Lead Finding for Acetyl Cholinesterase Inhibitors from Natural Origin: Structure Activity Relationship and Scope

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Abstract: Acetylcholinesterase (AChE) inhibitors are considered as promising therapeutic agents for the treatment of several neurological disorders such as Alzheimer's disease (AD), senile dementia, ataxia and myasthenia gravis. There are only few synthetic medicines with adverse effects, available for treatment of cognitive dysfunction and memory loss associated with these diseases. A variety of plants has been reported to possess AChE inhibitory activity and so may be relevant to the treatment of neurodegenerative disorders such as AD. Hence, developing potential AChE inhibitors from botanicals is the need of the day. This review will cover some of the promising acetylcholinesterase inhibitors isolated from plants with proven *in vitro* and *in vivo* activities with concern to their structure activity relationship.

Keywords: Acetylcholinesterase inhibitors, Alzheimer's, structure activity relationship, ethnomedical.

INTRODUCTION

Work on new bioactive compounds from medicinal plants has led to the isolation and structure elucidation of a number of exciting new pharmacophores. Alzheimer's disease (AD) is one of the most common forms of dementia affecting so many elderly people. Besides the neuropathologic hallmarks of this disease, namely neurofibrillary tangles and neuritic plaques, it is characterized neurochemically by a consistent deficit in cholinergic neurotransmission, particularly affecting cholinergic neurons in the basal forebrain [1]. Principal role of acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (ACh). Inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease [1]. Even though mankind always relies on nature for their basic requirements, rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics that include plant-derived pharmaceuticals, multicomponent botanical drugs, and plant-produced recombinant proteins. There is great interest in finding better AChE inhibitors from natural product showing low toxicity, good brain penetration and high bioavailability. Natural products have inspired many developments in organic chemistry leading to advances in synthetic methodologies in developing several therapeutically potential analogues of lead compounds. The research lead on ayurvedic drugs yielded numerous drug candidates that are prevailing in the market. There are several plants of ayurvedic origin with potential therapeutic activity, which are widely used as ayurvedic medicine. Nature has provided several leads as potential AChE inhibitors from plant sources, including those for memory disorders. This article highlights several aspects of lead identification for Acetyl cholinesterase Inhibitors, their structure activity relationship and potential uses.

NATURAL REMEDIES FOR AD

Natural sources genetic codes contain the recipes for chemical compounds of potential value in pharmaceutical products. Pharmaceutical research in natural products is more often intended to develop leads and to identify those plants, which can be used in unmodified form. Plant made pharmaceuticals (PMPs) are the result of a breakthrough application of biotechnology on plants to enable them to produce therapeutic proteins that could ultimately be used by the medical community to combat life-threatening illnesses [2]. The leads from plants are promising molecules that must be modified to increase efficacy or reduce side effects. Based on these aspects several works on the search for natural AChEI has been reported from our laboratory [3-6].

Alzheimer's disease (AD) is the fourth leading cause of death among the elderly worldwide, accounting for the most common form of dementia diagnosed after the age of 60. Currently, several kinds of AChE inhibitors, such as donepezil (Fig. (**1a**)), galantamine (Fig. (**3b**)), and rivastigmine (Fig. (**2e**)) are available for the symptomatic treatment of patients with mild-to-moderate AD.

There are a number of approaches to the treatment of the cholinergic deficit in Alzheimer's disease, most of which have initially focused on the replacement of ACh precursors (choline or lecithin) but these agents failed to increase central cholinergic activity. Other studies have investigated the use of AChE inhibitors that reduce the hydrolysis of ACh for example, physostigmine. Further researches are being conducted in order to find a suitable remedy for this ailment. The most recent investigational compounds for treatment of cholinergic deficit include specific M1 muscarinic or nicotinic agonists, M2 muscarinic antagonists, or improved "sec-

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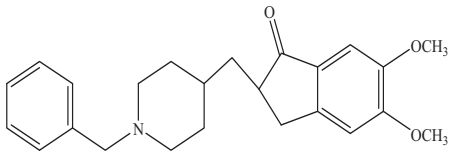
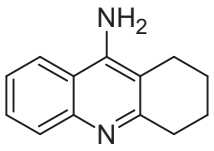


(a) Donepezil



(c) Tacrin, (IC_{50} 1 μM)

Fig. (1).

ond generation" AChE inhibitors [7]. This type of cholinesterase inhibitors are found in abundance in natural products which encourages the researchers towards lead finding for AD from the natural products.

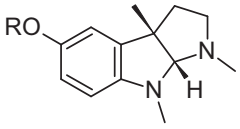
Although a variety of AChE inhibitors have been developed as potential treatments for Alzheimer's disease, their pharmacological activities differ. One of the most fundamental differences between them is in the mechanism of ChE inhibition. For example, enzyme kinetic studies have shown that tacrine (Fig. (**1c**)), an acridine compound, and donepezil, a novel piperidine class agent, are "mixed type" reversible inhibitors of ChE. These compounds inhibit ChE *via* both non-competitive (by blockade of the deacetylation process) and ACh competitive mechanisms [7].

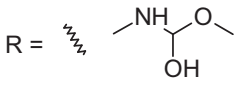
STRUCTURE-ACTIVITY RELATIONSHIP OF NATURAL ACETYLCHOLINESTERASE (AChE) INHIBITORS

The ethno medicinal plant used in the treatment of AD contains numerous molecules, they exhibit inhibitory effect due to the potential structural characteristics of the individual molecule and due to the synergistic action of the molecules collectively. As the phytochemicals can be structurally classified based on their chemical groups, it will be useful to study the effect of these molecules based on the groups under which it can be classified. Several potential molecules has been isolated from various ethnomedical plants and studied for their AChE inhibitory activity. Activities of some therapeutically active phytochemical groups have been discussed in the following sections:

ALKALOIDS

Many alkaloids like physostigmine (Fig. (**2a**)), eseroline (Fig. (**2b**)), neostigmine (Fig. (**2c**)), pyridostigmine (Fig. (**2d**)), rivastigmine (Fig. (**2e**)), eptastigmine (Fig. (**2f**)), as shown in Fig. (**2**) have been proved to possess AChE inhibitory activity. The plant alkaloid galantamine is a phenanthrene similar to codeine, which has been isolated from *Galanthus nivalis* L., the European daffodil or common snowdrop [8, 9]. Galantamine is the last drug approved for the treatment of AD. It is a tertiary alkaloid with a unique, dual mode of action. It is a reversible, competitive AChE inhibitor, and also an allosteric modulator of nicotinic acetylcholine receptors [10, 11]. The efficacy of galantamine has been

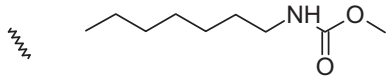




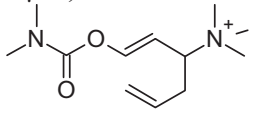
(a) Physostigmine (IC_{50} 0.25 μM)

R = H

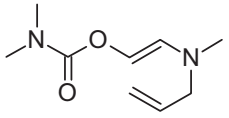
(b) Eseroline



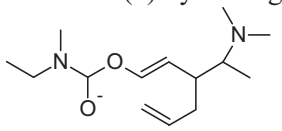
(c) Neostigmine



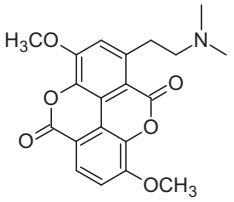
(d) Pyridostigmine



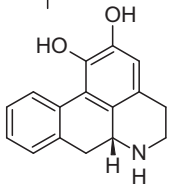
(e) Rivastigmine



(f) Eptastigmine



(g) Taspine
IC_{50} 0.33± 0.07 μM



(h)Asimilobine

Fig. (2).

extensively studied in clinical trials that have demonstrated that a dosing regimen of 16–24 mg/day consistently produced beneficial effects on cognitive and non-cognitive AD symptoms. Galantamine exhibits favorable pharmacokinetic characteristics including predictable linear elimination kinetics at the recommended maintenance doses (16 and 24 mg/day), a relatively short half-life (approximately 7 h), and high bioavailability, and side effects include the predictable gastrointestinal upset which is transient with mild intensity, and easily controllable using the recommended slow dose-escalation scheme [12,13].

