Molecules **2014**, *19*, 19172-19179; doi:10.3390/molecules191119172

OPEN ACCESS

molecules

ISSN 1420-3049

www.mdpi.com/journal/molecules

Article

Anti-Cholinesterase Activity of *Lycopodium* Alkaloids from Vietnamese *Huperzia squarrosa* (Forst.) Trevis

Nguyen Ngoc Chuong [1], Nguyen Thi Thu Huong [1], Tran Manh Hung [2,*] and Tran Cong Luan [1,*]

[1] Research Center of Ginseng and Medicinal Materials, 41 Dinh Tien Hoang, District 1, Ho Chi Minh City 700-00, Vietnam

[2] Faculty of Chemistry, University of Science, Vietnam National University-HoChiMinh City, 227 Nguyen Van Cu, District 5, Ho Chi Minh City 227-01, Vietnam

* Authors to whom correspondence should be addressed; E-Mails: tmhung801018@gmail.com (T.M.H.); congluan53@gmail.com (T.C.L.); Tel./Fax: +84-8-3827-4377 (T.C.L).

External Editor: Isabel C. F. R. Ferreira

Received: 6 October 2014; in revised form: 14 November 2014 / Accepted: 17 November 2014 / Published: 19 November 2014

Abstract: A series of *Lycopodium* alkaloids, namely lycosquarosine A (**1**), acetylaposerratinine (**2**), huperzine A (**3**), huperzine B (**4**), 8α-hydrophlemariurine B (**5**), and huperzinine (**6**), has been isolated from Vietnamese *Huperzia squarrosa*. Among them, lycosquarosine A (**1**) is the new metabolite of the natural source. Lycosquarosine A completely inhibited AChE activity in a dose dependent manner with an IC_{50} value of 54.3 μg/mL, while acetylaposerratinine (**2**) showed stronger inhibitory activity than **1** with an IC_{50} value of 15.2 μg/mL. This result indicates that these alkaloids may be a potent source of AChE inhibitors.

Keywords: *Huperzia squarrosa*; Lycopodiaceae; lycosquarosine A; acetylcholinesterase; *Lycopodium* alkaloids

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease and the most frequent and predominant cause of dementia among the elderly, provoking progressive cognitive decline, psychobehavioral disturbances, memory loss, presence of senile plaques, neurofibrillary tangles and a decrease in cholinergic transmission [1,2]. Neuropathological evidence has demonstrated that cholinergic functions decline in the basal forebrain and cortex in senile dementia of the Alzheimer type [3]. Accordingly, the enhancement

of cholinergic neurotransmission has been considered as one potential therapeutic approach against AD. Although the pathogenesis of AD is complicated and involves numerous pathways, two major hypotheses are currently under consideration regarding the molecular mechanism: the cholinergic hypothesis and the amyloid cascade hypothesis. Thus, the focus herein is upon selective cholinesterase (ChE) inhibitors in order to alleviate cholinergic deficits and improve neurotransmission. Pursuant to this, both could be established as viable therapeutic targets for AD [3–5]. One treatment strategy to enhance the cholinergic function is the use of acetylcholinesterase (AChE, EC 3.1.1.7) inhibitors to increase the amount of acetylcholine, which is present in the synapses between cholinergic neurons [6]. AChE inhibitors such as donepezil, rivastigmine and galantamine, which are the most extensively studied AChE inhibitors, have been shown to significantly improve cognitive function in AD [7,8].

