Fitoterapia 107 (2015) 44–48

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Hexacyclic monoterpenoid indole alkaloids from *Rauvolfia verticillata*

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ARTICLE INFO

Article history:
Received 21 August 2015
Received in revised form 8 October 2015
Accepted 10 October 2015
Available online 22 October 2015

Keywords:
Rauvolfia verticillata
Monoterpenoid indole alkaloid
Rauvovertine
Cytotoxicity

ABSTRACT

Five new hexacyclic monoterpenoid indole alkaloids, rauvovertine A (**1**), 17-*epi*-rauvovertine A (**2**), rauvovertine B (**3**), 17-*epi*-rauvovertine B (**4**), and rauvovertine C (**5**) together with 17 known analogues were isolated from the stems of *Rauvolfia verticillata*. Compounds **1**/**2** and **3**/**4** were obtained as C-17 epimeric mixtures due to rapid hemiacetal tautomerism in solution. The structures of **1**–**5** were established by spectroscopic analysis and with the aid of molecular modeling. The new alkaloids were evaluated for their cytotoxicity in vitro against human tumor HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines.

1. Introduction

Plants of the genus Rauvolfia (Apocynaceae), comprising approximately 60 species, are trees or shrubs mainly distributed in America, Africa, Asia, and Oceania, of which seven species are present in China [1]. *Rauvolfia verticillata* is a prolific source of heterocyclic indole alkaloids, and also an indigenous medicinal plant used in China to treat snake poisoning, hypertension, malaria, insanity, and typhus [1, 2]. As part of a BioBioPha [http://www.chemlib.cn] objective to assemble a large-scale natural product library valuable in the discovery of new drug leads from nature [3,4], phytochemical investigation on the stems of *R. verticillata* led to the isolation of five new hexacyclic monoterpenoid indole alkaloids, rauvovertine A (**1**), 17-*epi*-rauvovertine A (**2**), rauvovertine B (**3**), 17-*epi*-rauvovertine B (**4**), and rauvovertine C (**5**) (Fig. 1), together with 17 known analogues, peraksine [5], vellosimine [6], tombozine [6], 3-hydroxysarpagine [7], macusine B [8], dihydroperaksine [9], sarpagine [10], spegatrine [11], akuammidine [12], 10-hydroxy-16-epiaffinine [13], pelerine [14], β-yohimbine [15], α-yohimbine [15], antirhine [16], strictosamide [17], sitsirikine [18], and reserpine [19]. The present paper describes the isolation and structure elucidation of the new alkaloids **1**–**5**, and their cytotoxic evaluation against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines.

2. Results and discussion

Compound **1**/**2** was isolated as an inseparable C-17 epimeric mixture (3:2) where some of the signals appeared as duplicate in the NMR spectra (Tables 1 and 2). Its molecular formula was determined as $C_{19}H_{22}N_2O_3$ from HREIMS at *m/z* 326.1628 $[M]^+$ (calcd 326.1630), requiring 10 degrees of unsaturation. The IR spectrum suggested the presence of OH/NH (3421 cm^{-1}) functionalities. The UV spectrum showed absorptions at λ_{max} 222, 277 and 288 nm typical of an unsubstituted indole chromophore [20]. The ^1H NMR spectrum (Table 1) displayed one NH group at δ_H 10.72/10.74 (1H, s), two active hydroxy protons at δ_H 6.49/6.09 (1H, d, $J = 4.0/3.5$ Hz) and 5.74 (1H, br s), four aromatic protons at δ_H 7.33/7.32 (1H, d, $J = 7.1$ Hz), 7.24/7.25 (1H, d, $J = 8.0$ Hz), 6.98 (1H, t-*like*, $J = 7.5$ Hz), and 6.90/6.91 (1H, t-*like*, $J = 7.4$ Hz), and one secondary methyl at δ_H 1.14/1.08 (3H, d, $J = 6.6$ Hz). The ^{13}C NMR and DEPT spectrum (Table 2) exhibited 19 carbon signals of each epimer, consisting of one methyl, two sp^3 methylenes, eight sp^3 methines, four sp^2 methines, and four sp^2 quaternary carbons. Combined analysis of its ^1H and ^{13}C NMR data indicated that **1**/**2** had a framework of sarpagine-type alkaloid [21]. The signals assignable to a hemiacetal methine (δ_H 4.55/4.82; δ_C 96.83/93.76) and an oxygenated methine (δ_H 3.49/4.02; δ_C 71.08/65.35) suggested the presence of 17,19-epoxy moiety, which was further confirmed by the HMBC correlation from H-17 to C-19 (Fig. 2). And the HMBC correlations from H-17 to C-5, C-15 and C-19, and from H-21 to C-3 and C-19 permitted the assignment of hydroxy groups at C-17 and C-21, respectively.