Physostigmine, prototype acetylcholinesterase inhibitor (also known as serine) is isolated from the seeds of *Physostigma venenosum* Balf (Papilionaceae). The structure of physostigmine was determined and shown to have a pyrroloindole skeleton. Physostigmine is distributed throughout the body and produce general cholinergic effect. Because of its polarity it is not distributed in large concentrations in the CNS. In bovine erythrocytes, it showed AChEI activity with IC_{50} of 0.25 μM. it was found that the carbamate portion is essential for cholinesterase inhibitory activity. When the ester link is hydrolyzed to the product eseroline inhibitory activity was not observed. However, the carbonyl group interacts with the hydroxyl (-OH) of a serine to form ester in the AChE with urethane part of the molecule. The ester form is slowly hydrolyzed and regenerates the active parent form, which interfere the AChE activity of the enzyme. The carbamate moiety has been a key factor in the use of eserine as a lead molecule for dramatization or synthesis of longer-lasting or more selective drugs, insecticides and other agents. To make a possible binding the presence of an aromatic ring and an N atom is required.

Two alkaloids, taspine (Fig. (**2g**)) and asimilobine (Fig. (**2h**)) were detected in *Magnoliax soulangiana* Hort extract. Of these two, taspine showed significantly higher effect on AChE than galantamine and selectively inhibited the enzyme with time and concentration-dependant manner with an IC_{50} value of 0.33± 0.07 μM. Further studies also have showed that the ligand bind in an alternative orientation when compared to galanthamine. It was also found to be stabilized by sandwich like π stacking interaction between the planar aromatic ligand and the Trp84 and Phe330 of the enzyme, esteratic site anchoring with the amino acid side chain and hydrogen bonding network [14-16].

1. Amaryllidaceae Alkaloids

Twenty three pure amaryllidaceae alkaloids shown in (Fig. (**3a-3w**)) and 26 extracts from different species of the genus *Narcissus* were tested for their acetylcholinesterase inhibitory activity using galanthamine as a standard. Among them seven alkaloids, belonging to the galantamine and lycorine skeleton types, exhibited cholinesterase inhibitory effect. When compared with galantamine (IC_{50}= 0.33 μM), sanguinine (Fig. (**3a**)) was observed to be most active (IC_{50} = 0.72 μM). An *in vitro* structure-activity relationship study involving sanguinine and other synthetic analogues of galantamine indicated that properly placed hydrophilic groups on galantamine contribute to its effective binding to the AChE molecule [15-17].

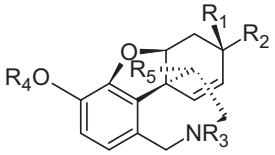
The structural features of the amaryllidaceae alkaloids have been explained by Lo´pez *et al*, [19]. It has been shown that the relatively tight binding in the structure appears to come from a number of moderate to weak interactions with the protein, including classical and non-classical hydrogen bonds. Therefore, the extra hydroxyl group of sanguinine available for potential interaction with AChE can explain the strong inhibitory activity of this alkaloid. Assoanine, oxoassoanine has been reported as the most active amongst the lycorine-type alkaloids, which could be due to the aromatic ring C which gives a certain planarity to those molecules [18, 19].

The acetylcholinesterase inhibitory effect of 23 amaryllidaceae alkaloids has been reported [20]. Among them, the alkaloid, 1-O-acetyllycorine (Fig. (**$3a_1$**)) (IC_{50}: 0.96±0.04) was found to show significant inhibitory activity in micro molar concentration. Beside these, compounds shown in (Fig. (**$3b_2$**), (**$3c_3$**), (**$3d_4$**), (**$3e_5$**), (**$3f_6$**), (**$3g_7$**), (**$3h_8$**), (**$3i_9$**), (**$3j_{10}$**), (**$3k_{11}$**), (**$3l_{12}$**), (**$3m_{13}$**), (**$3n_{14}$**) and (**$3a_1$**) were also found to have weak activity. It seems that lycorine was found to be a potent inhibitor of AChE because of the different ring type when compared with that of other alkaloids. Further the presence of an acetoxy group and a hydroxyl group at positions 1 and respectively are required in 1-O-acetyllycorine for proper binding and inhibition of activity of the enzyme. On the other hand, the aromatic ring C that gives certain planarity to assoanine and oxoassoanine explains the higher activity of the molecules in comparison with lycorine type alkaloids. In Crinine type alkaloids, the stereochemistry of 5,10 b-ethano bridge has no effect whereas 15-carbon ring system of crinine is important for their activity.

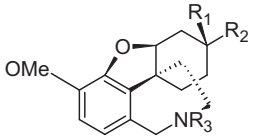
2. Indole Alkaloids

Andrade *et al.* (2005) reported ten indole alkaloids from the chloroform extract of stalk of *Tabernaemontana australis* Miers, as depicted in (Fig. (**4a-4j**)) were studied for their acetylcholinesterase inhibitory activity. Of these the first four showed potent acetylcholinesterase activity. The activity of these alkaloids seems to be related to their antagonist effect in subtype a3b4 nicotinic receptors (nChRs), binding with low affinity to other types of receptors, including a4β2 nChRs. [21].

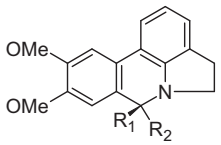
Compounds shown in (Fig. (**4k-4q**)) are the fungal metabolite isolated from *Aspergillus terreus*. Among this territrem B (TRB) (Fig. (**4l**)), shown to be a potent and irreversible inhibitor of acetylcholinesterase (AChE). Omura's group additionally showed that several analogs of territrem isolated from rice culture broth of Penicillium sp. FO-4259, called arisugacins, which are highly specific and potent AChE inhibitors [22, 23]. Both territrems and arisugacins are composed of a basic structure that includes a benzyl group, a pyran, and a terpenoid. The mechanism of TRB AChE inhibition using both kinetic and molecular modeling studies indicates that TRB does not form a covalent bond with the enzyme. It is consistent with its lack of a carbamate and a phosphate moiety, which could otherwise react with the active serine of the enzyme. By searching for a probable binding mode between TRB and AChE through extensive docking simulations, a structural model of their complex was derived. The notable absence of nitrogen in these compounds is



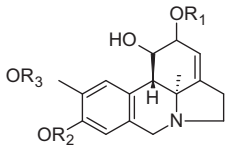
(a) Sanguinine: R^1=OH, R^2=H, R^3=Me, R^4=H, R^5=H, (IC_{50} = 0.72 μM)
(b) Galanthamine: R^1=OH, R^2=H, R^3=Me, R^4=Me, R^5=H, (IC_{50} 0.33 μM)
(c) 11-Hydroxygalanthamine: R^1=OH, R^2=H, R^3=Me, R^4=Me, R^5=OH
(d) Epinorgalanthamine: R^1=H, R^2=OH, R^3=H, R^4=Me, R^5=H



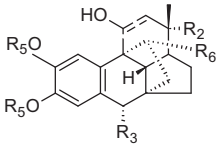
(e) Lycoramine: R^1=OH, R^2=H, R^3=Me
(f) Epinorlycoramine: R^1=H, R^2=OH, R^3= H



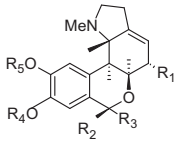
(g) Assoanine:R^1=R^2=H
(h) Oxoassoanine:R^1+R^2=O



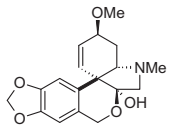
(i) Lycorine: R^1=H,R^2+R^3=CH_2
(j) Pseudolycorine:R^1=H,R^2=Me,R^3=H
(k) 2-O-Acetylpseudolycorine: R^1=Ac,R^2=Me,R^3=H



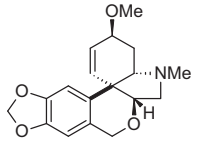
(l) Haemanthamine: R^1=OMe,R^2=H,R^3=H,R^4+R^5=CH_2,R^6=OH
(m) Crinamine: R^1=H,R^2=OMe,R^3=H,R^4+R^5=CH_2,R^6=OH, (IC_{50}: 697±12)
(n) Papyramine: R^1=OMe,R^2=H,R^3=OH,R^4=Me,R^5=Me,R^6=H
(o) Haemanthidine: R^1=OMe,R^2=H,R^3=OH,R^4+R^5=CH_2,R^6=OH