Club moss (Lycopodiaceae) species are well-known to be a rich source of *Lycopodium* alkaloids possessing a complex heterocyclic ring system and wide ranging biological properties that have attracted great interest from biogenetic, synthetic, and biological perspectives [9–11]. Huperzine A, a famous $C_{16}N_2$-type alkaloid isolated from the Chinese folk medicinal herb *Huperzia serrata*, has been shown to be a highly potent, specific, and reversible inhibitor of AChE [10,12]. Until now, more than 300 *Lycopodium* alkaloids were reported [9–11]. Most of the *Lycopodium* alkaloids possessing AChE inhibitory activity such as huperzine A, huperzine B, and *N*-methylhuperzine B belong to the lycodine class [12,13]. In our continuing efforts to search for structurally interesting and bioactive *Lycopodium* alkaloids, especially in *Lycopodium* spp. from Vietnam, a new $C_{16}N_1$-type alkaloid, lycosquarosine A was isolated together with five known *Lycopodium* alkaloids from the club moss *Huperzia squarrosa* (Forst.) Trevis. Previously, *Lycopodium squarrosum* (*H. squarrosa*) originally from Thailand, was phytochemically investigated and several fawcettimine related alkaloids were described [14]. In this paper, we describe the isolation and structure elucidation of lycosquarosine A (**1**) and the other *Lycopodium* alkaloids **2**–**6** as well as their anti-cholinesterase activity.

2. Result and Discussion

The MeOH extract of the club moss *H. squarrosa* was partitioned into *n*-hexane-, EtOAc-, and *n*-BuOH-soluble fractions and a H_2O layer. Chromatographic purification of the EtOAc-soluble fraction led to the isolation of six compounds **1**–**6** (Figure 1).

Lycosquarosine A (**1**) was obtained as a colourless amorphous solid and its molecular formula was deduced from HR-EI-MS analysis to be $C_{18}H_{25}NO_4$. IR absorptions indicated the presence of a carboxylate functionality (1582 cm^{-1}). Its ^{13}C-NMR and DEPT spectra displayed signals for one methyl at δ_C 22.9 (C-16), two N-bearing methylenes at δ_C 50.6 (C-1 and C-9, two peak in overlap), six high-field methylenes at δ_C 18.4 (C-2), 22.1 (C-3), 37.4 (C-6), 25.1 (C-10), 30.3 (C-11) and 32.7 (C-14), one oxygenated methine at δ_C 79.6 (C-8), two methines at δ_C 45.0 (C-7) and 29.6 (C-15), together with four sp^2 quaternary carbons at δ_C 205.7 (C-5), 173.1 (C-13), 169.8 (C-12) and 142.7 (C-4), indicating a phlegmariurine B-type related framework [15]. In addition, the carbon signals of one carbonyl δ_C 169.7 (C-17) and one methyl carbon at δ_C 20.9 (C-18) were ascribed to an acetoxyl group. The ^{1}H-NMR spectrum of **1** displayed signals for a tertiary methyl of the acetoxyl group at δ_H 2.19 (3H, *s*, H-18), a secondary methyl at δ_H 1.08 (3H, *d*, J = 6.3 Hz, H-16), and one oxymethine proton at δ_H 5.06 (1H, *brd*, J = 5.0 Hz, H-8) (Table 1). By comparison with literature ^{1}H- and ^{13}C-NMR data [15,16], **1** could be assigned a phlegmariurine B carbon skeleton with a rearranged five member ring of a >C12=C4-

C5(C=O)-C6-C7 type (Figure 1). The complete NMR assignments and connectivity of **1** were further determined by analysis of the COSY, HMQC and HMBC spectroscopic data. ^{1}H–^{1}H COSY and HSQC analyses indicated the presence of three carbon chains between H-1/H-2/H-3 (**a**), H-6/H-7/H-8/H-15/H-14 (**b**), and H-9/H-10/H-11 (**c**) shown by the bold lines in Figure 2. The long-range ^{1}H–^{13}C coupling (HMBC) observed between oxygenated methine H-8 and carbonyl carbon at δ_C 169.7 (C-17) confirmed the position of the acetoxyl group to be at C-8 (Figure 2 and Supplementary data). The ROESY correlation between H-16 and H-7 indicated that **1** had an α-oriented methyl group at C-15, which was similar to phlegmariurine type of *Lycopodium* alkaloids [15]. Furthermore, the β-orientation of the acetoxyl function located at C-8 was deduced from the ROESY experiment, showing ROE correlations between H_α-7/H-16 and H-8. Thus, compound **1** was proved to be an 8β-acetoxyl derivative of phlegmariurine B, and was named lycosquarosine A

Figure 1. Chemical structures of isolated compounds **1**–**6**.

