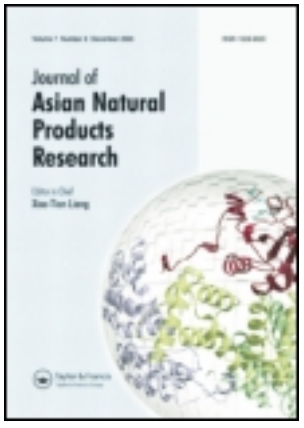

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[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China

[b] College of Horticulture and Landscape, Yunnan Agricultural University, Kunming, 650201, China

[c] Lijiang Forest Ecosystem Research Station, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China

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Indole Alkaloids from Leaves and Twigs of *Rauvolfia verticillata*

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[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;* [b]*College of Horticulture and Landscape, Yunnan Agricultural University, Kunming 650201, China;* [c]*Lijiang Forest Ecosystem Research Station, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*

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Seven new indole alkaloids, rauverines A–G (**1—7**), and 19 known indole alkaloids were isolated from the leaves and twigs of *Rauvolfia verticillata*. All compounds showed no cytotoxicity against five human cancer cell lines, human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW480) cells.

Keywords: *Rauvolfia verticillata*; monoterpenoid indole alkaloid; rauverines A–G; structure elucidation

1. Introduction

Because of vincristine derivatives, which are well-known antitumor bioactive agents, monoterpenoid indole alkaloid (MIA) dimers have attracted more attention in search of new antitumor compounds. Plants of the genus *Tabernaemontana* are rich in dimeric forms and good research objects for active compounds [1]. During our studies on MIAs from *T. divaricata*, cytotoxic divaricatines A–D possessing a new six-membered ring via an ether linkage between C-17/C-21 and C-17/C-22, respectively, were found [2]. As a continuation of our studies on the structure–activity relationship of cytotoxicity, this study aimed to examine whether monomers with the same ring system are active. The plant of the genus *Rauvolfia*, family Apocynaceae, is a good source of MIAs, especially akuammidine group, which is a precursor of possible active monomers [3]. Phytochemical investigation on *Rauvolfia verticillata* led to 7 new alkaloids (**1**–**7**), together with the 19 known alkaloids, namely perakine (**8**) [4], alstoyunine F (**9**) [5], 19,20-dihydrovomilenine (**10**) [4], vinorine (**11**) [6], vomilenine (**12**) [7], serpinine (**13**) [8], ajmaline (**14**) [9], sitsirikine (**15**) [10], 18,19-dihydro-sitsirikine (**16**) [11], tombozine (**17**) [12], vellosimine (**18**) [13], vallesiachotamine (**19**) [14], isovallesiachotamine (**20**) [14], 10-hydroxy-16-epiaffinine (**21**) [15], 1-methyl-β-carboline (**22**) [16], 1-methoxycarbonyl-β-carboline (**23**) [17], pseudoyuhimbine (**24**) [18], yuhimbine (**25**) [19], and β-yuhimbine (**26**) [20].

2. Results and discussion

The MeOH extract of *R. verticillata* leaves and twigs was partitioned between H_2O and EtOAc after acid–alkali treatment, and column chromatography (CC) over silica and C_{18}-silica gel was used to separate the alkaloidal fraction into 26 alkaloids including 7 new alkaloids (Figure 1).

Alkaloid **1** had a molecular formula of $C_{20}H_{24}N_2O_2$, indicated by high-resolution

