

Huperphlegmines A and B, two novel *Lycopodium* alkaloids with an unprecedented skeleton from *Huperzia phlegmaria*, and their acetylcholinesterase inhibitory activities

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Abstract

Two novel *Lycopodium* alkaloids, huperphlegmines A and B (**1** and **2**), were isolated from the aerial parts of *Huperzia phlegmaria* collected in Vietnam, together with the five known compounds lycophlegmariol A (**3**), phlegmariurine B (**4**), 5-hydroxymethyl-2-furaldehyde (**5**), rhexanone C (**6**), and loliolide (**7**). The chemical structures of the present compounds were elucidated by means of 1D and 2D NMR and HRESIMS spectroscopy, and by comparisons to the reported data in the literature. Compounds **1** and **2** showed moderate acetylcholinesterase inhibitory activities, with IC_{50} values of 25.95 ± 0.67 and 29.14 ± 0.77 $\mu\text{g/ml}$, respectively.

Keywords: *Huperzia phlegmaria*, *Lycopodium* alkaloid, huperphlegmine, acetylcholinesterase inhibitory activity.

1. Introduction

Huperzia phlegmaria (L.) Rothm. (Lycopodiaceae) [synonym: *Lycopodium phlegmaria* L.] is an epiphytic club moss found on tree trunks and rocks in forests located at heights of 100-2,400 m in China, Cambodia, India, Japan, Laos, Nepal, Thailand, Vietnam, Pacific islands, Paleotropics, and South America [1]. All parts of this plant have been used in traditional Chinese medicine for the treatment of rheumatic pain, arthritis, traumatic injury, sore throat, edema, and urticaria for many years [2]. Furthermore, this plant is also used as a potential medicinal herb for the treatment of several brain diseases, such as Alzheimer's disease [3]. Previous phytochemical investigations of *H. phlegmaria* revealed that its main constituents are serratane-type triterpenes [4–6], *Lycopodium* alkaloids [7–9], and abietane diterpenes [6]. Huperzine A, the *Lycopodium* alkaloid being tested as an anti-Alzheimer's disease drug candidate [10, 11], has also been isolated from *H. phlegmaria*, as in the case of the other *Huperzia* species. Remarkably, *H. phlegmaria* reportedly contains high concentrations of huperzine A, ranging from 44-345 $\mu\text{g g}^{-1}$ [12], which may account for the effectiveness of this plant in the treatment of several brain diseases. However, the presence of other constituents that are also effective for brain diseases in this plant has not been excluded, since the efficacy of the crude drug is generally associated with multiple components.

In our previous search for secondary metabolites with acetylcholinesterase (AChE) inhibitory activity in Vietnamese *H. phlegmaria*, we isolated several terpenoids and a known alkaloid with fawcettimine-related structures [13]. Furthermore, we recently identified two *Lycopodium* alkaloids, huperphlegmines A and B (**1–2**), with an unprecedented skeleton and a 2,5-substituted dihydrofuran moiety, together with five known compounds, lycophlegmariol A (**3**) [6], phlegmariurine B (**4**) [14], 5-hydroxymethyl-2-furaldehyde (**5**) [15], rhemanone C (**6**) [16], and loliolide (**7**) [17], from this plant (Fig. 1). Herein, we describe the isolation and

structural elucidation of **1** and **2**, as well as the AChE inhibitory activities of the isolates, except for **4**. Remarkably, the new compounds **1** and **2** exhibited moderate AChE inhibitory activities.

2. Experimental

2.1. General experimental procedures

UV spectra were recorded on a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Infrared spectra were recorded on an IR Prestige-21 spectrometer (Shimadzu, Kyoto, Japan). Optical rotations were measured on a JASCO P-2100 polarimeter (Hachioji, Tokyo, Japan). High-resolution electron spray ionization mass spectrometry (HRESIMS) data were acquired, using a micrOTOF-Q 10187 mass spectrometer (Bruker, Massachusetts, USA). NMR spectra were recorded using a Bruker Avance 500 spectrometer (^1H NMR for 500 MHz, ^{13}C NMR for 125 MHz) (Bruker, MA, USA), with TMS as an internal reference. Column chromatography was performed using silica gel (60 N, spherical, neutral, 40-50 μm , Kanto Chemical Co., Inc., Tokyo, Japan), Cosmosil 75C18-OPN (Nacalai Tesque Inc., Kyoto, Japan), YMC RP-18 (Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan), and Sephadex LH-20 (Dowex[®] 50WX2-100, Sigma–Aldrich, USA). Analytical TLC was performed on pre-coated silica gel 60F₂₅₄ and RP-18 F₂₅₄ plates (0.25 or 0.50 mm thickness, Merck KGaA, Darmstadt, Germany). AChE, acetylthiocholine (ACTI), 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB), and galanthamine hydrobromide were purchased from Sigma-Aldrich (USA). Dimethyl sulfoxide (DMSO) was purchased from Merck (Darmstadt, Germany). All chemicals used were of the highest grade available.