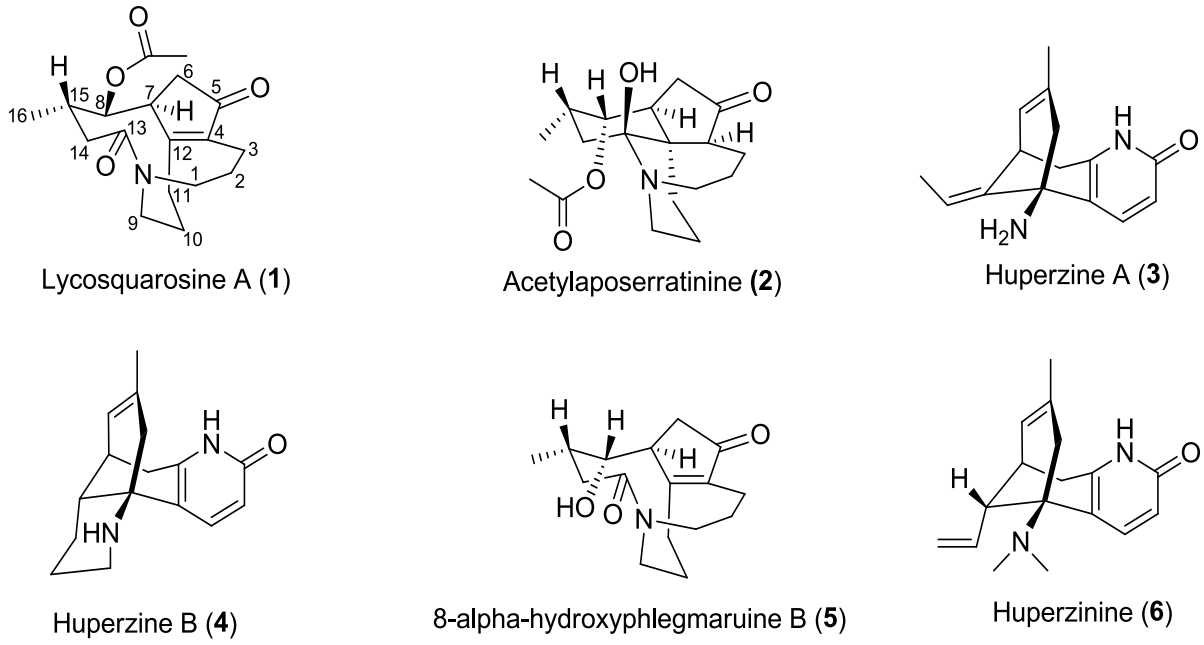


Table 1. ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of lycosquarosine A (**1**).

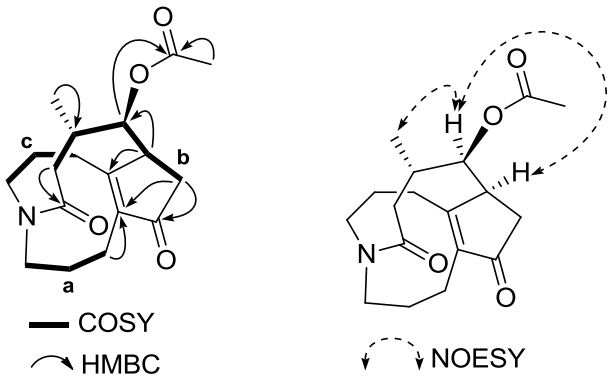
Position	**1** [a]	
	δ_H (*J* in Hz) [b]	**δ_C**
1	4.06 (1H, dd, 13.6, 3.6), 2.90 (1H, dt, 13.6, 3.0)	50.6
2	2.39 (1H, m), 1.41 (1H, m)	18.4
3	2.53 (1H, m), 2.46 (1H, m)	22.1
4		142.7
5		205.7
6	2.55 (1H, m), 2.03 (1H, brd, 19.0)	37.4
7	3.04 (1H, m)	45.0
8	5.06 (brd, 5.0)	79.6

