New Anti-Alzheimer Drugs from Biodiversity: The Role of the Natural Acetylcholinesterase Inhibitors

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative pathology with severe economic and social impact. There is currently no cure, although cholinesterase inhibitors provide effective temporary relief of symptoms in some patients. Nowadays, drug research and development are based on the cholinergic hypothesis that supports the cognition improvement by regulation of the synthesis and release of acetylcholine in the brain. There are only four commercial medicines approved for treatment of AD, and natural products have played an important alternative role in the research for new acetylcholinesterase inhibitors, as exemplified through the discovery of galantamine. This profile conducts us to give in this paper an overview relating the several classes of natural products with anti-cholinesterasic activity as potential templates to the design of new selective and powerful anti-Alzheimer drugs.

Alzheimer's disease (AD) is a neurodegenerative disorder with enormous social and economic impact, which is responsible for 50-60 % of total cases among people over age 65. It is estimated that 15 million people around the world are suffering from some symptoms of AD [1].

In the United States, AD is considered one of the most important health problems due to its impact not only on the individuals, but also on their families, on the health care system and the society as a whole. Although half of the total cases are cared on public health institutions, the remaining patients are taken care at home, involving parents and friends in the health caring. This situation is accompanied by a dramatic psychological, emotional and financial stress. The treatment is very expensive, representing about $100 billion annually in direct and indirect costs of caring, and patients gradually lose motor functions and cognition, being incapable of recognizing close parents on the most advanced stages of the disease [1,2].

Scientists estimate that more than 4 million people have AD and that its incidence duplicates every 5 years beyond age 65 [2,3]. Furthermore, 4 million Americans are 85 years old or older, and in the most industrialized countries, this age group is increasing fast and will number nearly 19 million by the year 2050. Some experts suggest that half of all these people will develop some form of dementia [4].

The German pathologist Alois Alzheimer first described this progressive degenerative process of cognitive and psychomotor functions in 1907. The disease is an age-related irreversible brain disorder that occurs gradually and results in memory loss, behavior and personality changes, and a decline in thinking abilities, which varies from person to person. Patients with AD could live from 8 to 10 years after the first symptoms, despite the fact that the disease can last up to 20 years [1]. The memory and cognitive losses are consequence of the breakdown of the connections between nerve cells in the brain and the eventual death of many of these cells. The brain regions associated to the superior mental functions, particularly the neocortex and hippocampus, are the most affected by the biochemical changes resultant from AD [1,5,6].

Among the most evident causes of the disease genesis are the occurrences of extra cellular deposition of β-amyloid peptide (APP) in senile platelets and the abnormal formation of intracellular neurofibrillary tangles. In healthy neurons, microtubules form structures like train tracks, which guide nutrients and molecules from the bodies of the cells down to the ends of the axon. A protein called TAU, the major component of these tangles, has its structure chemically changed in patients with AD, reflecting in a modification of the structural shape from twists to paired helical filaments. In this situation, TAU no longer holds the railroad tracks together and the microtubules fall apart. The probable reason for this structural alteration in TAU protein seems to be dependent on the abnormal phosphorylation of the serine 199 residue [7], which occurs predominantly in both early and late stages of AD due to over-activation of certain protein kinases [8]. This profile collapses the transport system and may first result in malfunctions in communication between nerve cells, loss of neuronal function and synaptic damage and may later affect short memory, cognition and learning ability, leading to dementia [1,6].

At biochemical level, the cognitive deficit in AD has been correlated with a functional derangement of the central cholinergic system [1,9]. Recent studies also demonstrated

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the reduction in the number of acetylcholine nicotinic and muscarinic receptors (M_2) located at pre-synaptic cholinergic terminations, whereas pos-synaptic muscarinic receptors (M_1 and M_2) in patients with AD were preserved [1]. In this context, the efficacy of cholinergic therapies in this disease validates and supports the cholinergic hypothesis of AD [10,11].

CHOLINOMIMETIC THERAPY FOR AD

The scientific support of the cholinergic hypothesis is correlated with the ability of drugs to restore the cholinergic function, improve cognition and attenuate some behavior effects associated to AD. There are several alternatives to revert the cholinergic deficit and most of them consider the substitution of ACh precursors (lecithin and choline). However, these agents have showed inefficiency in the improvement of central cholinergic activity [1]. Several reports has also investigated the use of cholinesterase inhibitors (IChEs) that can reduce AChE hydrolysis (*e.g.* physostigmine). Recently, therapeutic approaches have explored the involvement of specific muscarinic (M_1) and nicotinic receptors, and muscarinic antagonists as well [1,6]. As a matter of fact, the knowledge concerning the evolution and molecular pathology of AD has showed that the use of IChEs must be the most efficient approach to treat AD [1,7,8,12,13].

One therapeutic approach to AD is the development of direct M_1 postsynaptic muscarinic receptors agonists (Fig. **1**). Stimulation of these receptors was able to produce a cognition improvement in animals. Nevertheless, despite the efforts to the development of M_1 agonist ligands, many of the compounds tested showed low selectivity, and muscarinic side effects due to the activation of M_3 receptors in intestine, urinary bladder and lung. M_1 non-selective agonists may also interact with M_4 and M_5 receptors subtypes in central nervous system (CNS), producing consequences still unknown [14].

Another cholinergic approach would be the development of M_2 postsynaptic muscarinic autoreceptors antagonists (Fig. **1**). Ethnopharmacological data revealed that these receptors blockade lead to increased levels of ACh and an improvement in cognition on animal models. However, in despite of the large number of potent M_2 antagonists reported, little had showed selectivity faced with other muscarinic receptors subtypes [14].

The initial observation that scopolamine-type muscarinic antagonists produce short-term memory deficits led to the development of a proposal in which that the source of the cholinergic deficit in AD was predominantly correlated with the modulation of muscarinic receptors. However, this viewpoint has changed because of some evidences including hystochemical and autoradiographic autopsy studies of brain tissue. Cerebral image studies in patients with AD corroborated such data and showed specific loss of nicotinic receptors in higher extension than the muscarinic ones [12,15,16].

Nowadays, there are many evidences indicating that nicotinic drugs affect memory and learning process. Nicotine and other nicotinic agonists can improve psychomotor cognition function, whereas nicotinic antagonists cause deficiency in cognition. Beside this fact, AD incidence in smokers is lower than that in non-smoker people, and this fact could be related to the increasing of acetylcholinesterase nicotinic receptors (nAChRs) expression levels observed in smokers' brain. Consequently, nicotinic drugs could show chronic and acute effects on cognitive function and, chronic effects could possibly include neuroprotection [15].

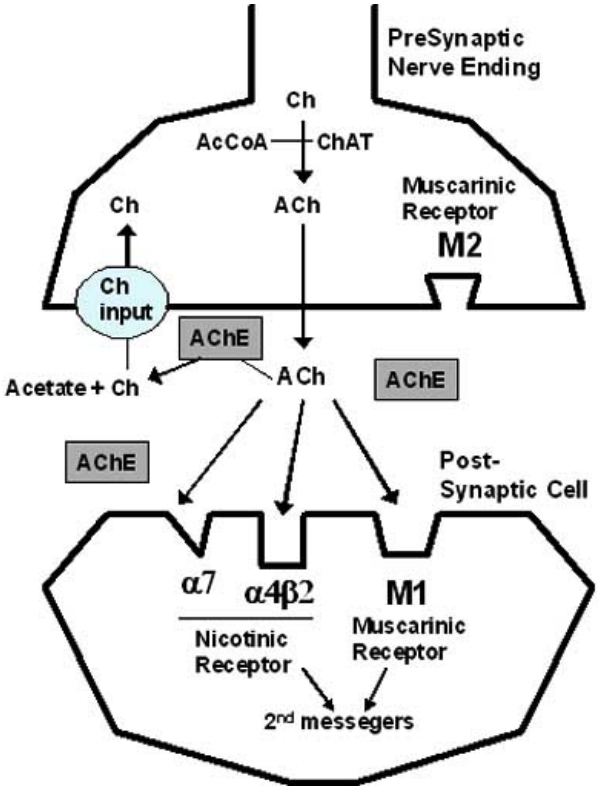


Fig. (1). Schematic vision of cholinergic hypothesis and muscarinic receptors location.