2.2. Plant material

The aerial parts of *Huperzia phlegmaria* (L.) Rothm. were collected from Quang Tri province, Vietnam, 16°55'42.5"N 106°36'30.3"E; 16°56'40.4"N 106°33'49.9"E, in June 2016,

and were identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST, Vietnam. A voucher specimen (No. HP01) was deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Vietnam.

2.3. Extraction and isolation

The dried aerial parts of *H. phlegmaria* (1.5 kg) were extracted with MeOH (3 times, 5.0 L each) at room temperature to yield 75 g of a dark solid extract. This extract was then dissolved in 3% tartaric acid (1.0 L) and filtered to separate the solid residues (solid A). The remaining acidic solution was adjusted to pH 10 with saturated aqueous Na₂CO₃, and partitioned with CH₂Cl₂ (3 times, 2.0 L each) to obtain the total alkaloidal fraction (HC, 8 g), after removal of the solvent *in vacuo*.

The alkaloidal fraction was chromatographed on a normal phase silica gel column, eluted with a stepwise gradient of CH₂Cl₂–MeOH (40:1 to 10:1, v/v) to obtain 6 sub-fractions, Fr.1–Fr.6. Fr.5 (2.87 g) was subjected to Sephadex LH-20 column chromatography, eluted with MeOH to obtain 5 sub-fractions, Fr.5.1–Fr.5.5. Fr.5.3 (1.6 g) was then applied to a YMC RP-18 column, eluted with acetone–MeOH–water (1:3:1, v/v) to yield 7 fractions, Fr.5.3.1–Fr.5.3.7. Fr.5.3.4 (789 mg) was chromatographed on a normal phase silica gel column, eluted with CH₂Cl₂–acetonitrile–ethylamine (10:1:0.1, v/v), followed by a Sephadex LH-20 column eluted with MeOH–water (4:1, v/v), and then subjected to preparative reversed phase HPLC (Zorbax SB – C18 5µm, 9.4 × 250mm) using MeOH–water (60:40, flow rate 2 mL/min) as the eluent, to afford **1** (38.0 mg) and **2** (21.0 mg). Fr.3 (1.32 g) was separated on a Sephadex LH-20 column eluted with CH₂Cl₂–MeOH (9:1, v/v) to obtain 4 sub-fractions, Fr.3.1 – Fr.3.4, and further purification of HC3.3 (660 mg) yielded compound **4** (10.2 mg).

The solid A (57 g) was washed with distilled water until neutral, and extracted with EtOAc to yield the EtOAc fraction (HE, 38 g). The EtOAc fraction was chromatographed on

a normal phase silica gel column, eluted with a gradient of CH₂Cl₂–MeOH (100:0, 50:50, 0:100, v/v), to obtain 3 fractions (HE1–HE3), according to their TLC profiles. The fraction HE2 (4.92 g) was subjected to Sephadex LH-20 column chromatography, eluted with CH₂Cl₂-MeOH (9:1, v/v), to obtain 5 sub-fractions, HE2.1-HE2.5. The purification of HE2.2 (1.81 g) afforded **5** (3.4 mg), **6** (4.5 mg), and **7** (5.7 mg). A portion (7.5 g) of HE3 was chromatographed on a normal phase silica gel column, eluted with a gradient of CH₂Cl₂–acetone (60:0 to 1:1, v/v), to obtain 12 sub-fractions, HE3.1–HE3.12, and the purification of HE3.8 (1.02 g) yielded **3** (15.0 mg).