Table 1. *Cont.*

Position	1 [a]	
	δ_H (*J* in Hz) [b]	δ_C
9	3.96 (1H, td, 15.0, 3.0), 3.23 (1H, brd, 15.0)	50.6
10	2.78 (1H, m), 1.93 (1H, m)	25.1
11	2.98 (1H, m), 2.78 (1H, m)	30.3
12		169.8
13		173.1
14	1.59 (1H, d, 15.5), 3.06 (1H, dd, 8.5, 15.5)	32.7
15	2.56 (1H, m)	29.6
16	1.08 (3H, d, 6.3)	22.9
17		169.7
18	2.19 (3H, s)	20.9

[a] Measured in mixture of MeOD and $CDCl_3$; [b] Chemical shift may be overlapped signals which were confirmed by DEPT-135, HMQC, and HMBC experiments.

Figure 2. Selected 2D NMR correlations of **1**.



Compound **2** showed a pseudo-molecular ion peak at *m*/*z* 322 $[M+H]^+$ in the ESI-MS, and the molecular formula, $C_{18}H_{27}NO_4$, was established by HR-ESI-MS *m*/*z* 322.2051, $[M+H]^+$. IR absorptions (1585 cm^{-1}) implied the presence of a carboxylate functionality. ^{1}H-, ^{13}C-NMR and DEPT data revealed eighteen carbon signals due to four sp^2 quaternary carbons, four sp^3 methines, eight sp^3 methylenes, and two methyl groups. Among them, two methylenes [(δ_C 54.9, δ_H 3.90 and 2.99) and (δ_C 50.9, δ_H 3.58 and 3.27), belonging to C-1 and C-9, respectively] were ascribed to those bearing a nitrogen. The ^{1}H-NMR spectrum of **2** displayed signals for a tertiary methyl of the acetoxyl group at δ_H 2.14 (3H, *s*, H-18), a secondary methyl at δ_H 1.02 (3H, *d*, *J* = 6.5 Hz, H-16) which are similar with those of positions in **1**. Since no IR bands and ^{13}C-NMR signals indicated a double bond in comparison with **1**, compound **2** must be pentacyclic, which suggested the possibility that a new ring was formed. Extensive NMR analyses spectra of **2** resembled those of **1** except for the presence of one carbinolamine moiety at δ_C 94.5 (C-13), a sp^2 quaternary carbon at δ_C 47.5 (C-12) and a sp^3 methine at C-4 position (δ_C 47.6, δ_H 2.40) instead of three quaternary carbons at the same positions in **1** (Table 1). Combination of HMQC and ^{1}H–^{1}H COSY also indicated the presence of three fragment carbon chains (**a**) –$CH_2CH_2CH_2CH$– (C-1–C-4), (**b**) –$CH_2CHCHCHCH_2$– (C-6–C-8–C-15–C-14) and (**c**) –$CH_2CH_2CH_2$–(C-9–C-11) (Figure 2). The long-range ^{1}H-^{13}C coupling (HMBC) observed between oxygenated methine H-8 and

carbonyl carbon at δ_C 170.4 (C-17) confirmed the position of the acetoxyl group to be at C-8. The coupling pattern of the oxymethine resonance at δ_H 5.04 (1H, *brs*, H-8) and its oxygenated carbon at δ_C 72.3 (C-8) differed from that in **1** with δ_H 5.08 (1H, *brd*, J = 5.0 Hz) and δ_C 80.8, indicating for the α-orientation of the acetoxyl group which is in accordance with the orientation of phlegmariurine B [14]. Additionally, the relative stereochemistry of **2** was elucidated from NOESY correlations. From that, the α-orientation of the acetoxyl function was confirmed from the enhancement of the signals for H-6β methylene hydrogen and H-15 by H-8 irradiation. Other key NOESY correlations were observed between H-4 and H-7, and H_3-16 indicating that they are on the same α-orientation. Thus, the relative stereochemistry of **2** was assigned. This compound named acetylaposerratinine [14].