The relative configuration of each epimer was established spectroscopically based on computer-generated 3D drawing with minimized energy by MM2 calculation (Fig. 3). In the ROESY spectrum of **1**/**2**, the correlation of H-16 ↔ H-6β demonstrated that H-16 and H-6β were in

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http://dx.doi.org/10.1016/j.fitote.2015.10.004
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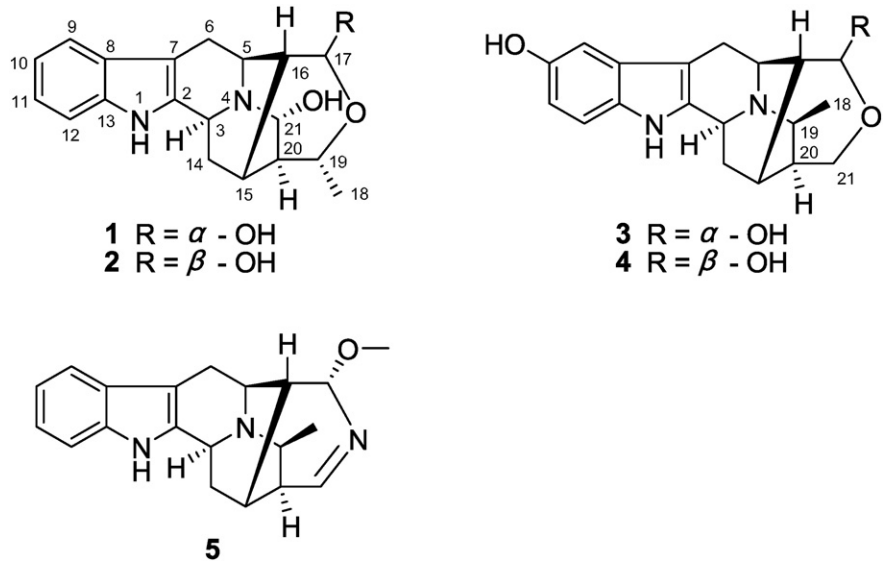


Fig. 1. The structures of compounds **1**–**5**.

the same side, and the correlations of H-21 ↔ H-5/Me-18, 21-OH ↔ H-3, and H-20 ↔ H-14α revealed the α-orientation of H-3, H-5, H-20, 21-OH and Me-18. The hemiacetal methine proton H-17 showed the significant ROESY correlations with H-15/H-19 in **1**, but with H-5, not H-15/H-19 in **2** (Fig. 3), which revealed that the orientations of H-17 were assigned as β in **1** and α in **2**. Another noteworthy observation is that the chemical shift of H-5 (δ_H 3.22) in **1** was relatively deshielded compared to that of H-5 (δ_H 2.87) in **2**, while the shifts of H-15 (δ_H 2.00) and H-19 (δ_H 4.02) in **2** were relatively deshielded compared to those of H-15 (δ_H 1.52) and H-19 (δ_H 3.49) in **1**, which is attributed to paramagnetic deshielding effects caused by the hemiacetal hydroxy group. Conformationally, the pyran ring in this mixture (**1**:**2** ≈ 3:2) adopted a chair form, in which 17α-OH in the major epimer occupied an equatorial position. As a result, the major epimer is lower in energy and more is present at the equilibrium since a molecule favors the conformation in which larger substituent is in equatorial position. Hence, the structures of **1**/**2** were unambiguously established as 17,19-epoxy-19,20-dihydrosarpagane-17α/β,21α-diol, and given the trivial names rauvovertine A and 17-*epi*-rauvovertine A, respectively.

