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ARTICLE *in* JOURNAL OF ETHNOPHARMACOLOGY · SEPTEMBER 2007

Impact Factor: 3 · DOI: 10.1016/j.jep.2007.05.030 · Source: PubMed

Huperzine A from *Huperzia* species—An ethnopharmacolgical review

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Abstract

Huperzine A (HupA), isolated originally from a traditional Chinese medicine *Qiang Ceng Ta*, whole plant of *Huperzia serrata* (Thunb. ex Murray) Trev., a member of the Huperziaceae family, has attracted intense attention since its marked anticholinesterase activity was discovered by Chinese scientists. Several members of the Huperziaceae (*Huperzia* and *Phlegmariurus* species) have been used as medicines in China for contusions, strains, swellings, schizophrenia, myasthenia gravis and organophosphate poisoning. HupA has been marketed in China as a new drug for Alzheimer's disease (AD) treatment and its derivative ZT-1 is being developed as anti-AD new drug candidate both in China and in Europe. A review of the chemistry, bioactivities, toxicology, clinical trials and natural resources of HupA source plants is presented.

Keywords: Huperzine A; ZT-1; Alzheimer's disease; *Huperzia serrata*; Huperziaceae; Drug discovery; Bioactivities; Clinical trials; Traditional Chinese medicine

Contents

Abbreviations: HupA, huperzine A; HupB, huperzine B; SIMM, Shanghai Institute of Materia Medica; CAS, Chinese Academy of Sciences; ChEI, cholinesterase inhibitor; AChE, acetylcholinesterase; AChEI, acetylcholinesterase inhibitor; AD, Alzheimer's disease; OP, organophosphate; PYR, pyridostigmine; s.l., sensu lato; VD, vascular dementia; DSM IV-R, Diagnostic and Statistical Manual of Mental Disorders—Fourth Revision; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke; FDA, Food and Drug Administration; NGF, nerve growth factor; MMSE, Mini Mental State Examination; ADL, activity of daily living; CDR, clinical dementia rating; AAMD, American Association of Mental Deficiency; HDS, Hasegawa dementia scale; IC_{50}, 50% inhibitory concentration; DM, double-mimic; MC, multicenter; DB, double blind; R, randomized; PC, placebo-controlled; MQ, memory quotient; WMS, the Wechsler Memory Scale

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1. Introduction

Qian Ceng Ta, a traditional Chinese medicine produced from the whole plant of the club moss *Huperzia serrata* (Thunb. ex Murray) Trev. (synonym *Lycopodium serratum* Thunb. ex Murray, a member of Huperziaceae), has been used for over 1000 years in China for treatment of a number of ailments, including contusions, strains, swellings, schizophrenia, myasthenia gravis and now organophosphate poisoning (College, 1985; Ma, 1997). It has become known worldwide as a medicinal plant since Chinese scientists discovered huperzine A (HupA) (Fig. 1) from it in the 1980s (Liu et al., 1986a, 1986b).

The earliest record of medicinal usage of Qian Ceng Ta can be traced back to an ancient Chinese pharmacopeia "Ben Cao Shi Yi", which was written by Zangqi Chen in 739 (during the Tang Dynasty). The herb was named Shi Song in this book and it was prescribed for relieving rheumatism and colds, to relax muscles and tendons and to promote blood circulation. The same herb with different names but similar usage prescriptions can be found in "Ben Cao Gang Mu" by Shizhen Li in 1578 (during the Ming Dynasty) and "Zhi Wu Ming Shi Tu Kao" by Qijun Wu in 1848 (during the Qing Dynasty). In fact, Shi Song was a confusing name for the medicinal herb in the ancient times because this name was used to describe several different medicinal herbs. Qian Ceng Ta is one of these Shi Song herbs and was popularly used in southern China. However, all of the Shi Song herbs are members of the genus *Lycopodium* (sensu latu) (e.g. *Lycopodium japonicum* Thunb., *Lycopodium annotinum* L., *Lycopodium* obscurum L., *Diphasiastrum complanaatum* (L.) Holub, *Phalhinhaea cernua* (L.) A. Franco et Vasc., and *Huperzia serrata*) (Ma, 1997).

In the early 1980s, Chinese scientists searched for new drugs for myasthenia gravis treatment. Lycopodium alkaloids extracted from *Lycopodium* (s.l.) species were lead drug targets based on the traditional use the herbs that contain them (Cheng et al., 1986). In vitro and in vivo pharmacological studies have demonstrated that Lycopodium alkaloids produce definite effects in the treatment of diseases that affect the cardiovascular or neuromuscular systems, or that are related to cholinesterase activity. These alkaloids have been shown to have positive effects on learning and memory (Liu et al., 1986b; Tang et al., 1986; Zhu and Tang, 1987). Of these, HupA, which was isolated from Qian Ceng Ta (*Huperzia serrata*) by Chinese scientist Liu and coworkers (Liu et al., 1986b) is the most well known, and appears to be the most potent. HupA has been extensively evaluated by the Chinese for bioactivity, especially for activity toward cholinesterases and for treatment of Alzheimer's disease (AD).

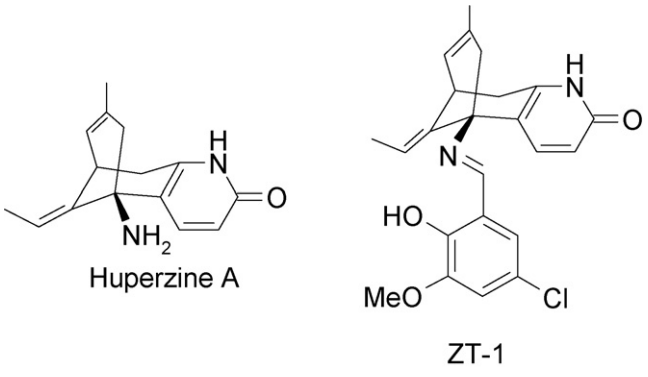

Fig. 1. Structures of HupA and ZT-1.

HupA has been proven to be a powerful, highly specific, and reversible inhibitor of acetylcholinesterase (AChE) (Tang et al., 1986, 1989; Cheng et al., 1996). Shuangyiping, a tablet form of HupA produced from extracts of *Huperzia serrata*, was developed in 1996 (by one of the authors, Zhu) as a new drug for symptomatic treatment of AD in China (Tang, 1996). HupA is also marketed in the USA as a dietary supplement (as powdered *Huperzia serrata* in tablet or capsule form). As the world's population lives longer, increasing numbers of people, especially in the developed world suffer from AD and other types of dementia. AD is the most common form of dementia, and is characterized by the loss of memory, motor ability and eventually muscle control. Phase IV clinical trials in China have demonstrated that HupA significantly improves memory deficits in elderly people with benign senescent forgetfulness and in patients with AD or vascular dementia (VD), with minimal peripheral cholinergic side effects and no unexpected toxicity (Xu et al., 1995, 1999; Zhang et al., 2002b). Several new drugs for treatment of AD symptoms have been approved by the U.S. Food and Drug Administration (FDA) in recent years, all of which are AChE inhibitors (AChEIs). These include tacrine (trade name: Cognex) in 1993, donepezil (trade name: Aricept) in 1996, rivastigmine (trade name: Exelon) in 2000, and galanthamine (trade name: Reminyl) in 2001. Tacrine produces some serious side effects (including liver toxicity) among >29% of patients, and has removed from the market. Donepezil appears to have better properties, both greater effect and lower toxicity, than tacrine. Rivastigmine also appears to have better properties than tacrine, in that it does not appear to cause liver damage. However, it can produce stomach-related side-effects such as nausea and vomiting. Galanthamine, unlike the other three FDA-approved AChEIs, is a natural plant alkaloid, produced by *Galanthus nivalis* L. and related plants (Amaryllidaceae family) (Heinrich and Lee Teoh, 2004). It has also been used to treat symptoms of other forms of dementia, such as VD (Farlow, 2003). Compared with the above acetylcholinesterase inhibitors (AChEIs),

including galanthamine, HupA has better penetration through the blood–brain barrier, higher oral bioavailability, and longer duration of AChE inhibitory action (Wang et al., 2006a). Most of these clinical trials have been performed in China, where an estimated 100,000 people have been treated with HupA (Chiu and Zhang, 2000). Results of these studies indicate that HupA is an effective and safe drug that alleviates AD and improves cognitive function. In addition, HupA can also be used as a protective (prophylactive) agent against organophosphate (OP) poisoning (Saxena et al., 1994; Rocha et al., 1998; Lallement et al., 2002b; Gordon et al., 2005; Liu and Sun, 2005; Eckert et al., 2006).

ZT-1 (Fig. 1), a semi-synthetic derivative of HupA, was originally synthesised by Zhu and coworkers at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Ma and Gang, 2004). Experimental data demonstrated that ZT-1 possesses AChEI activity similar to HupA. However, it is more selective and displays less BChEI activity as well as less toxicity in mice than HupA. ZT-1 has similar properties to HupA regarding the ability to cross the blood–brain barrier, its oral bioavailability, and its longevity of action (Ma and Gang, 2004). ZT-1 is a promising new drug candidate and is expected to be the first Chinese new drug, with independent intellectual property rights owned by Chinese scientists, to move into the international major pharmaceutical markets.