Huperphlegmine A (**1**): Yellow oil; $[\alpha]_D^{22}$ -65 (*c* 0.1, MeOH); IR (KBr) ν_{\max} (cm⁻¹): 3439, 2926, 1701, 1612, 1570, 1456, 1169, 1020, 901, 795; UV (MeOH) λ_{\max} (log ϵ): 347 (4.55); ¹H NMR (500 MHz, CD₃OD) and ¹³C NMR (125 MHz, CD₃OD): see Table 1; HRESIMS *m/z* 397.2122 [M+H]⁺ (calcd. for C₂₃H₂₉O₄N₂, 397.2127).

Huperphlegmine B (**2**): Yellow oil; $[\alpha]_D^{22}$ -162 (*c* 0.1, MeOH); IR (KBr) ν_{\max} (cm⁻¹): 3402, 2930, 1699, 1609, 1570, 1448, 1169, 1020, 901, 795; UV (MeOH) λ_{\max} (log ϵ): 349 (4.57); ¹H NMR (500 MHz, CD₃OD) and ¹³C NMR (125 MHz, CD₃OD): see Table 1; HRESIMS *m/z* 397.2122 [M+H]⁺ (calcd. for C₂₃H₂₉O₄N₂, 397.2127).

2.4. AChE inhibition assay

The acetylcholinesterase inhibitory assay was performed in triplicate in 96-well microplates, and the absorbance was recorded using an ELISA microplate reader (EMR-500, LABOMED INC., CA, USA). The AChE inhibitory assay was performed by the colorimetric method reported by Ellman, with slight modifications [18]. ACTI was used as the substrate to examine the inhibitory effect of the samples on the AChE activities. The reaction mixture, containing 140 μ L Tris-HCl buffer (pH 8.0), 20 μ L of the tested sample solution, and 20 μ L of the AChE solution (0.25 units/mL), was incubated at room temperature for 15 min, and the reaction was

then initiated by adding 10 μL of 0.24 mM ACTI, along with 10 μL of 0.24 mM Ellman's reagent (DTNB), which produces a yellow 5-thio-2-nitrobenzoate anion. The mixture was incubated further at room temperature for 15 min, and the optical density was measured at 405 nm to calculate the percentage inhibition. Galanthamine was used as a positive control. All tested samples and the positive control were dissolved in 10% DMSO (analytical grade). The percentage inhibition (I %) was calculated using the following equation, in which A_{sample} is the absorbance of the tested compound-containing reaction and A_{control} is the absorbance of the control reaction.

$$I \% = (1 - A_{\text{sample}} / A_{\text{control}}) \times 100$$

The AChE inhibitory activity of each compound was expressed as the concentration ($\mu\text{g/ml}$) required to inhibit the hydrolysis of the substrate by 50% (IC_{50} value). A range of concentrations of the tested compounds was used, so the IC_{50} value could be calculated from the logarithmic dose-inhibition curve.

3. Results and discussion

The methanol extract of the aerial parts of *H. phlegmaria* was treated with acid and purified by a combination of chromatographic methods, including normal and reverse phase open column chromatography, Sephadex LH-20 chromatography, and reversed phase HPLC, to furnish two unprecedented Lycopodium alkaloids, **1** and **2**, and five known compounds (**3–7**).

Compound **1** was obtained as a yellow oil, with negative optical rotation ($[\alpha]_{\text{D}}^{22} -65$ (c 0.1, MeOH)). The HRESIMS analysis of the molecular ion clusters $[\text{M}+\text{H}]^+$ (m/z 397.2122) revealed the molecular formula to be $\text{C}_{23}\text{H}_{28}\text{O}_4\text{N}_2$, in conjunction with the NMR data analysis, suggesting that **1** has eleven degrees of unsaturation. Its IR spectrum revealed the strong