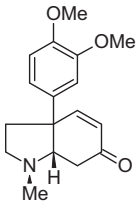
(p) 8-O-Demethylhomolycorine: R^1=H, R^2+R^3=O,R^4=H,R^5=Me
(q) 9-O-Demethyl-2α-hydroxyhomolycorine:R^1=OH,R^2+R^3=O, R^4=H,R^5=Me
(r) Dublusine: R^1=OAc,R^2+R^3=O, R^4=Me,R^5=OOC-CH2-CHOH-Me
(s) Hippeastrine: R^1=OH,R^2+R^3=O,R^4+R^5=CH_2
(t) O-Methyllycorenine: R^1=H,R^2=H,R_3=OMe,R^4=Me,R^5=Me



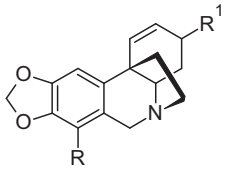
(u) Tazettine, (IC_{50}: 705±63)



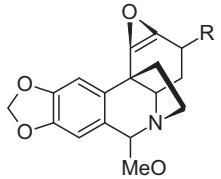
(v) Pretazettine



(w) Mesembrenone

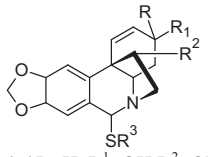


(b_2)R=H,R^1=OH Crinine, (IC_{50}: 461±140)
(c_3)R=H, R^1=OMe Epibuphanisine, (IC_{50}: 547± 5),

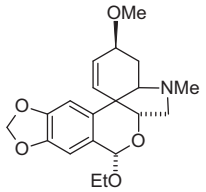


(d_4)R=OH Crinamidine, (IC_{50}:300±27)

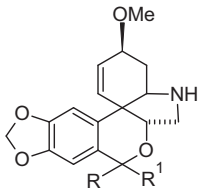
(Fig. 3). Contd…..



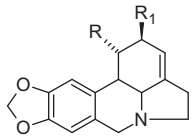
(e_5)R=H, R^1=OH,R^2=OH,R^3=H Hamayne, (IC_{50}: 533±3)
(f_6)R= R^3=H, R=H, R^1=OAc,R^2=OH 3-O-Acetylhamayne, (IC_{50}: 594±8)
(g_7)R=H, R^1=OH,R^2=R^3=H Epivittatine, (IC_{50}:239±9)
(h_8)R=H, R^1=OMe,R^2=OH,R^3=OH (α,β) 6-Hydroxycrinamine, (IC_{50}:490±27)



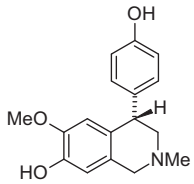
(i_9) 8α-ethoxypretazettine, (IC_{50}: 234±13)



(j_{10}) R= H, R^1= OEt N-desmethyl-N-desmethyl-8α-ethoxypretazettine, (IC_{50}: 234±13)
(k_{11}) R= OEt, R^1= H N-desmethyl-N-desmethyl-8β-ethoxypretazettine, (IC_{50}: 419±8)



(l_{12})R=R^1=OH Lycorine, (IC_{50}:213±1)
(a_1) R=OAc,R^1=OH 1-O-acetyllycorine, (IC_{50}: 0.96±0.04)
(m_{13})R=R^1=OAc 1,2-di-O-acetyllycorine, (IC_{50}:211±10)



(n_{14}) Cherylline, (IC_{50}:407±32)

Fig. (3). Amaryllidaceae alkaloids.

unlike other known AChE inhibitors. It was found that the inhibition of TRB on AChE is mediated by a tight non covalent binding that is kinetically irreversible, at least within the time duration of our experiments.

3. Isoquinoline Derivatives

Cocculus pendulus (J. R. & G. FORST.) results two new alkaloids namely kurramine-2'-β -N-oxide (Fig. (**5a**)), kurramine-2'-α-N-oxide (Fig. (**5b**)), and three known bisbenzylisoquinoline alkaloids (1,2-dehydroapateline (Fig. (**5 c)**), cocsoline (Fig. (**5d**)) and cocsuline (Fig. (**5e**)) during phytochemical investigation. Compounds (Fig. (**5d**)) (IC_{50} 6.1μM) and (Fig. (**5e**)) (IC_{50} 12.0 μM) were found to be active against butyrylcholinesterase, while compounds (Fig. (**5a**)) (IC_{50} 10.0μM) and (Fig. (**5d**)) (IC_{50} 47.6μM) have inhibited acetyl cholinesterase significantly [24]. The activity of the bisbenzylisoquinoline alkaloids is found to be associated with the quaternary nitrogen group present in the compounds. Quantitative structure-activity relationship studies have shown that the inhibitory potency of isoquinoline derivatives was determined by steric, rather than electrostatic, properties of the compounds. Tubocurarine isolated from *Chondodendron tomentosum* Ruiz & Pav (Menispermaceae) is a classical example for the potential activity of the benzylisoquinoline ring. A number of other bisbenzylisoquinoline (BBIQ) alkaloids; such as fangchinoline (Fig. (**5f**)), atherospermoline (Fig. (**5g**)), and fenfangjine E (Fig. (**5h**)) isolated from root of *Stephania tetrandra* S. Moore, Menispermaceae family, were also found to inhibit acetylcholinesterase enzyme in micro molar range [25].

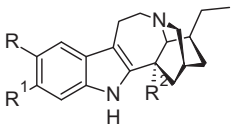
4. Lycopodium Alkaloids

Three lycopodium alkaloids isolated from the club moss *Lycopodium casuarinoides* Spring were tested for their inhibitory activity against acetylcholinesterase. Lycoparins A (Fig. (**6a**)) and lycoparins B (Fig. (**6b**)) possessing a carboxylic acid at C-15 and one or two N-methyl groups did not show such activity (IC_{50} > 200 μM) . Whereas lycoparin C (Fig. (**6c**)) was found to inhibit acetylcholinesterase with the IC_{50} 25 μM. [26]. Carinatumins A (Fig. (**6d**)), B (Fig. (**6e**)) and C (Fig. (**6f**)), another three lycopodium alkaloids isolated from the club moss *Lycopodium casuarinoides* were also studied. Among this, carinatumins A and B inhibited acetylcholinesterase with IC_{50} 4.6 and 7.0 μM, respectively, whereas carinatumin C did not show such activity (IC_{50} > 100 μM). Carinatumin A possessing a hydroxyl at C-10 showed less potent inhibition compared with huperzine A (IC_{50} 0.8 μM), while carinatumin B showed inhibition of acetylcholinesterase comparable to that of huperzine B, IC_{50} 8 μM [27].

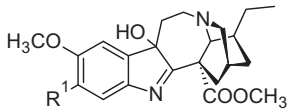
5. Pregnane Alkaloids

Choudhary *et al.* [28] reported that *Sarcococca hookeriana* contains four new pregnane type of alkaloids as shown in (Fig. (**7a -7c**).This has been reported by bioassay guided isolation and the same showed wide variation in AChE inhibitory activity with IC_{50} values ranging from 1.5 to 148.2 μM.