A historical outline of HupA's discovery and development, beginning with its original discovery from traditional Chinese medicine Qiang Ceng Ta, is presented in Table 1. The discovery and development of HupA and ZT-1 as new drugs present a successful model for development of new drugs from traditional Chinese medicine.

2. Chemistry

Lycopodium alkaloids, triterpenes, flavones and phenolic acids are commonly found natural products in Huperziaceae plants (Towers and Maas, 1965; Voirin et al., 1976; Voirin and Jay, 1978; Li et al., 1988; Ma, 1997; Lu et al., 2002; Tong et al., 2003; Ma and Gang, 2004; Zhou et al., 2004b; Shi et al., 2005). The most extensively investigated of these compounds are the Lycopodium alkaloids and the most important and intensively studied by far is HupA.

2.1. Lycopodium alkaloids from HupA source plants

A recent review covering advances in research on the Lycopodium alkaloids from the Spring of 1993 until August 2004 was published by Ma and Gang (2004). Lycopodium alkaloids can be divided into four major structural classes: lycopodine, lycodine, fawcettimine and miscellaneous. So far, the Lycopodium alkaloids with acetylcholinesterase inhibition activity, such as HupA, huperzine B (HupB) (Xu and Tang, 1987), *N*-methyl-huperzine B and huperzinine (Yuan and Wei, 1988) (see Fig. 2), all belong to the lycodine class (Ma and Gang, 2004). In total, 141 Lycopodium alkaloids have been isolated and reported from eleven HupA source plants: *Huperzia serrata*, *Huperzia selago* (L.) Bernh. ex Schrank et Mart., *Huperzia lucidula* (Michx.) Ching, *Huperzia chinensis* (Christ) Ching, *Huperzia miyoshiana* (Makino) Ching, *Huperzia saururus* (Lam.) Trevis., *Phlegmariurus carinatus* (Desv.) Ching, *Phlegmariurus sieboldii* (Miq.) Ching, *Phlegmariurus phlegmaria* (L.) Holub, *Phlegmariurus fordii* (Bak.) Ching, and *Phlegmariurus hamiltonii* (Sprengel) L. Löve et D. Löve (Table 2). Of these compounds, 40 belong to the lycopodine class, 19 belong to the lycodine class, 53 belong to the fawcettimine class, and 29 belong to the miscellaneous group. Lycopodine (see Fig. 2) was the first identified Lycopodium alkaloid and appears to be the most widely distributed (Ma and Gang, 2004). Between August 2004 and April 2007, 10 novel Lycopodium alkaloids have been discovered (see Fig. 2), including four of the lycopodine class: 4,6α-dihydroxylycopodine (1), 12-epilycodoline *N*-oxide (2), 7-hydroxylycopodine (3) and sauroine (4); three of the lycodine class: carinatumin A (5), carinatumin B (6) and nankakurine A (7); one of the fawcettine class: lycoposerramine B (8); and two of the miscellaneous group: carinatumin C (9) and lycoperine A (10). All of these compounds were identified from Lycopodium species. Lycoperine A and carinatumins A and B have been reported to possess potent inhibitory activity against AChE (Hirasawa et al., 2006; Choo et al., 2007).

2.2. Chemical synthesis and structure modification of HupA

Since the anti-AChE activity of HupA was reported in 1986 (Liu et al., 1986a, 1986b), many attempts have been made to produce analogs and derivatives of HupA that would possess higher and longer duration of activity, as well as lower toxicity in model systems. These have included development of several approaches for the total synthesis of HupA, its structure–activity relationships, and the affect of specific structural modifications to the HupA core molecule. The first total synthesis of HupA was reported by Qian and Ji (1989). A large number of analogues of HupA have been published by Kozikowski and coworkers since 1989 (Xia and Kozikowski, 1989; Xia et al., 1989; Kozikowski et al., 1990a, 1990b, 1991a, 1991b, 1992, 1993, 1994a, 1994b, 1995, 1996a, 1996b, 1996c, 1998; Hanin et al., 1991; Campiani et al., 1993; Saxena et al., 1993; Raves et al., 1997; Campiani et al., 1998; Rajendran et al., 2000, 2001; Kellar and Kozikowski, 2002). A comprehensive review of structure–activity data for these analogues was provided by Ma and Gang (2004). A recent paper by Lucey et al. (2007) described a modified approach that "allowed access to the full tricyclic skeleton of HupA by a concise and convergent route to the key ketoester used by Kozikowski in his ground-breaking synthesis". To date many analogs of HupA have been prepared. Among these, only a very few compounds have obvious anti-AChE activity (Ma and Gang, 2004).

Zhu's group has produced a large number of HupA analogs and derivatives. ZT-1, the most efficacious one, was finally selected by Zhu's laboratory from over 100 HupA derivatives. ZT-1 is a Schiff base made by a condensation reaction between HupA and 5-Cl-*O*-vanillin. This semi-synthesis pathway only requires two steps and the materials are readily available and

Table 1
History of HupA and ZT-1 discovery and development

Year	Development of HupA and ZT-1
Early 1980s	Chinese scientists from the Shanghai Institute of Materia Medica (SIMM) at the Chinese Academy of Sciences (CAS) and Zhejiang Medical Research Institute (ZMRI) screened for new drugs for myasthenia gravis treatment from the traditional Chinese medicine Shi Song, i.e. *Lycopodium* (s.l.) species, based on their traditional use for relieving rheumatism and cold, relaxing muscles and tending to promote blood circulation. Different degrees of cholinergic side effects were observed during folk applications and clinical trials. Chemical constituent investigation and pharmacological studies were then carried out to find the active compounds for myasthenia gravis treatment and the cholinergic side effect. Chaomei Yu et al. (Yu et al., 1982a, 1982b) isolated three Lycopodium alkaloids (lycodoline, lycoclavine, and serratinine) from She Zu Cao (*Lycopodium serratum* Thunb. ex Murray), a synonym of Qian Ceng Ta (*Huperzia serrata*). These three alkaloid components did not show marked muscle relaxant effect
1986	1. HupA was first isolated from Qian Ceng Ta (*Huperzia serrata*), one member of Huperziaceae family or *Lycopodium* (s. l.), by Jiasen Liu and co-workers at SIMM, CAS (Liu et al., 1986a) 2. The structures of HupA and B and their marked anticholinesterase activity were demonstrated by Liu and co-workers at SIMM, CAS (Liu et al., 1986b) 3. The first clinical trials of HupA for myasthenia gravis treatment were reported by Yuansen Cheng et al. from the 2nd Hospital, Zhejiang Medical University; Huashan Hospital, Shanghai Medical University; Zhejiang Jiaxing 1st Hospital; Zhejiang Taitzhou Hospital; and ZMRI, China (Cheng et al., 1986) 4. The first clinical trials of HupA for treatment of the aged patients with memory impairment were reported by Cilu Zhang from Zhejiang Taizhou Hospital, China (Zhang, 1986) 5. The first in vivo pharmacological study of the effect of HupA on learning and the retrieval process of discrimination performance in rats was reported by Xican Tang and co-workers at SIMM, CAS (Tang et al., 1986; Wang et al., 1986a) 6. The first in vitro pharmacological report on HupA anticholinesterase activity was published by Yuee Wang and Tang at SIMM, CAS (Wang et al., 1986b).
Late 1980s	HupA attracted worldwide attention after its anticholinesterase activity was revealed. For example, scientists from Georgetown University Medical School (GUMS), Pittsburgh University and Walter Reed Army Institute of Research in the USA, and at the Weizmann Institute of Science (WIS) in Israel showed great interest in HupA and started chemical synthesis, structure-activity relationship, pharmacology and related studies
1989	Total synthesis of HupA was initiated almost simultaneously by Ruyun Ji's (SIMM, CAS, China) and Alan P. Kozikowski's (GUMS, USA) groups. The first report was published by Ligang Qian and Ji at SIMM, CAS (Qian and Ji, 1989). Kozikowski's group soon published their own method (Xia and Kozikowski, 1989)
Early 1990s	To develop HupA derivatives that may serve as more efficacious candidates for use in clinical application, synthesis of HupA derivatives and further natural resource investigation of Qian Ceng Ta (*Huperzia serrata*) was initiated in Dayuan Zhu's group at SIMM, CAS
1991	The structure and absolute configuration of HupA were confirmed by X-ray crystallographic analysis by Steven J. Geib et al. from Kozikowski's group at GUMS, USA (Geib et al., 1991)
1995	Xiaoqiang Ma (Ph.D. candidate of SIMM, CAS, supervisor: Prof. Dayuan Zhu) started ethnopharmacological surveys, chemical analysis, and natural resource investigations of HupA source plants in China
1996	1. Shuangyiping (Huperzine A Tablet), the first HupA new drug approved by the State Administration of Traditional Chinese Medicine of the People's Republic of China, was developed by Zhu and co-works at SIMM, CAS. It has been marketed in China since 1996 (Tang, 1996) 2. ZT-1 (a HupA derivate) was selected as a new drug candidate by Zhu and Tang from over 100 HupA derivatives 3. Patents in China, Japan, the USA, Europe and internationally, for the synthesis and application/use of ZT-1, were been applied for by and later granted to Zhu and co-workers at SIMM, CAS. China Patent Certificate No. 54041, 3/17/2000; USA Patent No. 5,929,084, authorizing date 7/27/1999; European Patent Register No. Ep959415720; Japanese Patent Register No. 08-520102; International Patent No. WO96/20176; and International Patent Pact PCT/CN95/00100
1997	1. The structure of acetylcholinesterase complexed with HupA was reported by Mia L. Raves et al. from Joel L. Sussman's group at WIS, Israel (Raves et al., 1997) 2. The efficacy of HupA in preventing soman-induced seizures, neuropathological changes and lethality was reported by Guy Lallement and co-workers at Unite de Neuropharmacologie, CRSSA, France (Lallement et al., 1997)
1999	1. A cooperation intent on the development of ZT-1 as an anti-AD drug was initiated between SIMM, CAS and Debiopharm of Switzerland 2. HupA was marketed in USA as a dietary supplement (as powdered *Huperzia serrata* in tablet or capsule format)
2000	1. The first report about HupA clinical trials in USA by Mazurek (Mazurek, 2000) 2. A letter of intent about ZT-1 development cooperation was signed between SIMM and Debiopharm in April 2000 3. A signature ceremony for ZT-1 development collaboration in China was performed by SIMM, CAS and Jiangsu Yangtze River Pharmaceutical Group in August 2000
2000–2002	The first direct evidence of HupA protects neurons against aggregates of β-amyloid (Aβ) (one of the neuropathological hallmarks of AD) induced oxidative injury, cell toxicity, and apoptosis which might be beneficial for the treatment of AD, was reported by Xiaoqiu Xiao et al. from Tang's group at SIMM, CAS (Xiao et al., 2000a, 2000b, 2002)

