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Dois novos alcaloides tipo-licopódio, acetil-licoposerramina M e palcernina A foram isolados de extratos de plantas inteiras de *Palhinhaea cernua* L. juntamente com dez compostos previamente identificados. As estruturas dos dois novos compostos foram elucidadas por métodos espectroscópicos e difratometria de Raios X de monocristal usando o parâmetro Flack.

Two new *Lycopodium* alkaloids, acetyllycoposerramine M and palcernine A were isolated from whole plant extracts of *Palhinhaea cernua* L. together with ten previously identified compounds. The structures of the new compounds were elucidated by spectroscopic methods and single-crystal X-ray diffraction analyses using the Flack parameter.

Keywords: Lycopodiaceae, *Palhinhaea cernua*, *Lycopodium* alkaloids

Introduction

Palhinhaea cernua L. (syn. *Lycopodium cernuum* (L.); *Lepidotis cernua* (L.) P. Beauv.; *Lycopodiella cernua* (L.) Pic. Ser.), found in both the Flora of North America and the Flora of China,¹ is a member of the Lycopodiaceae family. The species is traditionally used to heal contusions, scalding and rheumatism by the Chinese people and is broadly distributed in Eastern and Southern China.² The chemical constituents of the genus *Lycopodium* (*sensu lato*) plants are mainly *Lycopodium* alkaloids which are quinolizine or pyridine and α -pyridone type alkaloids.³⁻¹⁰ Some of these alkaloids inhibit acetylcholinesterase (AChE) and provide a challenge for total synthesis.⁹⁻¹⁰ In our previous research on this plant, palhinine A, a novel C₁₆N-type alkaloid was isolated.⁹ We now report the isolation and structural elucidation of two new *Lycopodium* alkaloids (1-2) along with ten previously identified compounds (Figure 1), namely, lycoflexine

N-oxide (3)⁸, lycoflexine (4),¹¹ lycodine (5),¹² α -obscurine (6),¹² dehydroisofawcettiine (7),^{13,14} lycopodine (8),¹⁴ 5-acetyllycofoline (9),¹⁵ acetyllycofawcettiine (10),^{16,17} cernuine (11)¹⁸ and lycocernuine (12).¹⁸

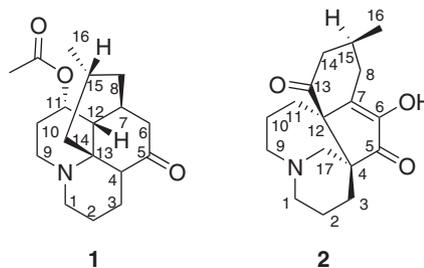


Figure 1. The structures of compounds 1-2.

Results and Discussion

Acetyllycoposerramine M (1) was obtained as a colorless columnar crystal (mp 197-198 °C). A molecular formula (C₁₈H₂₇NO₃) was assigned by HREIMS (electrospray

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ionization mass spectrometric) analysis (observed $[M]^+$ at m/z 305.1995, calcd. $[M]^+$: 305.1991), implying six degrees of unsaturation. The IR (infrared) spectrum showed absorptions for ester (1695 cm^{-1}) and keto (1733 cm^{-1}) carbonyl groups. One singlet (δ_{H} 2.09, 3H) and one doublet (δ_{H} 0.88, 3H, J 6.3 Hz, H₃-16) for two methyl groups were observed in ^1H NMR (nuclear magnetic resonance) spectrum of **1** (Table 1). Meanwhile, signals of 18 carbons were recorded in the ^{13}C NMR and DEPT (distortionless enhancement by polarization) spectra (Table 2), including the presence of one ester carbonyl (δ_{C} 170.3), one keto carbonyl (δ_{C} 214.4, C-5), one sp^3 quaternary, five sp^3 methine, eight sp^3 methylene and two methyl groups. Specifically, the signals of an oxygenated methine group were recorded at δ_{H} 5.23 (ddd, J 2.9, 2.9, 2.9 Hz, H-11) and δ_{C} 72.3 (C-11). The above mentioned analyses suggested that **1** was a lycopidine alkaloid, and shared a similar planar structure with the known *Lycopodium* alkaloid, lycoposerramine M.¹⁹ Differentiations between the two compounds were observed in that the additive signals (δ_{H} 2.09, 3H, s; δ_{C} 22.0 and 170.3) for an acetyl group appeared

