An Overview on Natural Cholinesterase Inhibitors - A Multi-Targeted Drug Class - and Their Mass Production

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Abstract: Cholinesterase enzyme family consisting of acetylcholinesterase (AChE) and butrylcholinesterase (BChE) is important in pathogenesis of Alzheimer's disease (AD), explained by "cholinergic hypothesis". Accordingly, deficiency of the neuromediator called "acetylcholine" excessive amount of BChE has been well-described in the brains of AD patients. Consequently, cholinesterase inhibition has become one of the most-prescribed treatment strategies for AD. In fact, cholinesterase inhibitors have been also reported for their effectiveness in some other diseases including glaucoma, myasthenia gravies, as well as Down syndrome, lately. They play a role in the action of mechanism of insecticidal drugs such as carbamate derivatives as well as nerve gases such as malathion and parathion. All these utilizations can make them a multi-targeted drug class putting a special emphasis on AD therapy in the first place. Several inhibitors of cholinesterases with synthetic and natural origins are available in drug market; however, the reasons including side effects, relatively low bioavailability, etc. limit their uses in medicine and there is still a great demand to discover new cholinesterase inhibitors. Galanthamine, an alkaloid derivative isolated from snowdrop (*Galanthus nivalis* L.), is the latest anticholinesterase drug used against AD. Huperzine A, isolated from *Huperzia serrata* (Thunb.) Trev. is the most-promising drug candidate with potent anticholinesterase effect and it is a licensed anti-AD drug in China. In this review, a short introduction will be given on known cholinesterase inhibitors and, then, galanthamine and huperzine A will be covered in regard with their cholinesterase inhibitory potentials and mass productions by organic synthesis and *in vitro* culture techniques.

Keywords: Cholinesterase inhibition, acetylcholinesterase, butyrylcholinesterase, Alzheimer's disease, galanthamine, huperzine A, organic synthesis, *in vitro* plant culture.

INTRODUCTION

Cholinesterase enzyme family consists of mainly two enzymes; acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). AChE (EC 3.1.1.7) (*syn*. RBC cholinesterase, acetylcholine acetylhydrolase, erythrocyte cholinesterase) can catalyze the hydrolysis of the neurotransmitter called "acetylcholine" (ACh) into choline and acetic acid, while BChE (EC 3.1.1.8) (*syn*. pseudocholinesterase) can function in the same way as AChE, differing only hydrolyzing another neurotransmitter "butyrylcholine" (BCh) instead of ACh. AChE and BChE exhibit analogous amphiphilic or soluble molecular forms in tissues and body fluids with different tissue distribution. For example, AChE, present in various molecular types referred to G1, G2, G3, and G4, is generally found in nerve tissue and red blood cells, whereas BChE exists primarily in the liver. BChE involved three different enzymatic activities in its structure like its sister enzyme, AChE: esterase, aryl acylamidase and peptidase (or protease) **[1]**. In fact, the role of AChE in cholinergic neurotransmission is well described; the real physiological function of BChE is yet to be explored more.

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Depending on their wide spectrum medical applications, ChIs have become quite attractive for scientists. Cholinesterase inhibitors (ChI) are currently being used in the treatment of several diseases such as Alzheimer's disease (AD) [2,3], myastenia gravis [4,5], glaucoma [6,7], and Down syndrome [8,9] more recently. Besides, reversible quaternary cholinesterase inhibitors such as neostigmine, pyridostigmine, and edrophonium, are mainly used to antagonize non-depolarizing neuromuscular blockade in general anesthesia [4,10]. Insecticidal drugs, especially carbamate derivatives, also act through mechanism of cholinesterase inhibition [11,12]. The organophosphorus nerve gases used as chemical warfare exert their effect through cholinesterase inhibition [13]. Interestingly, BChE is the only enzyme found in human serum that can hydrolyze heroin [14].