Nicotinic receptors are expressed as various subtypes in mammalian, although α4β7 and α7 are the most prominent and their occurrence includes the post-, pre-, peri- and extra synaptic regions. NAChRs α7 subtypes exert few different functions from that of α4β2 receptors, but show very high Ca^{++} permeability, faster desensitization and differ on pharmacology as well. This difference includes activation by Ch and blockade promoted by α-bungarotoxin, a snake venom toxin [16]. Due to their sensibility to Ch, α7 nicotinic receptors could be chemically excited even after the natural neurotransmitter has being cleaved. For this reason, this receptor subtype can respond not only to synaptic events from ACh liberation, but also to alterations on concentration volume of ACh/Ch. Furthermore, due to their high permeability to Ca^{++}, α7 receptors activation can produce metabotropics answers on excited cells, such as liberation of neurotransmitters controlled by Ca^{++}, stimulation of genetic transcription and protein biosynthesis [12,15,16].

At present time, there are three main approaches being used to balance nicotinic cholinergic deficits: stimulation of ACh synthesis, inhibition of ACh degradation and administration of nicotinic receptors agonists (Fig. **2**) [12,15,16].

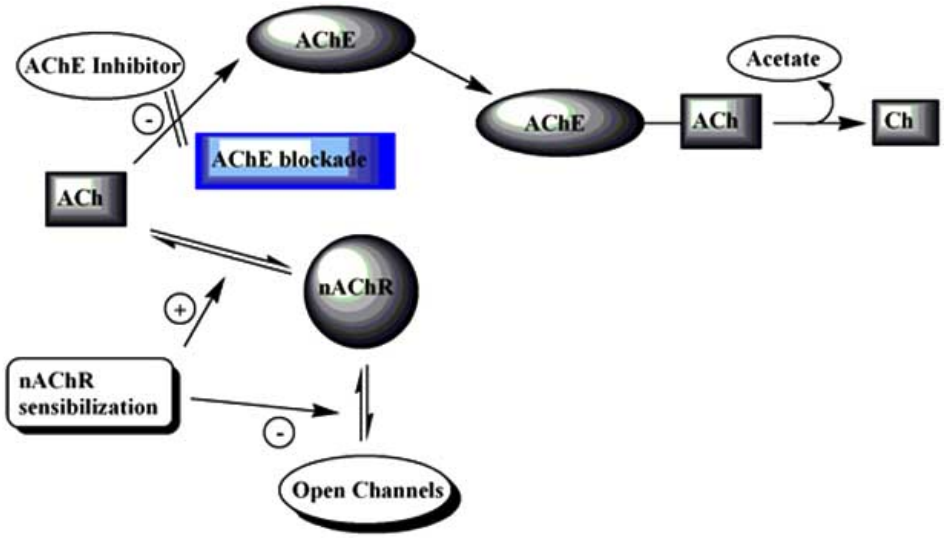


Fig. (2). Illustration of process and intervention manners from liberation to degradation of ACh in AD patients (adapted from Refs. [14,17,18]).

In practice, no therapeutic advance was obtained by ACh precursors administration. Actually, administration of choline esterase inhibitors has been the most common and effective therapeutic alternative [12]. Nevertheless, these inhibitors have limited therapeutic value and the majority of them is not capable of preventing the progression in any level of the disease [15,16].

Many nicotinic receptors agonists remain on pre-clinic and clinic development phases, but even so, they are difficult to dose. In higher levels, they may cause desensitization in bigger extension than the increasing in nicotinic receptors activation. Another point to be solved is the drug transport to the nicotinic receptor-target (specific receptor subtype) in the brain and the receptor subtype selectivity [15].

In consequence of recent advances concerning the physiology, biochemistry and gene expression of nicotinic receptors and their effective action on events related to AD, another strategy to AD treatment is the application of nicotinic allosteric modulators [12,15,16]. Allosteric modulators are substances that interact with receptors by binding sites distinct from that of AChE, nicotinic agonists and antagonists as well [16]. As a result of the association of AD and the reduction of nicotinic neurotransmission, allosteric modulators must potentialize the activity of nicotinic receptors channels responding to ACh. These properties originate a novel class of nAChRs ligands, the allosteric potentiating ligands (APL) [12,16].

COMMERCIAL DRUGS FOR AD TREATMENT

As depicted previously, among several mechanisms that act over cholinergic transmission, inhibition of acetylcholinesterase enzyme (AChE) is nowadays the most effective method to improve cholinergic deficit by increasing the ACh levels in CNS and then ameliorate AD symptoms.

Tacrine (THA, Cognex®, **1**, Fig. **3**) was the first synthetic drug approved by the FDA (Food and Drug Administration) in the United States to treat AD. Tacrine showed moderate effect in the relieving AD symptoms of medium and mild intensity. However, its application becomes limited due to serious side effects, like hepatotoxicity, which has forced the patients to discontinue the treatment [7]. Besides THA, there are three other commercial drugs available in the USA and Europe for AD treatment: donepezil (**2**, Aricept®), rivastigmine (**3**, Exelon®) and galantamine (**4**, Reminyl®) (Fig. **3**). Among these drugs, compounds **1**, **2** and **4** are reversible AChE inhibitors (AChEIs). Galantamine (**4**) is a natural product recently approved by the FDA that has been used as a prototype to the anti-cholinesterase drug development [5,7].

THE SEARCH FOR NEW CANDIDATES FROM NATURAL ORIGIN FOR AD TREATMENT

The structural diversity of known AChEIs and the possibility of exploring distinct modes of action have stimulated phytochemical studies with diverse plant and microorganisms species that can furnish new anti-cholinesterasic models. In this context, various plant and microorganisms species have been studied due to their popular uses or ethnobotanic data. One of the most disseminated examples of phytomedicines is the gingko extract.

Ginkgo biloba (Ginkgoaceae) is a fossil tree used for centuries in Chinese folk medicine to improve alertness. Nowadays, ginkgo is probably the most scattered plant extract used specifically to enhance cognition. Its use is prevalent in Europe, where it was recently approved by the *German Bundesgesundheit Association* for treatment of dementia [17]. Most of the results suggest that the property of improving cognition function is associated with the assays and the use of a specific standardized extract referred to as EGb 761. The most common experiments that demonstrated the enhancement in cognition have been made by learning and memory tests. Moreover, improvements were evident among patients treated with ginkgo and submitted to tests of various cognitive abilities such as attention, short-term memory, and reaction time related to

choosing something. The therapeutic profile for ginkgo, determined by this analysis, is comparable to that previously reported for the drug donepezil, which is currently the drug of choice for treating AD [17].

Some results obtained from short-term memory, attention and learning tests are not reproducible interpopulations. Moreover, many results are published in limited circulation journals, which make the information access difficult. In most of the cases, *in vivo* experiments on animals and humans were restricted to a small number of individuals, which makes a whole conclusive evaluation of the results extremely hard.