Table 1. ^1H NMR data of compounds **1-2** (δ in ppm, J in Hz)

No.	1 ^a	2 ^b
1a	3.42 (dd, 14.2, 3.4)	3.09 (m)
1b	2.64 (m)	2.93 (m)
2a	1.89 (m)	2.51 (dd, 14.8, 4.0)
2b	1.51 (m)	1.34 (d, 13.4)
3a	2.13 (m)	2.29 (m)
3b	1.59 (ddd, 13.6, 13.6, 4.4)	1.99 (dd, 14.8, 9.4)
4	3.24 (dd, 12.0, 2.9)	
6a	2.76 (dd, 16.6, 6.7)	
6b	2.23 (d, 16.6)	
7	2.33 (s)	
8a	1.69 (br. d, 13.0)	2.90 (m; β)
8b	1.35 (ddd, 12.7, 12.7, 3.4)	2.66 (dd, 15.1, 8.0; α)
9a	3.37 (m)	2.99 (m)
9b	2.61 (m)	2.82 (m)
10a	2.10 (m)	2.15 (m)
10b	1.98 (m)	1.41 (m)
11a	5.23 (ddd, 2.9, 2.9, 2.9)	2.28 (m; α)
11b		1.65 (dd, 14.7, 11.8; β)
12	1.85 (m)	
14a	2.66 (m)	2.49 (dd, 14.8, 4.0; α)
14b	1.09 (dd, 13.1, 13.1)	1.97 (dd, 14.8, 9.4; β)
15	1.54 (m)	2.19 (m)
16	0.88 (3H, d, 6.3)	0.90 (3H, d, 6.7)
17a		3.10 (m)
17b		2.99 (m)
OCCH ₃	2.09 (3H, s)	
6-OH		12.19 (br. s)

^aRecorded at 500 MHz in CDCl₃; ^brecorded at 500 MHz in C₃D₃N.

Table 2. ^{13}C NMR data of compounds **1-2** (δ in ppm)

No.	1 ^a	2 ^b
1	47.2 t	52.7 t
2	19.4 t	21.1 t
3	20.0 t	29.3 t
4	45.4 d	54.0 s
5	214.4 s	203.8 s
6	44.4 t	149.1 s ^c
7	35.1 d	145.0 s
8	44.3 t	28.4 t
9	42.1 t	57.7 t
10	31.0 t	26.1 t
11	72.3 d	34.0 t
12	46.8 d	61.7 s
13	58.9 s	210.0 s
14	43.8 t	48.9 t
15	25.5 d	27.4 d
16	22.8 q	21.7 q
17		57.8 t
OCCH ₃	170.3 s	
OCCH ₃	22.0 q	

^aRecorded at 125 MHz in CDCl₃; ^brecorded at 125 MHz in C₃D₃N; ^cdetected by HMBC.

in the ^1H and ^{13}C NMR spectra of **1**, and the presence of a chemical shift of H-11 in **1** was downshifted compared with that of the known compound (δ_{H} 4.22).¹⁹ Therefore, it could be speculated that **1** was the acetylated derivative of lycoposerramine M. This was confirmed by the presence of the cross peak between H-11 and ester carbon in the HMBC (heteronuclear multiple bond correlation) spectrum of **1** (Figure 2).

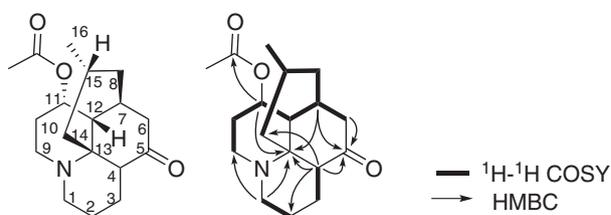


Figure 2. ^1H - ^1H COSY and HMBC correlations of **1**.