Compound **3**/**4** was also isolated as an inseparable C-17 epimeric mixture (≈2:1) with the same molecular formula as **1**/**2**, determined by HREIMS at *m*/*z* 326.1638 $[M]^+$ (calcd for $C_{19}H_{22}N_2O_3$, 326.1630). The NMR data (Tables 1 and 2) of **3**/**4** were very similar to those of peraksine [5], but it was evident that **3**/**4** had an additional hydroxy group located at C-10 since the methine signal of C-10 in peraksine was changed to an oxygenated quaternary carbon (δ_C 152.04) in **3**/**4**, which was confirmed by the HMBC correlation from the *m*-coupled broad singlet of H-9 to C-7 and the ROESY correlation of H-9 ↔ H-6β. The relative configuration of **3**/**4** was established on the basis of its ROESY spectrum. The correlations of H-16 ↔ H-6β, H-3 ↔ H-19 and

Table 1
^{1}H NMR spectroscopic data for **1**–**5**.

No.	1/2[a]	3/4[b]	5[b]
1	10.72/10.74, s		
3	4.23/4.25, dd, 10.5, 2.4	4.80, d, 10.2	4.09, br d, 10.1
5	3.22, t, 5.3/2.87[c]	4.27/4.09, t, 5.5	3.19, t, 5.6
6α	2.89, dd, 15.2, 6.1/2.86[c]	3.23/3.18, dd, 16.6, 5.5	2.88, dd, 15.3, 5.1
6β	2.46, d, 15.2/2.51[c]	2.83/2.87, d, 16.6	2.71, dd, 15.3, 1.1
9	7.33/7.32, d, 7.8	6.82/6.83, br s	7.38, d, 7.8
10	6.90/6.91, t-*like*, 7.4		6.97, t-*like*, 7.4
11	6.98, t-*like*, 7.5	6.71, br d, 8.6	7.04, t-*like*, 7.6
12	7.24/7.25, d, 8.0	7.18, d, 8.6	7.27, d, 8.1
14α	1.80/1.77, m	2.43/2.39, t, 11.6	2.01, ddd, 13.3, 10.1, 1.6
14β	1.21[c]/1.14[c]	1.86/1.78, br d, 13.0	1.56, ddd, 13.3, 4.4, 1.8
15	1.52/2.00, br s	2.04/2.54, br s	1.80, m
16	1.21[c]/1.12[c]	1.88/1.74, d, 5.5	1.97, m
17	4.55, br d, 4.0/4.82, br s	4.74/5.10, s	4.35, br s
18	1.14/1.08, d, 6.6	1.63, d, 6.9	1.23, d, 7.1
19	3.49/4.02, qd, 6.6, 1.8	3.91, m	3.35, dd, 9.3, 7.1
20	1.09[c]/1.14[c]	1.91/1.97, br d, 8.4	2.49, m
21α	4.31/4.38, br s	4.02, d, 12.6/3.69, d, 12.3	8.00, t, 2.9
21β		3.56, d, 12.6/4.05, d, 12.3	
17-OH	6.49, d, 4.0/6.09, d, 3.5		
21-OH	5.74, br s		
OMe			3.58, s

[a] Measured in DMSO-d_6 (2.49 ppm).
[b] Measured in methanol-d_4 (3.30 ppm).
[c] Overlapped signals.

Table 2
^{13}C NMR spectroscopic data for **1**–**5**.