*Corresponding author. Email: xhcai@mail.kib.ac.cn

electron impact mass spectrum (HR-EI-MS) at *m/z* 324.1845 $[M]^+$, in combination with the ^1H, ^{13}C NMR, and DEPT spectra. Its IR spectrum showed the presence of NH and OH (3418 and 3059 cm^{-1}) groups, and of benzene rings (1631 cm^{-1}). Absorption maxima at 225 and 280 nm in the UV spectrum of **1** were identical to indole alkaloids [4]. Its ^1H NMR spectrum (Table 1) displayed an indole NH (δ_H 9.91, s), an indole moiety [δ_H 7.43 (d, $J = 7.8$ Hz), 6.97 (t, $J = 7.8$ Hz), 7.03 (t, $J = 7.8$ Hz), and 7.31 (d, $J = 7.8$ Hz)], one nitrogen methyl group (δ_H 2.40, 3H, s), and one double bond (δ_H 5.14, q, $J = 6.6$ Hz). The ^{13}C NMR and DEPT spectra of **1** (Table 2) suggested 20 carbons, including two methyl (δ_C 41.8, 12.8), three methylene (δ_C 63.7, 33.8, 17.3), 10 methine (δ_C 118.6, 119.5, 121.5, 111.8, 115.2, 97.7, 55.1, 54.0, 53.1, 34.2), and five quaternary (δ_C 138.3, 137.1, 134.5, 127.9, 107.3) carbons. Alkaloid **1** was thus readily identified as an indole alkaloid similar to alstonerine [21]. A significant difference between both alkaloids was the presence of a hemiacetal and the absence of an α,β-unsaturated ester among C-19/20/21 in **1**. The HMBC correlations of H-17 at δ_H 4.79 with the carbons at δ_C 34.2 (C-15), 63.7 (C-21), 54.0 (C-5) could confirm a hemiacetal at C-17 (Figure 2). A double bond C-19/20 was confirmed by their typical chemical shifts (Tables 1 and 2), which was also supported by correlations of H-19 (δ_H 5.14 q, $J = 6.6$ Hz) with C-18 and C-21 in its HMBC spectrum. The correlations from H-15 to H-17 and H-19 in its ROESY spectrum indicated the relative configuration of **1** as shown in Figure 3. All the signals of ^1H and ^{13}C NMR were assigned by the HSQC and HMBC spectra, and it was named rauverine A.

The ^1H NMR spectrum of **2** showed signals of a mono-substituted MIA [δ_H 7.00 (d, $J = 2.0$ Hz), 6.83 (dd, $J = 8.8$, 2.0 Hz),

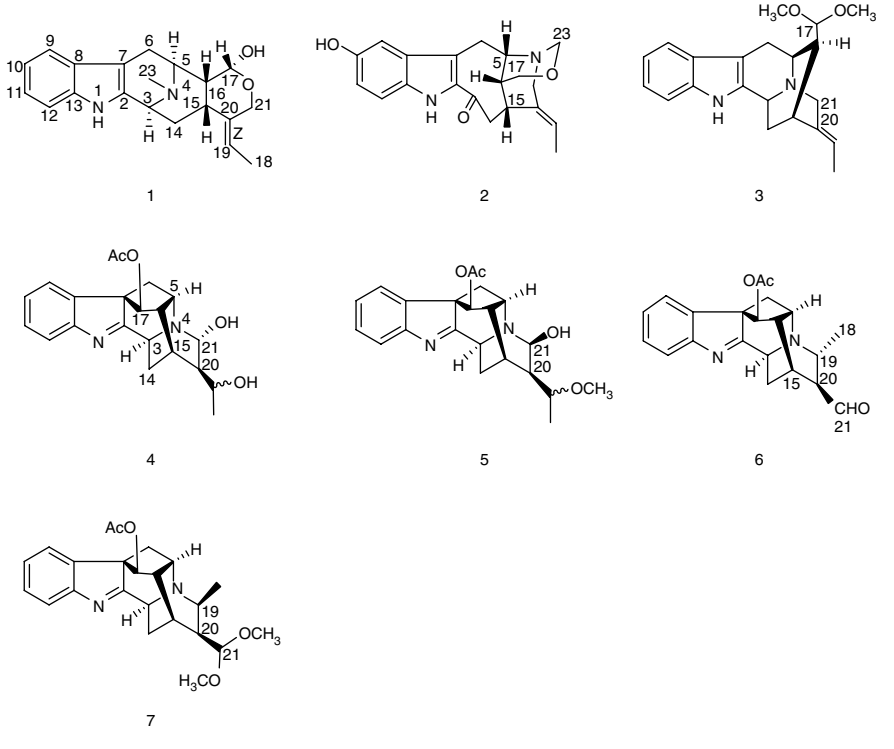


Figure 1. New alkaloids (**1**–**7**) isolated from *R. verticillata*.

Table 1. ^{1}H NMR spectral data of **1**–**7** in acetone-d_6 (δ in ppm, J in Hz).