To elucidate the absolute configuration, the crystals of **1** were isolated from a mixed solution of petroleum ether/Me₂CO (9:1), and single-crystal X-ray crystallographic analysis was performed (Figure 3). The results of the analysis revealed the absolute configuration of **1** to be 4*R*, 7*R*, 11*R*, 12*R*, 15*R* in light of the Flack parameter of 0.0(2), using anomalous dispersion with copper radiation.²⁰⁻²² Finally, **1** was elucidated as acetyllycoposerramine M.

Palcernine A (**2**) was obtained as a colorless block crystal (mp 98-101 °C). Its molecular formula (C₁₇H₂₄N₃O)

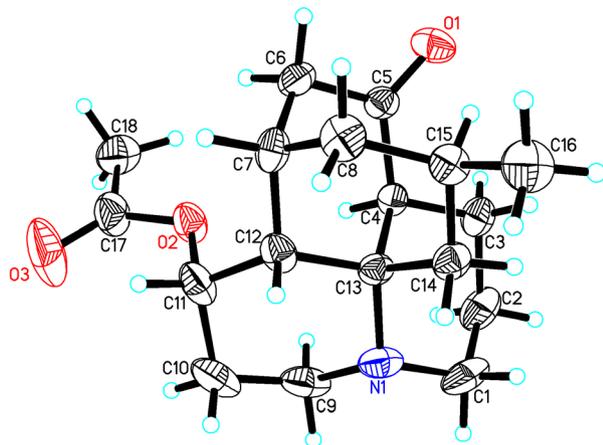


Figure 3. ORTEP drawing of the asymmetric unit of compound 1.

was established on the basis of HRESIMS for the $[M + H]^+$ ion at m/z 290.1754 (calcd. 290.1756), implying seven degrees of unsaturation. The IR spectrum showed the presence of hydroxy (3441 cm^{-1}), ketone (1709 cm^{-1}) and α,β -unsaturated ketone (1631 cm^{-1}) groups. A signal δ_{H} 0.90 (d, 3H, J 6.7 Hz) for a methyl group was observed in the ^1H NMR spectrum of **2** (Table 1), and 17 carbon signals for two ketone, one tetrasubstituted double bond, two sp^3 quaternary, one methine, nine methylene and one methyl groups were recorded in the ^{13}C NMR and DEPT of **2** (Table 2). Three fragments, **a** (C-1 to C-3), **b** (C-9 to C-12) and **c** (C-8–C-15–C-16) were elucidated by the ^1H - ^1H COSY (correlation spectroscopy) experiments (Figure 4). Based on the HMBC correlations (Figure 4) of H-2a/C-4, H-17a/C-2 & C-3, H-17a & H-3a/C-5, H-10b/C-12, H-11 β /C-7 & C-13, H-8 α /C-6, C-7 & C-12, H-15/C-13, and H₃-16/C-14 & C-13, a fawcettimine skeleton of **2** was determined, and the double bond was

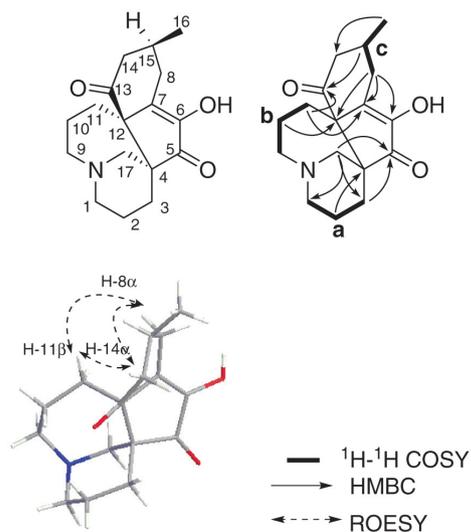


Figure 4. Key 2D NMR correlations of **2**.

located at C-6, and two ketone groups were located at C-5 and C-13, respectively. After analysis of the molecular formula of **2** the presence of a hydroxy functionality in **2** was confirmed, possibly being located at C-6.