The other compounds were identified as huperzine A (**3**), huperzine B (**4**) [12,13], 8α-hydrophlemariurine B (**5**) [15], and huperzinine (**6**) [13] by comparing their physiochemical and spectroscopic data with those reported in the corresponding literature.

AChE inhibitors increase the availability of acetylcholine in central cholinergic synapses and are currently the most promising available drugs for the treatment of Alzheimer's disease [17]. Cholinergic interneurons in the striatum are an even richer source of acetylcholinesterase and would also be affected strongly by such enzyme inhibitors [17,18]. The anti-cholinesterase activity of the isolated alkaloids was tested by the Ellman reaction [19]. Because the known compounds **3**–**6** were already reported to have cholineseterase inhibitory activity, in this experiment, we tested only compounds **1** and **2** for AChE and BuChE inhibition. The new compound, lycosquarosine A (**1**) showed potent AChE inhibitory activity with an IC_{50} value of 54.3 μg/mL. However, acetylaposerratinine (**2**) showed stronger inhibitory activity than **1**, with an IC_{50} value of 15.2 μg/mL. Both of them exhibited weak inhibitory effects on BuChE, with IC_{50} values over 100 μg/mL. The results showed that lycosquarosine A (**1**) and acetylaposerratinine (**2**) exhibited selective inhibition for AChE compared with BuChE. Berberine, which was used as positive control [20], exhibited AChE and BuChE inhibitory activity with IC_{50} values of 0.09 and 8.01 μg/mL, respectively.

3. Experimental Section

3.1. General Procedures

Optical rotations were measured with a DIP 370 digital polarimeter (JASCO, Tokyo, Japan). UV spectra were taken in MeOH using an Evolution™ 300 Thermo Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The NMR spectra were obtained on a Unity Inova 500 MHz spectrometer (Varian, McKinley Scientific Inc., Sparta Township, NJ, USA). Silica gel (63–200 μm particle size, Merck, Seoul, Korea) and RP-18 (75 μm particle size, Merck) were used for column chromatography. TLC was carried out using Merck silica gel 60 F_{254} and RP-18 F_{254} plates. HPLC was carried out using a Waters (Waters Corporation, Milford, MA, USA) system (515 pump) equipped with a UV detector (486 Tunable Absorbance) and an YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size, YMC Co., Ltd., Kyoto, Japan) and HPLC solvents were purchased from SK Chemicals, Seoul, Korea.

3.2. Plant Material

The *H. squarrosa* club moss was collected in Lam Dong Province, in the central area of Viet Nam on May 2012, and identified by Professor Luan TC, Department of Oriental Medicine, Ho Chi Minh City University of Medicine and Pharmacy. A voucher specimen (TCL 00116) was deposited at the Herbarium of the Research Center of Ginseng and Medicinal Materials, Ho Chi Minh City, Vietnam.