No.	**1**[a]	**2**[b,c]	**3**[b]	**4**[d]	**5**[d]	**6**[b]	**7**[b]
1	9.91 (s)	11.40 (s)	9.96 (s)				
2	4.17 (s)		4.16 (d, 10.4)	4.20 (d, 8.0)	4.10 (d, 9.2)	4.13 (d, 9.0)	4.11 (d, 9.6)
5	3. 40 (m)	3.17 (m)	3.44 (dd, 10.3, 4.8)	3.42 (dd, 12.0, 5.6)	3.93 (dd, 12.0, 5.6)	3.26 (m)	3.75 (m)
6a	3.04 (dd, 6.6, 16.8)	3.63 (m)	3.19 (d, 15.4)	2.80 (m)	2.80 (dd, 11.6, 4.9)	2.80 (dd, 14.0, 5.0)	2.82 (dd, 15.4, 6.0)
6b	2.69 (d, 16.8)	3.15 (m)	2.80 (dd, 15.4, 6.0)	1.45 (m)	1.44 (m)	1.56 (d, 14.0)	1.50 (d, 15.4)
9	7.43 (d, 7.8)	7.00 (d, 2.0)	7.45 (d, 7.7)	7.54 (d, 8.0)	7.55 (d, 7.8)	7.56 (d, 7.8)	7.54 (d, 7.8)
10	6.97 (t, 7.8)		6.98 (t, 7.7)	7.20 (t, 8.0)	7.21 (t, 7.8)	7.23 (t, 7.8)	7.21 (t, 7.8)
11	7.03 (t, 7.8)	6.83 (dd, 8.8, 2.0)	7.05 (t, 7.7)	7.36 (t, 8.0)	7.39 (t, 7.8)	7.35 (t, 7.8)	7.37 (t, 7.8)
12	7.31 (d, 7.8)	7.21 (d, 8.8)	7.33 (d, 7.7)	7.51 (d, 8.0)	7.52 (d, 7.8)	7.56 (d, 7.8)	7.54 (d, 7.8)
14a	1.88 (2H, m)	3.28 (m) 2.50 (m)	1.81 (m)	1.73 (m)	1.96 (m)	1.93 (2H, m)	1.51 (m)
14b		2.50 (m)	1.69 (m)		1.46 (m)		2.04 (m)
15	1.91 (m)	3.13 (m)	2.78 (m)	2.68 (m)	2.55 (m)	2.96 (m)	2.37 (t, 5.4)
16	1.71 (m)	1.31 (s)	2.16 (m)		2.39 (m)	2.34 (m)	2.44 (m)
17a	4.79 (d, 7.8)	3.71 (d, 11.2)	3.98 (d, 9.5)	5.10 (s)	4.98 (s)	5.05 (s)	4.99 (s)
17b		3.60 (d, 11.2)					
18	1.54 (3H, d, 6.6)	1.60 (3H, d, 6.6)	1.62 (3H, d, 6.8)	1.24 (d, 6.0, 3H)	1.24 (3H, d, 6.0)	1.38 (3H, d, 6.8)	1.29 (d, 7.2, 3H)
19	5.14 (q, 6.6)	5.11 (d, 6.6)	5.22 (q, 6.8)	3.72 (m)	3.43 (m)	3.73 (q, 6.8)	2.85 (m)
20				1.40 (m)	1.41 (m)	2.46 (m)	1.63 (t, 8.4)
21a	4.50 (d, 13.6)	4.28 (d, 12.0)	3.55 (2H, s)	4.31 (s)	4.25 (d, 7.2)	9.81 (s)	4.55 (d, 8.4)
21b	3.64 (d, 13.6)	3.20 (d, 12.0)					
23a	2.40 (3H, s)	4.51 (d, 9.6)					
23b		4.43 (d, 9.6)					
OH		9.00 (s)					
CH_3					2.15 (3H, s)	2.19 (3H, s)	2.15 (3H, s)
OCH_3			3.06 (3H, s)				3.36 (3H, s)
			3.08 (3H, s)				3.40 (3H, s)

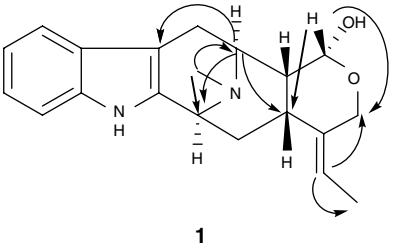
[a] At 500 MHz.
[b] At 600 MHz.
[c] In DMSO.
[d] At 400 MHz.