No.	1/2[a]	3/4[b]	5[b]
2	139.96/140.06, C	134.00/134.12, C	138.57, C
3	41.70/41.51, CH	54.13/54.07, CH	52.54, CH
5	46.59/50.33, CH	48.95/51.30, CH	46.36, CH
6	27.85/26.71, CH_2	27.60/26.26, CH_2	28.81, CH_2
7	102.56/102.13, C	102.47/102.18, C	104.98, C
8	127.34/127.30, C	128.32, C	128.74, C
9	117.50/117.46, CH	103.47/103.54, CH	118.71, CH
10	118.20/118.23, CH	152.04, C	119.82, CH
11	120.14/120.18, CH	113.02, CH	122.13, CH
12	111.01/111.04, CH	113.33, CH	112.08, CH
13	136.17, C	133.30, C	138.27, C
14	33.22/33.05, CH_2	31.99/32.07, CH_2	31.53, CH_2
15	29.80/23.74, CH	29.23/22.56, CH	27.29, CH
16	40.04/39.84, CH	42.38/41.43, CH	40.25, CH
17	96.83/93.76, CH	96.97/93.39, CH	93.30, CH
18	17.39/17.49, CH_3	11.95/12.29, CH_3	14.82, CH_3
19	71.08/65.35, CH	60.05/60.09, CH	55.91, CH
20	42.68/43.56, CH	33.22/34.27, CH	38.48, CH
21	81.67/80.94, CH	64.84/59.22, CH_2	169.98, CH
OMe			56.64, CH_3

[a] Measured in DMSO-d_6 (39.5 ppm).
[b] Measured in methanol-d_4 (49.0 ppm).

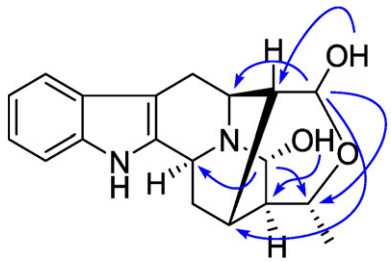


Fig. 2. Key HMBC correlations of **1**/**2**.

Me-18 ↔ H-5 indicated that these protons were spatially close to each other. Similarly, the orientation of H-17 was assigned as β in **3** and α in **4** according to the presence/absence of ROESY correlations of H-17 ↔ H-15/H-21β and H-17 ↔ H-5. This was also verified by the 17-OH induced downfield shifts for H-5 ($\Delta = 0.18$ ppm) in **3** and H-15 ($\Delta = 0.50$ ppm) and H-21β ($\Delta = 0.49$ ppm) in **4** compared to their counterparts. Thus, the structures of **3**/**4** were finally established as 17,19-epoxy-19,20-dihydro-21-methyl-18-norsarpagane-10,17α/β-diol and named rauvovertine B and 17-*epi*-rauvovertine B, respectively.

Compound **5** gave a molecular formula of $C_{20}H_{23}N_3O$ by HREIMS ($[M]^+$ *m/z* 321.1849, calcd 321.1841). Comparison of its ^{13}C NMR data (Table 2) with those of **3**/**4** revealed similarities except for the replacement of the hydroxy group at C-17 by a methoxy (δ_C 56.64), and of the oxygenated methylene (δ_C 64.84/59.22) at C-21 by an unusual olefinic methine (δ_C 169.98). The location of the methoxy was established by the HMBC correlation from OMe (δ_H 3.58) to C-17. Combined analysis of the NMR and MS information of **5** implied the presence of a rare imine group (δ_H 8.00; δ_C 169.98) between C-17 and C-20, confirmed by the HMBC correlations from H-21 to C-15, C-17 and C-20. The orientation of H-17 was assigned as β from the ROESY correlation of H-17 ↔ H-15. Thus, the structure of **5** was elucidated as shown in Fig. 1 and named rauvovertine C. It represents a new class of peraksine alkaloid with an unusual N bridge between C-17 and C-20. A possible biosynthetic pathway for **5** was proposed as shown in Scheme 1. The key intermediate product **6** could be enzymatically synthesized from perakine [9], an alkaloid isolated previously from the genus *Rauvolfia* [2,9]. Intermediate product **7** was proposed to be generated from **6** through the amination reaction with the participation of β-aminoethanol [22,23].