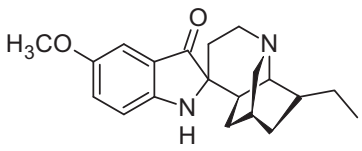
The mechanism of inhibition of acetylcholinesterase enzymes by 23 pregnane-type alkaloids including compounds



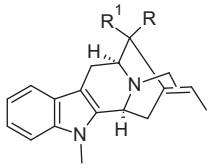
(a) Coronaridine: R=R^1=H,R^2=COOCH$_3$
(b)Voacangine:R=H,R^1=OCH$_3$,R^2=COOCH$_3$
(e) Ibogamine: R=R^1=R^2=H
(f) Ibogaine:R=OCH$_3$,R^1=R^2=H
(g) Ibogaline: R=R^1=OCH$_3$,R^2=H
(h) Desethyl-voacangine:R=H,R^1=OCH$_3$,R^2=COOCH$_3$



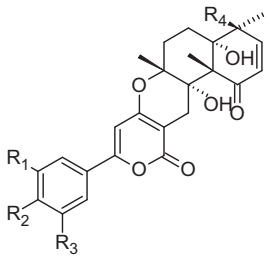
(c) Voacangine hydroxyindolenine



(d) Rupicoline



(i) Voachalotine: R=COOOH$_3$,R^1=CH$_2$OH
(j) Affinisine: R=CH$_2$OH,R^1=H



	R^1	R^2	R^3	R^4
(k)Territrem A (TRA)	-OCH$_2$O-		-OCH$_3$	–CH$_3$
(l)Territrem B (TRB)	–OCH$_3$	-OCH$_3$	OCH$_3$	–CH$_3$
(m)Territrem C (TRC)	-OCH$_3$	-OH	-OCH$_3$	-CH$_3$
(n)Arisugacin A	-H	-OCH$_3$	–H	-CH$_3$
(o)Arisugacin B	-H	-OCH$_3$	–H	-CH$_3$
(p)MB2	-OCH$_3$	-OCH$_3$	-OCH$_3$	-CH$_2$OH
(q)MB2-Succinate	–OCH$_3$	–OCH$_3$	–OCH$_3$	-CH$_2$OCOCH$_2$CH$_2$COOH

Fig. (4). Indole alkaloids.

as shown in Fig. (**7e-7z**), (**7z$_1$**) were isolated from the *Sarcococca saligna* (D. Don) Muell. From the study, it was established through SAR that, the major interaction of the enzyme-inhibitor complexes are due to the hydrophobic and cation - interactions inside the aromatic narrow passage of these cholinesterases.

Khalid *et al.* [29] has reported that the structures with amino nitrogens at C-3 and/or C-20 positions are the most important features that determine the inhibitory potency of these compounds, which are expected to be protonated at physiological pH. This finding has further established the fact that the replacement of any of these two amino substituents with oxygen function, as in compounds 2-dydroxysalignarine-E and salignamine exhibit decrease in the IC$_{50}$ and Ki values against AChE. C-20 amino group may remain close to the aromatic rings of Tyr-70 and Trp- 279, near the top of the narrow passage, thus allowing the substrate to be accommodated at the active site of the enzyme. The most active members of this series were found to be compounds axillaridine-A (Fig. (**7y**)), sarsalignone (Fig. (**7z**)) and sarsalignenone (Fig. (**7z$_1$**)). They have a carbonyl or acetoxy substituents at C-4 (29).

When compared to the compounds containing aliphatic side chain such as tigloyl or sencioyl groups at C-3, benzamide moieties at C-3 of compounds 2β-Hydroxyepipachysamine-D (Fig. (**7g**)), axillarine-C (Fig. (**7j**)) and epipachysamine-D (Fig. (**7u**)) appears to cause a steric hindrance, which may result in some decrease in the activity. The steric effect of C-3 benzamide substituent may be due to the limited possible flexibility of the molecule within the aromatic narrow passage of the enzyme. This study proves that the affinity of the compound which is bulkier than the existing inhibitors could be rationalized by their flexibility [29, 30].

6. Protopine

A total methanolic extract of tuber of *Corydalis ternate* Nakai (papaveraceae) was found to have anti cholinesterase activity. An alkaloid, protopine (Fig. (**8a**)) was isolated and it was found to be responsible for the acetylcholinesterase

inhibitory activity in a dose dependant manner. The IC_{50} value was 50 μM protopine was found to be specific, reversible and competitive inhibitor of acetylcholinesterase. Protopine was also proved to have an efficacy almost identical to a marketed tacrine derivative, velnacrine [31-34]. Similarly, protopine derived from Korean natural resource *Corydalis* speciosa through bioactivity guided isolation, showed acetylcholinesterase activity in a dose-dependent manner, with IC_{50} values of 16.1 μM [33].

7. Steroidal Alkaloids

Methanolic extract of the aerial parts of the *Sarcococca coriacea* (Hook f.) gave two new steroidal alkaloids, shown in (Fig. (**9a -9b**)) and two known compounds funtumafrine C [(20S)-20-(N,N-dimethylamino)-5a-pregna-3-one] (Fig. (**9c**)) and N-methylfuntumine (Fig. (**9d**)). The compounds epoxynepapakistamine-A (IC_{50}>200), funtumafrine C (IC_{50} 45.75±1.122) and N-methylfuntumine (97.61±1.731) were found to have cholinesterase inhibitory activity [35]. Two new cevanine steroidal alkaloids, impericine (Fig. (**9e**)) and forticine (Fig. (**9f**)) along with known bases delavine (Fig. (**9g**)), persicanidine A (Fig. (**9h**)), and imperialine (Fig. (**9i**)) were isolated from the bulbs of *Fritillaria imperialis* Rubra. These steroidal bases also showed anti-acetylcholinesterase and anti-butyrylcholinesterase inhibitory activity [36, 37].

FLAVANONES

Hispidone (Fig. (**10a**)), a new flavanone, has been isolated from *Onosma hispida* WALL. In addition, (2S)-5,2'-dihydroxy-7,5'- dimethoxyflavanone, benzoic acid, and 4-hydroxy benzoic acid are also reported for the first time from this species. Both compounds hispidone and (2S)-5,2'-dihydroxy-7,5'- dimethoxyflavanone were found to be potent cholinesterase inhibitors and inhibited enzymes in a concentration-dependent manner with the IC_{50} values 11.6 and 28.0 μM against AChE and 15.7 and 7.9 μM against BChE, respectively. The activity of these compounds depends upon the structure of the flavanone. The SAR of flavanones shows that the inhibitory efficiency of the compounds depends on the hydroxyl group and their potential [38].

PREGNANE GLYCOSIDE

It is reported that, cynatroside B was isolated from the methanol extract of the roots of *Cynanchum atratum* Bunge (Fig. (**11a**)), and it significantly inhibited the AChE activity. Cynatroside B was found to be the most potent of these isolated pregnane glycoside inhibitors and its mode of AChE inhibition was also characterized. Cynatroside B inhibited AChE activity in a dose-dependent manner and its IC_{50} value was 3.6 μM. The mode of AChE inhibition by cynatroside B was reversible and non-competitive. Therefore, cynatroside B has anti-AChE activity that may ultimately hold significant therapeutic value in curing certain memory impairments observed in Alzheimer's disease [39].

TERPENOIDS

1. Monoterpenoids

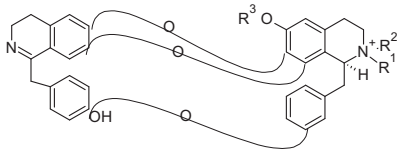
Monoterpenes were first reported to exhibit AChE inhibitory activity by Perry *et al* [40]. The essential oil of *Salvia lavandulaefolia* Vahl. was reported to exhibit uncompetitive, reversible inhibition of AChE in erythrocytes. This study confirmed, the low molecular weight compounds inhibit AChE. Similar reports of AChE inhibitory activity has been exhibited by oil of Salvia (Sage) species. The phytochemical constituents present in the oil (21.5%) such as 1,8 cineole exhibited marked AchE activity at IC_{50} value of 0.06±0.007 mg/ml. From the dose response curve plotted against 1,8 cineole, it was concluded that 1,8 cineole produced only 20% inhibition of AChE, hence it could be reasoned out that the synergistic activity exerted by other phytoconstituents could have provided a high IC_{50} values.