Table 2. ^{13}C NMR spectral data of **1**–**7** in acetone-d_6 (δ in ppm).

No.	**1**[a]	**2**[b,c]	**3**[b]	**4**[a]	**5**[d]	**6**[b]	**7**[b]
2	134.5 (s)	135.6 (s)	139.7 (s)	185.2 (s)	184.1(s)	184.2 (s)	184.1 (s)
3	55.1 (d)	190.7 (s)	50.7 (d)	50.5 (d)	55.2 (d)	51.7 (d)	57.6 (d)
5	54.0 (d)	55.6 (d)	52.9 (d)	55.7 (d)	50.9 (d)	59.9 (d)	51.9 (d)
6	17.3 (t)	26.2 (t)	23.5 (t)	38.7 (t)	38.0 (t)	38.6 (t)	38.1 (t)
7	107.3 (s)	117.5 (s)	106.3 (s)	66.1 (s)	66.2 (s)	65.2 (s)	65.9 (s)
8	127.9 (s)	128.1 (s)	127.4 (s)	138.1 (s)	137.9 (s)	136.3 (s)	137.7 (s)
9	118.6 (d)	102.7 (d)	118.5 (d)	124.4 (d)	124.8 (d)	123.9 (d)	124.9 (d)
10	119.5 (d)	151.1 (s)	119.6 (d)	125.7 (d)	126.1 (d)	125.6 (d)	126.3 (d)
11	121.5 (d)	117.0 (d)	121.6 (d)	128.9 (d)	129.1 (d)	128.8 (d)	129.3 (d)
12	111.8 (d)	113.2 (d)	112.0 (d)	121.1 (d)	121.3 (d)	121.1 (d)	121.3 (d)
13	137.1 (s)	131.7 (s)	137.7 (s)	157.9 (s)	157.9 (s)	157.5 (s)	157.4 (s)
14	33.8 (t)	43.6 (t)	29.2 (t)	28.2 (t)	22.7 (t)	27.4 (t)	22.7 (t)
15	34.2 (d)	34.2 (d)	27.4 (d)	26.7 (d)	27.6 (d)	27.5 (d)	27.3 (d)
16	53.1 (d)	32.4 (d)	42.4 (d)	42.7 (d)	50.1 (d)	43.6 (d)	49.7 (d)
17	97.7 (d)	73.2 (t)	102.8 (d)	78.8 (d)	78.4 (d)	78.2 (d)	78.5 (d)
18	12.8 (q)	11.1 (q)	13.1 (q)	22.5 (q)	17.4 (q)	21.2 (q)	19.5 (q)
19	115.2 (d)	113.7 (d)	113.4 (d)	67.8 (d)	76.2 (d)	51.5 (d)	53.9 (d)
20	138.3 (s)	139.4 (s)	141.3 (s)	55.2 (d)	50.4 (d)	56.3 (d)	45.3 (d)
21	63.7 (t)	50.0 (t)	57.2 (t)	84.9 (d)	83.5 (d)	203.2 (d)	106.2 (d)
23	41.8 (q)	86.9 (t)					
*C*H$_3$CO				21.1 (q)	21.0 (q)	21.1 (q)	21.2 (q)
CH$_3$*C*O				169.9 (s)	170.2 (s)	170.1 (s)	170.6 (s)
OCH$_3$			48.7 (q)		56.0 (q)		52.8 (q)
OCH$_3$			54.2 (q)				55.1 (q)

[a] At 125 MHz.
[b] At 150 MHz.
[c] In DMSO.
[d] At 100 MHz.

7.21 (d, J = 8.8 Hz), 11.40 (s, NH)]. Its UV spectrum (207, 290, and 325 nm) showed the presence of a conjugated system consistent with its yellow powder. The ^{13}C NMR and DEPT spectra (Table 2) suggested one methyl, five methylene, seven methine, and seven quaternary carbons. The above-mentioned data indicated that **2** was similar to 10-methoxy-16-de(methoxycarbonyl) pagicerine [3] except for the absence of a methoxyl group in **2**. The molecular formula as $C_{20}H_{22}N_2O_3$ from the HR-EI-MS (*m/z* 338.1621 $[M]^+$) of **2** further confirmed a hydroxyl group substituent, other than a methoxyl group. Furthermore, HMBC correlations between the proton at δ_H 9.00 (OH) with the carbons at δ_C 151.1 (C-10), 117.0 (C-11), and 102.7 (C-9) could confirm this assignment. Therefore, **2** was 10-hydroxy-16-de (methoxycarbonyl) pagicerine, and named as rauverine B, subsequently.



