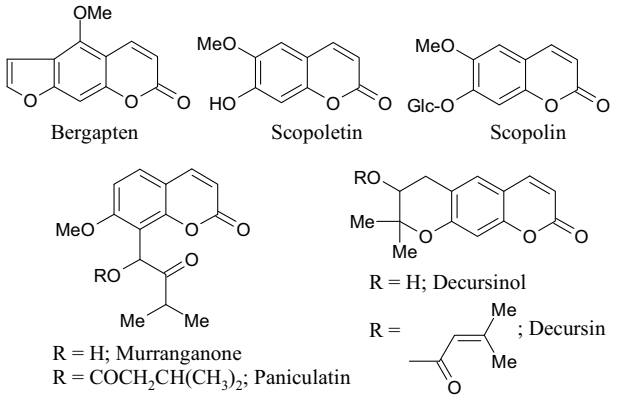


Fig. 11 Coumarins that inhibit AChE.

small groups (e.g. hydroxyl and methoxy) reduce AChE inhibition compared to larger substituents (e.g. benzyloxy) (Fallarero *et al*. 2008).

The coumarins murranganone and paniculatin (**Fig. 11**), isolated from *Murraya paniculata* (L.) Jack (Rutaceae) leaves, show moderate AChE and BChE inhibitory activity, with paniculatin showing some selectivity for AChE; neither compound was as potent as physostigmine (Choudhary *et al*. 2002). Twelve coumarins isolated from roots of *Angelica gigas* Nakai (Apiaceae), a plant used in traditional Korean medicine and reputed to have anti-amnesic effects, showed inhibitory activity against AChE, with the most potent of these being the dihydropyranocoumarin decursinol (**Fig. 11**) and the furanocoumarins isoimperatorin, nodakenin, marmesin and xanthotoxin (IC_{50} 2.8 × 10^{-5}, 6.9 × 10^{-5}, 6.8 × 10^{-5}, 6.7 × 10^{-5} and 5.4 × 10^{-5} M, respectively) (Kang *et al*. 2001). Both decursin (**Fig. 11**) and nodakenin reversed scopolamine-induced cognitive impairments in mice, an effect attributed to their ability to inhibit AChE (Kang *et al*. 2003; Kim *et al*. 2007b). Other naturally derived coumarins that inhibit AChE include marmesin and columbianetin (Tumiatti *et al*. 2008).

Psoralen and isopsoralen, furanocoumarins from *Psoralea* species (Leguminosae), alleviate scopolamine-induced amnesia in rats which was attributed to their AChE inhibitory effects (Wu *et al*. 2007), although the toxicity of these compounds may limit any therapeutic potential for cognitive disorders. The furanocoumarin, feronielloside, and three coumarin glycosides from *Feroniella lucida* Swingle. (Rutaceae) roots inhibited AChE *in vitro*, but this study did not compare the inhibitory activities of these coumarins with any positive controls such as physostigmine (Phoopichayanun *et al*. 2008). In general, the AChE inhibitory potency of furanocoumarins such as bergapten is considered to be moderate to low, but potency may be increased by substitution with large groups at positions 5 and 8 of the furanocoumarin nucleus (Fallarero *et al*. 2008).

As several coumarins have shown inhibitory activity against AChE, it is logical to explore the AChE inhibitory potential of synthetic coumarin derivatives, to optimise inhibitory potency. Ensaculin is a coumarin analogue that not only inhibits AChE but also modulates dopaminergic, serotonergic and adrenergic neurotransmitter systems in addition to being an *N*-methyl-D-aspartate (NMDA) receptor antagonist (Teismann and Ferger 2000). Consequently, ensaculin (as the HCl salt: KA-672) has been explored for clinical use in AD and has been used as a template for the development of a series of coumarin analogues including those with various substitutions of a phenylpiperazine moiety, which are inhibitors of AChE (Shen *et al*. 2005; Zhou *et al*. 2008). Although the synthetic coumarin derivative AP2243 showed promising anti-AChE activity *in vitro* (IC_{50} 0.018 μM), attempts to modify the structure and improve AChE inhibitory activity by synthesising amidic nonpeptidic derivatives yielded compounds that inhibited AChE less potently but were more potent inhibitors of β-

secretase, also a potential therapeutic target in AD (Piazzi *et al*. 2008). Another coumarin derivative, 3-chloro-7-hydroxy-4-methylcoumarin, also inhibits both AChE and BChE *in vitro* (Simeon-Rudolf *et al*. 1999).

OTHER PHENOLIC COMPOUNDS AS INHIBITORS OF ACHE

In traditional European medicine *Melissa officinalis* L. (Lamiaceae) has been used as a remedy for over 2000 years, and it is reputed to treat melancholia, neuroses and hysteria and the plant was acclaimed for promoting long life and restoring memory (McVicar 1994; Kenner and Requena 1996; Wichtl 2004). John Hill (1751) reported that *M. officinalis* was 'Good for disorders of the head and stomach' (Crellin and Philpott 1990). In other traditional practices of medicine *M. officinalis* is reputed to treat depression (Arabic medicine) (McVicar, 1994) and hysteria (Greek medicine) (Malamas and Marselos 1992). The possible modes of action, including ChE inhibition, to explain both reputed and clinically observed effects of *M. officinalis* on cognitive function have been investigated. Both an essential oil and an ethanolic extract from *M. officinalis* weakly inhibit AChE (Ferreira *et al*. 2006) but another study did not show an AChE inhibitory effect with aqueous and methanolic extracts of *M. officinalis* (Adsersen *et al*. 2006). Although chemical variation in *M. officinalis* plants from different sources may explain differences in AChE inhibitory activities, it could also be suggested that the active compounds may be concentrated in less polar extracts or the essential oil. To investigate this, an ethanolic extract of the leaves was fractionated and nine of twelve fractions inhibited AChE, with the majority being more potent than the original crude extract (Dastmalchi *et al*. 2009). Compounds that were tentatively identified in the most potent fraction included rosmarinic acid (**Fig. 12**) and two of its derivatives (Dastmalchi *et al*. 2009). A study using NMR spectroscopy suggested that rosmarinic acid, and also salvianolic acid, could bind to AChE (Yin *et al*. 2008). The ChE inhibitory activity of rosmarinic acid, also found in other members of the Lamiaceae such as *Rosmarinus officinalis* L., has been confirmed in other studies. Rosmarinic acid inhibited AChE and BChE (47 and 86% inhibition, respectively) but only at high concentrations (1 mg mL^{-1}) (Orhan *et al*. 2008b). Other studies also show rosmarinic acid (IC_{50} > 0.1 mM and 0.44 mg mL^{-1} in separate studies) and extracts from *Plectranthus* species (Lamiaceae) that contain rosmarinic acid, to moderately inhibit AChE (Choi *et al*. 2008; Falé *et al*. 2009). Ferulic acid (**Fig. 12**) is a competitive inhibitor of AChE and this action is one mechanism considered to explain the molluscicidal effect of plants containing ferulic acid (Kumar *et al*. 2009). Tacrine-ferulic acid hybrids have been synthesised, with the aim of exploiting the anti-AChE effect of tacrine with the antioxidant properties of ferulic acid, rather than its AChE inhibitory properties, to develop novel compounds for potential use in dementia, and some of these hybrids were more potent inhibitors of AChE than tacrine and were also antioxidant (Fang *et al*. 2008).

Of several polyphenolic compounds from *Hopea hainanensis* Merr. & Chun (Dipterocarpaceae) stem bark, hopeahainol A showed comparable AChE inhibitory activity to huperzine A (Ge *et al*. 2008). Other polyphenolic compounds are vitisin A and γ-viniferin, extracted from *Vitis vinifera* L. (Vitaceae), which have been suggested to be useful in the prevention and treatment of some diseases, as these compounds could inhibit AChE *in vitro* at relatively low concentrations (IC_{50} 7.5 and 15.6 μM, respectively) (Tumiatti *et al*. 2008). Polyphenols from green tea (*Camellia* species (Theaceae)) also inhibit AChE (Kim *et al*. 2004b; Chung *et al*. 2005) and when green tea extract was administered to aged rats it improved learning and memory and decreased AChE activity in the cerebrum (Kaur *et al*. 2008). A polyphenol-rich extract from blueberries (*Vaccinium angustifolium* Benth. (Ericaceae)) improved learning and memory in rodents administered (i.p.) this extract, an effect that was associated with both anti-oxidant and anti-AChE effects (Papandreou *et al*. 2009).

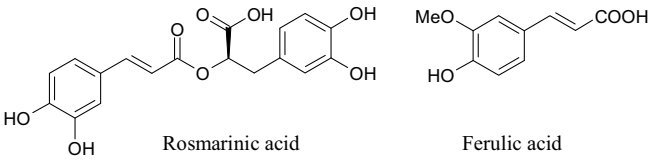


Fig. 12 Phenolic compounds that inhibit AChE.