The underlying principle of this review is to furnish an outline on the status of natural anticholinesterase drug molecules including galanthamine and huperzine A as well as their production by organic synthesis, derivatization, and *in vitro* plant culture techniques.

Cholinesterase Inhibitors (ChIs)

ChIs have been mostly used for the treatment of Alzheimer's type of dementia that directed the research to development of many drugs of both synthetic and herbal ori-

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gins for AD. There are approximately half dozen AChE inhibitors currently available in the market, which fall into three classes: tertiary amines, organophosphates, and carbamates. The first-discovered AChE inhibitor was physostigmine **(1)** (Synapton®, Antilirium®) (Fig. **1**), an alkaloid isolated in 1864 from the seeds of *Physostigma venenosum* L. (Fabaceae) (calabar bean) and it was used in therapy more than a half century before the discovery of ACh as a neurotransmitter. It also named as "eserine", was synthesized for the first time in 1935 by the chemists Percy Lavon Julian and Josef Pikl and was used against memory deficit (AD), myasthenia gravis, glaucoma, and γ-hydroxybutyrate toxicity [15]. In fact, clinical use of AChE inhibitors started in the 1980's with physostigmine, which, afterward, led to the synthesis of phenserine and eptastigmine. However, effect of physostigmine was reported to be too diminutive in duration and its cholinergic side effects were very recurrent and distinct, which later on caused its use in AD to be brought to an end. Although physostigmine is no longer applied for AD treatment, it has been recently suggested for the treatment of hypertension [16,17].

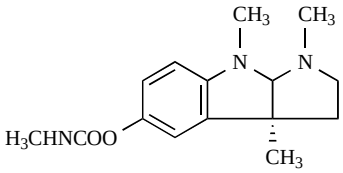

Fig. (1). Physostigmine.

Tacrine (Cognex®) was the first AChE inhibitor with synthetic origin approved by the US Food and Drug Administration (FDA) for the treatment of mild to moderate types of AD. Following tacrine, second-generation AChE inhibitors (post-physostigmine and post-tacrine compounds), *e.g.* rivastigmine, donepezil, and galanthamine, have been developed with better therapeutic profiles, despite their modest efficacy and replaced with tacrine [18]. Galanthamine is the latest anticholinesterase drug isolated from the snowdrop plant (*Galanthus nivalis* L.) and has been prescribed broadly for AD treatment [19]. On the other hand, huperzine A, is an alkaloid derivative isolated from *Huperzia serrata* (Thunb.) Trev.) and promise a strong hope to become a drug with potent anticholinesterase effect in the near future. Consequently, a particular prominence will be given to these two natural molecules; galanthamine and huperzine A in the following parts of the present review.

Galanthamine

Galanthamine **(2)** (Reminyl®), chemically known as [(4a*S*,6*R*,8a*S*)-5,6,9,10,11,12-hexahydro-3-methoxy-11-methyl-4a*H*-[1]-benzofuro[3a,3,2-ef] [2]benzazepin-6-ol], is the newest anticholinesterase drug in the market with a tertiary alkaloid structure having isoquinoline skeleton, firstly isolated from *Galanthus nivalis* (Amaryllidaceae) [20].

It is a selective, reversible, and competitive AChE inhibitor, currently approved for the symptomatic treatment of AD, with possible additional allosteric potentiating effects at the nicotinic ACh receptor (nAChR) [21]. It was later on also obtained from some other members of Amaryllidaceae such as *Leucojum* sp, *Narcissus* sp., and *Lycoris* sp. [22-24]. Galanthamine exerts more than a 10-fold selectivity towards AChE as compared to BChE [25], which is in contrast to non-selective agents such as tacrine and physostigmine. It is an allosteric potentiating ligand because it acts at a site on the nAChR that is different from the ACh-binding site [26]. Crystallographic studies documented the interaction between galanthamine and the *Torpedo* AChE at 2.3 °A resolution [27]. According to Bartolucci *et al*. [28], galanthamine binds at the base of the active site gorge of AChE, interacting with both the acyl-binding pocket and the principal quaternary ammonium-binding site of the enzyme.