2. Diterpenoids

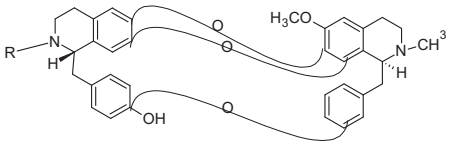
Four inhibitory compounds dihydrotanshinone (Fig. (**12a**)), cryptotanshinone (Fig. (**12b**)), tanshinone I (Fig. (**12c**)) and tanshinone IIA (Fig. (**12d**)) were isolated from the dried roots of *Salvia miltiorhiza* Bunge called as Danshen in China [41]. Among these, the activity of dihydrotanshinone and cryptotanshinone were found to be dose dependant with IC_{50} values of 1.0 and 7.0 μM respectively, while tanshinone I and tanshinone II A showed an weaker inhibition at >50 and >140 μM concentration. The structures of dihydrotanshinone differs only by double bond and dihydro furan ring with tanshinone, which reflects the activity of the compound. Cryptotanshinone and tanshionone IIA show similar difference of activity, so it proves that dihydro furan ring is accountable for the acetylcholinesterase activity. Further, the hydrophobicity clogP values of dihydrotanshinone, cryptotanshinone, tanshinone I and tanshinone IIA were calculated as 2.4, 3.4, 4.8 and 5.8 respectively. This indicates that the compounds have the potential to penetrate blood-brain barrier [41].

Seven abietane and seco-abietane diterpenes were isolated from the methanolic extract of aerial part of *Salvia candelabrum* Boiss [38]. Besides these several terpenes like those shown Fig. (**12e-12k**) were also isolated from *Salvia candelabrum.* The enzyme dependent assay were performed for diterpenes candesalvoquinone (Fig. (**12e**)), candelabroquinone (Fig. (**12f**)), candesalvone B methyl ester (Fig. (**12h**)), candelabrone (Fig. (**12i**)), candesalvone B (Fig. (**12j**)) and candesalvolactone (Fig. (**12k**)). The effects were measured for enzyme dependent assay and was found to indicate similar inhibitory activity, while 12-O-methylcandesalvone B (Fig. (**12g)**) showed weak inhibitory activity. The IC_{50} values were found to be 3.49-10.42 μM [42].

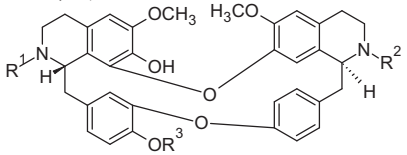
AChE inhibiting properties of natural products buxamine-B (Fig. (**12l**)) and buxamine-C (Fig. (**12m**)) isolated from *Buxus papillosa* C.K. Schneider and *Buxus hyrcana* Pojark species were studied. The buxamine-B and buxamine-C have been found to inhibit AChE noncompetitively in a concentration dependent fashion. The IC_{50} values of buxamine- B and buxamine-C were 74 and 7.5 μM respectively. Buxamine- B and buxamine-C have two amino groups situated at both ends of the cyclopentanophenanthrene ring system. The structures of these compounds differ only at the C-3 and C-20 amino substituents. Structurally similar triterpenoidal compounds lacking the C-3 and C-20 amino groups could not inhibit the AChE (*Torpedo californica*) in concen-



(a): R^1=α-CH_3, R^2= β-O, R^3=H kurramine-2'-β -N-oxide, (IC_{50} 10.0μM)
(b): R^1= β-CH_3, R^2=α-O, R^3=H kurramine-2'-α-N-oxide
(c): R^1=CH_3, R^2= R^3=CH_3 1,2-dehydroapateline

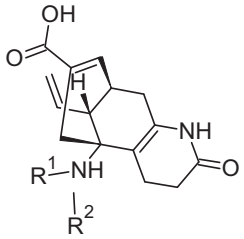


(d) R=H Cocsoline, (IC_{50} 47.6μM)
(e) R=CH_3 Cocsuline, (IC_{50} 12.0 μM)

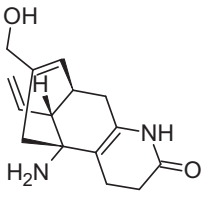


Compound	R^1	R^2	R^3	
Fangchinoline	CH_3	CH_3	CH_3	(f)
Atherospermoline	CH_3	CH_3	H	(g)
Fenfangline E	CH_3	H	CH_3	(h)

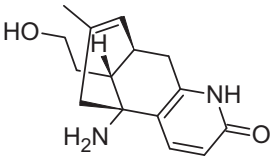
Fig. (5). Isoquinoline derivatives.

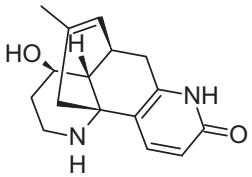


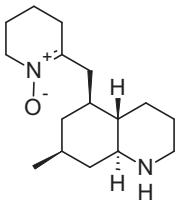
(a) R^1=R^2=CH_3 Lycoparin A
(b) R^1=CH_3,R^2=H Lycoparin B



(c) Lycoparin C , (IC_{50} 25 μM)







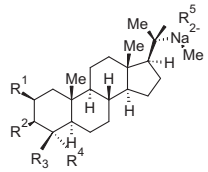
(d) Carinatumins A, IC_{50} 4.6 μM) (e) Carinatumins B, IC_{50} 7 μM) (f) Carinatumins C, (IC50 > 100 μM)

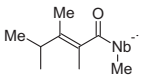
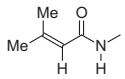
Fig. (6). Lycopodium alkaloids.

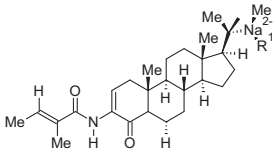
trations up to 1 μM, which demonstrates the importance of these amino groups on the inhibitory activities of these compounds. The distances of C-3 amino nitrogen in both alkaloids and quaternary ammonium of decamethonium are 2.8 and 1.2 A°, respectively. Therefore, it can be predicted that the C-3 tertiary ammonium of buxamine-C is better positioned as compared to secondary amino group of buxamine-B [43,15].

3. Triterpenoids

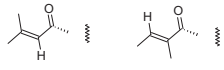
The ethanol extract of *Origanum majorana* L. was screened for its inhibitory activity on acetylcholinesterase. It showed the highest inhibitory effect on AChE *in vitro* among the herbs, edible plants and spices screened. By sequential fractionation of *Origanum majorana* L., the active component was identified as ursolic acid (3 -Hydroxyurs-12-en-28-oic acid) (Fig. (**13a**)). The ursolic acid of *Origanum*



	R^1	R^2	R^3	R^4	R^5	unsaturation
(a)	H		H	H	CHO	
(b)	OAc		H	H	Me	$\Delta^{14,15}$
(d)	H		OAc	H	Me	



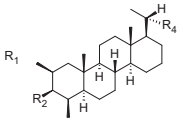
	R^1	unsaturation
(c)	H	$\Delta^{14,15}$



Senecioyl

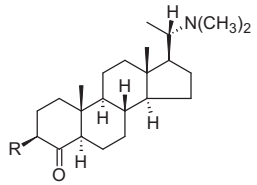
Tigloyl

Cinnamoyl



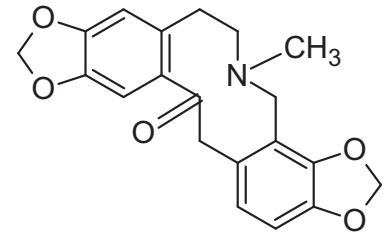
R_3

Compound	R^1	R^2	R^3	R^4	Unsaturation
(e) Salignenamide-C	OH	HN-Tigloyl	OAc	$N(CH_3)_2$	$\Delta^{14,15}$
(f) Salignenamide-D	α-OH	HN-Tigloyl	H	$N(CH_3)_2$	$\Delta^{4,5}$& $\Delta^{16,17}$
(g) 2β-Hydroxyepipachysamine-D	OH	HN-Benzoyl	H	$N(CH_3)_2$	
(h) Salignenamide-E	H	$NCH_3COCH=CCH_3CH(CH_3)_2$	H	$N(CH_3)2$	$\Delta^{16,17}$
(i) Salignenamide-F	H	$NCH_3COCH=CCH_3CH(CH_3)_2$	H	$N(CH_3)2$	
(j) Axillarine-C	OH	HN-Benzoyl	OAc	$N(CH_3)_2$	
(k) Axillarine-F	OH	HN-Tigloyl	OAc	$N(CH_3)_2$	
(l) Sarcorine	H	NHAc	H	$N(CH_3)_2$	
(m) Na-Demethylsaracodine	H	$NHCH_3$	H	NCH_3Ac	
(n) Saligcinnamide	H	CH_3N -Cinnamoyl	H	$N(CH_3)_2$	
(o) Salignenamide-A	H	$NHCOCH=C(CH_3)CH(CH_3)_2$	H	$N(CH_3)_2$	
(p) Vaganine-A	H	HN-Senecioyl	OAc	$N(CH_3)_2$	
(q) 5,6-Dehydrosarconidine	H	$NH(CH_3)$	H	N(CH3)2	$\Delta^{16,17}$
(r) 2-Dydroxysalignarine-E	OH	OCH_3	H	$N(CH_3)_2$	$\Delta^{5,6}$& $\Delta^{16,17}$
(s) Salignamine	H	OCH_3	H	$N(CH_3)_2$	$\Delta^{5,6}$& $\Delta^{16,17}$
(t) 2-Hydroxysalignamine-E	OH	HN-Tigloyl	H	$N(CH_3)_2$	$\Delta^{4,5}$
(u) Epipachysamine-D	H	HN- Benzoyl	H	$N(CH_3)_2$	
(v) Dictyophlebine	H	$NHCH_3$	H	$N(CH_3)_2$	
(w) Iso-N-formylchoneformine	OH	$N(CH_3)_2$	H	NHCHO	
(x) Sarcodinine	H	$N(CH_3)_2$	H	$N(CH_3)_2$	$\Delta^{5,6}$