2002–2003	1. Ma and Eckhard Wellmann succeeded in propagating *Phlegmariurus squarrosus* (Forst.) Löve et Löve, a potential HupA source plant, at the University of Freiburg, Germany. (Patent was applied for in Germany.) 2. Phase I daily oral clinical trials of ZT-1 in China were performed from September 2002 to July 2003 and were designed to evaluate the tolerance to dosage level and to study pharmacokinetics. Meanwhile, four phase I clinical trials were carried out in The Netherlands from March 2002 to April 2003 3. High concentrations HupA was detected from the cultures of *Phlegmariurus squarrosus* using LC/MS/MS analysis in David R. Gang's group at the University of Arizona (UA), USA by Ma, Wellmann and Gang
October 2003	Debiopharm started a Phase II trial for a once daily oral formulations of ZT-1 to treat AD. The multicenter trial was conducted in France, Belgium and Switzerland, and enrolled 180 patients suffering from mild to moderate AD
May 2004	Debiopharm exercised its option and signed a license agreement with SIMM for the development and commercialization of ZT-1
2004	1. Liquid suspension tissue cultures of *Phlegmariurus squarrosus* were started at UA by Ma and Gang 2. The first hypothesis regarding the biosynthetic pathway leading directly to HupA was outlined by Ma and Gang (Ma and Gang, 2004)
2005	Phase II daily oral clinical trials of ZT-1 were completed in 35 hospitals in six European countries by Debiopharm
2006	1. Haiyan Zhang and Tang at SIMM, CAS proposed that a multitarget approach might be a more accurate description of how HupA affects AD (Zhang and Tang, 2006) 2. The first monthly sustained-release DEBIO-9902 SR implants (formerly ZT-1) were developed by Debiopharm for Alzheimer's patients 3. Phase I and II clinical trials of ZT-1 in China passed checking and accepting
2007	Phase II monthly implant clinical trials of DEBIO-9902 SR will be conducted in more than 20 hospitals on three continents. The study will measure the safety and efficacy of DEBIO-9902 SR implants, in comparison to oral donepezil, in a clinical trial named 'BRAINz' (Better Recollection for Alzheimer's patients with ImplaNts of ZT-1)

inexpensive. However, it relies on a source of the HupA molecule itself, which up to now has been provided by natural resources. We will return to this issue below.

2.3. Biosynthesis of HupA

Several studies have been performed in an effort to elucidate the biosynthesis of Lycopodium alkaloids (Conroy, 1960; Maki, 1961; Ayer et al., 1968; Gupta et al., 1968, 1970; Castillo et al., 1970a, 1970b, 1970c; Herbert, 1971; Ho et al., 1971; Braekman et al., 1972; Ayer, 1973; Marshall, 1973; Marshall et al., 1975; Nyembo et al., 1978; Hemscheidt and Spenser, 1990, 1993). Based on these, we proposed a pathway leading directly to HupA (Ma and Gang, 2004). No direct biosynthetic studies have sought to identify the route to HupA due to the fact that HupA source plants have not been cultivated and are very difficult to be produced by in vitro propagation methods. So far, no enzymes have been identified in the Huperziaceae plants that might be involved in the production of the HupA. Only one enzyme, lysine decarboxylase, has been proposed as the entry point enzyme into the pathways to the Lycopodium alkaloids (Hemscheidt, 2000). However, investigations on this enzyme has not been performed with any Huperziaceae plants (Gerdes and Leistner, 1979). Nevertheless, the experiments that have been performed with Lycopodium species have established a very good framework for identifying the key enzymes involved in the biosynthesis of HupA and other Lycopodium alkaloids.

3. Bioactivities of HupA

Several bioactivities have been described for HupA. The most important of these relate to its effects on memory and its neuronal protection capabilities. HupA improved memory retention processes in cognitively impaired aged and adult rats (Lu et al., 1988). In multicentre, placebo-controlled, doubleblind and randomized trials, HupA significantly improved memory and behavior in AD patients (Zhang et al., 1991, 2002b; Xu et al., 1995). HupA was reported to be more selective for AChE than butyrylcholinestrase (BuChE) and less toxic than the synthetic AChEIs donepezil and tacrine (Wang and Tang, 1998). It decreased neuronal cell death induced by glutamate toxicity (Skolnick, 1997), affected other neurotransmitter systems to improve memory, protected cortical neurons against β-amyloid induced apoptosis in vitro (Ou et al., 2001), and attenuated cognitive deficits and brain injury after hypoxia–ischemic brain damage in neonatal rats (Wang et al., 2003). Antagonizing effects of HupA on *N*-methyl-D-aspartate receptors and potassium currents may also contribute to its neuroprotection as well (Wang and Tang, 2005).

3.1. Treatment of AD

AD is the most common form of dementia and is characterized by profound memory loss sufficient to interfere with social and occupational functioning. The disease affects more than 20 million people worldwide, as a very conservative estimate. Common symptoms include an insidious loss of memory, associated

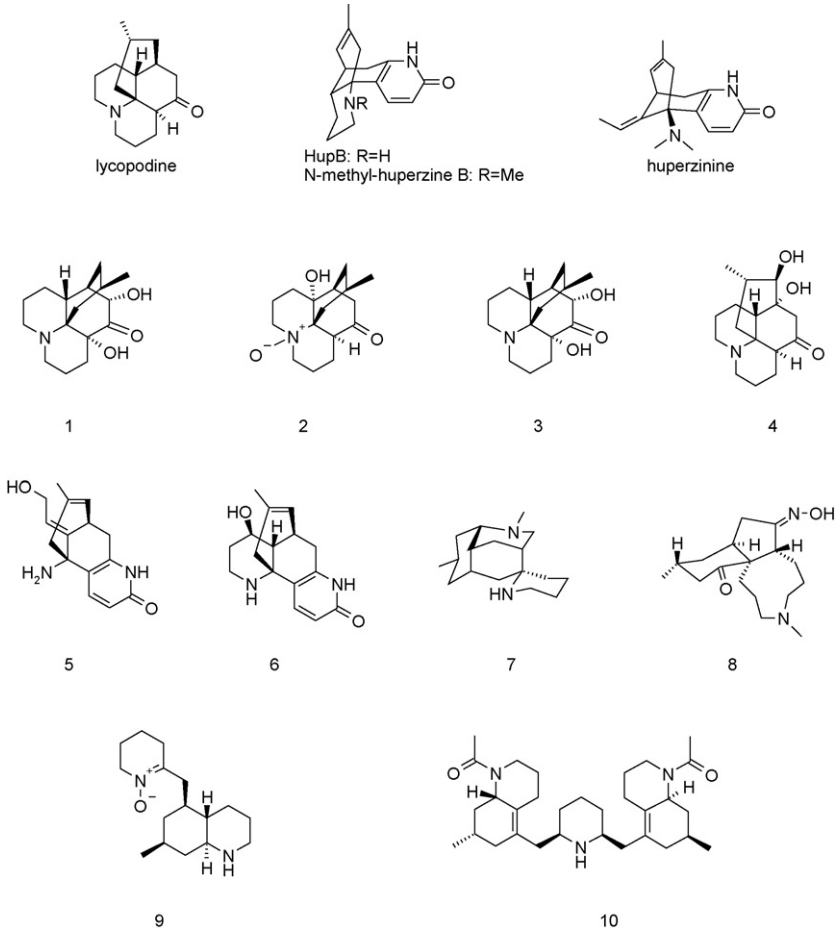

Fig. 2. Structures of lycopodine, huperzine B, *N*-methyl-huperzine B, huperzinine, and 10 novel Lycopodium alkaloids from HupA source plants.

functional decline, and behavioral disturbances (Akhondzadeh and Abbasi, 2006). The areas of the brain that control memory and thinking skills are affected first, but as the disease progresses, cells die in other regions of the brain. If the individual has no other serious illness, the loss of brain function itself will eventually cause death. The etiology of these diseases is not fully understood (Sivaprakasam, 2006). However, it is know that levels of neurotransmitters are affected. For example, in AD patients, the level of acetylcholine in the brain is reduced and this accompanies pathological changes to the brain tissue (Cervenka and Jahodar, 2006). Several neurodegenerative disorders such as AD, cerebral ischemia-reperfusion injuries, and head injuries are thought to be related to changes in oxidative metabolism. Increased oxidative stress, resulting from free radical damage to cellular function, can be involved in the events leading to AD (Wang et al., 2006a). Organic and toxic damage of the brain, formation of free radicals, and other changes participate in the development of AD (Shang et al., 1999; Xiao et al., 2000a, 2000b; Grundman and Delaney, 2002; Wang et al., 2003, 2006b; Tang et al., 2005; Cervenka and Jahodar, 2006; Wang and Tang, 2007). Therefore, drugs as such as nootropics, cognitives, and neuroprotectives are commonly used to treat AD (Cervenka and Jahodar, 2006).