Generally, the alkaloids **1**–**5** represent two types of carbon skeletons related to sarpagine and peraksine. Since the absolute stereochemistry of sarpagine and peraksine were assigned previously [24,25], biogenetically, the absolute stereochemistry of **1**–**5** could be assumed as (3*S*,5*S*,15*S*,16*R*,17*R*/*S*,19*R*,20*R*,21*R*) for **1**/**2**, (3*S*,5*S*,15*S*,16*R*,17*R*/*S*,19*S*,20*S*) for **3**/**4** and (3*S*,5*S*,15*S*,16*R*,17*S*,19*S*,20*S*) for **5**, as shown in Fig. 1.

All new alkaloids were evaluated for their in vitro growth inhibitory effects toward five human cancer cell lines with cisplatin and taxol as positive controls by the MTS method. The results showed that compound **5** exhibited moderate cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7 and SW-480 with IC_{50} values of 10.76, 15.02, 15.70, 12.63 and 14.02 μM, respectively, while the remaining were noncytotoxic ($IC_{50} > 40$ μM).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Jasco P-1020 automatic digital polarimeter. UV spectra were obtained in an HPLC (Agilent 1200, DAD). IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with a Bruker Avance III 600 instrument at room temperature. ESI-MS and EI-MS (including HR-EI-MS) and were measured on Bruker HCT Esquire 3000 and Waters AutoSpec Premier P776 spectrometers. Silica gel (200–300 mesh) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Medium pressure liquid chromatography (MPLC) was performed on a Büchi Sepacore System equipping with pump manager C-615, pump modules C-605, and fraction collector C-660 (Büchi Labortechnik AG, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Japan). Fractions were monitored by TLC and HPLC (Agilent 1200, Extend-C18 column, 5 μm, 4.6 × 150 mm).

3.2. Plant material

The stems of *R. verticillata* were purchased from Kunming Chrysanthemum Village medicine market of China, in February 2012 and identified by Mr. Yu Chen of Kunming Institute of Botany, Chinese Academy of Sciences. The voucher specimen (No. BBP0447019RV) was deposited at BioBioPha Co., Ltd.

3.3. Extraction and isolation

The stems of *R. verticillata* (40 kg) were powdered and extracted with EtOH-H_2O (95:5, v/v; 3 × 70 L, each 5 days) at room temperature, and the solvent was removed under reduced pressure to give crude extract (*ca.* 1.7 kg), then dissolved in 2% HCl (5 L) and filtered. The filtration was basified using 10% ammonia-water to pH 9–10 and then partitioned with EtOAc to give a total alkaloidal extract (*ca.* 280 g), which was fractionated by silica gel CC successively eluted with $CHCl_3$/MeOH (1:0 → 0:1 gradient) to give six fractions A–F.

Fraction B was subjected to MPLC, eluted with MeOH/H_2O (10% → 100%), to obtain fractions B1–B4. Fraction B2 was separated by silica gel CC ($CHCl_3$/MeOH, 100:1 → 80:1) to obtain vellosimine (279 mg), akuammidine (10 mg) and reserpine (2 mg). Fraction B3 was purified by MPLC (MeOH/H_2O, 80%) and then Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to afford α-yohimbine (54 mg). Fraction B4 was separated by silica gel CC using petroleum ether (PE)/acetone (5:1 → 1:1) to afford pelirine (41 mg), β-yohimbine (115 mg) and sitsirikine (13 mg). Fraction C was separated by repeated silica gel CC ($CHCl_3$/MeOH, 80:1 → 10:1) into fractions C1–C3. Peraksine (364 mg) was crystallized from fraction C2, and the mother liquid was passed through a silica gel CC, eluted with $CHCl_3$/MeOH (80:1 → 10:1), to yield tombozine (477 mg) and an inseperable mixture **1**/**2** (27 mg). Fraction D was separated by silica gel CC ($CHCl_3$/MeOH, 60:1 → 10:1) into fractions D1–D4. Fraction D2