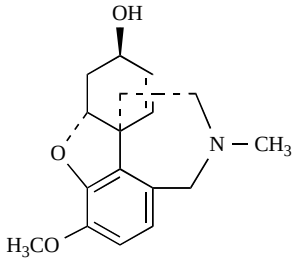

Fig. (2). Galanthamine.

On the other hand, galanthamine has been demonstrated to cause an improvement in cognition, behavior, and functioning in various randomized, double-blind, placebo-controlled clinical trials in patients with mild to moderate AD [29-33]. The serum half-life is 4 to 6 h, which is slightly longer than tacrine but much shorter than donepezil. Dosing of 16 to 24 mg/day was verified favorable for cognitive and non-cognitive AD symptoms [34].

Total synthesis of galanthamine was successfully achieved by different methods including biomimetic oxidative bisphenol coupling, phenol oxidation, remote asymmetric induction, intramolecular Heck reaction, semipinacol rearrangement, chiral synthesis, a tandem C3-selective Stille coupling-IMDA cycloaddition using 3,5-dibromo-2-pyrone reactions [35-41]. In fact, galanthamine was produced in kilogram-scale by Czollner *et al.* [42] using narwedine, another natural alkaloid, as the starting material.

On the other hand, several ester and carbamate derivatives of galanthamine having different side chains were synthesized and investigated against AChE [43]. It was concluded that side chain length as well as branching affected the AChE inhibitory activity and esters were generally less effective than carbamates.

In Mary *et al.*'s study [44], novel *bis*-interacting ligands of galanthamine were synthesized. *In vitro* inhibitory activity of the ligands possessing various alkyl linkers with a terminal ammonium or phtalimido groups connected to the terminal nitrogen of *N*-demethylgalanthamine (norgalanthamine) and to the oxygen of 6-*O*-demethylgalanthamine (sanguinine) was assayed against AChE from *Torpedo californica*. It was stated that the length of the alkyl linkers had a vital role in enhancing their inhibitory potentials. The derivatives having twelve methylene ($-CH_2-$) groups displayed the best inhibition. In addition, *N*-alkylammonium derivatives were superior to *N*-phthalimido derivatives. According to the findings, the iminium moiety and *N*-alkylation of the nitro-

gen atom of galanthamine caused to increase in inhibitory effect of these compounds against AChE. The homodimeric and heterodimeric alkylene linked *bis*-galanthamine derivatives synthesized by the same research group were tested for their AChE inhibitory and heterodimeric derivatives were found to be more potent than homodimeric ones [45]. Some researchers stated that the alkylene moieties attached to galanthamine promote the AChE inhibitory effect depending on lipophilicity of the alkyl and alkylene groups [45,46]. On the other hand, the inhibitory activity of a new synthetic series of galanthamine was tested towards AChE and the alkyl chain was again found to be contributory to the AChE inhibition [47]. Additionally, all of the new compounds exerted high inhibitory activities, among which the derivative containing an *N*-hexyl-benzyl piperidine substituent on the nitrogen atom displayed the best inhibitory activity on AChE.

In another study [48], *in vitro* tests against AChE of *Electrophorus electricus* origin and BChE of human serum origin were performed to establish inhibitory potential of a series of open D-ring synthesized analogs of galanthamine. Accordingly, the ring opening caused a clear decrease in AChE inhibitory potency. Another synthetic derivative of galanthamine, (-)-9-dehydrogalanthaminium bromide, was found to have a better inhibition than that of galanthamine [49].

There have also been a number of studies on galanthamine production by *in vitro* culture techniques. *Leucojum aestivum* L. (snowflake) is a plant species distributed in the Mediterranean region and Eastern Europe. One of the richest herbal sources of galanthamine is known as *L. aestivum* L. and *in vitro* culture and propagation of this plant as an alternative source of galanthamine production has been imposed since 1989 particularly under license in Bulgaria. The populations of snowflake growing in the south-east Bulgaria were found to possess the richest galanthamine content, dominated by galanthamine type synthesis [50].