Compound	R	Unsaturation
(y) Axillaridine-A	HN-Benzoyl	$\Delta^{2,3}$
(z) Sarsalignone	HN-Tigloyl	$\Delta^{5,6}$
(z_1) Sarsalignenone	HN-Tigloyl	$\Delta^{5,6}$ & $\Delta^{14,15}$

Fig. (7). Pregnane alkaloids.



(a) Protopine, IC_{50} 50 μM

Fig. (8). Protopine.

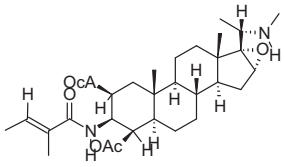
majorana L. inhibited AChE activity in a dose-dependent and competitive/non-competitive type. The Ki value (representing the affinity of the enzyme and inhibitor) of *Origanum majorana* L. ursolic acid was 6 pM, and that of tacrine was 0.4 nM. The IC_{50} value of the active compound is 7.5 nM and tacrine was 1 μM.

From the Km and Vmax value it was found that the compound inhibited the acetyl cholinesterase in a mixed (competitive / non-competitive) manner [44].

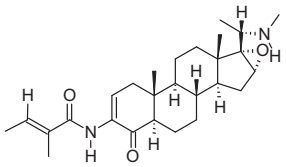
The methanolic extract of the twigs of *Vaccinium oldhami* Miquel, a Korean natural product was found to have significant acetylcholinesterase (AChE) inhibitory activity. Bioassay-guided fractionation of the extract resulted in the isolation of two compounds, taraxerol (Fig. (**13b**)) and scopoletin as active constituents. These compounds were found to inhibit AChE in a dose-dependent manner, and the IC_{50} values of compounds taraxerol and scopoletin were 33.6 (79 μM) and 10.0 (52 μM) mg/mL, respectively. These compounds exhibit good activity due to their low molecular weight, as they can easily reach the site of action by crossing the blood-brain barrier [45].

4. Meroterpenoid

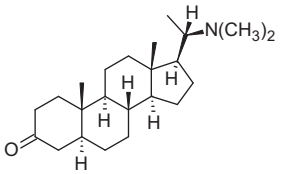
A seven-membered lactone type meroterpenoid isoterreulactone A (Fig. (**14a**)), was isolated from the solid state fermentation of *Aspergillus terreus* [46]. Meroterpenoids such as pyripyropene and oxalicine, and seven- membered lactone type terpenoids such as andilesins, anditomin, fumigatonin, and obacunol have been isolated from fungi [47,48]. Isoter-



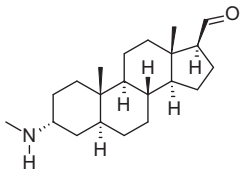
(a) Epoxynepapakistamine-A



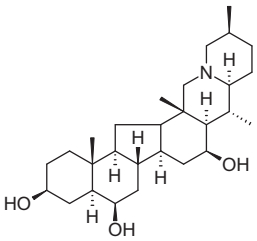
(b) Epoxysarcovagenine-D



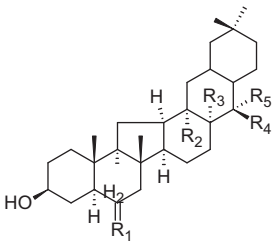
(c) Funtumafrine C, (IC_{50} 45.75±1.122)



(d) N-methylfuntumine, (IC_{50} 97.61±1.731)

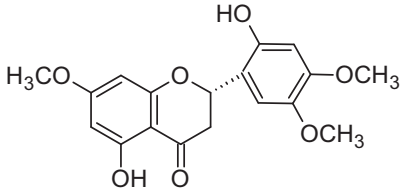


(e) Impericine



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	
(f)	OH / H	β -H	α-H	CH_3	H	CH_3	H	α-H	Forticine
(g)	OH / H	β -H	β -H	H	CH_3	CH_3	H	α-H	Delavine
(h)	OH / H	α-H	β -H	H	CH_3	H	CH_3	α-H	Persicanidine A
(i)	=O	β -H	β -H	OH	CH_3	CH_3	H	α-H	Imperialine

Fig. (9). Steroidal Alkaloids.



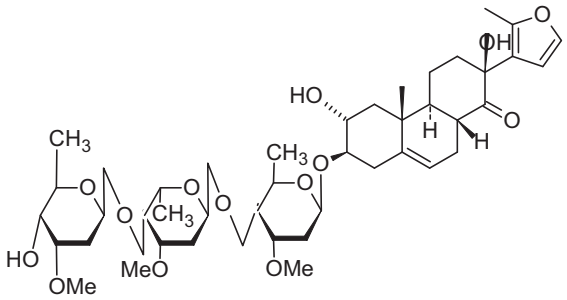
(a) Hispidone, (IC_{50} 11.6 μM)

Fig. (10). Flavanones.

reulactone A inhibited acetylcholinesterase with an IC_{50} value of 2.5 μM while did not inhibit butyrylcholinesterase even at 500 μM. Isoterreulactone A inhibited acetylcholinesterase in a dose-dependent mode with an IC_{50} (μM) value of 2.5. Anti-acetylcholinesterase activity of isoterreulactone A was 10 times weaker than that (0.23 μM) of terreulactone A, which suggested the important role of the ring A in acetylcholinesterase inhibitory activity.

STEROLS

Haloxysterols A (Fig. (**15a**)), B (Fig. (**15b**)), C (Fig. (**15c**)) and D (Fig. (**15d**)) have been isolated from the chloroform soluble fraction of *Haloxylon recurvum* Bunge ex Boiss, along with five known sterols, and all of them were found to inhibit AChE and BChE enzymes in a concentration-dependent manner. They found a non-competitive type