The relative configuration of partial chiral center of **2** could be elucidated, integrating ROESY (rotating-frame Overhauser effect spectroscopy) experiment and analysis on coupling constant. In ROESY spectrum (Figure 4), the cross peaks of H-8 α (δ_{H} 2.66, dd, J 15.1, 8.0 Hz)/H-11 β (δ_{H} 1.65, dd, J 14.7, 11.8 Hz), H-8 α /H-14 α (δ_{H} 2.49, dd, J 14.8, 4.0 Hz) and H-11 β /H-14 α were observed. H-14 α and H-15 should be in *cis* configuration due to the coupling constant of $J_{\text{H-14}\alpha/\text{H-15}}$ 4.0 Hz (Table 1). Therefore, the 16- CH_3 in **2** was β -oriented, which is rare because the 16- CH_3 is α -oriented in most of the *Lycopodium* alkaloids.³⁻⁵ Based on the single-crystal X-ray crystallographic analysis (Figure 5), the absolute configuration of **2** was determined to be 4*S*, 12*S*, 15*S* in light of the Flack parameter of 0.1(2), using anomalous dispersion with copper radiation.²⁰⁻²² Accordingly, **2** was elucidated as shown in Figure 2 and named palcernine A.

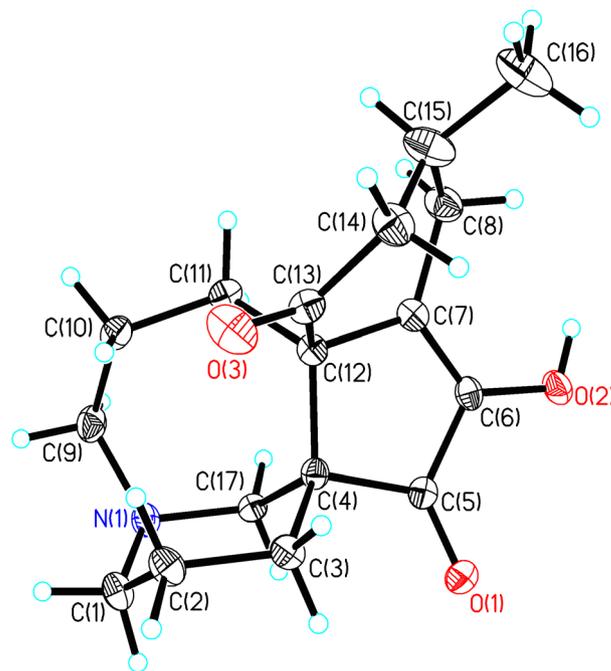


Figure 5. ORTEP drawing of the asymmetric unit of compound 2.

Compounds **1-3** were tested for their inhibition activities against acetylcholinesterase, butyrylcholinesterase and human chronic myelogenous leukemia K562 cells, respectively by the improved Ellman's method,²³⁻²⁷ and the MTT method.²⁸ However, using these methods no inhibitory activities for the compounds were detected.

Conclusions

Two new *Lycopodium* alkaloids, acetyllycposerramine M and palcernine A were isolated from whole plant extracts of *Palhinhaea cernua* L. together with ten previously identified compounds. The structures of the new compounds were elucidated by spectroscopic methods and single-crystal X-ray diffraction analyses using the Flack parameter. The two new *Lycopodium* alkaloids along with lycoflexine *N*-oxide were tested for their inhibition activities against acetylcholinesterase, butyrylcholinesterase and human chronic myelogenous leukemia K562 cells, respectively by the improved Ellman's method, and the MTT method. However, using these methods, none of the inhibitive activities of the compounds were detected.