Compared with tacrine, donepezil, rivastigmine, and galanthamine, HupA has better penetration through the blood–brain barrier, higher oral bioavailability, and longer duration of AChE inhibitory action (Wang et al., 2006a). It has been widely shown to reverse or attenuate loss of cognition in several behavioral models and different animal species including non-human primates, in addition to ameliorating deficits in learning and memory in human (Zhang and Tang, 2006). The most recent experimental data indicate that HupA, especially as its 5-Cl-*O*-vanillin derivative called ZT-1, shows particularly great promise to be the next internationally marketed new drug for AD treatment (Tang, 1996; Wang and Tang, 1998; Tang and Han, 1999; Ma and Gang, 2004; Orgogozo et al., 2006; Wang et al., 2006a; Zangara et al., 2006).

3.2. Effects on cholinesterase activity and inhibition mechanism

The key symptoms of AD are primarily caused by cholinergic dysfunction. A significant correlation has been found between a decrease in cortical cholinergic activity and the deterioration of mental test scores in patients with AD (Perry et al., 1978). Based on the cholinergic hypothesis of AD, cholinergic enhancement strategies have been at the forefront of efforts to pharmacologically palliate the cognitive impairments associated with AD. Among the various therapeutic approaches investigated to enhance cholinergic transmission, cholinesterase

Table 2
Lycopodium alkaloids reported from Huperziaceae plants (through April 2007)[a]

Popular name (chemical name)	Source[b]
I. Lycopodine class	
Acrifoline [$\Delta^{11,12}$,8-oxo-dihydrolycopodine (5β-OH)]	*Lycopodium selago* (Rodewald and Grynkiewicz, 1967)
Anhydrodihydrolycopodine	*Lycopodium phlegmaria* (Southon and Buckingham, 1989), *Lycopodium saururus* (Southon and Buckingham, 1989)
Annotinine	*Lycopodium selago* (Achmatowicz and Rodewald, 1955)
Clavolonine (8β-hydroxylycopodine)	*Huperzia miyoshiana* (Tong et al., 2003), *Lycopodium saururus* (Braekman et al., 1974), *Lycopodium serratum* (Southon and Buckingham, 1989), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967b), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967b), *Lycopodium sieboldii* (Southon and Buckingham, 1989)
Deacetylfawcettiine	*Lycopodium serratum* (Southon and Buckingham, 1989; Takayama et al., 2003)
Dihydrolycopodine (5α-OH)	*Lycopodium saururus* (Southon and Buckingham, 1989)
4,6α-Dihydroxylycopodine [(6α,15R)-4,6-dihydroxy-15-methyllycopodan-5-one] **(1)**	*Huperzia serrata* (Tan and Zhu, 2004)
4α,6α-Dihydroxyserratidine	*Huperzia serrata* (Tan et al., 2002g)
12-Epilycodoline (isolycodoline or pseudoselagine)	*Huperzia miyoshiana* (Tong et al., 2003), *Lycopodium lucidulum* (Ayer et al., 1969; Southon and Buckingham, 1989), *Lycopodium selago* (Achmatowicz and Rodewald, 1955, 1956)
12-Epilycodoline *N*-oxide [(12α,15R)-12-hydroxy-15-methyllycopodan-5-one *N*-oxide] **(2)**	*Huperzia serrata* (Tan and Zhu, 2004)
Fawcettiine (β-lofoline,5β-*O*-acetyl-8β-hydroxydihydro-lycopodine)	*Lycopodium saururus* (Braekman et al., 1974; Southon and Buckingham, 1989)
Flabelliformine (4α-hydroxylycopodine)	*Huperzia miyoshiana* (Tong et al., 2003), *Lycopodium lucidulum* (Ayer et al., 1969; Southon and Buckingham, 1989)
Gnidioidine ($\Delta^{11,12}$,8β-hydroxylycopodine)	*Lycopodium phlegmaria* (Southon and Buckingham, 1989)
Huperzine E	*Huperzia serrata* (Zhu et al., 1996; Wang et al., 2001)
Huperzine F	*Huperzia serrata* (Zhu et al., 1996; Wang et al., 2001)
Huperzine G	*Huperzia serrata* (Wang et al., 1998; Wang et al., 2000)
Huperzine O	*Huperzia serrata* (Wang et al., 2000)
6α-Hydroxylycopodine	*Huperzia serrata* (Yuan et al., 1995), *Lycopodium lucidulum* (Ayer et al., 1963)
7-Hydroxylycopodine [(15S)-7-hydroxy-15-methyllycopodan-5-one] **(3)**	*Huperzia serrata* (Tan and Zhu, 2004)
4α-Hydroxyserratidine	*Huperzia serrata* (Tan et al., 2002g)
6α-Hydroxyserratidine	*Huperzia serrata* (Tan et al., 2002g)
Lucidioline ($\Delta^{11,12}$-5β,6α-dihydroxydihydro-lycopodine)	*Huperzia serrata* (Zhou et al., 1993; Ma et al., 1998), *Lycopodium lucidulum* (Ayer et al., 1969), *Lycopodium serratum* (Takayama et al., 2003)
Lycoclavine (5β-*O*-acetyl-6α-hydroxy-dihydrolycopodine)	*Lycopodium serratum* (Yu et al., 1982a, 1982b)
Lycodoline	*Huperzia miyoshiana* (Tong et al., 2003), *Huperzia serrata* (Yuan et al., 1995), *Lycopodium lucidulum* (Ayer et al., 1969), *Lycopodium phlegmaria* (Southon and Buckingham, 1989), *Lycopodium saururus* (Southon and Buckingham, 1989), *Lycopodium selago* (Rodewald and Grynkiewicz, 1968), *Lycopodium serratum* (Yu et al., 1982a, 1982b; Takayama et al., 2003), *Lycopodium serratum* var. *serratum* (Morita et al., 2000), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967b), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967b), *Phlegmariurus fordii* (Ayer and Iverach, 1962; Inubushi et al., 1967b; Tong and Xiang, 1984)
Lyconesidine C	*Lycopodium chinense* (Hirasawa et al., 2002)
Lycopodine	*Huperzia miyoshiana* (Tong et al., 2003), *Huperzia serrata* (Yuan et al., 1995), *Lycopodium lucidulum* (Manske and Marion, 1946; Ayer et al., 1969), *Lycopodium phlegmaria* (Rouffiac, 1963), *Lycopodium saururus* (Southon and Buckingham, 1989), *Lycopodium selago* (Achmatowicz and Rodewald, 1956; Rodewald and Grynkiewicz, 1968), *Lycopodium serratum* (Takayama et al., 2003), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967b), *Lycopodium serratum* var *serratum f. intermedium* (Inubushi et al., 1967b)
Lycoposerramine G	*Lycopodium serratum* (Takayama et al., 2003)
Lycoposerramine H	*Lycopodium serratum* (Takayama et al., 2003)
Lycoposerramine I	*Lycopodium serratum* (Takayama et al., 2003)
Lycoposerramine K	*Lycopodium serratum* (Takayama et al., 2003)
Lycoposerramine L	*Lycopodium serratum* (Takayama et al., 2003)
Lycoposerramine M	*Lycopodium serratum* (Takayama et al., 2003)