absorption bands at 3439 and 1701 cm^{-1} , corresponding to hydroxyl and carbonyl groups. The UV spectrum indicated the presence of the aromatic ring (λ_{max} 347 nm). The ^1H NMR spectrum of **1** (Table 1) showed typical proton signals for an olefinic methine [δ_{H} 7.09 (d, $J = 1.5$ Hz, H-1'), two heteroaromatic methines [δ_{H} 6.88 (d, $J = 3.5$ Hz, H-3'), 6.52 (d, $J = 3.5$ Hz, H-4')], an oxymethylene [δ_{H} 4.60 (2H, s, H₂-6')], an *N*-methyl group [δ_{H} 2.51 (3H, s, H₃-17)], and a secondary methyl group [δ_{H} 1.24 (3H, d, $J = 7.0$ Hz, H₃-16)], along with 5 methine and 5 methylene groups. The ^{13}C NMR and HSQC spectra of **1** (Table 1) revealed twenty-three signals, including two carbonyl carbons [δ_{C} 213.8 (C-14), 210.5 (C-5)], two oxygenated sp^2 quaternary carbons [δ_{C} 160.1 (C-5'), 152.2 (C-2')], an sp^2 quaternary carbon [δ_{C} 138.9 (C-6)], an sp^2 methine carbon [δ_{C} 120.9 (C-1')], two heteroaromatic sp^2 methine carbons [δ_{C} 120.5 (C-3'), 111.5 (C-4')], two sp^3 quaternary carbons [δ_{C} 81.6 (C-13), 63.6 (C-12)], five sp^3 methine carbons [δ_{C} 66.2 (C-9), 64.7 (C-3), 61.3 (C-4), 46.9 (C-7), 43.5 (C-15)], an oxygenated methylene carbon [δ_{C} 57.6 (C-6')], an *N*-methyl carbon [δ_{C} 47.0 (C-17)], five methylene carbons [δ_{C} 46.5 (C-1), 37.1 (C-2), 36.7 (C-11), 34.7 (C-8), 20.5 (C-10)], and a methyl carbon (δ_{C} 18.6 (C-1), as well as the proton signals for five methylenes [δ_{H} 3.16 (dt, $J = 12.5, 3.5$ Hz, H-1a), 2.56 (overlap, H-1b), 2.27 (2H, m, H₂-8), 2.19 (m, H-10a), 1.79 (m, H-10b), 2.09 (m, H-11a), 1.58 (dt, $J = 13.0, 10.5$ Hz, H-11b), 1.99 (m, H-2a), 1.70 (m, H-2b)] and five methines [δ_{H} 4.26 (dd, $J = 9.5, 6.5$ Hz, H-9), 3.67 (q-like, $J = 3.0$ Hz, H-3), 3.44 (dt, $J = 9.0, 2.0$ Hz, H-7), 2.59 (d, $J = 3.5$ Hz, H-4), 2.40 (m, H-15)].

In the COSY spectrum (Fig. 2), the correlations of H-1a/H₂-2, H-1b/H-2a, H-2b/H-3/H-4, H-7/H₂-8/H-15/H₃-16, H-9/H-10a/H-11a, H-10b/H-11b, and H-3'/H-4' were observed. The HMBC correlations of H-3' to C-1'/C-2'/C-4'/C-5', H-4' to C-2'/C-3'/C-5', and H₂-6' to C-4'/C-5' (Fig. 2) suggested the presence of a 2,5-disubstituted dihydrofuran moiety with olefinic and hydroxymethylene groups as the side chains in the structure of **1**. The presence of a 16-methyl-14-indenone ring (numbering according to **1**) involving C-7–C-16 was verified by the HMBC

correlations from H-9 to C-13/C-14, H-11a to C-12/C-13, H-7 to C-12, and H₃-16 to C-8/C-14/C-15. Furthermore, the HMBC correlations from H-4 to C-5/C-6/C-12, H-7 to C-5/C-6, H₂-8 to C-6, and H-1' to C-5/C-6/C-7/C-2'/C-3' led not only to the construction of a cyclopentanone ring involving C-4, C-5, C-6, C-7, and C-12, but also to the connection of this ring to the 5-hydroxymethylenedihydrofuran moiety via a Δ^6 double bond at C-2. In addition, the COSY correlations of H-1a/H₂-2/H-3/H-4 and H-1b/H-2a, and the HMBC correlations from H-17 to C-1/C-9 and from H-4 to C-3, indicated that the methylated nitrogen atom and C-3 were connected to C-9 on the cyclopentane ring of the indenone ring and C-4 on the cyclopentanone ring, respectively, suggesting the presence of an *N*-methylazonane ring formed by the linkage of the nitrogen atom, C-1–C-4, and C-9–C-12. On the basis of the molecular formula and the observed NMR data mentioned above, the remaining nitrogen atom should be a secondary amine and one more ring was needed to fulfill the degree of saturation. Thus, the amine moiety was attached to C-3 and C-13, which is also supported by the observation of the HMBC correlation from H-3 to C-13, and a pyrrolidine ring involving the secondary amine, C-3, C-4, C-12, and C-13 was constructed in the structure of **1**. Based on these data, the planar structure of **1** was determined, as shown in Fig. 2.