Experimental

General experimental procedures

Melting points (mp) were determined using an X-4 melting point apparatus (Yingyu Yuhua Apparatus Factory, Gongyi, P. R. China), and was not adjusted. Bruker SMART APEX-II and Bruker APEX DUO diffractometers using graphite-monochromated Cu K_{α} radiations were employed for the intensity data collection, and the structures of compounds were solved by direct methods (SHELXS97). Optical rotations were determined on a JASCO DIP-370 automatic digital polarimeter. UV spectra were recorded on a Shimadzu double-beam 210A spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on Bruker AM-400, DRX-500 and Bruker Avance 600 spectrometers with chemical shifts in ppm relative to TMS (tetramethylsilane) as internal standard. ESIMS and HRESIMS were measured using an API QSTAR Pulsar 1 spectrometer, as well as EIMS and HREIMS using a VG Autospec Premier spectrometer. Column chromatography was performed over Al_2O_3 (200-300 mesh, Wusi Lt. Co., Shanghai), silica gel G (80-100 and 300-400 mesh), silica gel H (10-40 μ m) and Sephadex LH-20 (40-70 μ m, Amersham Pharmacia Biotech AB, Uppsala, Sweden). HPLC (high-performance liquid chromatographic) separations were performed using an Agilent 1200 series pump equipped with a diode array detector and a semi-preparative Zorbax SB-C₁₈ (5 μ m, ϕ 9.4 \times 250 mm) column. TLC (thin layer chromatography) was conducted on precoated GF₂₅₄ silica gel plates (Qingdao).

Plant material

Whole plant samples of *P. cernua*, a nodding club-moss was collected from Liping County of Guizhou Province, P. R. China and identified by Dr. Guang-Wan Hu at the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. SZ09048) was deposited in the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany.

Extraction and isolation

The air-dried and powdered sample (16.5 kg) was three times extracted with MeOH (4, 4, and 3 h) at 70 °C. The extracts were partitioned between EtOAc and 1% HCl/H₂O. Water-soluble materials were adjusted to pH 10 with 17% NaOH/H₂O solution and extracted with CHCl₃, producing an alkaloid-rich extract (82.0 g). The latter was submitted to a silica gel column (CHCl₃/MeOH, 1:0 to 0:1) to produce four fractions: Fr. 1-Fr. 4.

Fr. 1 was partitioned over C₁₈ column eluting with MeOH/H₂O (20% to 100%, v/v). The fraction by 50% MeOH/H₂O was chromatographed over Sephadex LH-20 (MeOH), Al_2O_3 [petroleum ether/Me₂CO/NH₃·H₂O, 80:10:1 (v/v/v)] and silica gel [petroleum ether/Me₂CO/NH₃·H₂O, 80:10:1 (v/v/v)] columns to obtain two sub-fractions: Fr. 1-50-1 and Fr. 1-50-2. Fr. 1-50-1 was deposited at room temperature, and compound **1** (246.2 mg) was crystallized out [petroleum ether/Me₂CO, 9:1 (v/v)]. The mother solution was evaporated and the residue was subjected to silica gel with petroleum ether/Me₂CO (8:1, v/v) to yield compound **7** (26.5 mg). Fr. 1-50-2 was submitted to Al_2O_3 and silica gel columns and preparative TLC [petroleum ether/Me₂CO/NH₃·H₂O, 80:10:1 (v/v/v)] to yield compound **3** (11.0 mg). The fraction by 60% MeOH/H₂O was purified by Sephadex LH-20 (MeOH) and preparative TLC [petroleum ether/Me₂CO/NH₃·H₂O, 80:10:1 (v/v/v)] to yield compound **5** (14.1 mg).

Fr. 2 was chromatographed over C₁₈ column eluting with MeOH/H₂O (30% to 100%, v/v), and the fraction by 50% MeOH/H₂O was subjected to Sephadex LH-20 (MeOH) and silica gel [petroleum ether/(C₂H₅)₂NH, 100:1 (v/v)] columns to give compounds **4** (98.6 mg) and **8** (16.5 mg).