Apparently, many of the CNS protective effects associated with the chronic use of ginkgo extracts are related to the presence of terpenes and flavonoids with antioxidant and anti-inflammatory properties. These substances can act in different ways, contributing to the integrity of the neuronal tissue: a) by inhibiting the activity of superoxide dismutase and monoamine oxidase, two enzymes that contribute to the production of free radicals in the brain and body; b) by scavenging free radicals that could cause neuron damage and thereby retard age-related changes in brain and other functions; c) by reducing the release of arachidonic acid, a particular toxic by-product of lipid metabolism that appears in the brain following an ischemic episode [17].

The imperative necessity to conduct the search for new compounds from natural origin more objective and less expensive lead to the development of many useful techniques to chemical and biochemical screening and selection of extracts, fractions and pure substances of bio/pharmacological interest. In the case of AChEIs, two bioautographic tests were developed for use in TLC experiments [18]. Marston & co-workers [19] utilized an azoic dye to identify AChE activity over 1-naphtyl acetate; in the other case, Rhee & co-workers [20] suggested the utilization of 5,5'-dithiobis-(nitrobenzoic acid) (Ellman's reagent) to visualize the enzymatic activity. It seems that the only restriction to the use of Ellman's reagent is the low visual detection limit, because in both cases white inhibition spots are formed over a blue (Marston's technique) and a yellow (Rhee's technique) dyed plate [18].

A recent work with Brazilian plants [21] utilized the bioautographic test developed by Rhee & co-workers and the microplate Elmann's test [21,22] to identify extracts that could be constituted by compounds able to inhibit AChE. In this work, the authors evaluated 58 extracts from 30 plant species and considered as a selection criteria for further chemical fractionation only those that showed at least 50% of inhibition of AChE. *Paullinia cupana, Amburana cearensis* and *Lippia sidoides* were the plants that showed the better results, inhibiting 65-100% of AChE activity in both bioassays. In the case of *Paullinia cupana*, a positive effect on enhancing memory after chronic and acute administration was evident. The bio-guided fractionation of *A. cearensis* and *L. sidoides* extracts led to the isolation of 12 coumarins to date [21], which made evident the utility of this kind of assay to the bioprospection of new acetylcholinesterase inhibitors.

Galantamine (**4**) is an alkaloid isolated from various plant species of the Amaryllidaceae family, which showed to be a selective, reversible, competitive and long duration AChE inhibitor. This substance was discovered by Bulgarian and Soviet researchers in 1953 and its best source is *Galanthus worownii*, a relative of the common snowdrop, which is limited to the Caucasus and certain regions of Bulgaria [23]. Its therapeutic effects remain even after the end of the treatment [9]. These effects are a consequence of a dual mechanism of action: inhibition of AChE and interaction with nicotinic receptors in the brain. Modulation of these receptors amplifies the ACh signal neurotransmission [7] and represents a great advance in drug design for AD treatment [12,13]. Galantamine acts by bonding to the cerebral AChE active site and also by activating pre- and pos-synaptic nicotinic receptors. As a consequence, there is an increasing in the release of neurotransmitters like ACh and glutamate, and direct stimulation of neuronal function [24].

The galantamine content within the Amaryllidaceae can vary widely among species, from trace amounts to 0.5% (dry weight-DW). A few examples of high-galantamine containing species are *Leucojum aestivum* (0.3%), *Galanthus woronowii* (0.9%) and *Ungernia victoris* (0.6%) [25]. More recently, an investigation conducted by Lopez & co-workers [25] determined the distribution and seasonal variations of galantamine and four other alkaloids in different organs of *Narcissus confusus*. The aim of that research was to evaluate the possibility of starting a new crop of *N. confusus* plants for the large scale production of galantamine, by discovering in which developmental stage and organ occurs the main accumulation of that metabolite and other four related alkaloids, *i.e. N*-formylnorgalantamine, haemanthamine, homolycorine and tazetine. Alkaloids were present in all organs (bulbs, leaves, stems, flowers and roots) and developmental stages of ontogenic cycle, with the exception of haemanthamine, which did not occur in senescent flowers [25].

In general, galantamine was found to be the main alkaloid in all organs of *N. confusus*, while its higher accumulation was found in the bulbs. Depending on the phase of the ontogenic cycle, galantamine content ranged from 2.32 mg/g (DW) in the roots (phase IV) to 25.42 mg/g in the bulbs (phase I). The proportion of galantamine between the aerial parts and the underground ones in senescent plants was 2.6-fold higher in the aerial parts [25]. The most interesting result showed the higher level of galantamine accumulated in the aerial parts of the plant (leaves, stems and flowers), at the end of the ontogenic cycle (phase IV), which can reach up to 2.5% referred to dry matter. This percentage can even rise up to 4.4% (DW) when the contents of both galantamine and *N*-formylnorgalantamine are considered jointly, noting that the latter alkaloid can be easily transformed into galantamine. The results of this research clearly indicated that it is of higher interest to start agronomical studies for the rational cultivation of this wild species, which would allow the aerial parts of the plant to be harvested when they are senescent and accumulate a high content of galantamine-type alkaloids, thus leaving the bulbs for the next growth season [25].

Galantamine has been investigated for Alzheimer's disease by many pharmaceutical companies, including Shire

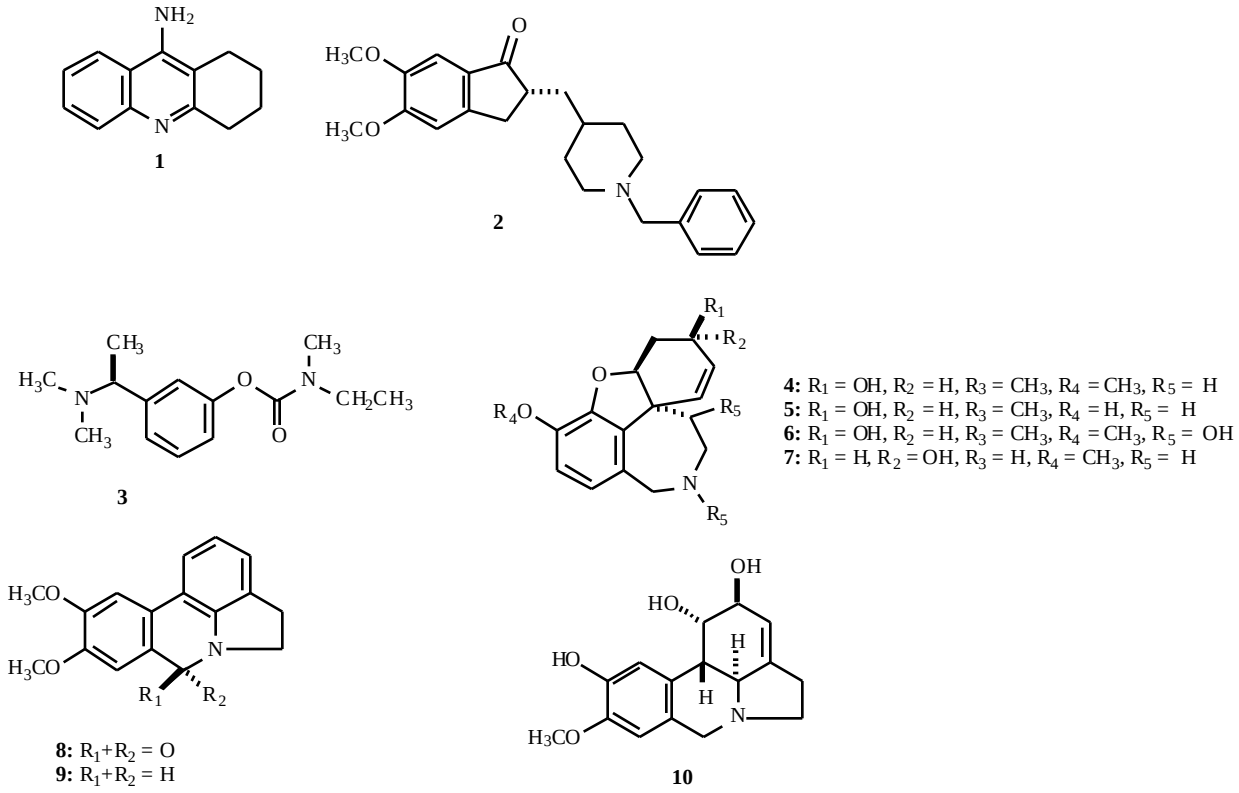


Fig. (3). Commercial drugs for AD treatment and some lycorine-type alkaloids isolated from *Narcissus* species.