The relative configuration of **1** was elucidated on the basis of the NOESY experiment (Fig. 3). The NOESY correlations from H-7/H₂-8 to H-3' indicated the *E* configuration of the Δ^6 double bond. The cross peaks from H-4/H-10b, H-4/H-11a, and H-7/H-11b in the NOESY spectrum suggested the α -orientations of H-4 and H-7, and the C-10 and C-11 dimethylene moieties. In contrast, the NOESY correlations of H-3/H-8 and the lack of correlations of H-3/H₂-10 and H-3/H₂-11 indicated that H-3 and the C-8 methylene group are β -oriented. Furthermore, the NOESY spectra indicated that the C-16 methyl moiety is located on the opposite side of H-7, as verified by observations of the NOESY correlation of H-7/H-15, as

well as the lack of that of H-7/H₃-16. Consequently, the structure of **1** was elucidated as depicted in Fig. 1, and named hyperphlegmine A.

Compound **2** was isolated as a yellow oil, with negative optical rotation ($[\alpha]_{\text{D}}^{22}$ -162 (c 0.1, MeOH). The HRESIMS analysis indicated the same molecular formula of C₂₃H₂₈O₄N₂ as that of **1**. IR spectrum of **2** showed the strong absorption bands at 3402 and 1699 cm⁻¹, corresponding to hydroxyl and carbonyl groups and the UV spectrum indicated the presence of the aromatic ring (λ_{max} 349 nm), as in the case of **1**. The ¹H and ¹³C NMR spectroscopic data of **2** (Table 1) were quite similar to those of **1**, except for those of the C-7 methine (δ_{H} 3.51, δ_{C} 43.5), C-14 carbonyl (δ_{C} 215.4), C-15 methine (δ_{H} 2.77, δ_{C} 40.0), and C-16 methyl (δ_{H} 16.5, δ_{C} 16.5) groups. However, the 2D-NMR analysis indicated that **2** possesses the same planar structure as that of **1**, suggesting that **2** is a stereoisomer of **1**. In order to clarify this hypothesis, the relative configuration of **2** was determined by a NOESY experiment (Fig. 3). Remarkably, in contrast to the NOESY data of **1**, H-7 (δ_{H} 3.51) correlated with H₃-16 (δ_{H} 1.32), but not with H-15 (δ_{H} 2.77). Compound **2** was thus suggested to be the 15-epimer of **1**. This proposal may also be supported by the significant difference of the chemical shift values of C-7 between **1** and **2**, due to the shielding effect of the C-13 methyl group on the C-7 position. In addition, the H₂-8/H-3' cross peak observed in the NOESY spectrum of **1** was not present, and instead, a correlation of H-1' (δ_{H} 7.06)/H-3' (δ_{H} 6.85) appeared in the NOESY spectrum of **2**. Furthermore, the NOESY spectrum showed a correlation between H₃-16 and H-6' (δ_{H} 4.60), which was not observed in that of **1**. This evidence suggested that the oxygen atom in the furan ring of **2** is oriented toward C-8. The differences in the orientations of the furan ring between **1** and **2** might be explained by the rotation around the C-1'/C-2' bond of these moieties. Thus, the structure of **2** was elucidated as shown in Fig. 1, and was named hyperphlegmine B.

The structures of the isolated known compounds **3–7** were identified by comparisons of their spectroscopic data with those reported in the literature. The Lycopodium alkaloids,

slycopalhine A [19] and obscurumine H [20], with the C-9–N-2' linkage-containing six ring C₁₆N₂ skeleton similar to those of **1** and **2**, were isolated from *Palhinhaea cernua* (syn.: *Lycopodium cernua*) and *L. obscurum*, respectively. However, to the best of our knowledge, this is the first demonstration of the isolation of Lycoposium alkaloids with the novel C₁₆N₂-type skeleton consisting of five rings, including the new C-9–C-13 linkage. Presumably, **1** and **2** are generated from obscurinine via (1) its oxidation at C-14 to form the 15-epimer intermediates **A**, (2) the hydrogenation and oxidation of **A** for conversion to the intermediates **B**, (3) the Polonovski-type reaction of **B** followed by a sequential cyclization of their intermediates **C** for the formation of the intermediates **D**, (4) the aldol condensation of **D** with rhexanone C (**6**), which was also isolated from this plant in the present study, and the dihydroxylation to connect the 2,5-disubstituted dihydrofuran side chain (Fig. 4).