3.3. Extraction and Isolation

The dried sample (2.5 kg) was extracted with MeOH (5 L) by refluxing three times for. The combined extracts were concentrated under reduced pressure to give the crude extract (502 g), which was then suspended in 5% HCl and partitioned with CH_2Cl_2. The aqueous layer was alkalinized until pH~10 with aqueous ammonia, preparing for submitting to Diaion HP 20 macroporous resin column chromatography (750 × 1000 mm). The separation by resin-based column was executed as following: extract solution (pH 10) was loaded onto the column. After adsorption, the column was washed with deionised water to remove the polar impurities, and then eluted with 100% MeOH to obtain the crude alkaloid extracts. The combined extract was dried by rotator evaporation at 40 °C. This residue was further separated by silica gel column chromatography using a system of CH_2Cl_2/MeOH (100%→0%) gradient, 80% CH_2Cl_2/MeOH, saturated with ammonia, to give eight subfractions. After solvent removal sub-fraction 2 (242 mg) gave a single spot. The residue was a colourless amorphous solid (16.3 mg, compound **1**). Sub-fraction 3 (870 mg), a single spot after removal of the solvent, was recovered from methanol-acetone to give **2** (26 mg). Sub-fraction 7 (218 mg) was chromatographed by MPLC on an ODS column using MeOH–H_2O (5:1) with addition of 0.1% trifluoroacetic acid (TFA) to afford **3** (8.5 mg). From sub-fraction 4 (115 mg), compounds **4** (3.6 mg, t_R = 18.6 min) and **5** (3.6 mg, t_R = 21.1 min) were purified by semi-preparative HPLC (using a gradient solvent system of MeOH-0.1% TFA (25:75 → 85:15; flow rate 5 mL/min) over 90 min; UV detection at 210 nm; YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size)]. Compound **6** (5.5 mg; t_R = 28.6 min) was isolated from fraction 5 (212 mg) by semi-preparative HPLC (using a gradient solvent system of MeOH-0.1% TFA (20:80 → 80:20; flow rate 5 mL/min) over 90 min; UV detection at 210 nm; YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size)].

3.4. Lycosquarosine A (**1**)

White amophous powder; $[\alpha]_D^{25}$ = −6.54 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 255 (3.50) nm; IR ν_{max} (KBr): 3397, 1685, 1630, 1452, 865 cm^{-1}; HR-ESI-MS *m/z* 320.1868 $[M+H]^+$ (calcd for $C_{18}H_{25}NO_4$, 320.1885); ^{1}H- ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data are listed in Table 1.

3.5. In Vitro Cholinesterase Inhibitory Activity Assay

The AChE and BChE inhibitory activities were measured using the spectrophotometric method developed by Ellman *et al.* with a slight modification. ACh and BCh were used as the substrates to detect the inhibition of AChE and BChE, respectively. The reaction mixture contained sodium phosphate buffer (pH 8.0, 140 μL); tested sample solution (20 μL); and either AChE or BChE solution (20 μL), which were mixed and incubated for 15 min at room temperature. All tested samples and the

positive control (berberine) were dissolved in 10% analytical grade dimethyl sulfoxide. The reactions were started with the addition of DTNB (10 μL) and either ACh or BCh (10 μL), respectively. The hydrolysis of ACh or BCh was monitored by following the formation of the yellow 5-thio-2-nitrobenzoate anion at 412 nm for 15 min, which resulted from the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of either ACh or BCh, respectively. All reactions were performed in triplicate and recorded in 96-well microplates, using a VERSA max instrument (Molecular Devices, Sunnyvale, CA, USA). Percent inhibition was calculated from the expression (1 − S/E) × 100, where E and S were the respective enzyme activities without and with the tested sample, respectively. The ChE inhibitory activity of each sample was expressed in terms of the IC_{50} value (μM required to inhibit the hydrolysis of the substrate, ACh or BCh, by 50%), as calculated from the log-dose inhibition curve [19].

4. Conclusions

Six lycopodium alkaloids, including a new natural product, lycosquarosine A, were isolated from the club moss *H. squarrosa*. This is the first report on the alkaloid constituents of *H. squarrosa* from Vietnam and the potential cholinesterase inhibitory activity of these compounds might suggest new sources of anti-Alzheimer disease agents.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/19/11/19172/s1.

Acknowledgments

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.99-2011.19.

Author Contributions

N.N.C., T.M.H., T.C.L. carried out conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the manuscript and revising. N.N.T.H. carried out acquisition of data, analysis and interpretation of data, statistical analysis. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Scarpini, E.; Scheltens, P.; Feldman, H. Treatment of Alzheimer's disease: Current status and new perspectives. *Lancet Neurol.* **2003**, *2*, 539–547.
2. Parihar, M.S.; Hemnani, T. Alzheimer's disease pathogenesis and therapeutic interventions. *J. Clin. Neurosci.* **2004**, *1*, 456–467.
3. Whitehouse, P.J.; Price, D.L.; Struble, G.R.; Clarke, A.W.; Coyle, J.T.; DeLong, M.R. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science* **1982**, *15*, 1237–1239.