CONCLUSION

Numerous species particularly from the plant kingdom have been used in various traditional practices of medicine to alleviate symptoms associated with cholinergic dysfunction, including some cognitive disorders such as memory impairment, and have been used for their medicinal properties for a long time, and some of these continue to be used for these and other purposes. Some of these species have been subjected to scientific studies to establish any pharmacological basis for their historical uses, and for a number of these, their ability to inhibit ChE has been proposed to explain their uses in traditional medicine. One example is a TCM remedy prepared from *Huperzia serrata* which is reputed to alleviate memory loss (Howes and Houghton 2003). It was discovered that some alkaloids, huperzines A and B, isolated from *H. serrata* can inhibit AChE which might explain the traditional use of this moss, and since huperzine A in particular has been extensively studied and showed therapeutic potential for use in cognitive disorders, it was developed as a pharmaceutical in China to provide symptomatic treatment of AD (Ma *et al*. 2007). There are also many examples of plants that have been used traditionally for other purposes, including other medicinal uses not considered to be related to cholinergic function, and as poisons. Many of these plant species have also been investigated for their biological activities and some have been shown to inhibit ChE. For example, *Galanthus* species and *Physostigma venenosum* were not used traditionally for cognitive disorders, yet the alkaloid galantamine from *Galanthus* species is one of the most potent and well-characterised inhibitors of AChE, and physostigmine from *P. venenosum* showed interesting anti-AChE activity and modification of its chemical structure enabled the development of a more therapeutically promising compound, rivastigmine. Both galantamine and rivastigmine are now in clinical use to alleviate cognitive symptoms in dementia.

The extent to which species that show AChE inhibitory activity have been investigated varies considerably. For some species, some of the active constituents have been isolated and characterised but for many plants the compounds responsible for any observed inhibitory effects on AChE remain to be elucidated. The majority of naturally derived inhibitors of AChE are alkaloids and most investigations to identify new AChE inhibitors with therapeutic potential have focused on this structural class of compounds. There have been numerous attempts to chemically modify alkaloid structures to optimise AChE inhibitory potency and reduce toxicity, and in some cases compounds have been synthesised to achieve a dual mode of action that combines anti-AChE activity with another action that may be relevant to alleviate a particular cognitive disorder.

In recent years there has been an interest in developing new AChE inhibitors from natural origin for both therapeutic and insecticidal purposes and consequently a wide range of plants and some marine organisms have been investigated and a diverse array of structural types of compounds have been described as inhibitors of AChE. There are potentially many other natural sources of AChE inhibitors that have yet to be explored, but advances in the understanding of the pathology of diseases and their treatments will influ-

ence how useful AChE inhibitors will be for therapeutic use in the future.

REFERENCES

Adsersen A, Gauguin B, Gudiksen L, Jager AK (2006) Screening of plants used in Danish folk medicine to treat memory dysfunction for acetylcholinesterase inhibitory activity. *Journal of Ethnopharmacology* **104**, 418-422

Agid Y, Dubois B, Anand R, Gharabawi G (1998) Efficacy and tolerability of rivastigmine in patients with dementia of the Alzheimer type. *Current Therapeutic Research – Clinical and Experimental* **59**, 837-845

Ahmad VU, Khan A, Farooq U, Kousar F, Khan SS, Nawaz SA, Abbasi MA, Choudhary MI (2005) Three new cholinesterase-inhibiting *cis*-clerodane diterpenoids from *Otostegia limbata. Chemcal and Pharmaceutical Bulletin* **53**, 378-381

Ahmed E, Nawaz SA, Malik A, Choudhary MI (2006) Isolation and cholinesterase-inhibition studies of sterols from *Haloxylon recurvum. Bioorganic and Medicinal Chemistry Letters* **16**, 573-580

Alarcón J, Astudillo L, Gutierrez M (2008) Inhibition of acetylcholinesterase activity by dihydro-β-agarofuran sesquiterpenes isolated from Chilean Celastraceae. *Zeitschrift für Naturforschung C* **63**, 853-856

Alcalá MDM, Vivas NM, Hospital S, Camps P, Muñoz-Torrero D, Badia A (2003) Characterisation of the anticholinesterase activity of two new tacrine-huperzine A hybrids. *Neuropharmacology* **44**, 749-755

Andrade MT, Lima JA, Pinto AC, Rezende CM, Carvalho, MP, Epifanio RA (2005) Indole alkaloids from *Tabernaemontana australis* (Müell. Arg) Miers that inhibit acetylcholinesterase enzyme. *Bioorganic and Medicinal Chemistry* **13**, 4092-4095

Ashani Y, Peggins JO, Doctor BP (1992) Mechansim of inhibition of cholinesterases by huperzine A. *Biochemical and Biophysical Research Communications* **184**, 719-726

Ata A, Ackerman J, Bayoud A, Radhika P (2004) Bioactive chemical constituents of *Cladiella* species. *Helvetica Chimica Acta* **87**, 592-597

Atta-ur-Rahman, Feroz F, Naeem I, Zaheer-ul-Haq, Nawaz SA, Khan N, Khan MR, Choudhary MI (2004) New pregnane-type steroidal alkaloids from *Sarcococca saligna* and their cholinesterase inhibitory activity. *Steroids* **69**, 735-741

Atta-ur-Rahman, Akhtar MN, Choudhary MI, Tsuda Y, Sener B, Khalid A, Parvez M (2002) New steroidal alkaloids from *Fritillaria imperialis* and their cholinesterase inhibiting activities. *Chemical and Pharmaceutical Bulletin* **50**, 1013-1016

Atta-ur-Rahman, Parveen S, Khalid A, Farooq A, Choudhary MI (2001) Acetyl and butyrylcholinesterase-inhibiting triterpenoid alkaloids from *Buxus papillosa. Phytochemistry* **58**, 963-968

Atta-ur-Rahman, Fatima N, Akhtar F, Choudhary MI, Khalid A (2000) New norditerpenoid alkaloids from *Aconitum falconeri. Journal of Natural Products* **63**, 1393-1395

Babar ZU, Ata A, Meshkatalsadat MH (2006) New bioactive steroidal alkaloids from *Buxus hyrcana*. *Steroids* **71**, 1045-1051

Badia A, Banos JE, Camps P, Contreras J, Gorbig DM, Munoz-Torrero D, Simon M, Vivas NM (1998) Synthesis and evaluation of tacrine-huperzine A hybrids as acetylcholinesterase inhibitors of potential interest for the treatment of Alzheimer's disease. *Bioorganic and Medicinal Chemistry* **6**, 427-440

Barten DM, Albright CF (2008) Therapeutic strategies for Alzheimer's disease. *Molecular Neurobiology* **37**, 171-186

Berkov S, Codina C, Viladomat F, Bastida J (2007) Alkaloids from *Galanthus nivalis*. *Phytochemistry* **68**, 1791-1798

Berman SB, Greenamyre JT (2006) Update on Huntington's disease. *Current Neurology and Neuroscience Reports* **6**, 281-286

Bhattacharya SK, Kumar A, Ghosal S (1995) Effects of glycowithanolides from *Withania somnifera* on an animal model of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. *Phytotherapy Research* **9**, 110-113

Bianchi DA, Hirschmann GS, Theoduloz C, Bracca ABJ, Kaufman TS (2005) Synthesis of tricyclic analogs of stephaoxocanidine and their evaluation as acetylcholinesterase inhibitors. *Bioorganic and Medicinal Chemistry Letters* **15**, 2711-2715

Bickel U, Thomsen T, Weber W, Fischer JP, Bachus R, Nitz M, Kewitz H (1991) Pharmacokinetics of galanthamine in humans and corresponding cholinesterase inhibition. *Clinical Pharmacology and Therapeutics* **50**, 420-428

Bourne RS, Tahir TA, Borthwick M, Sampson EL (2008) Drug treatment of delirium: Past, present and future. *Journal of Psychosomatic Research* **65**, 273-282

Braida D, Sala M (2001) Eptastigmine: Ten years of pharmacology, toxicology, pharmacokinetic, and clinical studies. *CNS Drug Reviews* **7**, 369-386

Brimijoin S (1983) Molecular forms of acetylcholinesterase in brain, nerve and muscle: Nature, localisation and dynamics. *Progress in Neurobiology* **21**, 291-322

Brühlmann C, Marston A, Hostesttmann K, Carrupt PA, Testa B (2004) Screening of non-alkaloidal natural compounds as acetylcholinesterase inhibitors. *Chemistry and Biodiversity* **1**, 819-829

Butler MS (2008) Natural products to drugs: Natural product derived compounds in clinical trials. *Natural Product Reports* **25**, 475-516

Camps P, Cusack B, Mallender WD, El Achab R, Morral J, Muñoz-Torrero D, Rosenberry TL (2000) Huprine X is a novel high-affinity inhibitor of acetylcholinesterase that is of interest for treatment of Alzheimer's disease. *Molecular Pharmacology* **57**, 409-417

Cardoso CL, Castro-Gamboa I, Siqueira Silva DH, Furlan M, Epifanio RDA, Da Cunha Pinto Â, De Rezende CM, Lima JA, Da Silva Bolzani V (2004) Indole glucoalkaloids from *Chimarrhis turbinata* and their evaluation as antioxidant agents and acetylcholinesterase inhibitors. *Journal of Natural Products* **67**, 1882-1885

Carey GJ, Costall B, Domeney AM, Gerrard PA, Jones DNC, Naylor RJ, Tyers MB (1992) Ondansetron and arecoline prevent scopolamine-induced cognitive deficits in the marmoset. *Pharmacology Biochemistry and Behavior* **42**, 75-83

Carlier PR, Du DM, Han YF, Liu J, Pang YP (1999) Potent, easily synthesized huperzine A-tacrine hybrid acetylcholinesterase inhibitors. *Bioorganic and Medicinal Chemistry Letters* **9**, 2335-2338

Castro A, Martinez A (2006) Targeting β-amyloid pathogenesis through acetylcholinesterase inhibitors. *Current Pharmaceutical Design* **12**, 4377-4387