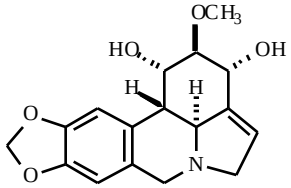
Pharmaceuticals and Janssen Pharmaceutica, and remained a critical resource available at prices around $40,000 per Kg until 1997 [23], when Sanochemia Pharmazeutika optimized an industrial process with about 18% overall yield. While Janssen's Reminyl, launched for treatment of Alzheimer's disease in the US and Europe, is still based on galantamine extracted from *Narcissus* spp, the fully synthetic version is expected to replace natural galantamine [23].

Another alkaloid, sanguinine (9-*O*-demethylgalantamine, **5**), isolated from *Narcissus* L. (Amaryllidaceae) was up to 10-fold more active than galantamine in *in vitro* assays. The search for other AChEIs from this plant genus led to the isolation of two additional bioactive galantamine derivatives, 11-hydroxygalantamine (**6**) and epinorgalantamine (**7**). Several structural lycorine-type alkaloids were isolated from this genus and the more active constituents were oxoassoanine (**8**), assoanine (**9**) and pseudoassoanine (**10**) [9] (Fig. **3**).

In a preliminary screening for AChE inhibitory activity, the ethanolic extract of *Narcissus* 'Sir Winston Churchill' showed mild activity in the microplate assay [26]. The combination of centrifugal partition chromatography (CPC) and on-line HPLC-UV/MS-biochemical detection techniques led to the isolation of a new active compound, ungiminorine (**11**) (Fig. **4**), together with galantamine (**4**). The IC_{50} value of ungiminorine (**11**) was 86 ± 7 μM, while galantamine (**4**) was more than 80-fold more potent (IC_{50} value of 0.98 ± 0.07 μM) [26].

Further twenty Amaryllidaceae alkaloids of different ring types (compounds **12**–**32**, Fig. **4**) isolated from *Crinum moorei* [27], *C. macowanii* [28], *C. bulbispermum* [29] and *Cyrtanthus falcatus* [30], were recently evaluated for their AChE enzyme inhibitory activity [31]. Galantamine (**4**) and cherylline (**32**) were used as positive control and the alkaloid 1-*O*-acetyllycorine (**31**) was the most potent inhibitor (IC_{50}: 0.96 ± 0.04 μM). Crinine (**12**, IC_{50}: 461 ± 14), crinamidine (**15**, IC_{50}: 300 ± 27 μM), epivittatine (**19**, IC_{50}: 239 ± 9 μM), 6-hydroxycrinamine (**21**, IC_{50}: 490 ± 7 μM), *N*-desmethyl-8α-ethoxypretazettine (**28**, IC_{50}: 234 ± 13 μM), *N*-desmethyl-8β-ethoxypretazettine (**29**, IC_{50}: 419 ± 8 μM) and lycorine (**30**, IC_{50}: 213 ± 1 μM) had weak activity, indicating that lycorine-type alkaloids were the most active compounds with 1-*O*-acetyllycorine (**31**) exhibiting inhibitory effects two-fold more potent than that of galantamine [31] in the Ellman's test.

Studies of several plant species of current use by folk medicine in China and Turkey revealed many bioactive alkaloids. An important example is *Huperzia serrata* (sin. *Lycopodium serratum*), which provides a tea prescribed for centuries in China for the treatment of fever and inflammation. Phytochemical studies of this plant led to the discovery of huperzine A (**33**, Fig. **5**), an interesting candidate for the treatment of CNS disorders and epilepsy, whose effects diminish neuronal death caused by high concentrations of glutamate. It is a selective and very potent AChEI, whose systemic use increases the release of ACh, dopamine and norepinefrine. Increased concentration of ACh persists for about 6 hours after administration and practically do not affect butyrylcholinesterase (BuChE) [32,33]. The continuing interest in new active metabolites from *H. serrata* led Tan & co-workers to isolate two new lycopodium-type alkaloids, huperzine P (**34**) [34] and huperzine R (**35**) [35] (Fig. **5**). Neverthless, these two compounds were less active than huperzine A (**33**).



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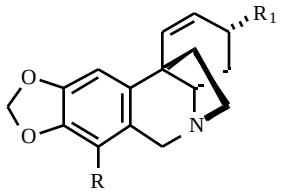
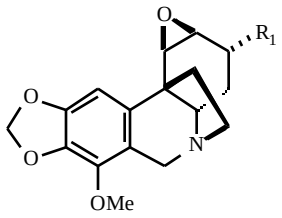
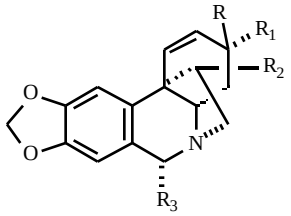


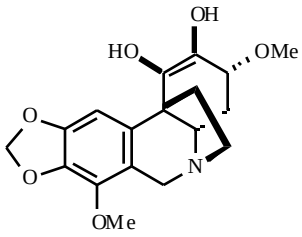
12-R = H, R_1 = OH
13-R = H, R_1 = OMe
14-R = OMe, R_1 = OH



15-R = OH
16-R = OMe



17-R = H, R_1 = OH, R_2 = OH, R_3 = H
18-R = R_3 = H, R_1 = OAc, R_2 = OH
19-R = H, R_1 = OH, R_2 = R_3 = H
20-R = H, R_1 = OMe, R_2 = OH, R_3 = OH (α, β)



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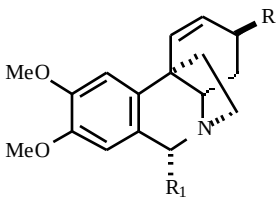
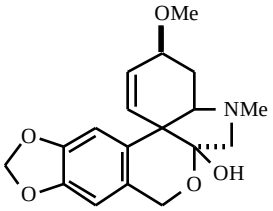


23-R = OMe, R_1 = OH (α, β)
24-R = OH, R_1 = H
25-R = OMe, R_1 = H



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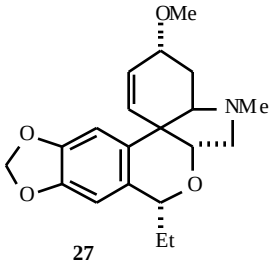

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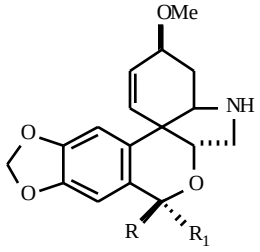
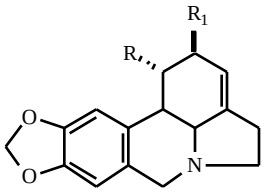


28-R = H, R_1 = OEt
29-R = OEt, R_1 = H



30-R, R_1 = OH
31-R = OAc, R_1 = OH

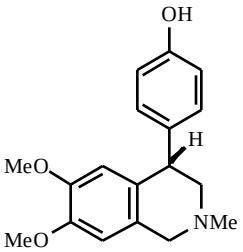


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Fig. (4). Some active Amaryllidaceae alkaloids isolated from *Narcissus*, *Crinum* and *Cyrtanthus* plant species.