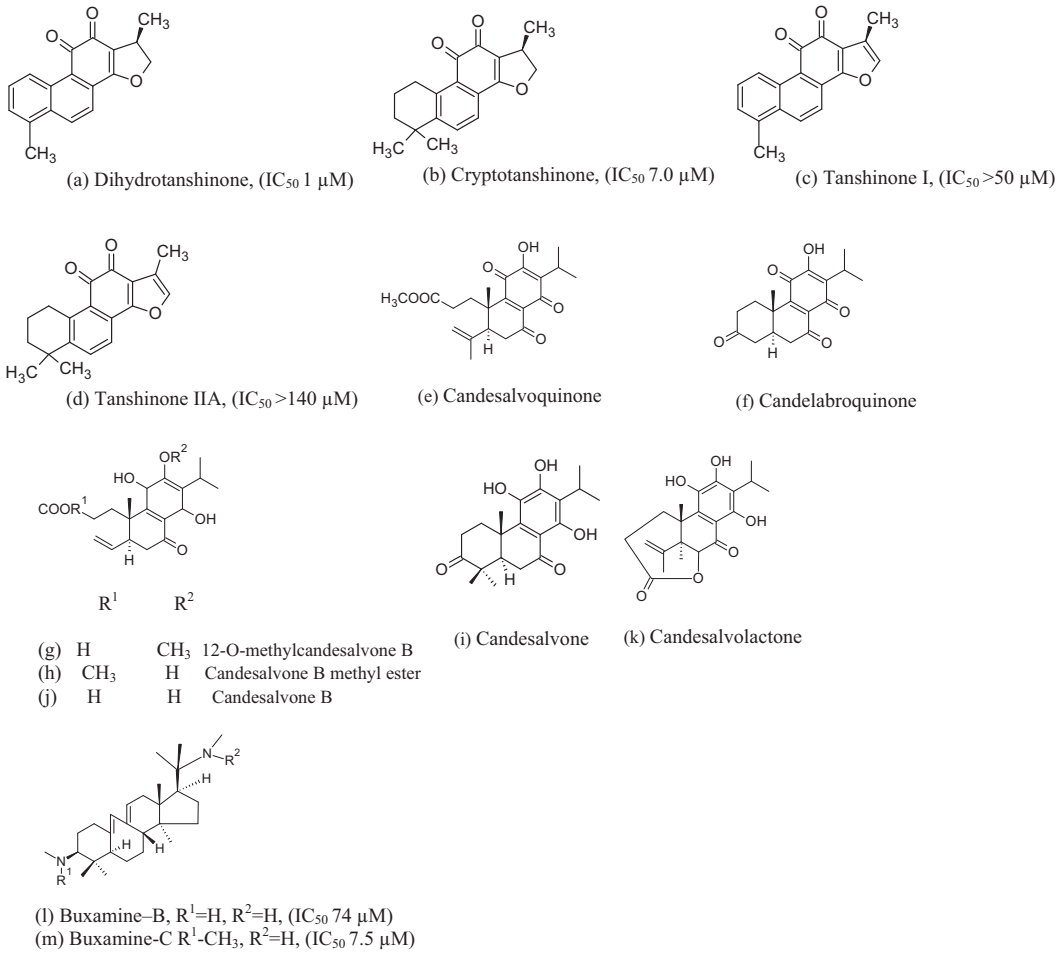
(a) Cynatroside B, (IC_{50} 3.6 μM)

Fig. (11). Pregnane glycoside.

of inhibition. Their similar binding mode is not surprising because all of them have almost similar structures with minor differences only in the basic skeleton of the compounds. The inhibitory potential of these compounds can be the cumulative effect of hydrogen bonding and π-π stacking interactions. The hydroxyl moieties present can be involved in hydrogen bonding with the amino acid residues of the active site of the AChE [49].

STILBENES

Two active stilbene oligomers α –viniferin (Fig. (**16a**)) and kobophenol A (Fig. (**16b**)) were isolated from the un-

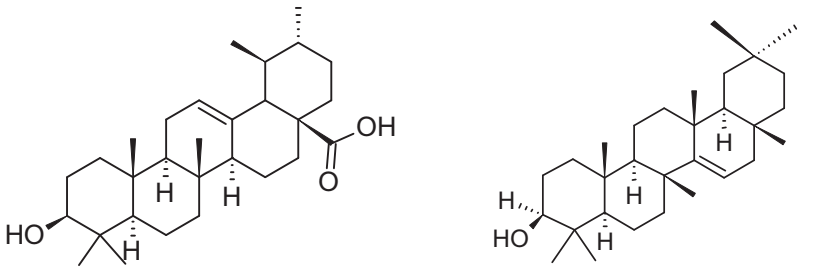


(a) Dihydrotanshinone, (IC_{50} 1 μM)
(b) Cryptotanshinone, (IC_{50} 7.0 μM)
(c) Tanshinone I, (IC_{50} >50 μM)
(d) Tanshinone IIA, (IC_{50} >140 μM)
(e) Candesalvoquinone
(f) Candelabroquinone

	R^1	R^2	
(g)	H	CH_3	12-O-methylcandesalvone B
(h)	CH_3	H	Candesalvone B methyl ester
(j)	H	H	Candesalvone B

(i) Candesalvone
(k) Candesalvolactone
(l) Buxamine–B, R^1=H, R^2=H, (IC_{50} 74 μM)
(m) Buxamine-C R^1-CH_3, R^2=H, (IC_{50} 7.5 μM)

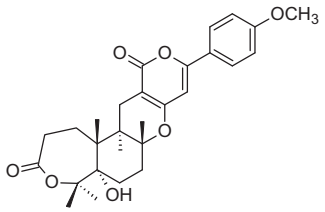
Fig. (12). Diterpenoid.



(a) Ursolic acid, (IC_{50} 7.5 nM) (b) Taraxerol, (IC_{50} 79 μM)

Fig. (13). Triterpenes.

derground parts of *Caragana chamlague* LAMRK (Leguminosae). Both α –viniferin and kobophenol A inhibited AChE activity in a dose-dependent manner, and the IC_{50} values were found to be 2.0 and 115.8 mM respectively. Among the two, the AChE inhibitory activity of α –viniferin was found to be specific, reversible and noncompetitive. α – viniferin has an appropriately bulky structure that masks AChE and was supposed to prevent acetylthiocholine iodide from binding to AChE in a noncompetitive manner. In contrast, in the case of kobophenol A, while it has a bulky structure, its activity may be lowered due to the simple difficulty of accessibility to AChE [50].



(a) Isoterreulactone A,(IC_{50} 2.5 μM)

Fig. (14). Meroterpenoid.

WITHANOLIDES

The withanolides are a group steroids present in, with a lactone-containing side chain of nine carbons attached at C-17. A total of six withanolides were isolated from the whole plant of *Withania somnifera* (L.) Dunal. Their structures were characterized as shown Fig. (**17a-17$_f$**), respectively. Compounds 5b, 6b -epoxy-4b, 17a, 27-trihydroxy-1-oxowitha- 2, 24-dienolide, withaferin-A, 6a,7a-epoxy-5a, 20b -dihydroxy-1-oxowitha- 2,24-dienolide and 5b, 6b -epoxy-4b-hydroxy-1-oxowitha-2,14,24-trienolide displayed inhibitory potential against butyrylcholinesterase, but only compounds withaferin-A, 2,3-dihydrowithaferin-A and 5b ,6b -epoxy-4b -hydroxy-1-oxowitha-2,14,24-trienolide were found to be active against acetylcholinesterase. Compounds (**17a-17f**) were screened for their anti-cholinesterase activity in a mechanism-based assay. Compounds 5b, 6b -epoxy-4b, 17a, 27-trihydroxy-1-oxowitha- 2,24-dienolide (IC_{50} 161.5 μM), withaferin-A (IC_{50} 84.0 μM), 6a,7a-epoxy-5a,20b -dihydroxy-1-oxowitha- 2,24-dienolide (IC_{50} 50.5 μM), and 5b ,6b -epoxy-4b -hydroxy-1-oxowitha-2,14,24-trienolide (IC_{50} 124.0 μM) were found to be active against AChE. Similarly, compounds withaferin-A (IC_{50} 125.0 μM), 2,3-dihydrowithaferin-A (IC_{50} 500. μM), and 5b,6b -epoxy-4b -hydroxy-1-oxowitha-2,14,24-trienolide (IC_{50} 62.5μM) inhibited the activity of BchE significantly [51].