Castro NG, Costa RS, Pimentel LS, Danuello A, Romeiro NC, Viegas C, Barreiro EJ, Fraga CAM, Bolzani VS, Rocha MS (2008) CNS-selective noncompetitive cholinesterase inhibitors derived from the natural piperidine alkaloid (-)-spectaline. *European Journal of Pharmacology* **580**, 339-349

Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Recanatini M, Melchiorre C (2008) Multi-target-directed ligands to combat neurodegenerative diseases. *Journal of Medicinal Chemistry* **51**, 347-372

Cavin A-L, Hay A-E, Marston A, Stoeckli-Evans H, Scopelliti R, Diallo D, Hostettmann K (2006) Bioactive diterpenes from the fruits of *Detarium microcarpum*. *Journal of Natural Products* **69**, 768-773

Céspedes CL, Alarcón J, Aranda E, Becerra J, Silva M (2001) Insect growth regulator and insecticidal activity of β-dihydroagarofurans from *Maytenus* spp. (Celastraceae). *Zeitschrift für Naturforschung C* **56**, 603-613

Chattipakorn S, Pongpanparadorn A, Pratchayasakul W, Pongchaidacha A, Ingkaninan K, Chattipakorn N (2007) *Tabernaemontana divaricata* extract inhibits neuronal acetylcholinesterase activity in rats. *Journal of Ethnopharmacology* **110**, 61-68

Chen YL, Hsieh CL, Wu PHB, Lin JG (2004) Effect of *Polygala tenuifolia* root on behavioral disorders by lesioning nucleus basalis magnocellularis in rat. *Journal of Ethnopharmacology* **95**, 47-55

Chinese Pharmacopoeia Commission (2005) *Pharmacopoeia of the People's Republic of China*, People's Medical Publishing House, China, pp 93, 213-214, 271-272

Cho KM, Yoo ID, Kim WG (2006) 8-Hydroxydihydrochelerythrine and 8-hydroxydihydrosanguinarine with a potent acetylcholinesterase inhibitory activity from *Chelidonium majus* L. *Biological and Pharmaceutical Bulletin* **29**, 2317-2320

Choi S-H, Hur J-M, Yang E-J, Jun, M, Park H-J, Lee K-B, Moon E, Song K-S (2008) β-secretase (BACE1) inhibitors from *Perilla frutescens* var. *acuta. Archives of Pharmacal Research* **31**, 183-187

Choo CY, Hirasawa Y, Karimata C, Koyama K, Sekiguchi M, Kobayashi J, Morita H (2007) Carinatumins A-C, new alkaloids from *Lycopodium carinatum* inhibiting acetylcholinesterase. *Bioorganic and Medicinal Chemistry* **15**, 1703-1707

Choudhary MI, Nawaz SA, Zaheer-ul-Haq, Azim MK, Ghayur MN, Lodhi MA, Jalil S, Khalid A, Ahmed A, Rode BM, Atta-ur-Rahman, Gilani AU, Ahmad VU (2005a) Juliflorine: A potent natural peripheral anionic-site-binding inhibitor of acetylcholinesterase with calcium-channel blocking potential, a leading candidate for Alzheimer's disease therapy. *Biochemical and Biophysical Research Communications* **332**, 1171-1179

Choudhary MI, Devkota KP, Nawaz SA, Ranjit R, Atta-ur-Rahman (2005b) Cholinesterase inhibitory pregnane-type steroidal alkaloids from *Sarcococca hookeriana*. *Steroids* **70**, 295-303

Choudhary MI, Nawaz SA, Zaheer-ul-Haq, Lodhi MA, Ghayur MN, Jalil S, Riaz N, Yousuf S, Malik A, Gilani AH, Atta-ur-Rahman (2005c) Withanolides, a new class of natural cholinesterase inhibitors with calcium antagonistic properties. *Biochemical and Biophysical Research Communications* **334**, 276-287

Choudhary MI, Yousuf S, Nawaz SA, Ahmed S, Atta-ur-Rahman (2004) Cholinesterase inhibiting withanolides from *Withania somnifera*. *Chemical and Pharmaceutical Bulletin* **52**, 1358-1361

Choudhary MI, Azizuddin, Khalid A, Sultani SZ, Atta-ur-Rahman (2002) A new coumarin from *Murraya paniculata*. *Planta Medica* **68**, 81-83

Chung JH, Kim M, Kim HK (2005) Green tea polyphenols suppress nitric oxide-induced apoptosis and acetylcholinesterase activity in human neuroblastoma cells. *Nutrition Research* **25**, 477-483

Claassen JA, Jansen RW (2006) Cholinergically mediated augmentation of cerebral perfusion in Alzheimer's disease and related cognitive disorders: The cholinergic-vascular hypothesis. *Journal of Gerontology. A. Biological Sciences and Medical Sciences* **61A**, 267-271

Cometa MF, Lorenzini P, Fortuna S, Volpe MT, Meneguz A, Palmery M (2005) *In vitro* inhibitory effect of aflatoxin B_1 on acetylcholinesterase activity in mouse brain. *Toxicology* **206**, 125-135

Crellin JK, Philpott J (1990) *Herbal Medicine Past and Present, A Reference*

Guide to Medicinal Plants (Vol 2), Duke University Press, Durham, USA

Cui Y-M, Ao M-Z, Li W, Yu L-J (2008) Effect of glabridin from *Glycyrrhiza glabra* on learning and memory in mice. *Planta Medica* **74**, 377-380

Cutler NR, Veroff AE, Anand R, Hartman R, Mancione L (1999) Correlation between cognitive effects and level of acetylcholinesterase inhibition in a trial of rivastigmine in Alzheimer patients. *Neurology* **52**, A173

Dastmalchi K, Ollilainen V, Lackman P, Gennäs GB, Dorman HJD, Järvinen PP, Yli-Kauhaluoma J, Hiltunen R (2009) Acetylcholinesterase inhibitory guided fractionation of *Melissa officinalis* L. *Bioorganic and Medicinal Chemistry* **17**, 867-871

Decker M (2005) Novel inhibitors of acetyl- and butyrylcholinesterase derived from the alkaloids dehydroevodiamine and rutaecarpine. *European Journal of Medicinal Chemistry* **40**, 305-313

Denholm I, Pickett JA, Devonshire AL (Eds) (1999) *Insecticide Resistance: From Mechanisms to Management*, CABI Publishing, Oxon, UK, pp 9-17

Desgranges B, Baron J-C, de la Sayette V, Petit-Taboué M-C, Benali K, Landeau B, Lechevalier B, Eustache F (1998) The neural substrates of memory systems impairment in Alzheimer's disease. *Brain* **121**, 611-631

Devkota KP, Lenta BN, Fokou PA, Sewald N (2008) Terpenoid alkaloids of the Buxaceae family with potential biological importance. *Natural Product Reports* **25**, 612-630

Devkota KP, Lenta BN, Choudhary MI, Naz Q, Fekam FB, Rosenthal PJ, Sewald N (2007) Cholinesterase inhibiting and antiplasmodial steroidal alkaloids from *Sarcococca hookeriana*. *Chemical and Pharmaceutical Bulletin* **55**, 1397-1401

Dodel R, Csoti I, Ebersbach G, Fuchs G, Hahne M, Kuhn W, Oechsner M, Jost W, Reichmann H, Schulz JB (2008) Lewy body dementia and Parkinson's disease with dementia. *Journal of Neurology* **255**, 39-47

Edwards K, Royall D, Hershey L, Lichter D, Hake A, Farlow M, Pasquier F, Johnson S (2007) Efficacy and safety of galantamine in patients with dementia with Lewy bodies: A 24-week open-label study. *Dementia and Geriatric Cognitive Disorders* **23**, 401-405

Eidi M, Eidi A, Bahar M (2006) Effects of *Salvia officinalis* L. (sage) leaves on memory retention and its interaction with the cholinergic system in rats. *Nutrition* **22**, 321-326

Eldeen IMS, Van Heerden FR, Van Staden J (2007) Biological activities of cycloart-23-ene-3,25-diol isolated from the leaves of *Trichilia dregeana*. *South African Journal of Botany* **73**, 366-371

Elgorashi EE, Stafford GI, van Staden J (2004) Acetylcholinesterase enzyme inhibitory effects of Amaryllidaceae alkaloids. *Planta Medica* **70**, 260-262

Eskander MF, Nagykery NG, Leung EY, Khelghati B, Geula C (2005) Rivastigmine is a potent inhibitor of acetyl- and butyrylcholinesterase in Alzheimer's plaques and tangles. *Brain Research* **1060**, 144-152

Falé PL, Borges C, Madeira PJA, Ascensão L, Araújo MEM, Florêncio MH, Serralheiro MLM (2009) Rosmarinic acid, scutellarein 4′-methyl ether 7-*O*-glucuronide and (16*S*)-coleon E are the main compounds responsible for the antiacetylcholinesterase and antioxidant activity in herbal tea of *Plectranthus barbatus* ("falso boldo"). *Food Chemistry* **114**, 798-805

Fallarero A, Oinonen P, Gupta S, Blom P, Galkin A, Mohan CG, Vuorela PM (2008) Inhibition of acetylcholinesterase by coumarins: The case of coumarin 106. *Pharmacological Research* **58**, 215-221

Fan P, Hay A-E, Marston A, Hostettmann K (2008) Acetylcholinesterase-inhibitory activity of linarin from *Buddleja davidii*, structure-activity relationships of related flavonoids, and chemical investigation of *Buddleja nitida*. *Pharmaceutical Biology* **46**, 596-601