Pavlov *et al*. [51] studied the callus production using the young fruits of *L. aestivum* on MS medium supplemented with 4 mg/L of 2,4-D and 2 mg/L of 6-benzylaminopurine (BAP). The maximum growth and galanthamine production (2.5 mg/L) were achieved by thirty-figth day after the shoot cultivation was initiated. Galanthamine accumulation was found to be dependent on the level of differentiation. To sum up, it was suggested that this result can be exploited for the mass production of galanthamine in industrial bioreactors. Tissue and hairy root cultures of *L. aestivum* with different stages of morphogenesis were studied to enhance galanthamine accumulation [52]. The tissue cultures prepared from the bulblets of *L. aestivum* were controlled by various exogenous hormonal conditions, while the hairy root cultures were subjected to *Agrobacterium rhizogenes* strain LBA 9402 application, whose ABC genes can modulate the action of the plant cell endogenous growth regulator [53]. Then, the extracts obtained from the *in vitro* cultures in this study consisting of 3-month old bulblets, 4-month old cultures, bifide roots (6 months after induction), and hairy roots (3 months after induction) as well as the leaves and scales from the market bulbs were analyzed by reversed phase-high pressure liquid chromatography (HPLC) in order to quantify galanthamine, which led to observation of spectacular variations in galanthamine content. It was stated by the authors that the bulbs used in preparation of all *in vitro* cultures did not contain galanthamine, whereas all *in vitro* bulblets, initiated with or without growth regulators, produced this alkaloid ranging between 1.14 - 6.79 $\times 10^{-3}$ % on dry weight basis. Nevertheless, galanthamine was not detected in *in vitro* roots. It was realized by these results that *in vitro* organogenesis caused an augmentation in galanthamine quantity in bulblets, whereas the transformed roots did not produce it.

Another study [54] investigating effects of some nutrients such as NH_4^+, NO_3^-, KH_2PO_4, and sucrose in Murashige-Skoog (MS) medium suggested that the modified MS nutrient medium consisting of 4.50 g/L KNO_3, 0.89 g/L NH_4NO_3, 1.25 g/L $(NH_4)_2SO_4$, 0.10 g/L KH_2PO_4, and 60 g/L sucrose gave a better result in terms of the production yield and the relative content of galanthamine in the alkaloid mixture. In a study on the intact plants, calli, and shoot-clump cultures of *L. aestivum* [50], twenty-four alkaloids were detected by gas chromatography-mass spectrometry (GC-MS) and the shoot-clumps cultures of the plant, which exhibited a similar profile to that of the intact plant, were observed to produce more abundant alkaloid content than the calli. Another finding obtained from this study was that the shoot-clump strains cultivated under light were found to accumulate approximately 2-fold more galanthamine (an average of 74 mg/g of dry weight) than those cultivated in darkness (an average of 39 mg/g of dry weight).

In vitro cultures of *L. aestivum* grown with a precursor (1-aminocyclopropane-1-carboxylic acid-ACC), inhibitors ($AgNO_3$, silver thiosulfate-STS) or an absorber ($KMnO_4$) of ethylene were obtained and analyzed by GC-MS and the maximum yield of galanthamine (0.002%) and lycorine was obtained in tissue cultures containing $KMnO_4$ [55]. In order to understand alkaloid metabolism in Amaryllidaceae, the shoot culture of *L. aestivum* was prepared in MS medium supplemented with auxin α-naphthalene acetic acid (NAA) (10 μM), 3% sucrose, and cytokinin benzylaminopurine (BAP) (5 μM) along with labeled precursor "4′-*O*-methyl-d3-norbelladine" at various concentrations (0.05, 0.10, and 0.20 g/L) was maintained at 25±2°C in the darkness and subcultured every 4 weeks [56]. Following harvesting the shoot cultures, the precursor and other alkaloids extracted from the tissues and medium were analyzed by HPLC. The findings pointed out that the labeled galanthamine was observed after 15 days of incubation with 0.10 g/L of the precursor up to 40 days of incubation. GC-MS analysis performed in the same study demonstrated that a total of six labeled alkaloids (d_3-demethylnarwedine, d_3-demethylgalanthamine, d_3-galanthamine, d_2-lycorine, d_2-crinine, and d_3-demethylmarithidine) were identified indicating that 4'-*O*-methyl-d_3-norbelladine is incorporated into three different groups of Amaryllidaceae alkaloids that are biosynthesized by three modes of intramolecular oxidative phenol coupling.