The results obtained from huperzine A stimulated Orhan & co-workers [36] to study other five *Lycopodium* species, looking for AChEI metabolites. After a preliminary evaluation by Ellman's spectrophotometric method [22], an extract from aerial parts of *L. clavatum* was selected and a bio-guided fractionation resulted in the isolation of α-oncocerin (**36**). The results of AChE activity showed that α-oncocerin (IC_{50}: 5.2 μM) was better than donepezil at concentrations of 1 and 3 mg/mL and was practically equipotent at 5 mg/mL. However, at any dose, the potency of galantamine was supplanted [36].

Hirasawa & co-workers [37] isolated sieboldine A (**37**) from the club moss *Lycopodium sieboldii*. It is a new alkaloid with an unprecedented fused-tetracyclic ring system and it inhibited AChE from electric eel with an IC_{50} value of 2.0 μM, which was comparable to that (IC_{50} 1.6 μM) displayed by (±)-huperzine A.

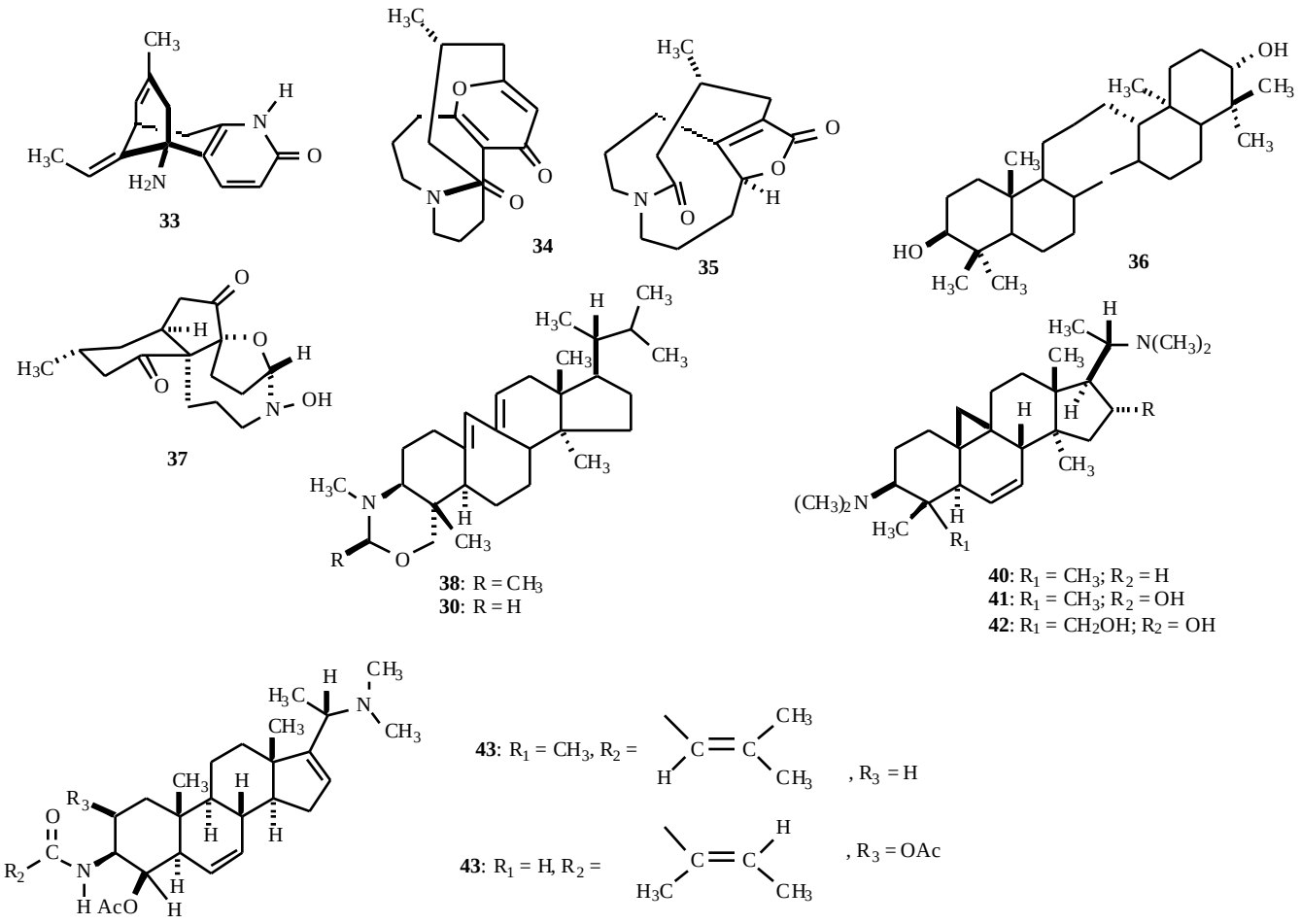


Fig. (5). Some bioactive alkaloids isolated from *Lycopodium* and *Buxus* species.

Some triterpene alkaloids were isolated from *Buxus hyrcana.* Among these, homomoenjodaramine (**38**) and moenjodaramine (**39**) showed the most promising results as AChEIs [38]. From the same family, *B. papillosa* furnished another three steroidal selective AChEI alkaloids, cycloprotobuxine C (**40**), cyclovirobuxein A (**41**) and cyclomicrofilin A (**42**) [39] (Fig. **5**).

Aconitum species (Ranunculaceae) produce diterpenoid and norditerpenoid alkaloids that are generally of the aconitine and lycoctonine types. *Aconitum falconeri* is widely distributed in Pakistan, the Himalayan and Garhwall regions of India, and it is used in indigenous medicine for its cardiotonic and sedative properties. The roots of this plant are frequently employed in the treatment of rheumatism and neuralgia [40]. Recently, Rahman & co-workers isolated faleoconitine (**43**, IC_{50}: 293 ± 3.8 μM, Ellman's test) and pseudaconitine (**44**, IC_{50}: 278 ± 3.6 μM, Ellman's test) (Fig. **6**) as mild anti-acetylcholinesterase metabolites from the roots of *A. falconeri* [40].

The traditional Chinese medicine has used the dried root of *Salvia miltiorhiza* for the treatment of cerebrovascularand CNS deterioration in old age for over a thousand years [41]. The acetone extract from the roots of this plant furnished four inhibitory compounds, dihydrotanshinone (**45**), cryptotanshinone (**46**), tanshinone I (**47**) and tanshinone IIA (**48**) (Fig. **6**). The inhibitory activities of compounds **45** (IC_{50}: 1.0 μM) and **46** (IC_{50}: 7.0 μM) in the Ellman's test, were dose dependent. These compounds, which were the major components of the extracts of dried roots, *i.e.* 0.054% w/w and 0.23% w/w, respectively. In the mixture they appear to be less active than as isolated compounds [41].

Zeatin (**49**, Fig. **6**) was first described as a growth inductor agent for plantlets, and was isolated from *Fiotoua villosa*, whose methanolic extract was selected after AChE activity screening [42]. Pure compound **49,** was tested according to the colorimetric Ellman's test [20], using acetylcholine iodide as a substrate and PC12 cell cultures as enzyme source, and inhibited AChE activity in a dose-dependent mode with IC_{50} value of 1.09×10^{-4} M [42].