The AChE inhibitory activities of the isolates, except for the previously reported compound **4** [21], were assessed at various concentrations. Galanthamine was used as a positive control. The results are summarized in Table 2. Among the tested compounds, only the novel compounds **1** and **2** showed moderate inhibitory activities against AChE, with IC₅₀ values of 25.95 ± 0.67 and 29.14 ± 0.77 µg/ml, respectively. These observations suggest that **1** and **2** contribute to the previously reported efficacy of this plant for the treatment of several brain diseases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at ---

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Figure legends.

Fig. 1. Structures of compounds **1-7** isolated from the aerial parts of *H. phlegmaria*.

Fig. 2. Key HMBC ($^1\text{H}\rightarrow^{13}\text{C}$, arrows) and COSY (bold lines) correlations of **1**.

Fig. 3. Key NOESY correlations (dashed arrows) of compounds **1** and **2**.

Fig. 4. Plausible biogenetic pathway of **1** and **2**.

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data for **1** and **2** in CD_3OD

Position	1		2	
	δ_{C}	δ_{H} (mult., J in Hz)	δ_{C}	δ_{H} (mult., J in Hz)
1a	46.5	3.16 (dt, 12.5, 3.5)	46.6	3.18 (dt, 12.5, 3.0)
1b		2.56 ^a		2.60*
2a	37.1	1.99 (m)	37.0	2.00 (m)
2b		1.70 (m)		1.71 (m)
3	64.7	3.67 (q-like, 3.0)	64.8	3.76 (q-like, 3.0)
4	61.3	2.59 (d, 3.5)	61.1	2.57 (d, 3.0)
5	210.5	–	210.3	–
6	138.9	–	139.3	–
7	46.9	3.44 (dt, 9.0, 2.0)	43.5	3.51 (ddd, 12.0, 5.5, 1.5)
8	34.7	2.27 (m)	34.6	2.80 (m)
8b				2.00*
9	66.2	4.26 (dd, 9.5, 6.5)	65.8	4.35 (dd, 9.5, 6.0)
10a	20.5	2.19 (m)	20.7	2.20 (m)
10b		1.79 (m)		1.79 (m)
11a	36.7	2.09 (m)	36.4	2.07 (m)
11b		1.58 (td, 13.0, 10.5)		1.49 (td, 13.5, 10.5)
12	63.6	–	65.7	–
13	81.6	–	81.8	–
14	213.8	–	215.4	–
15	43.5	2.40 (m)	40.0	2.77 (m)
16	18.6	1.24 (d, 7.0)	16.5	1.32 (d, 7.5)
17	47.0	2.51 (s)	47.1	2.52 (s)
1'	120.9	7.09 (d, 1.5)	120.4	7.06 (d, 1.5)
2'	152.2	–	152.3	–
3'	120.5	6.88 (d, 3.5)	120.6	6.85 (d, 3.5)
4'	111.5	6.52 (d, 3.5)	111.5	6.52 (d, 3.5)
5'	160.1	–	160.1	–
6'	57.6	4.60 (s)	57.7	4.60 (s)

^aOverlapping resonances within the same column.

Table 2. *In vitro* AChE inhibitory activities of compounds **1–3** and **5–7** from *H. phlegmaria*

Compounds	IC ₅₀ ± SD (µg/ml)
1	25.95 ± 0.67
2	29.14 ± 0.77
3	>1,000
5	>1,000
6	>1,000
7	>1,000
Galanthamine*	0.33 ± 0.01

*Positive control.