1

Figure 2. Key HMBC correlations of **1**.

Alkaloid **3** was found to possess the molecular formula $C_{21}H_{26}N_2O_2$, as evidenced by HR-ESI-MS at *m/z* 338.1987 $[M]^+$. The UV absorption maxima at 202, 225, and 281 nm and the IR spectrum (3426 and 1629 cm^{-1}) also indicated an indole ring [10]. The ^{1}H and DEPT NMR spectra

showed signals of a non-substituted MIA [δ_H 7.45 (d, $J = 7.7$ Hz), 6.98 (*t*, $J = 7.7$ Hz), 7.05 (*t*, $J = 7.7$ Hz), 7.33 (d, $J = 7.7$ Hz), 9.96 (s, NH)]. The ^{13}C NMR and DEPT spectra of **3** (Table 2) indicated 21 carbons, 3 methyls including 2 methoxyls, 3 methylene, 10 methine, and 5 quaternary carbons. The above-mentioned data showed **3** is similar to 16-epi-vellosimine and vellosimine [22] with the exception that an aldehyde at C-16 in 16-epi-vellosimine and vellosimine was substituted by a methylal in **3**. This presumption was supported by HMBC correlations of two methoxyl group signals at δ_H 3.06 and 3.08 with C-17 at δ_C 102.8. Its ROESY correlations of H-16/H-5 suggested H-16 at α-orientation. H-21/H-19 indicated that the configuration of C-19/20 was E (Figure 3). This stereo-chemistry was same to that of 16-epi-vellosimine other than the latter(vellosimine).

The ^{13}C NMR and DEPT spectra of **4** indicated an indole fraction with an imine moiety [δ_C 185.2 (s, C-2), 157.9 (s, C-13), 138.1 (s, C-8), 128.9 (d, C-11), 124.4 (d, C-9), 125.7 (d, C-10), 121.1 (d, C-12), 66.1 (s, C-7)]. Other signals at δ_C 38.7 (t, C-6), 28.2 (t, C-14), 22.5 (q, C-18), an acetyl group [δ_C 21.1 (q) and 169.9 (s)], and especially eight upfielded methines at δ_C 84.9, 78.8, 67.8, 55.7, 55.2, 50.5, 42.7, and 26.7 confirmed that **4** belongs to ajmaline skeleton [23]. Molecular formula, $C_{21}H_{24}N_2O_4$, of **4** from the HR-ESI-MS at *m/z* 368.1737 $[M]^+$ indicated two hydroxyls in **4**. Its HMBC correlations from H-5 at δ_H 3.42 to C-7 at δ_C 66.1, C-3 at δ_C 50.5 and C-21 at δ_C 84.9, and from H-19 at δ_H 3.72 to C-15 at δ_C 26.7, C-18 at δ_C 22.5 and C-21 placed hydroxyl groups at C-19 and C-21. ROESY correlations of H-21/H-5 (Figure 3) placed H-21 at β-orientation (Figures 1 and 3), and the singlet of H-21 indicated dihedral angle between H-21 and H-20 near to 90°, placing H-20 at α-orientation (Figure 1). Thus, **4** was named as rauverine D.

Alkaloid **5** showed similar ^{1}H, ^{13}C NMR, and DEPT spectral patterns to **4** except for the presence of additional methoxyl group. This methoxyl group was placed at C-19 by the HMBC correlations of H-19 at δ_H 3.43 with C-18 at δ_C 17.4, OMe at δ_C 56.0, C-15 at δ_C 27.6, and C-21 at δ_C 83.5. The ROESY correlation between H-21 and H-3, and the doublet ($J = 7.2$ Hz) of H-21 placed both H-20 and H-21 at α-orientation (Figures 1 and 3), and **5** was named as rauverine E.