The shoot culture of *L. aestivum* was also used to produce galanthamine and some other related alkaloids using a temporary immersion technology [57]. The findings obtained from this study indicated that biomass accumulation and the yields of galanthamine and other related alkaloids were significantly influenced by both immersion frequency and temperature applications. The cultivation of *L. aestivum* shoot

culture led to galanthamine production at the maximal yield in temporary immersion RITA® system under the conditions of immersion frequency 15 min flooding and 8 h stand-by periods at 26 °C.

Narcissus sp. (daffodil) is another member of Amaryllidaceae with remarkable galanthamine content and also used as a source for galanthamine production by *in vitro* culture techniques. In Colque *et al*.'s study [58], effect of the biotic elicitors; methyl jasmonate, arachidonic acid, chitosan, and salicylic acid was examined in liquid-shake cultured shoot-clumps of *Narcissus confusus* Pugsley. The results indicated that high concentrations of these elicitors had a negative effect on the growth of the explants, salicylic acid in particular. Quite the opposite, the addition of methyl jasmonate, mainly at 25 μM, promoted the release of galanthamine and other related alkaloids to the liquid medium in proportions of up to 300 % in relation to the control explants, and also their accumulation in tissues. On the other hand, *in vitro* culture protocols were developed to produce alkaloids specifically galanthamine [59,60]. First of all "shoot-clumps" culture was established from *N. confusus*, which is a suitable method for micropropagation of this plant [59]. The culture method combines three steps; first step is being twin scales starting from bulbs, the second culture of the formed shoots in bud proliferation medium (MS with BAP), and the third step is "shoot-clumps" in a liquid medium. In this study, effects of *trans*-cinnamic acid addition, which is the precursor of galanthamine in the biosynthetic pathway, were analyzed on the production and/or increase of galanthamine and associated alkaloid formation in the culture. Surprisingly, the precursor retarded the galanthamine formation but promoted the production of *N*-formyl-norgalanthamine. The total formation of galanthamine was 2.50 mg *per* culture of which 1.97 mg was released into the liquid medium.

Later on, callus induction, somatic embryogenesis, and organogenesis for two strains of *N. confusus* starting from their mature seeds were launched and their alkaloids, galanthamine in particular, were quantified by HPLC [61]. Somatic embryogenesis followed by plant regeneration and investigation of galanthamine formation. As a result, alkaloid content was observed to increase by tissue differentiation, *in vitro* produced plantlets demonstrated 1.43 μg/g of galanthamine.

Some *in vitro* culture techniques have been also applied to some *Lycoris* species. For instance; influence of abiotic and biotic elicitor application such as yeast elicitor, methyl jasmonate, salicylic acid, and sodium nitroprusside as nitric oxide donator was examined on growth and galanthamine accretion of *L. chinensis* seedlings [62]. The results showed that the addition of methyl jasmonate, nitric oxide, and yeast elicitor promoted galanthamine accumulation in the seedlings. The greater accumulation occurred after treatments at higher concentrations of nitric oxide (100 μM), where the release of galanthamine was 1.72-fold higher than that of the control at the 10th day of culture.