Fang L, Kraus B, Lehmann J, Heilmann J, Zhang Y, Decker M (2008) Design and synthesis of tacrine-ferulic acid hybrids as multi-potent anti-Alzheimer drug candidates. *Bioorganic and Medicinal Chemistry Letters* **18**, 2905-2909

Ferheen S, Ahmed E, Afza N, Malik A, Shah MR, Nawaz SA, Choudhary MI (2005) Haloxylines A and B, antifungal and cholinesterase inhibiting piperidine alkaloids from *Haloxylon salicornicum*. *Chemical and Pharmaceutical Bulletin* **53**, 570-572

Ferreira A, Proença C, Serralheiro MLM, Araújo MEM (2006) The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *Journal of Ethnopharmacology* **108**, 31-37

File SE, Jarrett N, Fluck E, Duffy R, Casey K, Wiseman H (2001) Eating soya improves human memory. *Psychopharmacology* **157**, 430-436

Fink DM, Palermo MG, Bores GM, Huger FP, Kurys BE, Merriman MC, Olsen GE, Petko W, O'Malley GJ (1996) Imino 1,2,3,4-tetrahydrocyclopent[*b*]indole carbamates as dual inhibitors of acetylcholinesterase and monoamine oxidase. *Bioorganic and Medicinal Chemistry Letters* **6**, 625-630

Förstl H, Hentschel F, Sattel H, Geiger-Kabisch C, Besthorn C, Czech C, Mönning U, Beyreuther K (1995) Age-associated memory impairment and early Alzheimer' disease. *Arzneimittelforschung* **45**, 394-397

Fulton B, Benfield P (1996) Galantamine. *Drugs Aging* **1**, 60-65

Ge HM, Zhu CH, Shi DH, Zhang LD, Xie DQ, Yang J, Ng SW, Tan RX (2008) Hopeahainol A: An acetylcholinesterase inhibitor from *Hopea hainanensis*. *Chemistry* **14**, 376-381

Ghosal S, Lal J, Srivastava R, Bhattacharya SK, Upadhyay SN, Jaiswal AK, Chattopadhyay U (1989) Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycowithanolides from *Withania somnifera*. *Phytotherapy Research* **3**, 201-206

Giacobini E (2004) Cholinesterase inhibitors: New roles and therapeutic alternatives. *Pharmacological Research* **50**, 433-440

Giacobini E (1990) The cholinergic system in Alzheimer disease. *Progress in Brain Research* **84**, 321-332

Gilani AH, Khalid A, Zaheer-ul-Haq, Choudhary MI, Atta-ur-Rahman (2005) Presence of antispasmodic, antidiarrheal, antisecretory, calcium antagonist and acetylcholinesterase inhibitory steroidal alkaloids in *Sarcococca saligna*. *Planta Medica* **71**, 120-125

Gilani AH, Ghayur MN, Saify ZS, Ahmed SP, Choudhary MI, Khalid A (2004) Presence of cholinomimetic and acetylcholinesterase inhibitory constituents in betel nut. *Life Sciences* **75**, 2377-2389

Greenblatt HM, Kryger G, Lewis T, Silman I, Sussman JL (1999) Structure of acetylcholinesterase complexed with (-)-galanthamine at 2.3 angstrom resolution. *FEBS Letters* **463**, 321-326

Greig NH, Utsuki T, Yu QS, Zhu XX, Holloway HW, Perry T, Lee B, Ingram DK, Lahiri DK (2001) A new therapeutic target in Alzheimer's disease treatment: Attention to butyrylcholinesterase. *Current Medical Research and Opinion* **17**, 159-165

Grossberg G, Desai A (2001) Review of rivastigmine and its clinical applications in Alzheimer's disease and related disorders. *Expert Opinion on Pharmacotherapy* **2**, 653-666

Grossman H, Bergmann C, Parker S (2006) Dementia: A brief review. *Mount Sinai Journal of Medicine* **73**, 985-992

Gutiérrez M, Theoduloz C, Rodriguez J, Lolas M, Schmeda-Hirschmann G (2005) Bioactive metabolites from the fungus *Nectria galligena*, the main apple canker agent in Chile. *Journal of Agricultural and Food Chemistry* **53**, 7701-7708

Halldorsdottir ES, Olafsdottir ES (2006) Alkaloids from the club moss *Lycopodium annotinum* L. – Acetylcholinesterase inhibitory activity *in vitro*. *Planta Medica* **72**, 961

Hao HP, Wang GJ, Cui N, Li J, Xie L, Ding ZQ (2006) Pharmacokinetics, absorption and tissue distribution of tanshinone IIA solid dispersion. *Planta Medica* **72**, 1311-1317

Hassall KA (1990) *The Biochemistry and Uses of Pesticides* (2nd Edn), MacMillan Press Ltd., London, pp 83-154

He X-C, Feng S, Wang Z-F, Shi Y, Zheng S, Xia Y, Jiang H, Tang X-C, Bai D (2007) Study on dual-site inhibitors of acetylcholinesterase: Highly potent derivatives of bis- and bifunctional huperzine B. *Bioorganic and Medicinal Chemistry* **15**, 1394-1408

Heinrich M, Teoh HL (2004) Galanthamine from snowdrop – The development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *Journal of Ethnopharmacology* **92**, 147-162

Heo HJ, Kim MJ, Lee JM, Choi SJ, Cho HY, Hong BS, Kim HK, Kim E, Shin DH (2004) Naringenin from *Citrus junos* has an inhibitory effect on acetylcholinesterase and a mitigating effect on amnesia. *Dementia and Geriatric Cognitive Disorders* **17**, 151-157

Hirasawa Y, Kato E, Kobayashi J, Kawahara N, Goda Y, Shiro M, Morita H (2008) Lycoparins A-C, new alkaloids from *Lycopodium casuarinoides* inhibiting acetylcholinesterase. *Bioorganic and Medicinal Chemistry* **16**, 6167-6171

Hirasawa Y, Kobayashi J, Morita H (2006) Lycoperine A, a novel CN-type pentacyclic alkaloid from *Lycopodium hamiltonii*, inhibiting acetylcholinesterase. *Organic Letters* **8**, 123-126

Högenauer K, Baumann K, Enz A, Mulzer J (2001) Synthesis and acetylcholinesterase inhibition of 5-desamino huperzine A derivatives. *Bioorganic and Medicinal Chemistry Letters* **11**, 2627-2630

Hornick A, Schwaiger S, Rollinger JM, Vo NP, Prast H, Stuppner H (2008) Extracts and constituents of *Leontopodium alpinum* enhance cholinergic transmission: Brain ACh increasing and memory improving properties. *Biochemical Pharmacology* **76**, 236-248

Houghton PJ, Howes M-JR (2005) Natural products and derivatives affecting neurotransmission relevant to Alzheimer's and Parkinson's disease. *Neurosignals* **14**, 6-22

Houghton PJ, Howes M-JR, Lee CC, Steventon G (2007) Uses and abuses of *in vitro* tests in ethnopharmacology: Visualizing an elephant. *Journal of Ethnopharmacology* **110**, 391-400

Houghton PJ, Ren YH, Howes MJ (2006) Acetylcholinesterase inhibitors from plants and fungi. *Natural Product Reports* **23**, 181-199

Houghton PJ, Agbedahunsi JM, Adegbulugbe A (2004) Choline esterase inhibitory properties of alkaloids from two Nigerian *Crinum* species. *Phytochemistry* **65**, 2893-2896

Howes M-JR, Houghton PJ (2003) Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. *Pharmacology Biochemistry and Behavior* **75**, 513-527

Howes M-JR, Perry NSL, Houghton PJ (2003) Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytotherapy Research* **17**, 1-18

Huang KC (1993) *The Pharmacology of Chinese Herbs*, CRC Press Ltd., Boca Raton, USA

Hung TM, Na M, Dat NT, Ngoc TM, Youn U, Kim HJ, Min B-S, Lee J, Bae K (2008a) Cholinesterase inhibitory and anti-amnesic activity of alkaloids from *Corydalis turtschaninovii*. *Journal of Ethnopharmacology* **119**, 74-80

Hung TM, Ngoc TM, Youn UJ, Min BS, Na M, Thuong PT, Bae K (2008b)

Anti-amnesic activity of pseudocoptisine from *Corydalis* tuber. *Biological and Pharmaceutical Bulletin* **31**, 159-162

Inestrosa NC, Dinamarca MC, Alvarez A (2008) Amyloid-cholinesterase interactions – Implications for Alzheimer's disease. *FEBS Journal* **275**, 625-632

Ingkaninan K, Changwijit K, Suwanborirux K (2006a) Vobasinyl-iboga bisindole alkaloids, potent acetylcholinesterase inhibitors from *Tabernaemontana divaricata* root. *Journal of Pharmacy and Pharmacology* **58**, 847-852

Ingkaninan K, Phengpa P, Yuenyongsawad S, Khorana N (2006b) Acetylcholinesterase inhibitors from *Stephania venosa* tuber. *Journal of Pharmacy and Pharmacology* **58**, 695-700

Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W (2003) Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *Journal of Ethnopharmacology* **89**, 261-264

Ingkaninan K, Hazekamp A, de Best CM, Irth H, Tjaden UR, van der Heijden R, van der Greef R, Verpoorte R (2000) The application of HPLC with on-line coupled UV/MS-biochemical detection for isolation of an acetylcholinesterase inhibitor from *Narcissus* 'Sir Winston Churchill'. *Journal of Natural Products* **63**, 803-806

Jaiswal P, Singh VK, Singh DK (2008) Enzyme inhibition by molluscicidal component of *Areca catechu* and *Carica papaya* in the nervous tissue of vector snail *Lymnaea acuminata*. *Pesticide Biochemistry and Physiology* **92**, 164-168