Fr. 3 was chromatographed over C₁₈ column eluting with MeOH/H₂O (30% to 100%, v/v). The fraction by 50% MeOH/H₂O was separated into two sub-fractions (Fr. 3-50-1 and Fr. 3-50-2). Fr. 3-50-1 was successively subjected to Sephadex LH-20 (MeOH) and Al_2O_3 [CHCl₃/MeOH, 30:1 (v/v)] columns to afford compound **2** (12.5 mg). Fr. 3-50-2 was submitted to

Sephadex LH-20 (MeOH) and silica gel [CHCl₃/MeOH/NH₃·H₂O, 300:20:1 (v/v/v)] columns to give compound **9** (26.4 mg). The fraction by 60% MeOH/H₂O was subjected Sephadex LH-20 (MeOH) and silica gel [CHCl₃/MeOH, 30:1 (v/v/v)] columns to obtain compounds **6** (138.6 mg) and **10** (407.3 mg).

Fr. 4 was chromatographed over C₁₈ column eluting with MeOH/H₂O (30% to 100%, v/v). The fraction by 40% MeOH/H₂O was purified by Sephadex LH-20 (MeOH), and compound **12** (7.4 mg) was obtained in light of recrystallization [petroleum ether/MeOH, 9:1 (v/v)]. The fraction by 50% MeOH/H₂O was separated on Sephadex LH-20 (MeOH) and silica gel [CHCl₃/MeOH, 20:1 (v/v)] columns to yield compound **11** (16.9 mg).

Acetylylcosposerramine M (**1**)

Colorless columnar crystal; mp. 197-198 °C (from petroleum ether/Me₂CO, 9:1); [α]_D^{23.6} + 45.9 (*c* 0.35, CHCl₃); UV (CHCl₃) λ_{\max} /nm (log ϵ) 241 (2.94); IR (KBr) ν_{\max} /cm⁻¹ 1733, 1695, 1235 and 1196; ¹H and ¹³C NMR data, see in Table 1 and Table 2; EIMS *m/z* (%) 305 (M⁺, 83), 262 (38), 246 (95); HREIMS *m/z* (%) 305.1995 (M⁺, C₁₈H₂₇NO₃⁺, calcd. 305.1991).

Crystal data for **1**

C₁₈H₂₇NO₃, MW = 305.41; colorless columnar, size 0.58 × 0.42 × 0.36 mm³, orthorhombic, space group *P*21 21 21; *a* = 8.43070 (10) Å, *b* = 10.9756 (2) Å, *c* = 17.8783 (3) Å, *a* = *b* = γ = 90.00°, *V* = 1655.31 (5) Å³, *T* = 296(2) K, *Z* = 4, ρ_{calcd} = 1.226 Mg m⁻³, $\mu(\text{Cu K}\alpha)$ = 0.7 mm⁻¹, *F*(000) = 664, 10967 reflections in *h*(-10/10), *k*(-13/12), *l*(-21/21), measured in the range 4.73° ≤ θ ≤ 66.50°, completeness θ_{\max} = 99.1%, 2843 independent reflections, *R*_{int} = 0.0386, 2878 reflections with $|F|^2 \geq 2\sigma |F|^2$, 202 parameters, 0 restraints, GOF = 1.116. Final *R* index: *R*₁ = 0.0424, *wR*₂ = 0.1089. *R* index (all data): *R*₁ = 0.0425, *wR*₂ = 0.1092. Flack parameter 0.0(2), largest difference peak and hole = 0.222 and -0.277 e Å⁻³. The intensity data for **1** were collected on a Bruker APEX DUO diffractometer with a graphite monochromator, Cu K α radiation. The structure of **1** was solved by direct methods (SHELXS97), expanded using difference Fourier techniques, and refined by the program and full-matrix least-squares calculations. The non-hydrogen atoms were anisotropically refined, and hydrogen atoms were fixed at calculated positions. Crystallographic data for the structure of **1** were deposited in the Cambridge Crystallographic Data Center (deposition No. CCDC 817948). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

Palcernine A (**2**)

Colorless block crystal; mp. 98-101 °C (from Me₂CO/MeOH, 5:1); [α]_D^{21.7} - 21.0 (*c* 0.06, MeOH); UV (MeOH) λ_{\max} /nm (log ϵ) 270 (3.12), 205 (3.44); IR (KBr) ν_{\max} /cm⁻¹ 3441, 1709, 1631, 1384 and 1102; ¹H and ¹³C NMR data, see in Table 1 and Table 2; ESIMS *m/z* 290 [M + H]⁺, 601 [2M + Na]⁺, 890 [3M + Na]⁺; HRESIMS *m/z* 290.1754 [M + H]⁺ (C₁₇H₂₄NO₃, calcd. 290.1756).