Table 2 *Continued*

Lycoposerramine N	*Lycopodium serratum* (Takayama et al., 2003)
Lycoposerramine O	*Lycopodium serratum* (Takayama et al., 2003)
Miyoshianine A (=lycoposerramine F)	*Huperzia miyoshiana* (Tong et al., 2003), *Lycopodium serratum* (Takayama et al., 2003)
Miyoshianine B (=lycoposerramine J)	*Huperzia miyoshiana* (Tong et al., 2003), *Lycopodium serratum* (Takayama et al., 2003)
Sauroine (7,8-dihydroxylycopodine) **(4)**	*Huperzia saururus* (Ortega et al., 2004)
Selagoline	*Huperzia selago* (Staerk et al., 2004)
Serratezomine C (6α,12β-dihydrolycopodine)	*Lycopodium serratum* var. *longipetiolatum* (Morita et al., 2000)
Serratidine (7α-hydroxyanhydrolycodoline)	*Huperzia serrata* (Tan et al., 2002a), *Huperzia selago* (Staerk et al., 2004), *Lycopodium serratum* (Takayama et al., 2003), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967a, 1968a), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967a, 1968a)
II. Lycodine class	
Carinatumin A **(5)**	*Lycopodium carinatum* (Choo et al., 2007)
Carinatumin B **(6)**	*Lycopodium carinatum* (Choo et al., 2007)
Des-*N*-methyl-β-obscurine	*Huperzia serrata* (Yuan et al., 1995)
N,*N*-Dimethylhuperzine A	*Huperzia serrata* (Hu et al., 1992)
Himeradine A	*Lycopodium chinense* (Morita et al., 2003)
Huperzine A	*Huperzia serrata* (Liu et al., 1986a), *Huperzia selago* (Staerk et al., 2004), *Lycopodium selago* (Ayer and Kasitu, 1989)
Huperzine B	*Huperzia serrata* (Liu et al., 1986a)
Huperzine C	*Huperzia serrata* (Liu and Huang, 1994)
Huperzine D	*Huperzia serrata* (Liu and Huang, 1994)
Huperzine U (2,3-dihydro-12-hydroxyhuperzine B)	*Huperzia serrata* (Tan et al., 2003a)
Huperzinine	*Huperzia serrata* (Yuan and Wei, 1988)
6β-Hydroxyhuperzine A	*Huperzia serrata* (Yuan and Zhao, 2000), *Lycopodium selago* (Ayer and Kasitu, 1989)
Lycodine	*Huperzia serrata* (Yuan et al., 1994), *Lycopodium lucidulum* (Southon and Buckingham, 1989), *Lycopodium phlegmaria* (Rouffiac, 1963), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967b), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967b), *Lycopodium serratum* var. *thunbergii* (Southon and Buckingham, 1989)
N-Methyl-huperzine B	*Huperzia serrata* (Yuan and Wei, 1988; Southon and Buckingham, 1989)
Nankakurine A **(7)**	*Lycopodium hamiltonii* (Hirasawa et al., 2004)
α-Obscurine (2,3-dihydro-β-obscurine)	*Lycopodium selago* (Rodewald and Grynkiewicz, 1968)
β-Obscurine	*Lycopodium selago* (Rodewald and Grynkiewicz, 1968)
Phlegmariurine M	*Phlegmariurus fordii* (Mou et al., 1989)
Sauroxine (12-epi-α-obscurine)	*Lycopodium saururus* (Deulofeu and de Langhe, 1942; Ayer et al., 1965; Southon and Buckingham, 1989)
III. Fawcettimine class	
8-Deoxy-13-dehydro-serratinine	*Lycopodium phlegmaria* (Inubushi and Harayama, 1982), *Lycopodium serratum* (Southon and Buckingham, 1989)
8-Deoxyserratinidine	*Lycopodium phlegmaria* (Inubushi and Harayama, 1982; Southon and Buckingham, 1989)
8-Deoxyserratinine	*Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967a; Ishii et al., 1970), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967b), *Huperzia serrata* (Li et al., 1987; Southon and Buckingham, 1989)
7α,11α-Dihydroxy-phlegmariurine B	*Huperzia serrata* (Tan et al., 2002b)
Epidihydrofawcettidine (5α-OH)	*Lycopodium phlegmaria* (Inubushi and Harayama, 1982; Harayama et al., 1984; Southon and Buckingham, 1989)
Fawcettidine	*Lycopodium phlegmaria* (Southon and Buckingham, 1989)
Fawcettimine	*Huperzia serrata* (Tan et al., 2000a)
Huperserratinine	*Huperzia serrata* (Zhu et al., 1994)
Huperzine H	*Huperzia serrata* (Gao et al., 1999)
Huperzine I (2α-hydroxyfawcettidine)	*Huperzia serrata* (Gao et al., 2000b)
Huperzine P	*Huperzia serrata* (Tan et al., 2000a)
Huperzine Q	*Huperzia serrata* (Tan et al., 2002f)
Huperzine R	*Huperzia serrata* (Tan et al., 2002e)
Huperzine S (2β,13β-epoxyalopecuridine)	*Huperzia serrata* (Tan et al., 2003a)
Huperzine T (5α-hydroxy-6-oxodihydrophlegmariurine A)	*Huperzia serrata* (Tan et al., 2003a)
Huperzine W	*Huperzia serrata* (Tan et al., 2002h)
7-Hydroperoxy-phlegmariurine B	*Huperzia serrata* (Tan et al., 2003b)
11α-Hydroperoxy-phlegmariurine B	*Huperzia serrata* (Tan et al., 2003b)
2α-Hydroxyphlegmariurine B	*Huperzia serrata* (Tan et al., 2002d)

Table 2 *Continued*

7α-Hydroxyphlegmariurine B	*Huperzia serrata* (Tan et al., 2002c)
8α-Hydroxyphlegmariurine B	*Huperzia serrata* (Tan et al., 2000b)
8β-Hydroxyphlegmariurine B	*Huperzia serrata* (Yuan and Zhao, 2003)
11α-Hydroxyphlegmariurine B	*Huperzia serrata* (Tan et al., 2002c)
Lycoflexine	*Lycopodium phlegmaria* (Inubushi and Harayama, 1982), *Lycopodium serratum* (Takayama et al., 2002), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967a), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967a)
Lyconesidine A	*Lycopodium chinense* (Hirasawa et al., 2002)
Lyconesidine B	*Lycopodium chinense* (Hirasawa et al., 2002)
Lycophlegmarine	*Lycopodium phlegmaria* (Inubushi and Harayama, 1981, 1982; Southon and Buckingham, 1989)
Lycoposerramine A	*Lycopodium serratum* (Takayama et al., 2001)
Lycoposerramine B **(8)**	*Lycopodium serratum* (Katakawa et al., 2005)
Lycoposerramine C	*Lycopodium serratum* (Takayama et al., 2002)
Lycoposerramine D	*Lycopodium serratum* (Takayama et al., 2002)
Lycoposerramine E	*Lycopodium serratum* (Takayama et al., 2002)
Lycoposerramine P	*Lycopodium serratum* (Takayama et al., 2002)
Lycoposerramine Q	*Lycopodium serratum* (Takayama et al., 2002)
Lycoposerramine S	*Lycopodium serratum* (Takayama et al., 2002)
Lycoposerramine U	*Lycopodium serratum* (Takayama et al., 2002)
Lycothunine ($\Delta^{11,12}$-fawcettimine)	*Lycopodium serratum* var. *longipetiolatum* (Inubushi and Harayama, 1981; Southon and Buckingham, 1989)
Macleanine	*Lycopodium serratum* (Ayer et al., 1994)
Neohuperzinine	*Huperzia serrata* (Yuan et al., 2002)
2-Oxophlegmariurine B	*Huperzia serrata* (Tan et al., 2002d)
11-Oxophlegmariurine B	*Huperzia serrata* (Tan et al., 2002d)
N-Oxyhuperzine Q	*Huperzia serrata* (Tan et al., 2002c)
Phlegmariurine A	*Huperzia serrata* (Tan et al., 2000b), *Phlegmariurus fordii* (Tong and Xiang, 1984; Wan et al., 1986; Chu and Li, 1988), *Lycopodium serratum* (Takayama et al., 2002)
Phlegmariurine B	*Huperzia serrata* (Yuan et al., 1994; Tan et al., 2000a, 2000b, 2002d), *Phlegmariurus fordii* (Tong and Xiang, 1984; Chu and Li, 1988)
Phlegmariurine C	*Phlegmariurus fordii* (Chu and Li, 1988)
Serratanidine (8α-hydroxy serratine)	*Lycopodium serratum* (Inubushi et al., 1968c; Southon and Buckingham, 1989), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967a), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967a)
Serratanidine (8α-hydroxy serratine)	*Lycopodium serratum* (Inubushi et al., 1968c; Southon and Buckingham, 1989), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967a), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967a)
Serratezomine A	*Lycopodium serratum* var. *longipetiolatum* (Morita et al., 2000)
Serratezomine B (serratinine *N*-oxide)	*Lycopodium serratum* var. *longipetiolatum* (Morita et al., 2000)
Serratine	*Huperzia serrata* (Zhang et al., 1990) *Lycopodium serratum* var. *thunbergii* (Inubushi et al., 1964), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967b), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967b)
Serratinidine (5α-NHAc,8α-OH-fawcettidine)	*Lycopodium serratum* (Yasui et al., 1966; Southon and Buckingham, 1989), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967b), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967b)
Serratinine	*Huperzia serrata* (Zhou et al., 1993; Ma et al., 1998), *Lycopodium serratum* (Yu et al., 1982a; Southon and Buckingham, 1989), *Lycopodium serratum* var. *serratum* (Morita et al., 2000), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967b), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967b), *Lycopodium serratum* var. *thunbergii* (Inubushi et al., 1962, 1968b)
Sieboldine A	*Lycopodium sieboldii* (Hirasawa et al., 2003b)
IV. Miscellaneous group	
Carinatumin C **(9)**	*Lycopodium carinatum* (Choo et al., 2007)
Cernuine (deoxylycocernuine)	*Lycopodium chinense* (Morita et al., 2001)
Dihydroluciduline (5α-OH)	*Lycopodium lucidulum* (Ayer et al., 1968; Southon and Buckingham, 1989)
Dihydrolycolucine (14,15,16, 17-tetradehydrolucidine B)	*Lycopodium lucidulum* (Ayer et al., 1979; Southon and Buckingham, 1989)
N,*N*-Dimethylphlegmarine	*Lycopodium phlegmaria* (Nyembo et al., 1978; Ayer et al., 1979; Inubushi and Harayama, 1982; Southon and Buckingham, 1989)
Huperzine J	*Huperzia serrata* (Gao et al., 2000a)
Huperzine K	*Huperzia serrata* (Gao et al., 2000a)
Huperzine L	*Huperzia serrata* (Gao et al., 2000a)
Huperzine V	*Huperzia serrata* (Liu et al., 2004)
Huperzinine B	*Huperzia serrata* (Yuan et al., 2001)
Lucidine A	*Lycopodium* lucidulum (Ayer et al., 1979; Tori et al., 2000)
Lucidine B (serratanine A)	*Lycopodium lucidulum* (Ayer et al., 1979; Tori et al., 2000), *Lycopodium serratum* var. *serratum f. serratum* (Inubushi et al., 1967b), *Lycopodium serratum* var. *serratum f. intermedium* (Inubushi et al., 1967b)