^{13}C NMR and DEPT spectral patterns of alkaloids **6** and **7**, especially five upfielded methines located from δ_C 60 to δ_C 40, suggested that both alkaloids belonged to perakine skeleton alkaloids

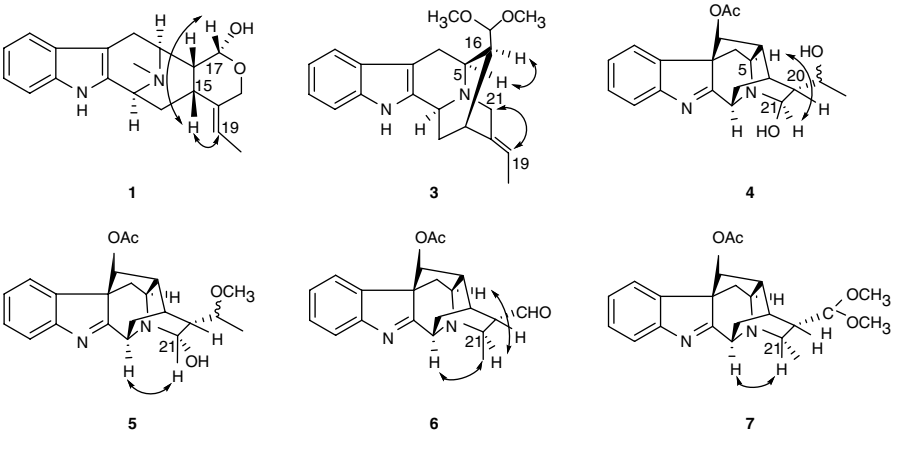


Figure 3. Key ROESY correlations of **1** and **3**–**7**.

[5,24]. ^{1}H and ^{13}C NMR spectra of alkaloid **6** were almost same as those of perakine, including 1 methyl, 2 methylene, 12 methine, 4 quaternary, and an acetyl carbon. Careful analysis of the ^{13}C NMR data indicated the difference was that methyl signals δ_H 1.38 and δ_C 21.2 in **6** are in place of the signals at δ_H 1.25 and δ_C 19.0 in perakine [5]. This difference suggested that **6** was an isomer of perakine. The ^{13}C NMR and DEPT spectra of **7** suggested that it was similar to methylal derivative of **6**. This presumption was supported by HMBC correlations of two methoxyls at δ_H 3.66 and 3.40 with C-21 at δ_C 106.2. The ROESY correlations of H-18/H-3, H-19/H-5 of **6** showed β-orientation of H-19 (Figures 1 and 3). Similar to **4**, the quartet (J = 6.8 Hz) of H-19 suggested dihedral angle of H-19/20 near to 90° and thus H-20 was α-oriented. However, ROESY correlations of H-19/H-3 of **7** suggested that the orientation of H-19 was contrary to that of **6**. Thus, **6** and **7** were named as rauverines F and G, respectively.

The remaining alkaloids were determined by comparison of their NMR spectroscopic data with those reported in the literature. Of the new alkaloids, **3** and **7** might be artificial products. Alkaloids **1**–**26** were evaluated for their cytotoxicity against five human cancer cell lines. None of them showed cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cells. Alkaloid **2** did not show cytotoxicity, suggesting that the active dimers might not be simplified as its monomer.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Jasco P-1020 digital polarimeter (Jasco International Co. Ltd., Tokyo, Japan). UV spectra were recorded on a Shimadzu UV-2401A spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR spectroscopy was performed on a Tenor 27 spectrophotometer using KBr pellets (Bruker Optics GmbH, Ettlingen, Germany). NMR spectra were recorded on Bruker Avance III-600, DRX-500, and AM-400 spectrometers with tetramethylsilane was used as an internal standard (Bruker BioSpin GmbH, Rheinstetten, Germany). HR-ESI-MS was performed on a Bruker HTC/Esquire spectrometer, and HR-EI-MS spectra were recorded on a Waters AutoSpec Premier P776 instrument (Waters Corp., Milford, MA, USA). CC was performed on silica gel (200–300 mesh) and C_{18}-silica gel (20–45 μm). Fractions were monitored by thin layer chromatography (TLC) on silica gel plates (GF_{254}), and spots were visualized with Dragendorff's reagent spray. Medium-pressure liquid chromatography (MPLC) was employed using a *Buchi* pump system coupled with a C_{18}-silica gel-packed glass column (15 × 230 and 26 × 460 mm, respectively). High-performance liquid chromatography (HPLC) was performed using a Waters 1525EF pump coupled with a Sunfire analytical, semi-preparative, or preparative C_{18} column (150 × 4.6, 150 × 10 mm, and 250 × 19 mm, respectively). The HPLC system employed a Waters 2998 photodiode array detector and a Waters fraction collector III.