Some glycoalkaloids present in high concentrations in tuber potato (*Solanum tuberosum* L.) are responsible for many cases of food poisoning. Observation of intoxicated patients revealed symptoms such as mental confusion, depression and weakness. These effects were attributed to AChE inhibition by α-solanine (**50**) and α-chaconine (**51**) (Fig. **6**), which correspond together for 95% of total glycoalkaloids present in *S. tuberosum* [43].

Other plant families, like Myristicaceae, Apocynaceae and Papaveraceae, were also investigated for metabolites with AChE inhibitory activity. The bark of *Iryanthera megistophylla* (Myristicaceae) furnished megislignan (**52**) and megislactone (**53**) (Fig. **7**) [44]. These two new compounds exhibited anti-acetylcholinesterase activity, with

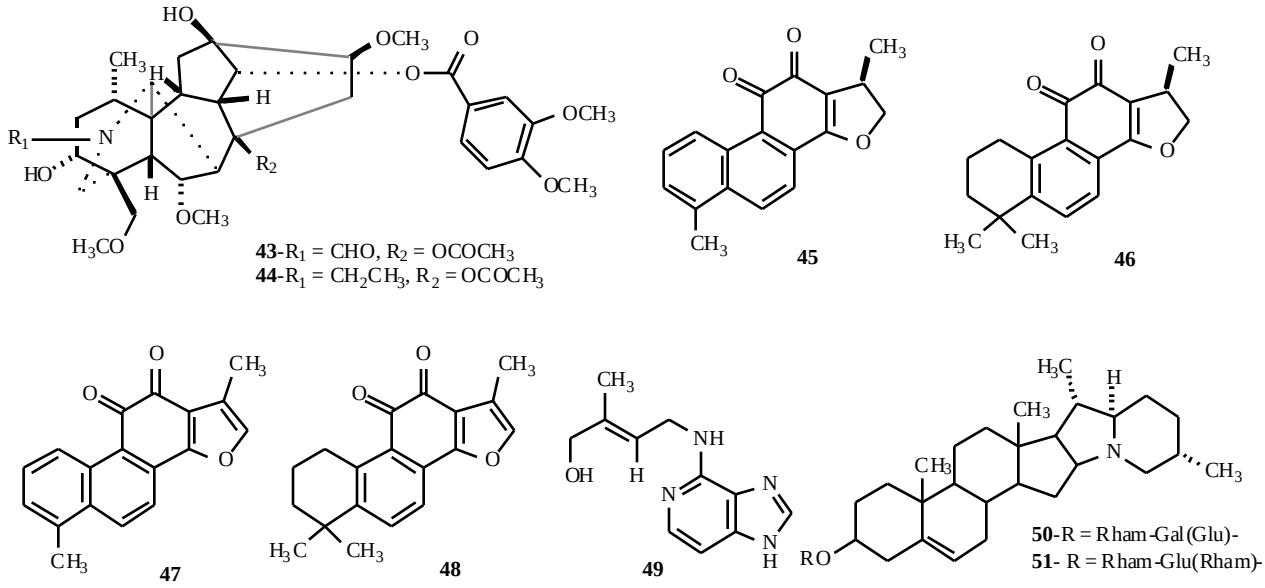


Fig. (6). Norditerpenoid alkaloids, diterpenoids, Zeatin and glycoalkaloids as AChE inhibitors.

52 and **53** showing inhibition rates of 67.6 ± 3.4% and 77.4 ± 6.1%, respectively. Each of them was tested at the concentration of 0.8 mg/mL, and both activities were considered moderate when compared to the 100.0 ± 0.0% inhibition displayed by galantamine [44]. From *Haplophyton crooksii* (Apocynaceae) were isolated 15 indole alkaloids [45-47]. All these compounds showed *in vitro* inhibition of AChE in the Ellman's test, at different concentration levels, but the most active metabolites, 10-methoxy-*N1*-methylpericyclivine (**54**, IC_{50}: 1.35 μM) and 16-decarbomethoxyvinervine (**55**, IC_{50}: 0.57μM) (Fig. **7**) were about 90 and 38 times less active than the positive control, eserine (physostigmine, IC_{50}: 0.015 μM) [45].

During the screening of natural products extracts in search of anticholinesterase activity, Kim & co-workers [48] found that a total methanolic extract from the tuber of *Corydalis ternata* (Papaveraceae) showed interesting inhibitory effects on acetylcholinesterase enzyme. Further fractionation of this extract led to the isolation of protopine (**56**) (Fig. **7**) [48]. This alkaloid inhibited AChE activity in a dose-dependent manner, with an IC_{50} value of 50μM. This inhibitory activity was specific, reversible and competitive. In addition, when mice were pretreated with protopine, the compound significantly alleviated scopolamine-induced memory impairment [48].

Microorganism cultures, especially fungus of several families and genus have been systematically studied as important sources of new useful drugs in the treatment of serious diseases as cancer, yellow fever, bacterial infections and so on.

Otoguro, Kuno & co-workers [49-51], looking for candidates for new drugs capable reestablishing the neurotransmission system through systematic screening of natural products produced by fungus, discovered a new class

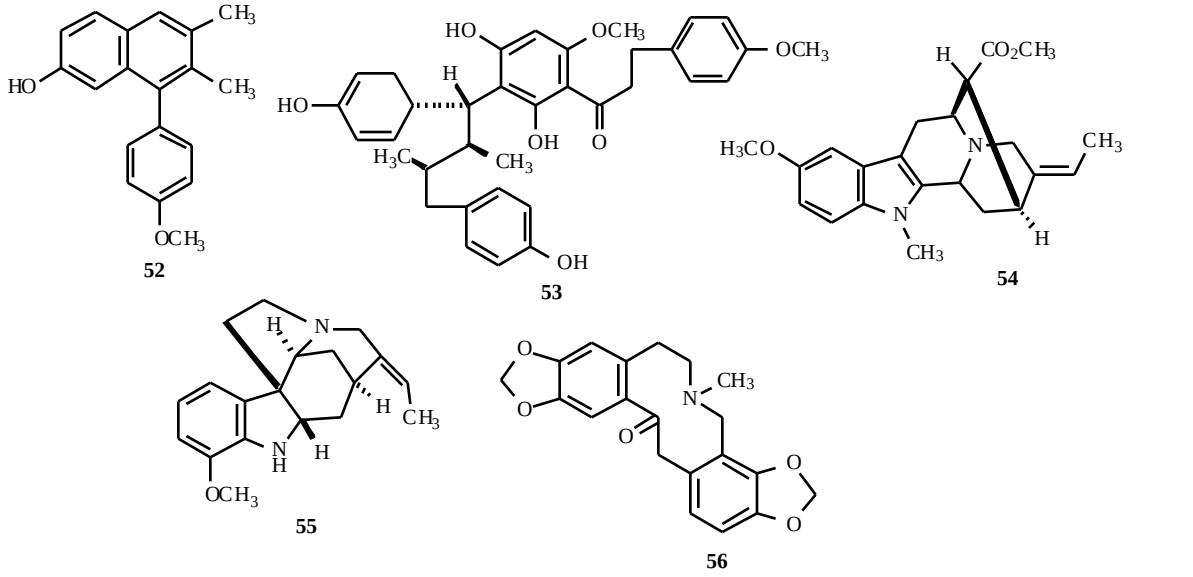


Fig. (7). Some active compounds isolated from Myristicaceae, Apocynaceae and Papaveraceae species.

of AChEIs, the arisugacins. From cultures of WK-4164 and FO-4259 soil fungus, cyclophostine (**57**), arisugacins A (**58**) and B (**59**) were obtained, besides the known territrems B (**60**), C (**61**) and cyclopenin (**62**, Fig. **8**). Enzymatic inhibition was measured by the Okabe's method, which incorporated some modifications that permitted the comparison between AChE and BuChE inhibition [49]. Among the isolated metabolites, arisugacins A (IC_{50}: 1.0 nM) and B (IC_{50}: 25.8 nM) did not inhibit BuChE, even at concentrations 200.000-fold higher to that of 50% AChE activity inhibition, which demonstrates their high selectivity [49-51].