Jett DA (2008) Cholinesterase research at the National Institutes of Health, USA. *Chemico-Biological Interactions* **175**, 22-25

Jiang H, Zhang XJ (2008) Acetylcholinesterase and apoptosis – A novel perspective for an old enzyme. *FEBS Journal* **275**, 612-617

Johnson G, Swart C, Moore SW (2008) Non-enzymatic functions of acetylcholinesterase – The question of redundancy. *FEBS Journal* **275**, 5129-5138

Joshi H, Parle M (2006) Antiamnesic effects of *Desmodium gangeticum* in mice. *Yakugaku Zasshi* **126**, 795-804

Julian PL, Pikl J (1935) Studies in the indole series. V. The complete synthesis of physostigmine. *Journal of the American Chemical Society* **51**, 755-757

Jung M, Park M (2007) Acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*. *Molecules* **12**, 2130-2139

Kadir A, Andreasen N, Almkvist O, Wall A, Forsberg A, Engler H, Hagman G, Lärksäter M, Winblad B, Zetterberg H, Blennow K, Långström B, Nordberg A (2008) Effect of phenserine treatment on brain functional activity and amyloid in Alzheimer's disease. *Annals of Neurology* **65**, 621-631

Kalauni SK, Choudhary MI, Khalid A, Manandhar MD, Shaheen F, Atta-ur-Rahman, Gewali MB (2002) New cholinesterase inhibiting steroidal alkaloids from the leaves of *Sarcococca coriacea* of Nepalese origin. *Chemical and Pharmaceutical Bulletin* **50**, 1423-1426

Kamal MA, Al-Jafari AA, Yu QS, Greig NH (2006a) Kinetic analysis of the inhibition of human butyrylcholinesterase with cymserine. *Biochimica et Biophysica Acta* **1760**, 200-206

Kamal MA, Klein P, Yu QS, Tweedie D, Li YZ, Holloway HW, Greig NH (2006b) Kinetics of human serum butyrylcholinesterase and its inhibition by a novel experimental Alzheimer therapeutic, bisnorcymserine. *Journal of Alzheimer's Disease* **10**, 43-51

Kamal MA, Greig NH, Alhomida AS, Al-Jafari AA (2000) Kinetics of human acetylcholinesterase inhibition by the novel experimental Alzheimer therapeutic agent, tolserine. *Biochemical Pharmacology* **60**, 561-570

Kaneko S, Shikano M, Katoh T, Terashima S (1997) Synthesis of (+/-)-12-fluorohuperzine A, a novel acetylcholinesterase inhibitor. *Synlett* **SI**, 447

Kaneko S, Nakajima N, Shikano M, Katoh T, Terashima S (1996) Synthesis and acetylcholinesterase inhibitory activity of fluorinated analogues of huperzine A. *Bioorganic and Medicinal Chemistry Letters* **6**, 1927-1930

Kang SY, Lee KY, Park MJ, Kim YC, Markelonis GJ, Oh TH, Kim YC (2003) Decursin from *Angelica gigas* mitigates amnesia induced by scopolamine in mice. *Neurobiology of Learning and Memory* **79**, 11-18

Kang SY, Lee KY, Sung SH, Park MJ, Kim YC (2001) Coumarins isolated from *Angelica gigas* inhibit acetylcholinesterase: Structure-activity relationships. *Journal of Natural Products* **64**, 683-685

Kaur T, Pathak CM, Pandhi P, Khanduja KL (2008) Effects of green tea extract on learning, memory, behavior and acetylcholinesterase activity in young and old male rats. *Brain and Cognition* **67**, 25-30

Kenner D, Requena Y (1996) *Botanical Medicine: A European Professional Perspective*, Paradigm Publications, Brookline, USA

Khadri A, Serralheiro MLM, Nogueira JMF, Neffati M, Smiti S, Araújo MEM (2008) Antioxidant and antiacetylcholinesterase activities of essential oils from *Cymbopogon schoenanthus* L. Spreng. Determination of chemical composition by GC-mass spectrometry and ^{13}C NMR. *Food Chemistry* **109**, 630-637

Khalid A, Azim MK, Parveen S, Atta-ur-Rahman, Choudhary MI (2005) Structural basis of acetylcholinesterase inhibition by triterpenoidal alkaloids. *Biochemical and Biophysical Research Communications* **331**, 1528-1532

Khalid A, Zaheer-ul-Haq, Anjum S, Khan MR, Atta-ur-Rahman, Choudhary MI (2004a) Kinetics and structure-activity relationship studies on pregnane-type steroidal alkaloids that inhibit cholinesterases. *Bioorganic and Medicinal Chemistry* **12**, 1995-2003

Khalid A, Zaheer-ul-Haq, Ghayur MN, Feroz F, Atta-ur-Rahman, Gilani AH, Choudhary MI (2004b) Cholinesterase inhibitory and spasmolytic potential of steroidal alkaloids. *Journal of Steroid Biochemistry and Molecular Biology* **92**, 477-484

Kim DK (2002) Inhibitory effect of corynoline isolated from the aerial parts of *Corydalis incisa* on the acetylcholinesterase. *Archives of Pharmacal Research* **25**, 817-819

Kim DH, Jeon SJ, Jung JW, Lee S, Yoon BH, Shin BY, Son KH, Cheong JH, Kim YS, Kang SS, Ko KH, Ryu JH (2007a) Tanshinone congeners improve memory impairments induced by scopolamine on passive avoidance tasks in mice. *European Journal of Pharmacology* **574**, 140-147

Kim DH, Kim DY, Kim YC, Jung JW, Lee S, Yoon BH, Cheong JH, Kim YS, Kang SS, Ko KH, Ryu JH (2007b) Nodakenin, a coumarin compound, ameliorates scopolamine-induced memory disruption in mice. *Life Sciences* **80**, 1944-1950

Kim DK, Lee KT, Baek N-I, Kim S-H, Park HW, Lim JP, Shin TY, Eom DO, Yang JH, Eun JS (2004a) Acetylcholinesterase inhibitors from the aerial parts of *Corydalis speciosa*. *Archives of Pharmacal Research* **27**, 1127-1131

Kim HK, Kim M, Kim S, Kim M, Chung JH (2004b) Effects of green tea polyphenol on cognitive and acetylcholinesterase activities. *Bioscience Biotechnology and Biochemistry* **68**, 1977-1979

Kim H, Xia H, Li L, Gewin J (2001) Actions of dietary soya vs. Premarin in mammalian brain. 21st ACS National Meeting, San Diego, US. *Biochemical Society Transactions* **29**, 216-222

Koyama K, Hirasawa Y, Kobayashi J, Morita H (2007) Cryptadines A and B, novel $C_{27}N_3$-type pentacyclic alkaloids from *Lycopodium cryptomerinum*. *Bioorganic and Medicinal Chemistry* **15**, 7803-7808

Kuang X, Du JR, Liu YX, Zhang GY, Peng HY (2008) Postischemic administration of *Z*-ligustilide ameliorates cognitive dysfunction and brain damage induced by permanent forebrain ischemia in rats. *Pharmacology Biochemistry and Behavior* **88**, 213-221

Kumar P, Singh VK, Singh DK (2009) Kinetics of enzyme inhibition by active molluscicidal agents ferulic acid, umbelliferone, eugenol and limonene in the nervous tissue of snail *Lymnaea acuminata*. *Phytotherapy Research* **23**, 172-177

Kumar V, Mukherjee K, Pal BC, Houghton PJ, Mukherjee PK (2007) Acetylcholinesterase inhibitor from *Clitoria ternatea*. *Planta Medica* **73**, 10.1055

Kumar V, Sugaya K, Messina J, Veach J (1999) Efficacy and safety of rivastigmine (Exelon) in Alzheimer's disease patients with vascular risk factors. *Neurology* **52**, A395-A396

Kuznetsova LP, Nikol'skaya EB, Sochilina EE, Faddeeva MD (2002) Inhibition of human blood acetylcholinesterase and butyrylcholinesterase by some alkaloids. *Journal of Evolutionary Biochemistry and Physiology* **38**, 35-39

Labraña J, Machocho AK, Kricsfalusy V, Brun R, Codina C, Viladomat F, Bastida J (2002) Alkaloids from *Narcissus angustifolius* subsp. *transcarpathicus* (Amaryllidaceae). *Phytochemistry* **60**, 847-852

Laganière S, Corey J, Tang X-C, Wülfert E, Hanin I (1991) Acute and chronic studies with the anticholinesterase huperzine A: Effect on central nervous system cholinergic parameters. *Neuropharmacology* **30**, 763-768

Langjae R, Bussarawit S, Yuenyongsawad S, Ingkaninan K, Plubrukarn A (2007) Acetylcholinesterase-inhibiting steroidal alkaloid from the sponge *Corticium* sp. *Steroids* **72**, 682-685

Lee KY, Yoon JS, Kim ES, Kang SY, Kim YC (2005) Anti-acetylcholinesterase and anti-amnesic activities of a pregnane glycoside, cynatroside B, from *Cynanchum atratum*. *Planta Medica* **71**, 7-11

Lee HJ, Ban JY, Koh SB, Seong NS, Song KS, Bae KW, Seong YH (2004a) Polygalae radix extract protects cultured rat granule cells against damage induced by NMDA. *American Journal of Chinese Medicine* **32**, 599-610