Figure 1

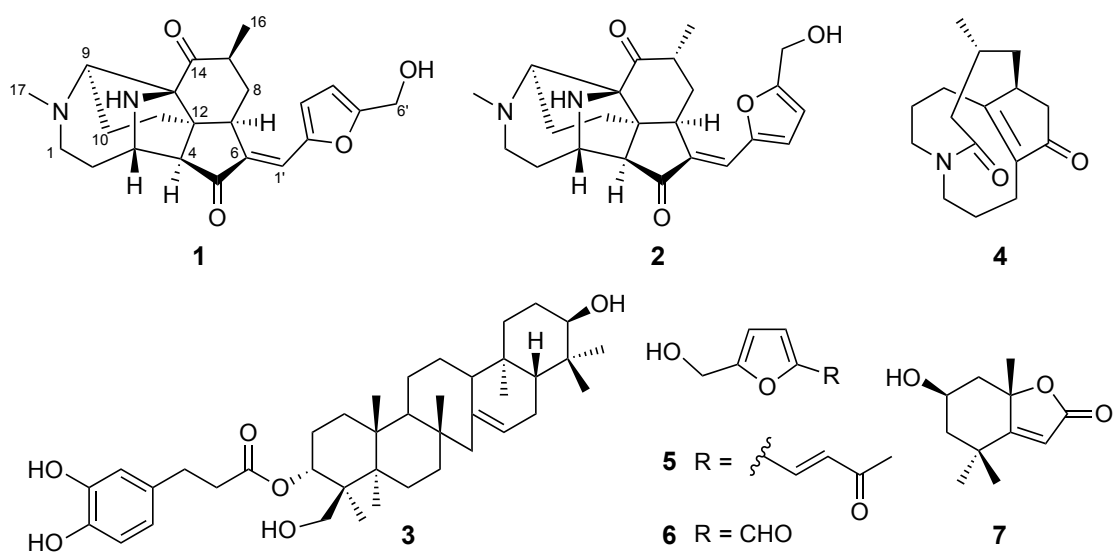


Figure 2

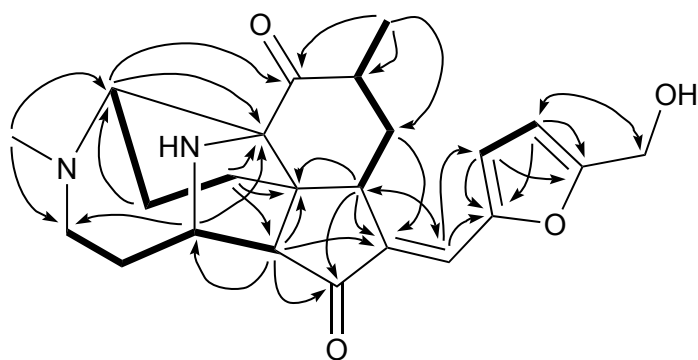


Figure 3

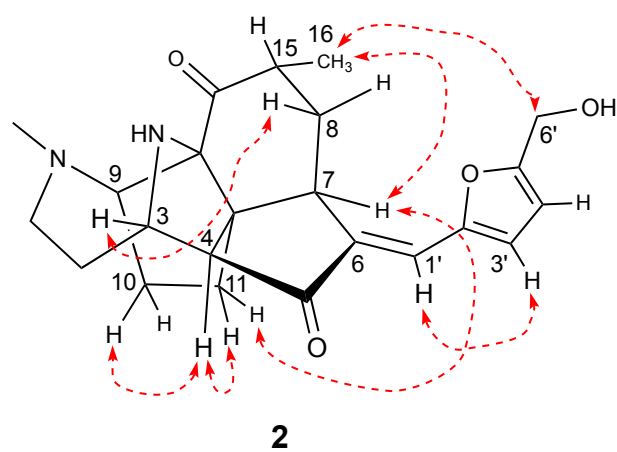
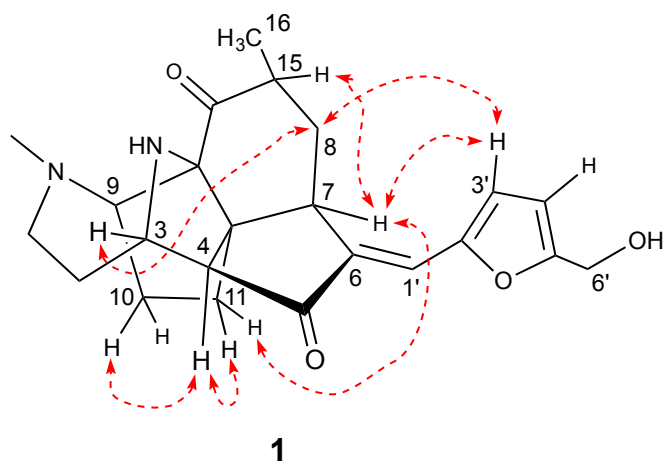


Figure 4