3.2 Plant material

Leaves and twigs of *R. verticillata* were collected in May 2009 in Longzhou, Guangxi Province, China, and identified by Dr De-Shan Deng. A voucher specimen (No. Cai090502) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

After being dried and powdered, 9.0 kg of *R. verticillata* leaves and twigs was extracted three times with methanol

(MeOH) at room temperature and the solvent was removed *in vacuo*. The residue was dissolved in 0.3% aqueous hydrochloric acid (v/v) and partitioned with ethyl acetate (EtOAc). The aqueous layer was basified with aqueous ammonia to pH 9–10, and partitioned with EtOAc. The EtOAc layer (110 g) was subjected to CC over silica gel (1.0 kg) and eluted with a gradient chloroform–acetone system (from 1:0 to 1:1, v/v) to produce six fractions (I–VI). Fraction I (2 g) was further purified on a preparative C_{18} MPLC column with a gradient flow of 70% and 80% aqueous MeOH to yield **23** (15 mg). Fraction II (16.5 g) was further chromatographed on C18-silica gel with a gradient flow of 50%, 65%, and 80% aqueous MeOH to yield subfrs II-1–3. II-1 (2.3 g) was further purified by C_{18} MPLC column with a gradient flow of 55%, 60%, and 65% to yield **4** (71.7 mg) and **7** (9.8 mg). II-2 (3.1 g) was purified by C_{18} MPLC column with a gradient flow of 60% and 65% to yield **11** (515.8 mg). II-3 (2.2 g) was further purified by silica gel with a chloroform–acetone mixture (15:1 to 8:1) to yield **9** (9.6 mg) and **12** (11.5 mg). Fraction III (2.0 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent (4:6 to 7.5:1.5, v/v) to yield subfr. III-1. Alkaloids **26** (51.3 mg), **15** (1.2 mg), and **16** (8.9 mg) were obtained from subfr. III-1 on a preparative C_{18} HPLC column with a gradient flow of 65% to 73% aqueous MeOH, respectively. Fraction IV (6 g) was purified by C_{18} MPLC column with a MeOH–H_2O gradient (3:7 to 3:2, v/v) to yield subfrs IV-1–5. Compound **25** (14.7 mg) was crystallized from subfr. IV-1. IV-2 (2.2 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent (4:6 to 7.5:1.5, v/v) to yield **18** (59.7 mg). Subfr. IV-3 (90 mg) was further separated on the same column with a gradient flow of 55% to 65% aqueous MeOH to yield **10** (4.2 mg). Subfr. IV-4 (1.3 g) was further separated on silica gel column with a gradient flow of chloroform–acetone mixture (15:1 to 8:1) to yield **1** (3.3 mg) and **24** (9.9 mg). Fr. V (13.5 g) was purified on a C_{18} MPLC column with a MeOH–H_2O gradient eluent (1:5 to 3:2, v/v) to yield subfrs V-1–3. Subfr. V-1 (230 mg) was further separated on a Sephadex LH-20 column with a gradient flow of 60% aqueous MeOH to afford **3** (4.4 mg). Alkaloid **8** (6.9 g) was crystallized from subfr. V-2. Subfr. V-3 (275 mg) was further separated on a preparative C_{18} column with a gradient flow of 30% to 50% aqueous MeOH to afford **5** (3.5 mg) and **6** (28.9 mg). Fr. VI (8.8 g) was purified on C_{18} column with a MeOH–H_2O gradient eluent (3:10 to 3:2, v/v) to yield subfrs VI-1–3. Subfr. VI-1 (2.1 g) was further separated on a preparative C_{18} column with a gradient flow of 30% to 55% aqueous MeOH to afford **2** (202.2 mg) and **13** (3.4 mg). Subfr. VI-2 (3.3 g) was further separated on a preparative C_{18} column with a gradient flow of 40% to 60% aqueous MeOH to produce **14** (1.66 g), **17** (14.4 mg), and **20** (4.5 mg). Subfr. VI-3 (1.2 g) was further separated on a preparative C_{18} HPLC column with a gradient flow of 40% to 60% aqueous MeOH to afford **19** (4.4 mg), **21** (22.4 mg), and **22** (7.5 mg).