For territrems B and C, the selectivities were much lower, despite the IC_{50} values (7.6 nM and 6.8 nM, respectively). Cyclopenin (**62**) was the less active (IC_{50}: 2040 nM), and although it was very selective, it did not inhibit BuChE at concentrations 2000-fold higher than its IC_{50}. Despite being very potent (IC_{50}: 1.3 nM), cyclophostine (**57**) was the substance that showed less selectivity, able to inhibit BuChE at doses 35-fold higher than its IC_{50} [49-52].

The incubation of territrem B (**60**) with rat liver microsomes led Peng [53] to produce four metabolites. The minor constituent of these, 4α-(hydroxymethyl)-4α-demethylterritrem B (**64**) (Fig. **8**), was evaluated together with compound **60** for inhibitory activity on eletric eel AChE. The IC_{50} value of **60** was 0.0026 μM and the IC_{50} value of **64** was 0.00423 x 10^{-3} μM, indicating that the metabolite **64** is 68 times more potent than **60** [53].

Territrems A (**63**), B (**60**) and C (**61**) were isolated from cultures of *Aspergillus terreus*, and despite the low selectivity showed by the studies of Otoguro & co-workers [51], territrem B was almost 20-fold more potent than neostigmine in the inhibition of AChE activity. These results have stimulated Peng [54] to prepare the territrems derivatives **65-70** (Fig. **8**) for a structure-activity relationship study. Evaluation of AChE activity inhibition by the Ellman's method [22] did not show potency increasing for any of the semi-synthetic territrems derivatives. However, the results permitted to recognize the C-2 double bond, C-1 carbonyl and the intact pirone subunit as indispensable pharmacophoric groups to the anticholinesterasic activity of this class of compounds [54].

The interest in AChE inhibitors from microbial metabolites led Kim & co-workers [55] to investigate the cultures of a new fungus, *Penicillium citrium* 90648. From the solid state fermentation of this microorganism there were isolated diastereoisomers quinolactacin A1 (**71**) and

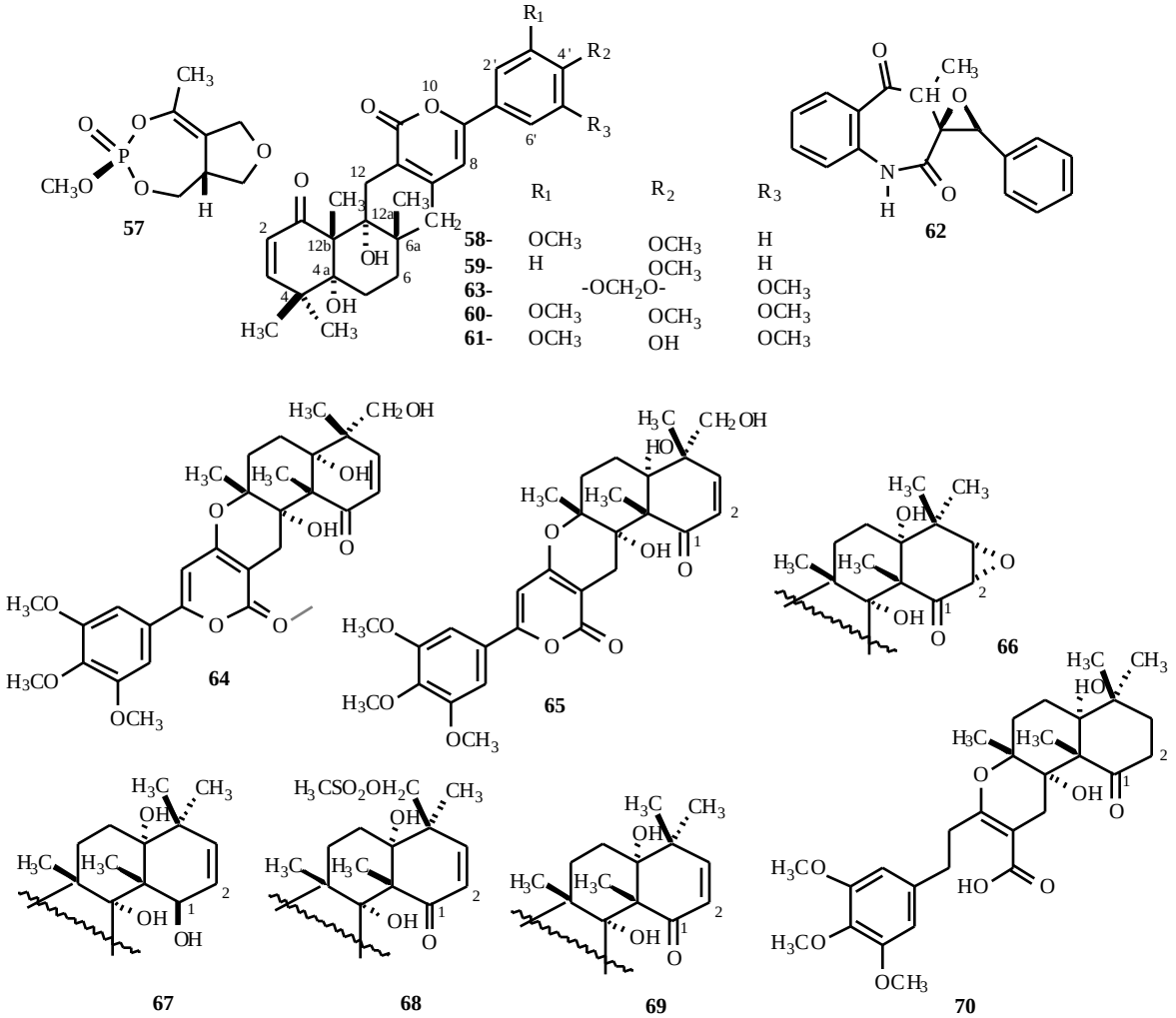


Fig. (8). Some AChEIs isolated from fungus and territrems semi-synthetic derivatives.

quinolactacin A2 (**72**) (Fig. **9**). Evaluation of these substances by the Ellman's method showed that isomer **71** (IC_{50}: 19.8 μM) was 14-times more potent than its diastereoisomer, *i.e.* compound **72** (IC_{50}: 280 μM) in a dose-dependent manner. Moreover, the eutomer **71** showed competitive and selective inhibitory activity over AChE versus BuChE ($IC_{50\ BuChE}$: 650 μM) in comparison with tacrine (**1**), used as a positive standard inhibitory agent in all assays ($IC_{50\ BuChE}$: 0.006 μM, $IC_{50\ AChE}$ 0.12 μM, very low selectivity) [55].