Lee JH, Lee KT, Yang JH, Baek NI, Kim DK (2004b) Acetylcholinesterase inhibitors from the twigs of *Vaccinium oldhami* Miquel. *Archives of Pharmacal Research* **27**, 53-56

Lee YB, Lee HJ, Won MH, Hwang IK, Kang TC, Lee JY, Nam SY, Kim KS, Kim E, Cheon SH, Sohn HS (2004c) Soy isoflavones improve spatial delayed matching-to-place performance and reduce cholinergic neuron loss in elderly male rats. *Journal of Nutrition* **134**, 1827-1831

Lim D-Y, Kim I-S (2006) Arecoline inhibits catecholamine release from perfused rat adrenal gland. *Acta Pharmacologica Sinica* **27**, 71-79

Lin BQ, Ji H, Li P, Fang W, Jiang Y (2006) Inhibitors of acetylcholine esterase *in vitro* – Screening of steroidal alkaloids from *Fritillaria* species. *Planta Medica* **72**, 814-818

Loizzo MR, Tundis R, Menichini F, Menichini F (2008) Natural products and their derivatives as cholinesterase inhibitors in the treatment of neurodegenerative disorders: An update. *Current Medicinal Chemistry* **15**, 1209-1228

López S, Bastida J, Viladomat F, Codina C (2002) Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and *Narcissus* extracts. *Life Sciences* **71**, 2521-2529

Louh GN, Lannang AM, Mbazoa CD, Tangmouo JG, Komguem J, Castilho P, Ngninzeko FN, Qamar N, Lontsi D, Choudhary MI, Sondengam BL (2008) Polyanxanthone A, B and C, three xanthones from the wood trunk of *Garcinia polyantha* Oliv. *Phytochemistry* **69**, 1013-1017

Lu W-H, Shou J, Tang X-C (1988) Improving effect of huperzine A in aged rats and adult rats with experimental cognitive impairment. *Acta Pharmaco-*

logica Sinica **9**, 11-15

Ma X, Tan C, Zhu D, Gang DR, Xiao P (2007) Huperzine A from *Huperzia* species – An ethnopharmacolgical review. *Journal of Ethnopharmacology* **113**, 15-34

Maggi L, Andreetta F, Antozzi C, Baggi F, Bernasconi P, Cavalcante P, Cornelio F, Muscolino G, Novellino L, Mantegazza R (2008) Thymoma-associated myasthenia gravis: Outcome, clinical and pathological correlations in 197 patients on a 20-year experience. *Journal of Neuroimmunology* **201-202**, 237-244

Malamas M, Marselos M (1992) The tradition of medicinal plants in Zagori, Epirus (northwestern Greece). *Journal of Ethnopharmacology* **37**, 197-203

Markmee S, Ruchirawat S, Prachyawarakorn V, Ingkaninan K, Khorana N (2006) Isoquinoline derivatives as potential acetylcholinesterase inhibitors. *Bioorganic and Medicinal Chemistry Letters* **16**, 2170-2172

Marshall IG, Parsons SM (1987) The vesicular acetylcholine transport system. *Trends in Neuroscience* **10**, 174-177

Massoulié J, Perrier N, Noureddine H, Liang D, Bon S (2008) Old and new questions about cholinesterases. *Chemico-Biological Interactions* **175**, 30-44

Massoulié J, Pezzementi L, Bon S, Krejci E, Vallette F-M (1993) Molecular and cellular biology of cholinesterases. *Progress in Neurobiology* **41**, 31-91

McGlacken GP, Fairlamb IJS (2005) 2-Pyrone natural products and mimetics: Isolation, characterisation and biological activity. *Natural Product Reports* **22**, 369-385

McKinney M, Miller JH, Yamada F, Tuckmantel W, Kozikowski AP (1991) Potencies and stereoselectivities of enantiomers of huperzine A for inhibition of rat cortical acetylcholinesterase. *European Journal of Pharmacology* **203**, 303-305

McVicar J (1994) *Jekka's Complete Herb Book*, Kyle Cathie Ltd., London

Mehndiratta MM, Kuntzer T, Pandey S (2008) *Anticholinesterase Treatment For Myasthenia Gravis (Protocol)*, The Cochrane Collaboration, John Wiley & Sons, NJ, pp 1-6

Mills C, Cleary BJ, Gilmer JF, Walsh JJ (2004) Inhibition of acetylcholinesterase by tea tree oil. *Journal of Pharmacy and Pharmacology* **56**, 375-379

Misra R (1998) Modern drug development from traditional medicinal plants using radioligand receptor-binding assays. *Medicinal Research Reviews* **18**, 383-402

Miyazawa M, Yamafuji C (2006) Inhibition of acetylcholinesterase activity by tea tree oil and constituent terpenoids. *Flavour and Fragrance Journal* **21**, 198-201

Miyazawa M, Yamafuji C (2005) Inhibition of acetylcholinesterase activity by bicyclic monoterpenoids. *Journal of Agricultural and Food Chemistry* **53**, 1765-1768

Mozayan M, Chen M-F, Si M, Chen PY, Premkumar LS, Lee TJF (2006) Cholinesterase inhibitor blockade and its prevention by statins of sympathetic α7-nAChR-mediated cerebral nitrergic neurogenic vasodilation. *Journal of Cerebral Blood Flow and Metabolism* **26**, 1562-1576

Mukherjee PK, Kumar V, Mal M, Houghton PJ (2007a) *Acorus calamus*: Scientific validation of Ayurvedic tradition from natural resources. *Pharmaceutical Biology* **45**, 651-666

Mukherjee PK, Kumar V, Mal M, Houghton PJ (2007b) *In vitro* acetylcholinesterase inhibitory activity of the essential oil from *Acorus calamus* and its main constituents. *Planta Medica* **73**, 283-285

Muñoz-Torrero D (2008) Acetylcholinesterase inhibitors as disease-modifying therapies for Alzheimer's disease. *Current Medicinal Chemistry* **15**, 2433-2455

Murebwayire S, Ingkaninan K, Changwijit K, Frédérich M, Duez P (2009) *Triclisia sacleuxii* (Pierre) Diels (Menispermaceae), a potential source of acetylcholinesterase inhibitors. *Journal of Pharmacy and Pharmacology* **61**, 103-107

Ndjakou Lenta B, Devkota KP, Ngouela S, Fekam Boyom F, Naz Q, Choudhary MI, Tsamo E, Rosenthal PJ, Sewald N (2008) Anti-plasmodial and cholinesterase inhibiting activities of some constituents of *Psorospermum glaberrimum. Chemical and Pharmaceutical Bulletin* **56**, 222-226

Neal MJ (1992) *Medical Pharmacology at a Glance*, Blackwell Scientific Publications, Oxford, pp 26-27

Nisar M, Ahmad M, Wadood N, Lodhi MA, Shaheen F, Choudhary MI (2009) New diterpenoid alkaloids from *Aconitum heterophyllum* Wall: Selective butyrylcholinesterase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry* **24**, 47-51

Nukoolkarn VS, Saen-oon S, Rungrotmongkol T, Hannongbua S, Ingkaninan K, Suwanborirux K (2008) Petrosamine, a potent anticholinesterase pyridoacridine alkaloid from a Thai marine sponge *Petrosia* n. sp. *Bioorganic and Medicinal Chemistry* **16**, 6560-6567

Ogane N, Giacobini E, Struble R (1992) Differential inhibition of acetylcholinesterase molecular forms in normal and Alzheimer's disease brain. *Brain Research* **589**, 307-312

Oinonen PP, Jokela JK, Hatakka AI, Vuorela PM (2006) Linarin, a selective acetylcholinesterase inhibitor from *Mentha arvensis. Fitoterapia* **77**, 429-434

Okello EJ, Dimaki C, Howes M-JR, Houghton PJ, Perry EK (2008) *In vitro* inhibition of human acetyl- and butyryl-cholinesterase by *Narcissus poeticus* L. (Amaryllidaceae) flower absolute. *International Journal of Essential Oil Therapeutics* **2**, 105-110

Ōmura S, Kuno F, Otoguro K, Sunazuka T, Shiomi K, Masuma R, Iwai Y (1995) Arisugacin, a novel and selective inhibitor of acetylcholinesterase from *Penicillium* sp. FO-4259. *Journal of Antibiotics* **48**, 745-746

Orhan I, Tosun F, Şener B (2008a) Coumarin, anthraquinone and stilbene derivatives with anticholinesterase activity. *Zeitschrift für Naturforschung C* **63**, 366-370

Orhan I, Aslan S, Kartal M, Şener B, Hüsnü Can Başer K (2008b) Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyrylcholinesterase enzymes. *Food Chemistry* **108**, 663-668

Orhan I, Terzioglu S, Şener B (2003) α-Onocerin: An acetylcholinesterase inhibitor from *Lycopodium clavatum*. *Planta Medica* **69**, 265-267

Otoguro K, Kuno F, Ōmura S (1997) Arisugacins, selective acetylcholinesterase inhibitors of microbial origin. *Pharmacology and Therapeutics* **76**, 45-54

Overshott R, Karim S, Burns A (2008) *Cholinesterase inhibitors for delirium (Protocol)*, The Cochrane Collaboration, John Wiley & Sons, NJ, pp 1-11

Pákáski M, Kálmán J (2008) Interactions between the amyloid and cholinergic mechanisms in Alzheimer's disease. *Neurochemistry International* **53**, 103-111

Pan Y, Anthony M, Watson S, Clarkson TB (2000) Soy phytoestrogens improve radial arm maze performance in ovariectomised retired breeder rats and do not attenuate benefits of 17β-estradiol treatment. *Menopause* **7**, 209-212