4. Vassar, R. β-secretase (BACE) as a drug target for Alzheimer's disease. *Adv. Drug Deliv. Rev.* **2002**, *54*, 1589–1602.
5. Yan, R.; Bienkowski, M.J.; Shuck, M.E.; Miao, H.; Tory, M.C.; Pauley, A.M. Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. *Nature* **1999**, *402*, 533–537.
6. Cummings, J.L. Cholinesterase inhibitors: Expanding applications. *Lancet* **2000**, *356*, 2024–2025.
7. Relman, A.S. Tacrine as a treatment for Alzheimer's dementia. *N. Engl. J. Med.* **1991**, *324*, 349–352.
8. Camps, P.; El-Achab, R.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E. New tacrine-huperzine A hybrids (huprines): Highly potent tight-binding acetylcholinesterase inhibitors of interest for the treatment of Alzheimer's disease. *J. Med. Chem.* **2000**, *43*, 4657–4666.
9. Kobayashi, J.; Morita, H. *The Alkaloids*; Cordell, G.A., Ed.; Academic Press: New York, NY, USA, 2005; Volume 61, pp. 1–57.
10. Ma, X.; Gang, D.R. The Lycopodium alkaloids. *Nat. Prod. Rep*. **2004**, *21*, 752–772.
11. Hirasawa, Y.; Kobayashi, J.; Morita, H. The Lycopodium alkaloids. *Heterocycles* **2009**, *77*, 679–729.
12. Liu, J.S.; Yu, C.M.; Zhou, Y.Z.; Han, Y.Y.; Qi, B.R.; Zhu, Y.L. Study on the chemistry of huperzine A and B. *Acta Chim. Sin.* **1986**, *44*, 1035–1040.
13. Shen, Y.C.; Chen, C.H. Alkaloids from *Lycopodium casuarinoides*. *J. Nat. Prod.* **1994**, *57*, 824–826.
14. Katakawa, K.; Mito, H.; Kogure, N.; Kitajima, M.; Wongseripipatana, S.; Arisawa, M.; Takayama, H. Ten new fawcettimine-related alkaloids from three species of *Lycopodium*. *Tetrahedron* **2011**, *67*, 6561–6567.
15. Tan, C.H.; Chen, G.F.; Ma, X.Q.; Jiang, S.H.; Zhu, D.Y. Three new phlegmariurine B type lycopodium alkaloids from *Huperzia serrata*. *J. Asian Nat. Prod. Res.* **2002**, *4*, 227–231.
16. Takayama, H.; Katakawa, K.; Kitajima, M.; Yamaguchi, K.; Aimi, N. Seven new Lycopodium alkaloids, lycoposerramines-C, -D, -E, -P, -Q, -S, and -U, from *Lycopodium serratum* Thunb. *Tetrahedron Lett.* **2002**, *43*, 8307–8311.
17. Giacobini, E. The cholinergic system in Alzheimer disease. *Prog. Brain Res.* **1990**, *84*, 321–332.
18. Houghton, P.J.; Ren, Y.; Howes, M.J. Acetylcholinesterase inhibitors from plants and fungi. *Nat. Prod. Rep.* **2006**, *23*, 181–199.
19. Ellman, G.L.; Courtney, K.D.; Andres, V., Jr.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–90.
20. Hung, T.M.; Na, M.; Dat, N.T.; Ngoc, T.M.; Youn, U.; Kim, H.J.; Min, B.S.; Lee, J.; Bae, K. Cholinesterase inhibitory and anti-amnesic activity of alkaloids from *Corydalis turtschaninovii*. *J. Ethnopharmacol.* **2008**, *119*, 74–80.

Sample Availability: Samples of the compounds are available from the authors.