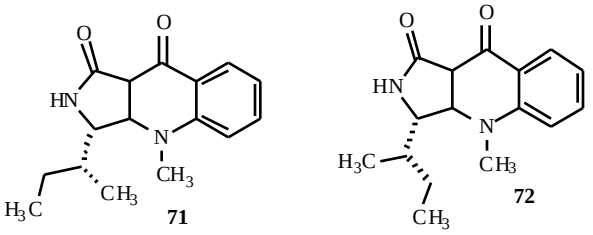
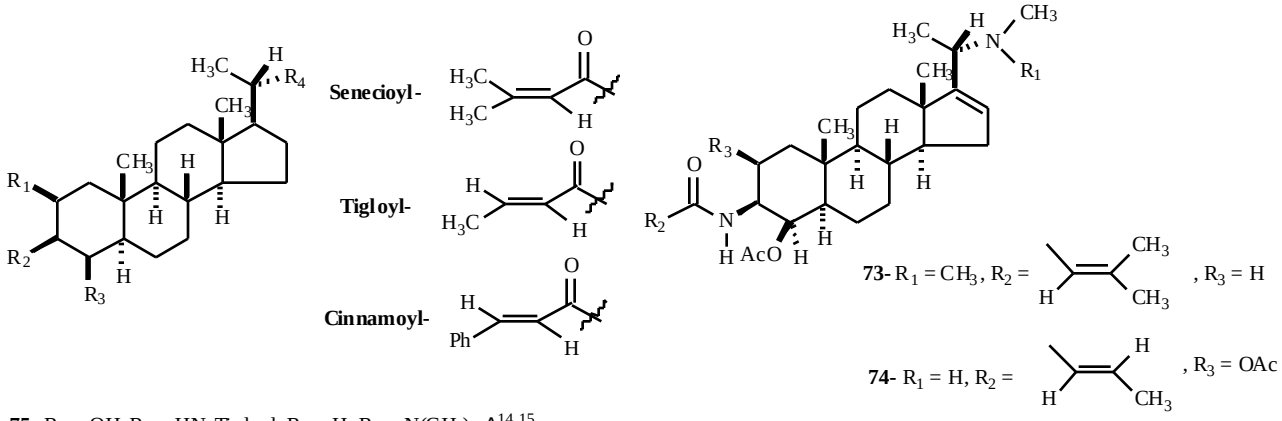


Fig. (9). Alkaloids isolated from cultures of *Penicillium citrinum*.

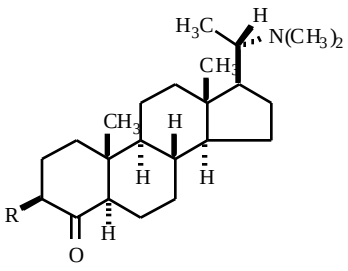
Sarcococca species (Buxaceae) are rich in steroidal alkaloids. Some species like *S. coriacea*, *S. hookeriana*, *S. saligna* and *S. wallichii* are reported from different ecological zones of Nepal and their extracts and metabolites have exhibited antiacetylcholinesterase, antibacterial, antiulcer and antitumor activities [56]. *S. coriacea* is an ever green shrub widely distributed in central Nepal, whose methanolic extract furnished two new anticholinesterasic steroidal alkaloids, vaganine D (**73**) and nepapakistamine A (**74**) (Fig. **10**). Additionally, these two new compounds showed greater selectivity toward butyrylcholinesterase enzyme [56]. *S. saligna* has been intensively investigated by Atta-ur-Rahman, Zaheer-ul-Haq & co-workers [57-60], furnishing a number of steroidal alkaloids. Examples of these active metabolites are compounds **75**-**89** (Fig. **10**), which were also described as inhibitors of acetyl and butyrylcholinesterase enzymes [60]. The inhibitory profile of these compounds against *Torpedo californica* AChE enzyme was studied. The goal of this study was to explore these steroidal alkaloids, which could represent new AChEI candidates, using docking experiments. All these compounds exhibit a similar binding mode, and therefore they are ideal targets for a systematic variation of the substituents [60].

Because of the docking results [60], comparative molecular field analysis (CoMFA) [61], kinetics and structure-activity relationship studies [62], it was possible to understand the inhibition profile of the ligands, which is mainly of noncompetitive nature. The subsequent molecular dynamics simulation showed that the complexation with these ligands does not have a considerable influence on the dynamics of the gorge width. One major observation found in the computational docking is that the ligands bind similar to what had been already observed for AChE inhibitors into the aromatic gorge of the enzyme. Although the ligands were completely buried in this gorge, the investigated compounds were not able to enter as deep as, for example, decamethonium [60].

Our interest in the search of new drugs from the Brazilian Atlantic Forest led us to select *Senna spectabilis* (sin. *Cassia spectabilis*, Leguminosae) for chemical and



75- R_1 = OH, R_2 = HN-Tigloyl, R_3 = H, R_4 = $N(CH_3)_2$ $\Delta^{14,15}$
76- R_1 = α-OH, R_2 = HN-Tigloyl, R_3 = H, R_4 = $N(CH_3)_2$ $\Delta^{4,5}$ & $\Delta^{16,17}$
77- R_1 = OH, R_2 = HN-Benzoyl, R_3 = H, R_4 = $N(CH_3)_2$
78- R_1 = H, R_2 = $NCH_3COCH{=}CCH_3CH(CH_3)_2$, R_3 = H, R_4 = $N(CH_3)_2$ $\Delta^{16,17}$
79- R_1 = H, R_2 = $NCH_3COCH{=}CCH_3CH(CH_3)_2$, R_3 = H, R_4 = $N(CH_3)_2$
80- R_1 = OH, R_2 = HN-Benzoyl, R_3 = OAc, R_4 = $N(CH_3)_2$
81- R_1 = OH, R_2 = HN-Tigloyl, R_3 = OAc, R_4 = $N(CH_3)_2$
82- R_1 = H, R_2 = NHAc, R_3 = H, R_4 = $N(CH_3)_2$
83- R_1 = H, R_2 = $NHCH_3$, R_3 = H, R_4 = $NHCH_3Ac$
84- R_1 = H, R_2 = CH_3N-Cinnamoyl, R_3 = H, R_4 = $N(CH_3)_2$
85- R_1 = H, R_2 = $NCH_3COCH{=}CCH_3CH(CH_3)_2$, R_3 = H, R_4 = $NCH_3)_2$
86- R_1 = H, R_2 = HN-Seneciolyl, R_3 = OAc, R_4 = $N(CH_3)_2$



87- R = *HN-Benzoyl* $\Delta^{2,3}$
88- R = *HN-Tigloyl* $\Delta^{5,6}$
89- R = *HN-Benzoyl* $\Delta^{5,6}$ & $\Delta^{14,15}$

Fig. (10). Active steroidal alkaloids isolated from *Sarcococca coriaceae and S. saligna*.

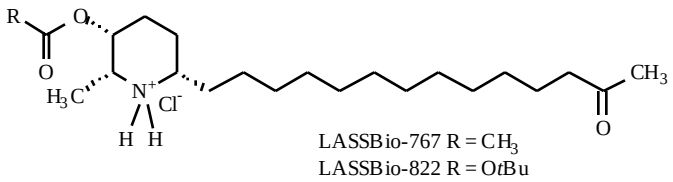


Fig. (11). Anticholinesterasic piperidine alkaloids obtained from *Senna spectabilis*.

pharmacological investigations. The ethanolic extracts from the leaves, flowers and green fruits of this plant were fractionated to furnish 12 piperidine alkaloids [63-65]. Some of these compounds exhibited selective citotoxity [63,64], analgesic and antiinflammatory properties [64,66]. Two of these metabolites also showed potent and selective antiacetylcholinesterase activity, with very low toxicity [67], representing a new class of AChEIs from natural origin. Further investigations are still being performed with the most active drug candidates, named LASSBio-767 and LASSBio-822, to clear its mechanism of action and to evaluate its toxic effects and mutagenicity (Fig. **11**).

CONCLUSION

As concluding remarks, we described in this overview the anticholinesterase activity of many natural products presenting different structural patterns, which were obtained from several different plants or fungi species. In this particular case, the strategy of look for natural bioactive templates helped by medicinal chemistry guided structural modifications is a powerful tool to discover of new potent, selective and safe drugs for Alzheimer disease treatment.

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