In another attempt to investigate apolar metabolic profile of *Pancratium maritimum*, another member of Amaryllidaceae, during *in vitro* organogenesis, presence of galanthamine was identified by GC-MS and it was found to mount up in the leaves of the plantlets at 17% on dry weight basis [63].

Huperzine A

Huperzine A **(3)**, chemically known as [(5*R*, 9*R*, 11*E*)-5-amino-11-ethylidine-5,6,9,10-tetrahydro-7-methyl-5,9-methanocycloocta-[*b*]-piridin-2 (1H)-one], is an alkaloid isolated from the pteridophyte *Huperzia serrata* (Thunb.) Trev.) (Huperziaceae) (*syn. Lycopodium serratum* Thunb. ex Murray), a club moss indigenous to China and known as "Qing Ceng Ta". The plant has been used in Chinese traditional medicine against memory deficits, contusions, strains, swellings, schizophrenia, and myasthenia gravis since ages [64] and its active component was identified to be huperzine A, which is a selective and well-tolerated inhibitor of AChE, by the researchers at Materia Medica Institute in China in the 1980's [65].

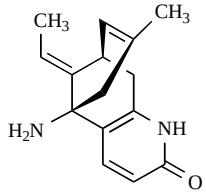

Fig. (3). Huperzine A.

The AChE inhibition potency of huperzine A has been observed to be similar or superior to those of other ChIs including galanthamine, donepezil, rivastigmine, and tacrine [66]. "Shuangyiping", a tablet form of huperzine A produced from the extracts of *H. serrata*, was developed in 1996 as a new drug for symptomatic treatment of AD in China [67]. Clinical trials at phase-IV in China showed that huperzine A significantly improved memory shortages in aged people with benign senescent forgetfulness and in patients with AD or vascular dementia, with minimal peripheral cholinergic side effects and no unexpected toxicity [68,69]. Moreover, it is also in clinical trials for the treatment of age-related memory deficiency in the United States [70].

On the other hand, *H. serrata*, the original herbal source of huperzine A, has some disadvantages such as low content of huperzine A (*ca.* 0.007%), ranging from 0.0047 to 0.025 % depending on the collecting seasons and growing regions, a limited geographical distribution, and very slow growth rate [71]. For instance; it takes minimum 15 years from spore germination through the gametophyte stage to finally reach the mature sporophyte stage. Besides, this species is about to extinct at near future. Therefore, organic total synthesis and *in vitro* culture have been applied for mass production of huperzine A. Because of these reasons, *in vitro* propagation of the plant material has attracted more interest in recent years. Unfortunately, efforts in this area have led to only limited success.

Total synthesis of the compound has been achieved up to date [72-76], however, the synthetic racemic mixture of huperzine A exhibited less AChE inhibitory activity than that of natural (-)-huperzine A [69,77].

Many analogs and derivatives of huperzine A were prepared and tested for their inhibitory activities towards AChE.

Unfortunately, neither the structurally simplified analogs nor the derivatives from the natural huperzine A exhibited the anti-AChE potency as huperzine A *per se* except 10-methyl huperzine A and a few of the Schiff bases of (–)-huperzine A [70]. Various derivatives of huperzine A were prepared using different moieties or halogens to increase the efficacy on AChE [78]. For instance, Kaneko *et al*. [79] synthesized novel fluorinated derivatives of huperzine A, containing one, three, and six fluorine atoms in the molecule, in which one-fluorine containing analog named as (±)-12-fluorohuperzine A was the most active. However, in the same experiment, natural (-)-huperzine A had a superior inhibitory effect to synthetic (+)-huperzine A having IC_{50} values of 0.05 and 10 μM, respectively. Consistent with this finding, (±)-14-fluorohuperzine A (IC_{50}=10 μM) synthesized by Zeng *et al*. [80] also displayed less potent inhibition than that of (-)-huperzine A. (±)-7-Ethylhuperzine A and its two regioisomeric derivatives were tested against AChE of bovine erythrocyte origin and (±)-7-ethyl derivative displayed a better inhibition [81]. Conversely, its effect was approximately 12-fold less than (-)-huperzine A.