Table 2 *Continued*

Luciduline	*Lycopodium lucidulum* (Ayer et al., 1968; Southon and Buckingham, 1989)
Lucidulinone (9-ketoluciduline)	*Lycopodium lucidulum* (Ayer et al., 1968; Tori et al., 2000)
Lycolucine (10,11,14,15,16, 17-hexadehydro-lucidine B)	*Lycopodium lucidulum* (Ayer et al., 1979; Southon and Buckingham, 1989)
Lycoperine A **(10)**	*Lycopodium hamiltonii* (Hirasawa et al., 2006)
*N*α-Methylphlegmarine	*Lycopodium phlegmaria* (Nyembo et al., 1978; Southon and Buckingham, 1989)
*N*β-Methylphlegmarine	*Lycopodium phlegmaria* (Nyembo et al., 1978; Southon and Buckingham, 1989)
Oxolucidine A	*Lycopodium lucidulum* (Ayer et al., 1979; Tori et al., 1999, 2000)
Oxolucidine B (or serratanine B,14β-hydroxy-lucidine B)	*Lycopodium serratum* (Ayer et al., 1979; Inubushi et al., 1980; Ayer et al., 1984; Southon and Buckingham, 1989)
Phlegmarine	*Lycopodium phlegmaria* (Nyembo et al., 1978)
Phlegmariurine N	*Huperzia serrata* (Miao et al., 1989; Yuan and Zhao, 2000)
Senepodine A	*Lycopodium chinense* (Morita et al., 2001)
Senepodine B	*Lycopodium chinense* (Hirasawa et al., 2003a)
Senepodine C	*Lycopodium chinense* (Hirasawa et al., 2003a)
Senepodine D	*Lycopodium chinense* (Hirasawa et al., 2003a)
Senepodine E	*Lycopodium chinense* (Hirasawa et al., 2003a)
Senepodine G	*Lycopodium chinense* (Morita et al., 2004)
Senepodine H	*Lycopodium chinense* (Morita et al., 2004)

[a] This table was modified from Table 1 of Ma and Gang (2004) published review (Ma and Gang, 2004).

[b] *Lycopodium selago* = *Huperzia selago*; *Lycopodium lucidulum* = *Huperzia lucidula*; *Lycopodium phlegmaria* = *Phlegmariurus phlegmaria*; *Lycopodium chinense* = *Huperzia chinensis*; *Lycopodium hamiltonii* = *Phlegmariurus hamiltonii*; *Lycopodium carinatum* = *Phlegmariurus carinatus*; *Lycopodium sieboldii* = *Phlegmariurus sieboldii*; *Lycopodium saururus* = *Huperzia saururus*. According to the taxonomic system of Flora of China, *Lycopodium serratum* var. *serratum f. serratum*, *Lycopodium serratum* var. *serratum f. intermedium*, *Lycopodium serratum* var. *longipetiolatum*, and *Lycopodium serratum* var. *thunbergii* are synonym of *Huperzia serrata*.

inhibitors (ChEI) are the first group of compounds that have shown some promise in the treatment of AD (Wang et al., 2006a).

Comparison studies with respect to in vitro and in vivo AChE inhibition showed that the potency of HupA was similar or superior to the inhibitors currently being used in AD treatment (Table 3) (Wang et al., 1986; Wang and Tang, 1998; Ogura et al., 2000; Zhao and Tang, 2002; Liang and Tang, 2004; Ma and Gang, 2004; Wang et al., 2004, 2006a). HupA causes a distinct concentration dependent inhibition in vitro of AChE and BuChE (Wang and Tang, 1998). The AChEI activity (IC_{50}) of HupA relative to other AChEIs is: donepezil > HupA > tacrine > physostigmine > galantamine > rivastigmine. Whereas HupA is the least potent BuChE inhibitor among the tested inhibitors and much more selective than these other compounds in its action (Wang et al., 1986; Cheng et al., 1996; Ogura et al., 2000). The nanomolar range of apparent inhibition constants (K_i value) for AChE indicates that these inhibitors have high affinity for the enzyme (Wang et al., 2006a). However, the doses of donepezil and tacrine used orally are much higher than that of HupA (Cheng and Tang, 1998), which might be related to their low bioavailability and/or by rapid metabolism (Wang et al., 2006a). Moreover, comparison studies of the effects of HupA, donepezil and rivastigmine on cortical acetylcholine levels and acetylcholinesterase activity in rats demonstrated that HupA was eight- and two-fold more potent in molar terms, respectively, than donepezil and rivastigmine in increasing cortical acetylcholine levels; and the effect of HupA was longer lasting (Liang and Tang, 2004). Furthermore, the inhibition of BuChE by tacrine is significantly higher than that by the other AChEIs (Table 3). The most obvious and severe side effect of tacrine among these drugs suggests that BuChE inhibition activity may contribute to these side-effects. It can be concluded that a high BuChE/AChE IC_{50} ratio is very desirable in AD drugs that target the cholinergic system (Ma and Gang, 2004).

HupA and other ChEIs can produce marked effects by inhibiting AChE, delaying hydrolysis of acetylcholine, and enhancing the level of acetylcholine in the synaptic cleft. These effects are thought to be the reason why these compounds are beneficial in treating AD symptoms (Ma and Gang, 2004). The mechanisms by which HupA inhibits AChE have been extensively studied using kinetics (Wang et al., 1986; Ashani et al., 1992), computer aided docking studies (Pang and Kozikowski, 1994; Dvir et al., 2002) and X-ray crystallographic approaches (Raves et al., 1997). Structural biology investigations (particularly by X-ray crystallography and computational modeling) have shown that HupA acts against AChE by directly binding to the opening of the active site of this enzyme, thus preventing access to the active site by the normal substrate (Raves et al., 1997). Kozikowski and coworkers hypothesized that the three-carbon bridge substructure of HupA was the prerequisite structure for the AChEI activity, and that the activity would be lowered if the double bond was eliminated from this portion of the molecule (Kozikowski et al., 1991b). The X-ray crystal structure of the (−)-huperzine A-AChE complex showed that this bridge in HupA was inserted into the hydrophobic area of AChE surrounded by aromatic residues (Raves et al., 1997). The other AChEIs function in a similar manner, but the HupA–AChE complex has a longer half-life than these and other prophylactic agents (Ma and Gang, 2004).

3.3. Neural cell protection

The most common pharmacological treatments for AD in recent years have focused on AChE inhibition. However, there is evidence that a multitarget approach might be more effective

Table 3
Effects of HupA and other cholinesterase inhibitors on AChE activity in the rat cortex and BuChE activity in rat serum in vitro

ChEI	IC_{50} (μM)		Ratio of IC_{50} BuChE/AChE	K_i (nM)
	AChE[a]	BuChE[b]		
Huperzine A	0.082	74.43	907.7	24.9
Tacrine	0.093	0.074	0.8	105.0
Donepezil	0.010	5.01	501.0	12.5
Physostigmine	0.251	1.26	5.0	–
Rivastigmine	181.39	31.07	0.17	–
Galanthamine	1.995	12.59	6.3	210.0

Data from Cheng et al. (1996) and Wang et al. (1986b), organized by Tang and Han (1999) and Wang et al. (2006).
[a] Rat cortex.
[b] Rat serum.

and accurate than an AChE single-target approach (Zhang and Tang, 2006). HupA has neuroprotective effects that go beyond the inhibition of AChE (Wang and Tang, 2005). Recent data have demonstrated that HupA can ameliorate the learning and memory deficiency in animal models and AD patients (Wang et al., 2006a; Zhang and Tang, 2006). Its potentially beneficial actions include modification of β-amyloid peptide processing, reduction of oxidative stress, neuronal protection against apoptosis, and regulation of the expression and secretion of nerve growth factor (NGF) and NGF signaling (Zhang and Tang, 2006).

3.4. Pretreatment drug for potential exposure to OP nerve agents

Organophosphate (OP) nerve agents are considered to be potential threats in both military and terrorism situations (Ricordel and Meunier, 2000; Lallement et al., 2002b). OP agents are potent irreversible inhibitors of central and peripheral AChE. Current pretreatment to ameliorate OP poisoning relies on the subchronic administration of the reversible AChE inhibitor pyridostigmine (PYR). However, PYR does not penetrate into the brain and afford protection against seizures and subsequent neuropathology induced by an OP agent such as soman. In contrast, HupA is a reversible AChEI that crosses the blood–brain barrier and has been successfully tested for pretreatment of OP poisoning (Lallement et al., 1997, 2002a, 2002b; Lallement, 2000; Tonduli et al., 2001). It can be expected that HupA will find wide-spread use in this area as well as in AD treatment.