Crystal data for **2**

C₁₇H₂₃NO₃, MW = 289.36; colorless blocks, size 0.22 × 0.12 × 0.08 mm³, orthorhombic, space group *P*21 21 21; *a* = 6.35940 (10) Å, *b* = 13.3290 (2) Å, *c* = 17.5422 (3) Å, *a* = *b* = γ = 90.00°, *V* = 1486.96 (4) Å³, *T* = 296(2) K, *Z* = 4, ρ_{calcd} = 1.293 Mg m⁻³, $\mu(\text{Cu K}\alpha)$ = 0.7 mm⁻¹, *F*(000) = 624, 2774 reflections in *h*(-6/6), *k*(-14/13), *l*(-19/15), measured in the range 4.17° ≤ θ ≤ 64.73°, completeness θ_{\max} = 71.1%, 1617 independent reflections, *R*_{int} = 0.0131, 1597 reflections with $|F|^2 \geq 2\sigma |F|^2$, 190 parameters, 0 restraints, GOF = 1.075. Final *R* index: *R*₁ = 0.0395, *wR*₂ = 0.1191. *R* index (all data): *R*₁ = 0.0400, *wR*₂ = 0.1203. Flack parameter 0.1(2), largest difference peak and hole = 0.318 and -0.201 e Å⁻³. The intensity data for **2** were collected on a Bruker SMART APEX-II diffractometer with a graphite monochromator using Cu K α radiation. The structure of **2** was solved by direct methods (SHELXS97), expanded using difference Fourier techniques, and refined by the program and full-matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at calculated positions. Crystallographic data for the structure of **2** were deposited in the Cambridge Crystallographic Data Center (deposition No. CCDC 831841). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

Bioactivity test *in vitro*

Acetylcholinesterase and tacrine were purchased from Sigma-Aldrich Corporation, *S*-butyrylthiocholine iodide and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Fluka Chemie GmbH and Acros Organics, respectively. Inhibitory activity of all new compounds against acetylcholinesterase was performed by the improved Ellman's method, and tacrine was used as positive (IC₅₀ = 0.20 μM).

Butyrylcholinesterase was obtained from the blood serum of SD rats which were purchased from Laboratory Animal Center, Guiyang Medical University, Guizhou Province, P. R. China. *S*-butyrylthiocholine iodide and

DTNB were purchased from Acros Organics. Inhibitory activity of all new compounds against butyrylcholinesterase was performed by the improved Ellman's method, and tetraisopropylpyrophosphoramidate was treated as positive ($IC_{50} = 1.35 \mu M$).

K562 human chronic myelogenous leukemia cell line was purchased from the China Center for Type Culture Collection, Hubei Province, P. R. China. K562 cells were maintained at 37 °C in RPMI-1640 medium (Hyclone) containing 10% fetal bovine serum (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd., P. R. China) in an atmosphere of humidified 5% CO₂. The cytotoxic assay was carried out in quintuplicate in 96-well microplates (Corning), and the amount of viable cells at the end of the incubation period was determined by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. K562 cells were exposed for 24 h to the solution of all new compounds. Non-treated culture cells were used as a negative control. IC_{50} was calculated by the GWBASIC software as the concentration (μM) of the compounds causing a 50% inhibition of cell viability. Adriamycin was treated as positive control ($IC_{50} = 0.96 \mu M$).

Supplementary Information

Supplementary information is available free of charge at <http://jbcs.sbq.org.br>, as PDF file.