Papandreou MA, Dimakopoulou A, Linardaki ZI, Cordopatis P, Klimis-Zacas D, Margarity M, Lamari FN (2009) Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. *Behavioural Brain Research* **198**, 352-358

Park CH, Choi SH, Koo JW, Seo JH, Kim HS, Jeong SJ, Suh YH (2002) Novel cognitive improving and neuroprotective activities of *Polygala tenuifolia* Willdenow extract, BT-11. *Journal of Neuroscience Research* **70**, 484-492

Park CH, Kim S, Choi W, Lee Y, Kim J, Kang SS, Suh YH (1996) Novel anticholinesterase and antiamnesic activities of dehydroevodiamine, a constituent of *Evodia ruraecarpa*. *Planta Medica* **62**, 405-409

Perry E (1986) The cholinergic hypothesis: 10 years on. *British Medical Bulletin* **42**, 63-69

Perry NSL, Houghton PJ, Jenner P, Keith A, Perry EK (2002) *Salvia lavandulaefolia* essential oil inhibits cholinesterase *in vivo*. *Phytomedicine* **9**, 48-51

Perry NSL, Houghton PJ, Sampson J, Hart S, Lis-Balchin M, Hoult JRS, Evans P, Jenner P, Milligan S, Perry EK (2001) *In vitro* activities of *S. lavandulaefolia* (Spanish sage) relevant to treatment of Alzheimer's disease. *Journal of Pharmacy and Pharmacology* **53**, 1347-1356

Perry NSL, Houghton PJ, Theobald A, Jenner P, Perry EK (2000) *In vitro* inhibition of human erythrocyte acetylcholinesterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. *Journal of Pharmacy and Pharmacology* **52**, 895-902

Perry N, Court G, Bidet N, Court J, Perry E (1996) European herbs with cholinergic activities: Potential in dementia therapy. *International Journal of Geriatric Psychiatry* **11**, 1063-1069

Perry E, Tomlinson E, Blessed G, Bergmann K, Gibson P, Perry R (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *British Medical Journal* **2**, 1457-1459

Phoopichayanun C, Phuwapraisirisan P, Tip-Pyang S, Jongaramruong J (2008) Complete NMR assignment and absolute configuration of feroniellosside, a new acetylcholinesterase inhibitor from *Feroniella lucida*. *Natural Product Research* **22**, 1297-1303

Piazzi L, Cavalli A, Colizzi F, Belluti F, Bartolini M, Mancini F, Recanatini M, Andrisano V, Rampa A (2008) Multi-target-directed coumarin derivatives: hAChE and BACE1 inhibitors as potential anti-Alzheimer compounds. *Bioorganic and Medicinal Chemistry Letters* **18**, 423-426

Picollo MI, Toloza AC, Mougabure Cueto G, Zygadlo J, Zerba E (2008) Anticholinesterase and pediculicidal activities of monoterpenoids. *Fitoterapia* **79**, 271-278

Polinsky RJ (1998) Clinical pharmacology of rivastigmine: A new-generation. *Clinical Therapeutics* **20**, 634-647

Raffaele KC, Asthana S, Beradi A, Haxby JV, Morris PP, Schapiro MB, Soncrant TT (1996) Differential response to the cholinergic agonist arecoline among different cognitive modalities in Alzheimer's disease. *Neuropsychopharmacology* **15**, 163-170

Rahman AU, Khalid A, Sultana N, Ghayur MN, Mesaik MA, Khan MR, Gilani AH, Choudhary I (2006) New natural cholinesterase inhibiting and calcium channel blocking quinoline alkaloids. *Journal of Enzyme Inhibition and Medicinal Chemistry* **21**, 703-710

Rajendran V, Saxena A, Doctor BP, Kozikowski AP (2002) Synthesis of more potent analogues of the acetylcholinesterase inhibitor, huperzine B. *Bioorganic and Medicinal Chemistry Letters* **12**, 1521-1523

Rajendran V, Prakash KRC, Ved HS, Saxena A, Doctor BP, Kozikowski AP (2000) Synthesis, chiral chromatographic separation, and biological activities of the enantiomers of 10,10-dimethylhuperzine A. *Bioorganic and Medicinal Chemistry Letters* **10**, 2467-2469

Rasoamiaranjanahary L, Guile D, Marstona A, Randimbivololona F, Hostettmann K (2003) Antifungal isopimaranes from *Hypoestes serpens*. *Phytochemistry* **64**, 543-548

Ren YH, Houghton PJ, Hider RC, Howes MJR (2004) Novel diterpenoid acetylcholinesterase inhibitors from *Salvia miltiorhiza*. *Planta Medica* **70**, 201-204

Rhee IK, Appels N, Hofte B, Karabatak B, Erkelens C, Stark LM, Flippin LA, Verpoorte R (2004) Isolation of the acetylcholinesterase inhibitor ungeremine from *Nerine bowdenii* by preparative HPLC coupled on-line to a flow assay system. *Biological and Pharmaceutical Bulletin* **27**, 1804-1809

Riaz N, Nawaz SA, Mukhtar N, Malik A, Afza N, Ali S, Ullah S, Muhammad P, Choudhary MI (2007) Isolation and enzyme-inhibition studies of the chemical constituents from *Ajuga bracteosa. Chemistry and Biodiversity* **4**, 72-83

Riekkinen P, Riekkinen M, Sirvio J (1993) Cholinergic drugs regulate passive avoidance performance via the amygdala. *Journal of Pharmacology and Experimental Therapeutics* **267**, 1484-1492

Roddick JG, Weissenberg M, Leonard AL (2001) Membrane disruption and enzyme inhibition by naturally-occurring and modified chacotriose-containing *Solanum* steroidal glycoalkaloids. *Phytochemistry* **56**, 603-610

Rollinger JM, Schuster D, Baier E, Ellmerer EP, Langer T, Stuppner H (2006) Tapsine: Bioactivity-guided isolation and molecular ligand-target insight of a potent acetylcholinesterase inhibitor from *Magnolia* x *soulangiana. Journal of Natural Products* **69**, 1341-1346

Rollinger JM, Ewelt J, Seger C, Sturm S, Ellmerer EP, Stuppner H (2005) New insights into the acetylcholinesterase inhibitory activity of *Lycopodium clavatum. Planta Medica* **71**, 1040-1043

Rollinger JM, Hornick A, Langer T, Stuppner H, Prast H (2004) Acetylcholinesterase inhibitory activity of scopolin and scopoletin discovered by virtual screening of natural products. *Journal of Medicinal Chemistry* **47**, 6248-6254

Ros E, Aleu J, de Aranda IG, Munoz-Torrero D, Camps P, Badia A, Marsal J, Solsona C (2001) The pharmacology of novel acetylcholinesterase inhibitors, (+/-)-huprines Y and X, on the *Torpedo* electric organ. *European Journal of Pharmacology* **421**, 77-84

Rozengart EV, Basova NE, Suvorov AA (2006) Comparative study of cholinergic activity of some tropolone and isoquinoline alkaloids. *Journal of Evolutionary Biochemistry and Physiology* **42**, 408-416

Ryan MF, Byrne O (1988) Plant-insect coevolution and inhibition of acetylcholinesterase. *Journal of Chemical Ecol*ogy **14**, 1965-1975

Sauvaître T, Barlier M, Herlem D, Gresh N, Chiaroni A, Guenard D, Guillou C (2007) New potent acetylcholinesterase inhibitors in the tetracyclic triterpene series. *Journal of Medicinal Chemistry* **50**, 5311-5323

Savelev S, Okello E, Perry NSL, Wilkins RM, Perry EK (2003) Synergistic and antagonistic interactions of anticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. *Pharmacology Biochemistry and Behavior* **75**, 661-668

Schliebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V (1997) Systemic administration of defined extracts from *Withania somnifera* (Indian ginseng) and shilajit differentially affects cholinergic but not glutamatergic markers in rat brain. *Neurochemistry International* **30**, 181-190

Schmeller T, Latz-Brüning B, Wink M (1997) Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. *Phytochemistry* **44**, 257-266

Schubert MH, Young KA, Hicks PB (2006) Galantamine improves cognition in schizophrenic patients stabilized on risperidone. *Biological Psychiatry* **60**, 530-533

Shen Q, Peng Q, Shao J, Liu X, Huang Z, Pu X, Ma L, Li Y-M, Chan ASC, Gu L (2005) Synthesis and biological evaluation of functionalized coumarins as acetylcholinesterase inhibitors. *European Journal of Medicinal Chemistry* **40**, 1307-1315

Shigeta K, Ootaki K, Tatemoto H, Nakanishi T, Inada A, Muto N (2002) Potentiation of nerve growth factor-induced neurite outgrowth in PC12 cells by a Coptidis Rhizoma extract and protoberberine alkaloids. *Bioscience Biotechnology and Biochemistry* **66**, 2491-2494

Shiomi K, Tomoda H, Otoguro K, Ōmura S (1999) Meroterpenoids with various biological activities produced by fungi. *Pure and Applied Chemistry* **71**, 1059-1064

Shu Y-Z (1998) Recent natural products based drug development: A pharmaceutical industry perspective. *Journal of Natural Products* **61**, 1053-1071

Simeon-Rudolf V, Kovarik Z, Radic Z, Reiner E (1999) Reversible inhibition of acetylcholinesterase and butyrylcholinesterase by 4,4'-bipyridine and by a coumarin derivative. *Chemico-Biological Interactions* **120**, 119-128