XANTHONES

The methanol extract of *Gentiana campstris* (L.) DC leaves were found to exhibit significant inhibition of AChE activity. Four xanthones as shown in Fig. (**18a-18$_d$**) were found to be responsible for the activity. It was found that all

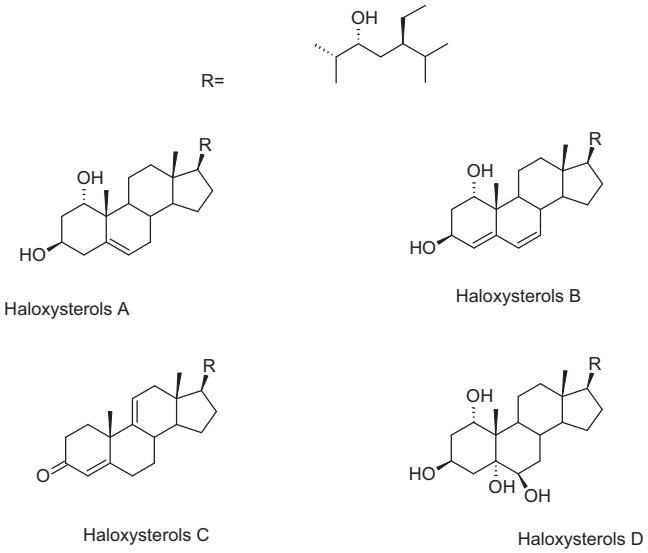
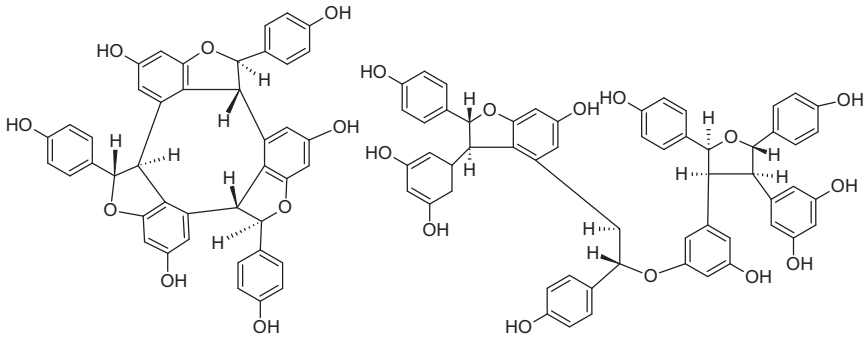
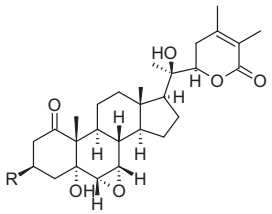


Fig. (15). Sterols.

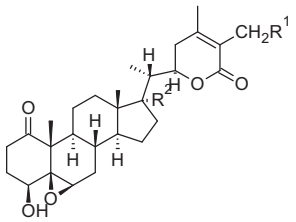


(a) α –Viniferin, (IC_{50} 2.0 mM) (b) Kobophenol A, (IC_{50} 115.8 mM)

Fig. (16) Stilbenes.



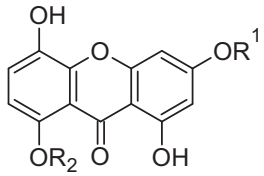
(a) R=OH (6a,7a-epoxy-3b,5a,20b -trihydroxy-1-oxowitha-24-enolide)
(e) R=H, $\Delta^{2,3}$ (6a,7a-epoxy-5a,20b -dihydroxy-1-oxowitha- 2,24-dienolide), (IC_{50} 50.5 μM)



(b) R^1=R^2=OH, $\Delta^{2,3}$ (5b ,6b -epoxy-4b ,17a,27-trihydroxy-1-oxowitha- 2,24-dienolide), (IC_{50}161.5 μM)
(c) R^1=OH, R^2=H, $\Delta^{2,3}$ (withaferin-A), (IC_{50} 84.0 μM)
(d) R^1=OH, R^2=H (2,3-dihydrowithaferin-A), (IC_{50} 50.5 μM)
(f) R^1=R^2=H, $\Delta^{2,3}$, $\Delta^{14,15}$ (5b ,6b -epoxy-4b -hydroxy-1-oxowitha-2,14,24-trienolide), (IC_{50} 124.0 μM)

Fig. (17). Withanolides.

the xanthones inhibited the enzyme at less than 0.5μg and bellidifolin was more active than bellidin and bellidin 8-O-βglucopyrnoside was more active than belllidin 8-O-β-glucopyranoisde suggesting the significance of methoxy group in position C-3 [52, 3].



(a) R^1 =CH_3, R_2 = β-glucopyranosyl
(b) R^1 = R_2 = H
(c) R^1 = CH_3, R_2 = H
(d) R^1= H, R_2 = β-glucopyranosyl

Fig. (18). Xanthone.

ZEATIN

Zeatin (Fig. (**19**)) is a member of the plant growth hormone family known as cytokinins. The methanol extract from *Fiatoua villosa* (Thunb.) Nakai among 100 traditional edible plants tested were showed the most potent inhibitory effect (51%) on acetylcholinesterase *in vitro*. Zeatin was isolated from this extract and tested for acetylcholinesterase inhibitory activity, which could easily reach the site of action after oral or transdermal administration, because the molecule could cross the blood-brain barrier. Thus this active component could slow down the decline of cognitive function and memory in some patients with mild or moderate AD [53].

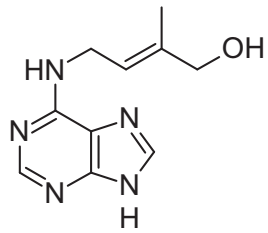


Fig. (19). Zeatin.

CONCLUSION

Alzheimer's disease (AD) is one of the most common forms of dementia affecting a large number of geriatric

population, which is due to the depletion of ACh. The ACh has a very short half-life due to the presence of large amounts of acetylcholinesterase (AChE), an enzyme which hydrolyses the ester bond in the molecule, thus leading to loss of stimulatory activity. Even though amyloid pathway is also a suggested pathway for the disease, AChE pathway is so far the most promising hypothesis. The cholinergic hypothesis of Alzheimer's disease is based on the presynaptic deficits found in the brains of patients with Alzheimer's disease and studies of the role of ACh in animal and human behaviour. Although it is now clear that cholinergic dysfunction may not cause cognitive impairment directly, but rather indirectly, by interfering with attentional processing, the hypothesis predicted that cholinomimetic drugs would improve cognitive function. This prediction was not fully realized with compounds because the emergence of side effects that may have constrained the dosing regimen to subefficacious doses. Poor tolerability seems to be less of an issue for the second generation compounds of the type now being licensed for the treatment of Alzheimer's disease. With improved diagnosis, careful patient selection, and fewer side effects, such compounds will establish its cholinomimetic therapy. Moreover, the emerging relation between neurotransmission and metabolism of two key proteins involved in Alzheimer's disease, APP and tau, raises the possibility that second generation ChE inhibitors may alter disease pathology and progression. However, acetylcholinesterase inhibitors have been accepted to be the most effective for the treatment of AD, up to the present. The effect on the activities of AChE not associated with nervous transmission is more difficult to predict, but inhibitors of these aspects of its function have been predicted as worth exploring in the search for novel drugs.

Ethno medicinal plants have been a huge resource for AChE inhibition activity [3, 54] and thus used widely for the treatment of Alzheimer's disease. The search for the plant derived molecules has accelerated in view of the benefits of these drugs not only in the treatement of AD but in other forms of dementia also [55-58]. More likely is the fact that discovery of AChE inhibitory compounds in traditional remedies, may explain their use in improving memory and other cognitive functions associated with cholinergic stimulation. Several phytomolecules from therapeutically potential plants play major role as AChE inhibitors. These phytomolecules of different chemical classes act by binding to various active sites of the receptor. The structure of these phytomolecules plays a major role in this type of inhibition. The use of the enzyme kinetics for determining the inhibition by isoenzymes with known variations and mutations in the amino acid sequence has helped the comparison of several binding areas. This has been especially facilitated by the use of molecular modeling programs. By using these techniques it has been clarified that the positively-charged nitrogen in these phytomolecules binds to the active site, even though several molecules have potential activities even in the absence of nitrogen. Further this was confirmed by earlier hypotheses about the structural features necessary for a molecule to have such type of inhibitory effects.

The data concerning AChE inhibitors isolated from the plants are complicated by the complexity of their molecular forms. In spite of the deficiencies in the literature concerning the correlation of structure with its activity the overall observation of current status in the research in this regard provide necessary inputs for boosting strategies to develop a valuable and more useful molecule from the ethno medicinal resources. Though a good number of AChE inhibitors have been developed from plants, the present drugs available for the treatment of AD possess some side effects and are effective only against the mild type of AD. Hence it is required to develop a potential phytomolecule from the yet to be explored ethnomedicinal plants. A potential molecule can be developed at ease when the relationship between the structure and their activity is well understood. With this in their mind scientists will have to look towards nature for another diverse molecule with a novel mode of action to tackle this alarming disease.

ACKNOWLEDGEMENTS

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