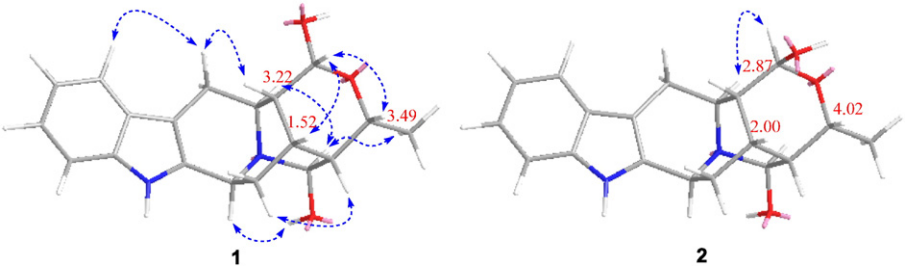
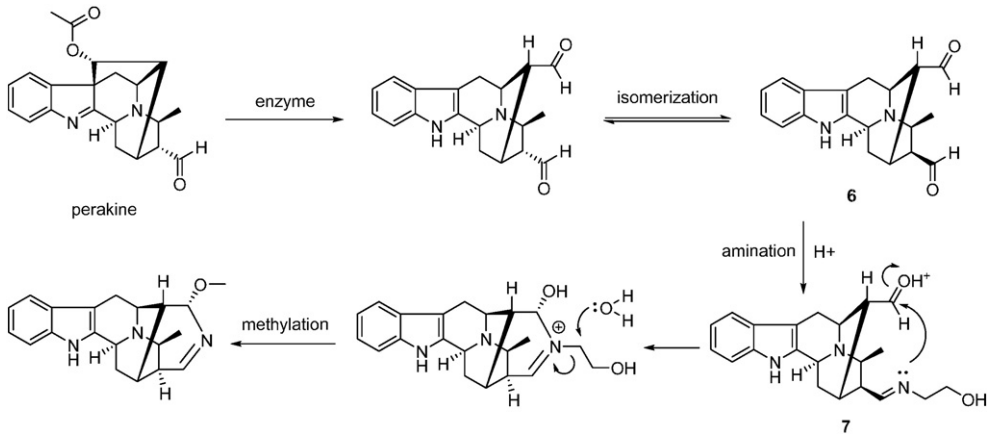


Fig. 3. Key ROESY correlations of **1**/**2**.



Scheme 1. Plausible biosynthetic pathway for **5**.

was further purified by preparative TLC ($CHCl_3$/MeOH, 20:1) to give **5** (37 mg). In the same way, 10-hydroxy-16-epiaffinine (216 mg) and antirhine (90 mg) were obtained from fractions D3 and D4, respectively. Fraction E was subjected to MPLC (MeOH/H_2O, 20% → 50%) and then by Sephadex LH-20 (MeOH) to give **3**/**4** (377 mg), 3-hydroxysarpagine (282 mg), dihydroperaksine (100 mg) and sarpagine (364 mg). Similarly, strictosamide (66 mg), macusine B (359 mg) and spegatrine (704 mg) were obtained from Fraction F.

3.3.1. Rauvovertine A (**1**)/17-epi-rauvovertine A (**2**)

White amorphous powder; $[\alpha]_D^{19}$ + 2.5 (*c* 0.13, MeOH); UV (MeOH) λ_{max}: 222, 277, 288 nm; IR (KBr) ν_{max} 3421, 2971, 2859, 1697, 1628, 1467, 1452, 1396, 1386, 1341, 1295, 1266, 1210, 1175, 1112, 1065, 1041, 1013 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; ESI-MS (pos.): *m/z* 327 $[M + H]^+$; HR-EI-MS: *m/z* 326.1628 (calcd for $C_{19}H_{22}N_2O_3$, 326.1630).

3.3.2. Rauvovertine B (**3**)/17-epi-rauvovertine B (**4**)

White amorphous powder; $[\alpha]_D^{19}$ + 32.2 (*c* 0.25, MeOH); UV (MeOH) λ_{max}: 222 (sh), 273, 296 (sh), 307 (sh) nm; IR (KBr) ν_{max} 3406, 2965, 2928, 1697, 1632, 1600, 1456, 1384, 1215, 1146, 1119, 1089, 1054, 1027 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; ESI-MS (pos.): *m/z* 327 $[M + H]^+$; HR-EI-MS: *m/z* 326.1638 (calcd for $C_{19}H_{22}N_2O_3$, 326.1630).