4. Toxicology

Toxicological studies conducted in different animal species indicated less severe undesirable side effects associated with cholinergic activation for HupA than for other ChEIs such as physostigmine and tacrine (Wang and Tang, 1998; Zangara, 2003). In mice, the LD_{50} doses were 4.6 mg (po), 3.0 mg (sc), 1.8 mg (ip), and 0.63 mg (iv). Histopathological examinations showed no changes in liver, kidney, heart, lung or brain after administration of HupA for 180 days, or in dogs (0.6 mg/kg, im) or rats (1.5 mg/kg, po). No mutagenicity was found in rats, and no teratogenic effect was observed in mice or rabbits (Zangara, 2003).

5. Clinical trials

5.1. Clinical trials with HupA

Clinical trials performed with HupA have demonstrated that HupA produces significant improvements in memory deficiencies in aged and AD patients. As mentioned in the Introduction, most of these studies have been performed in China, and an estimated 100,000 people have been treated with HupA (Chiu and Zhang, 2000). Several comprehensive reviews related to HupA clinical trials have been published recently and we recommend interested readers to refer to these sources for more detailed information (Jiang et al., 2003; Ma and Gang, 2004; Zhu et al., 2004; Wang et al., 2006a). A short summary of the major findings of HupA clinical trials in China and USA follows. Results of these trials indicate that HupA is an effective and safe drug that remarkably improves cognitive function, behavior, activity of daily life, and mood of AD patients (Table 4) (Zhang, 1986; Xu et al., 1999; Jiang et al., 2002; Zhang et al., 2002b; Kuang et al., 2004). HupA is also undergoing clinical trials in the USA for the treatment of age-related memory deficiency (http://www.clinicaltrials.gov/show/NCT00083590). The safety and efficacy of HupA were evaluated in 26 patients meeting the DSM IV-R (Diagnostic and Statistical Manual of Mental Disorders, Fourth Revision) and the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke) criteria for uncomplicated AD and possible or probable AD (Table 4) (Mazurek, 1999). Despite the small number of patients, the authors observed dose-related improvements with higher Mini Mental State Examination (MMSE) scores at higher dosage, and no serious side effects (Mazurek, 1999).

Combined therapy with HupA plus other medicines or mental training also showed positive results. For example, the superiority of combined therapy over HupA alone was suggested by significant favorable differences in MMSE, activity of daily living (ADL), and clinical dementia rating (CDR) scores after AD patients were treated with HupA plus nicergoline, conjugated estrogen, and nilestriol (for female AD patients) (Wang et al., 2003; Zhou et al., 2004a). Moreover, American Association of Mental Deficiency (AAMD) and ADL scores showed marked improved after treatment with HupA combined with training in daily life activities for 8 weeks for patients with mild to moderate AD (Miao, 2002). Furthermore, after AD and VD patients were

Table 4
Clinical trials performed using HupA to treat AD

Reference	Design[a]	Dose regime	Patient type[b]	No. of patients	Efficacy	Side effects
Zhang (1986)	DB	0.03 mg, im	Elderly patients with 17 probable cases of AD	100	Significant improvement in all rating scores as evaluated by Buschke Selective Reminding performance	No severe side effects were found
Jiang et al. (2002)	DB	0.03 mg, im, bid	Ageassociated memory impairment with MQ (WMS) < 100	120	The effective rates were 68.3% and 26.4%, respectively, in the treated and control groups for 14–15 days trials	No significant side effects were observed
		0.1 mg, po, qid	Cognitive deterioration, with MQ (WMS) < 100	88	The effective rates for the treated and control groups were 68.18% and 34.09% for 14–15 days trials, respectively	No significant side effects were observed except for minor complaints of gastric discomfort (2), dizziness (1), insomnia (1) and mild excitement (1) in the treated group
			VD AD	25 55	The effective rate of the treated group was 60%, significantly higher than the control (35%) for 14–15 days trials	No marked side effects were observed
Xu et al. (1999)	DB, DM, PC, R	0.2 mg, bid	AD	60	Significant differences on all the psychological evaluations between "before" and "after" the 60 days trials. Reducing the pathological changes of the oxygen free radicals in the plasma and erythrocytes of the patients	No severe side effects except mild to moderate nausea were observed
Zhang et al., (2002)	MC, DB, R, PC	0.4 mg/d	Possible or probable AD	202	Improved significantly at week 6, and further improved at week 12	Mild and transient adverse events (edema of bilateral ankles and insomnia) were observed in 3% of HupA treated patients
Kuang et al. (2004)	R	0.1 mg, po, tid	AD	61	Improvement in their memory, cognitive skills, and ability in their daily life	No severe side effects were found
Mazurek (2000)	Open label	0.05 mg, po, bid 0.1 mg, po, bid	Uncomplicated AD and possible or probable AD	22 4	Dose-related improvements with higher MMSE scores at higher dosage	No serious side effects were found

[a] DM: double-mimic; MC: multicenter; DB: double blind; R: randomized; PC: placebo-controlled.
[b] MQ: memory quotient; WMS: the Wechsler Memory Scale.

treated for 8 weeks with HupA complemented with a mental stimulation program consisting of reminiscence, reality orientation and remotivation, Hasegawa dementia scale (HDS), CDR and ADL scores were significantly improved (Wang et al., 2002). A clinical investigation involving 50 middle-aged and elderly patients demonstrated the efficacy of HupA in improving verbal recall, retention and retrieval in patients with mild and moderate degrees of dysmnesia (Chang et al., 2002; Zhang et al., 2002a; Wang et al., 2006a).

There is evidence that people with schizophrenia have neurocognitive impairments across multiple domains, including impairments in motor functioning, various aspects of attentional abilities, executive functions and memory functioning (Wang et al., 2006a). Ma et al. (2003) have recently studied the effect of HupA on memory disorders in schizophrenic patients and the results showed that memory functions of patients were significantly improved after treated with HupA (Ma et al., 2003). Similar results were also reported by Fang et al. (2002) and Yang (2003).

In addition, HupA may have application for younger people as well. Sun et al. (1999) reported that HupA enhanced the memory and learning performance of adolescent students. In a study conducted with children who had language delay and other developmental conditions, treatment with 0.05 mg HupA twice daily for more than 3 months improved language delay scores by a total of 67.56% (Liao et al., 2002).

Phase IV clinical trials conducted in China have demonstrated that HupA induces significant improvement in the memory of elderly people and patients with AD and VD without any notable side effects (Xu et al., 1995, 1999; Zhang et al., 2002b; Wang et al., 2006a).

5.2. Clinical trials with ZT-1

In vitro studies have shown ZT-1, through its biotransformation into HupA, to be a highly potent and selective AChE inhibitor. In vivo, ZT-1 resulted in a marked dose-dependent inhibition of AChE and increased acetylcholine brain cortical levels in rats and reversal of scopolamine-induced memory deficits in both rats and monkeys (Orgogozo et al., 2006).

ZT-1 has been developed as a new drug candidate by cooperation between the Shanghai Institute of Materia Medica at the Shanghai Academy of Sciences in China and Debiopharm in Switzerland. Clinical trials were performed with ZT-1 to determine its efficacy and safety in treating AD, and to see if it behaved the same as HupA in human subjects. These clinical trials of ZT-1 are currently in progress in China and Europe. Phase I and II clinical trials were initiated in 2000 and 2004, respectively.

Phase I studies involving young and elderly volunteers were used to evaluate safety. Phase II clinical trials for the treatment of AD are underway in 28 hospitals in 6 European countries are underway (http://www.debio.com/e/pdf/zt_1_e.pdf). So far, the results of these clinic trials are very encouraging and exciting. ZT-1 was generally safe and well tolerated. Most side effects corresponded to those known for currently marketed AChEIs, such as donepezil. However, ZT-1 exhibited marked improvement for some common gastrointestinal side effects. Controlled drug release may be a significant advantage for symptomatic AD treatment. Based on the cholinergic neurotransmitter deficit hypothesis, ZT-1 might prove useful for the symptomatic therapy of AD (Capancioni et al., 2006; Csajka et al., 2006; Orgogozo et al., 2006; Scalfaro et al., 2006; Zangara et al., 2006).