Siramon P, Ohtani Y, Ichiura H (2008) Biological performance of *Eucalyptus camaldulensis* leaf oils from Thailand against the subterranean termite *Coptotermes formosanus* Shiraki. *Journal of Wood Science* **55**, 41-46

Sitaram N, Weingartner H, Gillin JC (1978) Physostigmine: Improvement of long-term memory processes in normal humans. *Science* **201**, 272-276

Small G, Erkinjuntti T, Kurz A, Lilienfeld S (2003) Galantamine in the treatment of cognitive decline in patients with vascular dementia or Alzheimer's disease with cerebrovascular disease. *CNS Drugs* **17**, 905-914

Small GW, Rabins RV, Barry PP, Buckholtz NS, DeKosky ST, Ferros SH, Finkel SI, Gwyther LP, Khachaturian ZS, Lebowitz BD, McRae TD, Morris JC, Oakley F, Schneider LS, Streim JE, Sunderland T, Teri LA, Tune LE (1997) Diagnosis and treatment of Alzheimer's disease and related disorders. *Journal of the American Medical Association* **278**, 1363-1371

Soncrant TT, Raffaele KC, Asthana S, Berardi A, Morris PP, Haxby JV (1993) Rivastigmine – A review of its use in Alzheimer's disease. *Psychopharmacology* **112**, 421-427

Sugimoto H (2008) The new approach in development of anti-Alzheimer's disease drugs via the cholinergic hypothesis. *Chemico-Biological Interactions* **175**, 204-208

Tanaka R (1984) Effects of benzyl alcohol on adenosine triphosphate, *p*-nitrophenylphosphatase and acetylcholinesterase in rat erythrocyte membrane. *Journal of Toxicological Sciences* **9**, 109-116

Tang W, Eisenbrand G (1992) *Chinese Drugs of Plant Origin*, Springer-Verlag, Berlin, Germany, 891 pp

Taranalli AD, Cheeramkuzhy TC (2000) Influence of *Clitoria ternatea* extracts on memory and central cholinergic activity in rats. *Pharmaceutical Biology* **38**, 51-56

Tariot PN, Cohen RM, Welkowictz JA, Sunderland T, Newhouse PA, Murphey DL, Weingartner H (1988) Multiple-dose arecoline infusions in Alzheimer's disease. *Archives of General Psychiatry* **45**, 901-905

Teismann P, Ferger B (2000) Effects of ensaculin on dopamine metabolite levels and K^+-induced glutamate release. *European Journal of Pharmacology* **398**, 247-250

Thirugnanasampandan R, Jayakumar R, Narmatha Bai V, Martin E, Rajendra Prasad KJ (2008) Antiacetylcholinesterase and antioxidant *ent*-kaurene diterpenoid, melissoidesin from *Isodon wightii* (Bentham) H. Hara. *Natural Product Research* **22**, 681-688

Tumiatti V, Bolognesi ML, Minarini A, Rosini M, Milelli A, Matera R, Melchiorre C (2008) Progress in acetylcholinesterase inhibitors for Alzheimer's disease: An update. *Expert Opinion on Therapeutic Patents* **18**, 387-401

Urbain A, Marston A, Queiroz EF, Ndjoko K, Hostettmann K (2004) Xanthones from *Gentiana campestris* as new acetylcholinesterase inhibitors. *Planta Medica* **70**, 1011-1014

Viegas C, Bolzani VS, Pimentel LS, Castro NG, Cabral RF, Costa RS, Floyd C, Rocha MS, Young MC, Barreiro EJ, Fraga CA (2005) New selective acetylcholinesterase inhibitors designed from natural piperidine alkaloids. *Bioorganic and Medicinal Chemistry* **13**, 4184-4190

Vieira IJC, Medeiros WLB, Monnerat CS, Souza JJ, Mathias L, Braz-Filho R, Pinto AC, Sousa PM, Rezende CM, Epifanio RA (2008) Two fast screening methods (GC-MS and TLC-ChEI assay) for rapid evaluation of potential anticholinesterasic indole alkaloids in complex mixtures. *Anais da Academia Brasileira de Ciências* **80**, 419-426

Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, Padmaja R, Radhika S, Amit A, Venkateshwarlu K, Deepak M (2007) Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. *Journal of Ethnopharmacology* **109**, 359-363

Wang R, Yan H, Tang XC (2006) Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine. *Acta Pharmacologica Sinica* **27**, 1-26

Wang LM, Han YF, Tang XC (2000) Huperzine A improves cognitive deficits caused by chronic cerebral hypoperfusion in rats. *European Journal of Pharmacology* **398**, 65-72

Warrier PK, Nambiar VPK, Ramankutty C (1995) *Indian Medicinal Plants* (Vol 2), Orient Longman, India, 129 pp

Wen L, Lin Y-C, She Z-G, Du D-S, Chan W-L, Zheng Z-H (2008) Paeciloxanthone, a new cytotoxic xanthone from the marine mangrove fungus *Paecilomyces* sp. (Tree1-7). *Journal of Asian Natural Products Research* **10**, 133-137

Wichtl M (Ed) (2004) *Herbal Drugs and Phytopharmaceuticals*, Medpharm GmbH Scientific Publishers, Stuttgart, Germany, pp 382-386

Wilcock GK, Lilienfeld S, Gaens E (2000) Efficacy and safety of galantamine in patients with mild to moderate Alzheimer's disease: Multicentre randomised controlled trial. *British Medical Journal* **321**, 1445-1449

Wilkinson D, Murray J (2001) Galantamine: A randomised, double-blind, dose comparison in patients with Alzheimer's disease. *International Journal of Geriatric Psychiatry* **16**, 852-857

Wu C-R, Chang C-L, Hsieh P-Y, Lin L-W, Ching H (2007) Psoralen and isopsoralen, two coumarins of Psoralae Fructus, can alleviate scopolamine-induced amnesia in rats. *Planta Medica* **73**, 275-278

Yang YR, Chang KC, Chen CL, Chiu TH (2000) Arecoline excites rat locus coeruleus by activating the M_2 muscarinic receptor. *Chinese Journal of Physiology* **43**, 23-28

Yin G, Li YM, Wei W, Jiang SH, Zhu DY, Du WH (2008) Interactions of acetylcholinesterase with salvianolic acid B and rosmarinic acid from *Salvia miltiorhiza* water extract investigated by NMR relaxation rate. *Chinese Chemical Letters* **19**, 747-751

Yoo ID, Cho KM, Lee CK, Kim WG (2005) Isoterreulactone A, a novel meroterpenoid with anti-acetylcholinesterase activity produced by *Aspergillus terreus. Bioorganic and Medicinal Chemistry Letters* **15**, 353-356

Yoshida S, Suzuki N (1993) Antiamnesic and cholinomimetic side-effects of the cholinesterase inhibitors, physostigmine, tacrine and NIK-247 in rats. *European Journal of Pharmacology* **250**, 117-124

Yu XY, Lin SG, Chen X, Zhou ZW, Liang J, Duan W, Chowbay B, Wen JY, Chan E, Cao J, Li CG, Zhou SF (2007) Transport of cryptotanshinone, a major active triterpenoid in *Salvia miltiorrhiza* Bunge widely used in the treatment of stroke and Alzheimer's disease, across the blood-brain barrier. *Current Drug Metabolism* **8**, 365-377

Zaheer-ul-Haq, Wellenzohn B, Tonmunphean S, Khalid A, Choudhary MI, Rode BM (2003) 3D-QSAR studies on natural acetylcholinesterase inhibitors of *Sarcococca saligna* by comparative molecular field analysis (CoMFA). *Bioorganic and Medicinal Chemistry Letters* **13**, 4375-4380

Zeng FX, Jiang HL, Zhai YF, Zhang HY, Chen KX, Ji RY (1999) Synthesis and acetylcholinesterase inhibitory activity of huperzine A-E2020 combined compound. *Bioorganic and Medicinal Chemistry Letters* **9**, 3279-3284

Zeng FX, Jiang HL, Tang XC, Chen KX, Ji RY (1998) Synthesis and acetylcholinesterase inhibitory activity of (+/-)-14-fluorohuperzine A. *Bioorganic and Medicinal Chemistry Letters* **8**, 1661-1664

Zhang X (2004) Cholinergic activity and amyloid precursor protein processing in aging and Alzheimer's disease. *Current Drug Targets – CNS and Neurological Disorders* **3**, 137-152

Zhang H, Han T, Zhang L, Yu C-H, Wan D-G, Rahman K, Qin L-P, Peng C (2008) Effects of tenuifolin extracted from radix polygalae on learning and memory: A behavioral and biochemical study on aged and amnesic mice. *Phytomedicine* **15**, 587-594

Zhang YH, Chen XQ, Yang HH, Jin GY, Bai DL, Hu GY (2000) Similar potency of the enantiomers of huperzine A in inhibition of [^{3}H]dizocilpine (MK-801) binding in rat cerebral cortex. *Neuroscience Letters* **295**, 116-118

Zhou GC, Zhu DY (2000) Synthesis of 5-substituted analogues of huperzine A. *Bioorganic and Medicinal Chemistry Letters* **10**, 2055-2057

Zhou X, Wang X-B, Wang T, Kong L-Y (2008) Design, synthesis, and acetylcholinesterase inhibitory activity of novel coumarin analogues. *Bioorganic and Medicinal Chemistry* **16**, 8011-8021

Zhou J, Zhang HY, Tang XC (2001) Huperzine A attenuates cognitive deficits and hippocampal neuronal damage after transient global ischemia in gerbils. *Neuroscience Letters* **313**, 137-140