3.3.3. Rauvovertine C (**5**)

White amorphous powder; $[\alpha]_D^{26}$ + 9.6 (*c* 0.34, MeOH); UV (MeOH) λ_{max}: 220, 271, 280 (sh), 287 (sh) nm; IR (KBr) ν_{max} 3355, 2940, 2903, 1635, 1453, 1370, 1341, 1181, 1129, 1090, 1075, 1035, 817, 748 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EI-MS: *m/z* 321 $[M]^+$ (100), 306 (8), 29 (8), 208 (60), 195 (9), 168 (28); HR-EI-MS: *m/z* 321.1849 (calcd for $C_{20}H_{23}N_3O$, 321.1841).

3.4. Cytotoxicity assay

The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, MCF-7 and SW480. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. The cytotoxicity assay was performed according to an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] method with minor modifications, as described previously [26]. The IC_{50} value of each compound was calculated by the Reed and Muench's method [27].

Acknowledgments

This work was financially supported by the "Large-scale Compound Library" Project of National Development and Reform Commission of China and National Basic Research Program of China (973 Program, 2009CB522300).

References

[1] Editorial Committee of Flora of China, Flora of China, Science Press, Beijing, 1995 157–159.
[2] C.A. Geng, X.K. Liu, Five new indole alkaloids from the leaves of *Rauvolfia yunnanensis*, Fitoterapia 89 (2013) 42–47.
[3] Y. Gao, F. Wang, D.S. Zhou, Y. Li, J.K. Liu, Three new indole alkaloids from *Rauvolfia yunnanensis*, Nat. Prod. Bioprospect. 1 (2011) 104–107.
[4] Y. Gao, D.S. Zhou, L.M. Kong, P. Hai, Y. Li, F. Wang, et al., Rauvotetraphyllines A–E, new indole alkaloids from *Rauvolfia tetraphylla*, Nat. Prod. Bioprospect. 2 (2012) 65–69.
[5] H.R. Arthur, S.R. Johns, J.A. Lamberton, S.N. Loo, Alkaloids of *Rauwolfia verticillata* (Lour.) Bail. of Hong Kong. Identification of vellosimine and peraksine, and demonstration from NMR data that peraksine is a mixture of two epimers, Aust. J. Chem. 21 (1968) 1399–1401.
[6] J.M. Yu, T. Wang, X.X. Liu, J. Deschamps, J. Flippen-Anderson, X.B. Liao, et al., General approach for the synthesis of sarpagine indole alkaloids. Enantiospecific total synthesis of (+)-vellosimine, (+)-normacusine B, (−)-alkaloid Q3, (−)-panarine, (+)-Na-methylvellosimine, and (+)-Na-methyl-16-epipericyclivine, J. Org. Chem. 68 (2003) 7565–7581.
[7] A. Itoh, T. Kumashiro, M. Yamaguchi, N. Nagakura, Y. Mizushina, T. Nishi, et al., Indole alkaloids and other constituents of *Rauwolfia serpentina*, J. Nat. Prod. 68 (2005) 848–852.
[8] J. Quetin-Leclercq, L. Ancenot, L. Dupont, N.G. Bisset, Isolation and purification of panarine, A. Alkaloid from a Venezuelan Curare, Phytochemistry 27 (1988) 4002–4004.
[9] Y. Sheludko, I. Gerasimenko, H. Kolshorn, J. Stöckigt, New alkaloids of the sarpagine group from *Rauvolfia serpentina* hairy root culture, J. Nat. Prod. 65 (2002) 1006–1010.
[10] J.A. Martìnez, H. Velez, T. Santana, An alkaloid from two *Rauwolfia* spp. Phytochemistry 28 (1989) 961–962.
[11] M. Lin, D.Q. Yu, X. Liu, F.Y. Fu, Q.T. Zheng, C.H. He, et al., Chemical studies on the quaternary alkaloids of *Rauvolfia verticillata* (Lour.) Baill. F. ruberocarpa H. T. Chang. Mss, Acta Pharm. Sin. 20 (1985) 198–202.
[12] D. Ponglux, S. Wongseripipatana, S. Subhadhirasakul, S. Takayama, M. Yokota, K. Ogata, et al., Studies on the indole alkaloids of *Gelsemium elegans* (Thailand): structure elucidation and proposal of biogenetic route, Tetrahedron 44 (1988) 5075–5094.
[13] C. Lavaud, G. Massiot, J. Vercauteren, L.E. Men-olivier, Alkaloids of *Hunteria zeylanica*, Phytochemistry 21 (1982) 445–447.
[14] A.S.C. Wan, M. Yokota, K. Ogata, N. Aimi, S.I. Sakai, Structure of pelirine and chemical conversion of gardnerine to 11-methoxy-16-epiaffinine, Heterocycles 26 (1987) 1211–1214.
[15] E. Wenkert, C.J. Chang, H.P.S. Chawla, D.W. Cochran, E.W. Hagaman, J.C. King, et al., General methods of synthesis of indole alkaloids. 14. Short routes of construction of yohimboid and ajmalicinoid alkaloid systems and their ^{13}C nuclear magnetic resonance spectral analysis, J. Am. Chem. Soc. 98 (1976) 3645–3655.
[16] N. Boughandjioua, L. Bengaouer, F. Hotellier, E. Seguin, F. Tillequin, M. Koch, et al., Alkaloids of *Ochrosia alyxioides*, J. Nat. Prod. 52 (1989) 1107–1112.
[17] Z. Zhang, H.N. Elsohly, M.R. Jacob, D.S. Pasco, L.A. Walker, A.M. Clark, New indole alkaloids from the bark of *Nauclea orientalis*, J. Nat. Prod. 64 (2001) 1001–1005.