Campiani *et al*. [82] synthesized two new huperzine A derivatives by replacing the pyridone ring in the molecule with phenol and catechol moieties, which were tested against AChE purified from fetal bovine serum. Nevertheless, these derivatives displayed a lesser activity than huperzine A. Conversely, (*E*)-and (*Z*)-5-desamino derivatives of huperzine A were assayed against AChE from the brain of Sprague-Dawley rats and showed a substantial AChE inhibitory activity (IC_{50} values of 12.8 and 55.5 μM, respectively), even though their effects were found to be still lesser than that of (-)-huperzine A (IC_{50}=0.024 μM) [83].

Only the plants from the genus *Huperzia* (syn. *Lycopodium*) and *Phlegmariurus* have been found to produce huperzine A [84]. Actually, *in vitro* propagation studies were applied to some species of *Lycopodium* sp., such as *L. clavatum*, *L. cernnum*, *L. annotinum*, *L. complanatum*, and *L. selago* in the 1950s and 1960s [85,86].

Szypuła *et al*. [87] performed a study on *in vitro* shoot culture of the sporophytes and somatic embryogenesis in *H. selago*. Accordingly, the highest growth frequency was achieved on the MS medium at half-strength mineral salt content. The same medium was also used for the induction of somatic embryogenesis of this species. The findings pointed out that the cells of the callus, which developed from the apical meristem after 3 months of incubation, transformed into somatic embryos. HPLC analysis demonstrated the highest huperzine A content (3.33 mg/g on dry weight basis) in the shoots of the plants obtained from the somatic embryos. The yield of huperzine A from plants collected from a natural habitat differed from 0.54 (shoots harvested in spring) to 1.27 mg/g dry weight. In another study [88], *in vitro* propagation of the tissues of *Phlegmariurus squarrosus* from Huperziaceae led to production of comparably higher levels of huperzine A than the natural plant *per se*, and may represent an excellent source for this compound.

In a recent study [89], a novel endophytic fungus, named as *Shiraia* sp. Slf14, was isolated from *H. serrata* growing in China and found to produce huperzine A, which was identical to authentic huperzine A analyzed by thin layer chromatography (TLC). It also displayed the same level of inhibition against AChE. The authors concluded that this endophytic fungus may provide a promising alternative for large-scale production of this compound.

CONCLUSION

Cholinesterase inhibition is an important treatment strategy for a number of diseases including AD in the first place along with myasthenia gravis, glaucoma, etc. Multi-targeted actions of this drug class make them more attractive for the researchers. They are the most-prescribed drugs for AD treatment at the moment and an extensive research is still being conducted to find new inhibitors of both synthetic and natural origins. The natural cholinesterase inhibitors; galanthamine and huperzine A, have been discussed in this overview in respect to their cholinesterase inhibitory effects and production by organic synthesis and *in vitro* culture. The studies clearly show that galanthamine can be produced profitably by both techniques in large-scale. However, further studies should be performed immediately on huperzine A production by *in vitro* culture techniques as the source plant (*Huperzia serrata*) is under the danger of extinction.

CONFLICT OF INTEREST

No conflict of interest exists among the authors.

ABBREVIATIONS

ACC = 1-Aminocyclopropane-1-carboxylic acid

ACh = Acetylcholine

AChE = Acetylcholinesterase

AD = Alzheimer's disease

BAP = 6-Benzylaminopurine

BCh = Butyrylcholine

BChE = Butyrylcholinesterase

ChI = Cholinesterase inhibitors

FDA = US Food and Drug Administration

GC-MS = Gas chromatography-mass spectrometry

HPLC = High pressure liquid chromatography

IC = Inhibitory concentration

MS = Murashige-Skoog

NAA = α-Naphthalene acetic acid

nAChR = Nicotinic ACh receptor

STS = Silver thiosulfate

TLC = Thin layer chromatography

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