Acknowledgements

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References

- Zhang, X. C.; Zhang, L. B.; *Flora Reipublicae Popularis Sinicae*; Science Press: Beijing, China, 2004, ch. 6(3).
- Chinese Herbs* Edition Board; *Chinese Herbs*, Shanghai Science and Technology Press: Shanghai, China, 2005, ch. 2.
- Hirasawa, Y.; Kobayashi, J.; Morita, H.; *Heterocycles* **2009**, *77*, 679.
- Ayer, W. A.; *Nat. Prod. Rep.* **1991**, *8*, 455.
- Ma, X.; Gang, D. R.; *Nat. Prod. Rep.* **2004**, *21*, 752.
- Ishiyama, K.; Kubota, T.; Ishiyama, H.; Hayashi, S.; Shibata, T.; Kobayashi, J.; *Tetrahedron Lett.* **2011**, *52*, 289.
- Ishiyama, K.; Kubota, T.; Ishiyama, H.; Hayashi, S.; Shibata, T.; Mori, K.; Obara, Y.; Nakahata, N.; Kobayashi, J.; *Bioorg. Med. Chem.* **2011**, *19*, 749.
- Katakawa, K.; Mito, H.; Kogure, N.; Kitajima, M.; Wongseripipatana, S.; Arisawa, M.; Takayama, H.; *Tetrahedron* **2011**, *67*, 6561.
- Zhao, F. W.; Sun, Q. Y.; Yang, F. M.; Hu, G. W.; Luo, J. F.; Tang, G. H.; Wang, Y. H.; Long, C. L.; *Org. Lett.* **2010**, *12*, 3922.
- Kitajima, M.; Takayama, H.; *Topics in Current Chemistry*; Springer-Verlag: Heidelberg, 2011, vol. 1.
- Takayama, H.; Katakawa, K.; Kitajima, M.; Yamaguchi, K.; Aimi, N.; *Tetrahedron Lett.* **2002**, *43*, 8307.
- Nakashima, T. T.; Singer, P. P.; Browne, L. M.; Ayer, W. A.; *Can. J. Chem.* **1975**, *53*, 1936.
- Burnell, R. H.; Taylor, D. R.; *Tetrahedron* **1961**, *15*, 173.
- MacLean, D. B.; *Can. J. Chem.* **1963**, *41*, 2654.
- Anet, F. A. L.; Ahmad, M.; Khan, N. H.; *Can. J. Chem.* **1962**, *40*, 236.
- Burnell, R. H.; Mootoo, B. S.; Taylor, D. R.; *Can. J. Chem.* **1960**, *38*, 1927.
- Laemmerhold, K. M.; Breit, H.; *Angew. Chem., Int. Ed.* **2010**, *49*, 2367.
- Braekman, J. C.; Nyembo, L.; Bourdoux, P.; Kahindo, N.; Hootele, C.; *Phytochemistry* **1974**, *13*, 2519.
- Takayama, H.; Katakawa, K.; Kitajima, M.; Yamaguchi, K.; Aimi, N.; *Chem. Pharm. Bull.* **2003**, *51*, 1163.
- Flack, H.; *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *39*, 876.
- Flack, H. D.; Bernardinelli, G.; *J. Appl. Crystallogr.* **2000**, *33*, 1143.
- Sheldrick, G. M.; *SHELXS-97, Program for Crystal Structure Solution*; University of Gottingen: Gottingen, Germany, 1997.
- Ellman, G. L.; Courtney, K. D.; Andres, V. J.; Featherstone, R. M.; *Biochem. Pharmacol.* **1961**, *7*, 88.
- Rhee, I. K.; Appels, N.; Hofte, B.; Karabatak, B.; Erkelens, C.; Stark, L. M.; Flippin, L. A.; Verpoorte, R.; *Biol. Pharm. Bull.* **2004**, *27*, 1804.
- Sun, Q. Y.; Yang, F. M.; *Chin. Pharm. Bull.* **2008**, *24*, 1387.
- Vogel, H. G.; Vogel, W. H.; *Drug Discovery and Evaluation: Pharmacological Assays*; Springer: New York, 2008.
- Yang, F. M.; Sun, Q. Y.; *Chin. Pharm. Bull.* **2009**, *25*, 690.
- Mosmann, T.; *J. Immunol. Methods* **1983**, *65*, 55.

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