[18] J. Stöckigt, M. Rueffer, M.H. Zenk, G.A. Hoyer, Indirect identification of 4,21-dehydrocorynantheine aldehyde as an intermediate in biosynthesis of ajmalicine and related alkaloids, Planta Med. 33 (1978) 188–192.
[19] S. Hanessian, J. Pan, A. Carnell, H. Bouchard, L. Lesage, Total synthesis of (−)-reserpine using the chiron approach, J. Org. Chem. 62 (1997) 465–473.
[20] R. Verpoorte, Methods for the structure elucidation of alkaloids, J. Nat. Prod. 49 (1986) 1–25.
[21] J. Naranjo, M. Pinar, M. Hesse, H. Schmid, Alkaloids. 45. Indole alkaloids of *Pleiocarpa talbotii* Wernham, Helv. Chim. Acta 55 (1972) 752–771.
[22] J.Y. Zhang, W.M. Abdel-Mageed, M.M. Liu, P. Huang, W.N. He, L. Li, et al., Caesanines A–D, new cassane diterpenes with unprecedented N bridge from *Caesalpinia sappan*, Org. Lett. 15 (2013) 4726–4729.
[23] F.P. Wang, X.T. Liang, C_{20}-diterpenoid Alkaloids, in: G.A. Cordell (Ed.) The Alkaloids: Chemistry and Biology, 59, Academic Press, New York 2002, pp. 1–280.
[24] M.F. Bartlett, R. Sklar, W.I. Taylor, E. Schlittler, R.L.S. Amai, P. Beaket, et al., Rauwolfia alkaloids. XXXVIII.[1] Stereospecific degradations leading to the absolute configurations and structures of ajmaline, sarpagine and corynantheidine, J. Am. Chem. Soc. 84 (1962) 622–630.
[25] A.K. Kiang, S.K. Loh, M. Demanczyk, C.W. Gemenden, G.J. Papariello, W.I. Taylor, The structures of peraksine (RP-5) and RP-7 constituents of the leaves and stems of *Rauwolfia perakensis*, Tetrahedron 22 (1966) 3293–3300.
[26] Y. Gao, G.T. Li, Y. Li, P. Hai, F. Wang, J.K. Liu, Guajadials C–F, four unusual meroterpenoids from *Psidium guajava*, Nat. Prod. Bioprospect. 3 (2013) 14–19.
[27] L.J. Reed, H. Muench, A simple method of estimating fifty percent endpoint, Am. J. Hyg. 27 (1938) 493–497.