6. HupA source plants

The original source plant of HupA is *Huperzia serrata* (Chinese herb name: *Qian Ceng Ta*, *She Zu Cao*, *Jin Bu Huan*, *Shan Zhi*, etc.) (Liu et al., 1986a, 1986b; Ma, 1997; Ma et al., 2006). As discussed above, it has been extensively used for over 1000 years in different areas of China as a popular traditional Chinese medicine (Ma et al., 2006). *Huperzia serrata* belongs to the Huperziaceae family, according to the system of *Lycopodium* (s.l.) plants currently in use in China. This system was set up by Ching in 1978 (Table 5) (Ching, 1978). The Huperziaceae was originally separated from *Lycopodium* (s.l.) by Rothmaler (1944). *Lycopodium* (s.l.) once was a large genus with more than 500 club moss species, including all members of the order Lycopodiales. Three taxonomic systems have been proposed for *Lycopodium* (s.l.), including that of Ching (Ching, 1978), and those by Holub (Holub, 1985) and Ollgaard (Ollgaard, 1989) (Table 2). According to Ching's system (Ching, 1978), Lycophytina comprises two orders: Lycopodiales and Selaginellales. Selaginellales is a single family (Selaginellaceae) single genus (*Selaginella*) order. Lycopodiales includes two families, Huperziaceae and Lycopodiaceae, and seven genera: *Huperiza*, *Phlegmariurus*, *Lycopodium*, *Lycopodiella*, *Phahinhaea*, *Diphasiastrum*, and *Lycopodiastrum*. Zhang et al. have reported extensive investigations on the classical taxonomy of Chinese club mosses and their results have supported Ching's system (Zhang and Kung, 1998, 1999, 2000a, 2000b). The chemotaxonomic and DNA fingerprinting investigations of *Lycopodium* (s.l.) that were reported by Ma et al. (1998a, 1998b) also supported Ching's system. Ching's system has also been adopted in the recently published Flora of China (Zhang, 2004).

Table 5
The three most popular classifications of Lycopodiales

Ching (1978)	Holub (1985)	Ollgaard (1989)
Family Huperziaceae	Family Huperziaceae	Family Lycopodiaceae
Genus *Huperzia*	Genus *Huperzia*	Genus *Huperzia*
Genus *Phlegmariurus*	Family Lycopodiaceae	Genus *Lycopodium*
Family Lycopodiaceae	Genus *Lycopodium*	Genus *Lycopodiella*
Genus *Lycopodium*	Genus *Lycopodiella*	Genus *Phylloglossum*
Genus *Diphasiastrum*	Genus *Lycopodiostrum*	
Genus *Palhinhaea*	Genus *Diphasiopsis*	
Genus *Lycopodiella*	Genus *Pseudolycopodium*	
Genus *Lycopodiostrum*	Genus *Diphasium*	
	Genus *Diphasiastrum*	
	Genus *Palhinhaea*	
	Genus *Lateristochys*	
	Genus *Phylloglossum*	

Are there any plants that could be a better source of HupA than *Huperzia serrata*? We conducted a series investigations on the phylogeny, taxonomy, natural resources, chemotaxonomy, ethnopharmacology, and HupA content of 101 species of the subdivision Lycophytina that occur in China (Ma, 1997; Ma et al., 1998a, 1998b, 2005, 2006; Ma and Gang, 2004). Of these, 52 species, 5 varieties and 2 forma are from the Huperziaceae; 17 species are from the Lycopodiaceae; 15 species are from the Sellaginellaceae. A chemotaxonomic analysis of Lycopodium alkaloids in *Huperzia* and related genera of Lycopodiales was performed by Ma et al. (1998a). *Huperzia* (18 species, 1 variety and 2 forma) and related genera *Phlegmariurus* (8 species), *Lycopodium* (3 species), *Lycopodiella* (1 species), *Palhinhaea* (1 species), *Diphasiastrum* (2 species), and *Lycopodiastrum* (1 species) were examined by thin layer chromatography for the presence of 10 reference *Lycopodium* alkaloids: HupA, HupB, serratine, serratinine, lycodoline, lucidioline, lycopodine, lyconnotine, annotinine, and cernuine. The results indicated that HupA was present in species from both *Huperzia* and *Phlegmariurus*, but not from other genera. Using chemotaxonomic markers, the various genera could be distinguished from one another. These results also supported Ching's taxonomic system of the Lycopodiales as a reasonable classification system for Lycopodiales plants occurring in China.

Huperzia is a group of plants that are mostly terrestrial and erect, with gemmae present, and fertile leaves being normally the same size as sterile leaves. *Huperzia* plants were widely distributed in tropical, subtropical, and temperate zones in China. In contrast, *Phlegmariurus* plants are mostly epiphytic, pendent to erect, with gemmae absent, and fertile leaves present in strobili of smaller leaves. *Phlegmariurus* plants were found mainly in tropical and subtropical zones. Traditional uses of *Phlegmariurus* plants as medicinal herbs are very similar to uses of *Huperzia* plants, such that whole plants are used as remedies for the treatment of contusions, strains, swellings, schizophrenia, and myasthenia gravis (Ma and Gang, 2004). Purified alkaloid extracts from an Argentinean popular medicine made from *Huperzia saururus* also possesses AChEI activity (Ortega et al., 2004, 2006; Vallejo et al., 2007). However, no purified compound information has been reported for this plant.

To date, HupA has only been detected in species of the Huperziaceae. Species belonging to the Lycopodiaceae do not produce HupA or related compounds. Thus, HupA source plants appear to be limited to the plants of the Huperziaceae.

6.1. Natural resources

HupA can be chemically synthesized in the laboratory (Qian and Ji, 1989). Original synthesis methods were not capable of being industrialized. However, in recent years, Kozikowski's group has developed methods to synthesize HupA on an industrial scale. Nevertheless, HupA containing plants continue to be harvested from the wild. This practice will likely continue, as natural health products and dietary supplement forms of *H. serrata* and related species will continue to be in demand, placing further pressure on this group of increasingly threatened plants.

Huperziaceae plants are globally distributed with a total of about 150 species. Of these, 47 species have been found in China and 26 were endemic species of China (Ma, 1997; Zhang, 2004). The largest distribution Huperziaceae species is in tropical habitats of the Americas. In China, these plants are distributed mainly in the area along the Yangtze River and throughout the southern parts of China. *Huperzia serrata* is the only relatively common Huperziaceae species distributed in China. Nevertheless, these plants are not abundant, grow very slowly and are only found in very specialized habitats. Furthermore, *H. serrata* possesses a very low content of HupA (*ca*. 0.007%). Because of the low abundance of HupA in these small plants, the demand for HupA may soon lead to the decimation of all wild populations of *H. serrata* and related species (Ma and Gang, 2004).

An investigation of the natural resources of Huperzine alkaloid source plants in Anhui, Zhejiang, Jiangsu, Jiangxi, Fujian, Guangxi, Guangdong, Hainan, Guizhou, Yunnan, Xizang, Sichuan, Chongqing, Hubei, Hunan, Shaanxi, Xinjiang, Liaonin, Jilin and Heilongjiang provinces of China has been published (Ma, 1997; Ma et al., 2006). Morphological characters, distribution, chemical constituents, traditional medicinal use and preparation were determined through taxonomic comparison, field investigation, chemical tests and literature comparison (Ma et al., 2006). Analysis of the chemical constituents in Huperziaceae species focused on the Lycopodium alkaloids. The results of these investigations indicated that the natural resources of most Huperziaceae species are uncommon and rare.

The Huperziaceae is one of the oldest extant vascular plant lineages, dating back to the late Silurian (Ma and Gang, 2004). The habitats where these plants grow are very special and sensitive. These plants also grow very slowly, normally requiring 15–20 years of growth from spore germination to maturity. The sporophytes of *H. serrata* only reach a height of 5–15 cm and the whole plant body is harvested for HupA collection. HupA source plants are disappearing from many areas of their historical distribution. The local ecological environments have been destroyed due to farming, tree harvest, and removal of stones for building purposes. Over-harvesting of these plants from the wild currently has become a major problem for natural resources of HupA source plants. Huperziaceae species in China are in danger of becoming endangered or even extinct in the near future (Ma et al., 2006). A law dividing Huperziaceae natural resources into forbidden, limited, and collectable populations in different areas of China would be helpful for limitation and protection of Huperziaceae natural resources and allow their continued use in a sustainable manner (Ma et al., 2006).

6.2. In vitro propagation of HupA source plants

Cultivation of the HupA source plant *Huperzia serrata* is very difficult. So far, no successful mass production has been reported either for the cultivation or the tissue culture of HupA source plants, although a number of laboratories around the world have attempted to either introduce these plants into cultivation or propagate them via in vitro methods. Investment in and encouragement of in vitro micropropagation research of Huperziaceae

plants will be the best choice to rescue this threatened natural resource. Ma and Wellmann at the University of Freiburg, Germany succeeded in propagating *Phlegmariurus squarrosus* (Forst.) Löve et Löve. The in vitro produced cultures of this plant *Phlegmariurus squarrosus* contained significantly higher lever HupA than the parent plant or of *Huperzia serrata* plants (Ma and Gang unpublished results) (Ma et al., 2006). Such in vitro propagated plants will provide an invaluable resource for production of HupA in a non-synthetic, yet sustainable manner.

7. Conclusion

Because of the significant pharmacological activities of HupA and its derivative ZT-1, Huperziaceae species have recently been a hot target of many investigations related to the chemical, biological, pharmacological, and medical properties of these plants. Results from these investigations clearly show that there is a strong relationship between the ethnopharmacological use of these plants and the medicinal properties of important compounds identified from them, such as HupA. HupA has only been found in species belonging to the Huperziaceae. Because they are a major source for HupA, and the only natural source, the natural resources situation of the Huperziaceae is not optimistic. Methods to propagate HupA containing plants, either in an agronomic setting or in vitro, must be developed to help protect this very valuable, but increasingly threatened group of natural medicinal plants.

Acknowledgements

The authors would like to thank the financial support from the National Natural Science Foundation of China (NSFC, #39900013 to XM) for research related to this review and Professor M. Heinrich for critical review of the manuscript.

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