3.3.1 *Rauverine A (**1**)*

A white powder; $[\alpha]_D^{20}$ +16 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.22), 225 (3.40), 280 (2.70) nm; IR (KBr) ν_{max} 3418, 3088, 3059, 2924, 2853, 1631, and 1452 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; positive ESI-MS *m/z*: 325 $[M + H]^+$; HR-EI-MS *m/z:* 324.1845 $[M]^+$ (calcd for $C_{20}H_{24}N_2O_2$, 324.1838).

3.3.2 *Rauverine B (**2**)*

A yellow powder; $[\alpha]_D^{20}$ −45 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.19), 290 (3.09), 325 (2.88) nm; IR (KBr) ν_{max}: 3424, 2920, 2852, 1623, 1524, and 1466 cm^{-1}; for ^1H and ^{13}C NMR

spectral data, see Tables 1 and 2; positive ESI-MS *m/z*: 339 $[M + H]^+$; HR-EI-MS *m/z*: 338.1621 $[M]^+$ (calcd for $C_{20}H_{22}N_2O_3$, 338.1630).

3.3.3 *Rauverine C (**3**)*

A white powder; $[\alpha]_D^{20}$ 0 (*c* 0.44, MeOH); UV (MeOH) λ_{max} (log ε) 202 (2.54), 252 (2.65), 281 (1.96) nm; IR (KBr) ν_{max} 3426, 2928, 2855, 1629, 1452 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; positive ESI-MS *m/z*: 339 $[M + H]^+$; HR-EI-MS *m/z*: 338.1987 $[M]^+$ (calcd for $C_{21}H_{26}N_2O_2$, 338.1994).

3.3.4 *Rauverine D (**4**)*

A white powder; $[\alpha]_D^{20}$ +29 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.48), 258 (2.83) nm; IR (KBr) ν_{max} 3496, 3421, 3346, 2950, 2960, 1723, 1589, and 1453 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; positive ESI-MS *m/z*: 369 $[M + H]^+$; HR-EI-MS *m/z*: 368.1737 $[M]^+$ (calcd for $C_{21}H_{24}N_2O_4$, 368.1736).

3.3.5 *Rauverine E (**5**)*

A white powder; $[\alpha]_D^{20}$ +25 (*c* 0.46, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.63), 260 (3.02) nm; IR (KBr) ν_{max} 3424, 2970, 2935, 2903, 1744, 1620, 1594, 1468, and 1453 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; positive ESI-MS *m/z*: 383 $[M]^+$; HR-EI-MS *m/z*: 382.1895 $[M]^+$ (calcd for $C_{22}H_{26}N_2O_4$, 382.1893).

3.3.6 *Rauverine F (**6**)*

A white powder; $[\alpha]_D^{20}$ −8 (*c* 0.40, MeOH). UV (MeOH) λ_{max} (log ε) 219 (3.59), 261 (2.98) nm; IR (KBr) ν_{max} 3430, 2965, 2940, 1743, 1721, 1631, 1620, 1592, 1469, and 1453 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; positive ESI-MS *m/z*: 351 $[M + H]^+$; HR-EI-MS: *m/z* 350.1624 $[M]^+$ (calcd for $C_{21}H_{22}N_2O_3$, 350.1630).

3.3.7 *Rauverine G (**7**)*

A white powder; $[\alpha]_D^{20}$ +16 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.56), 260 (2.94) nm; IR (KBr) ν_{max} 2963, 2934, 2881, 1740, 1590, 1464, and 1449 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; positive ESI-MS *m/z*: 397 $[M + H]^+$; HR-EI-MS *m/z*: 396.2037 $[M]^+$ (calcd for $C_{23}H_{28}N_2O_4$, 396.2049).

3.4 *Cytotoxicity assay*

Five human cancer cell lines, breast cancer (MCF-7), hepatocellular carcinoma (SMMC-7721), human myeloid leukemia (HL-60), colon cancer (SW480), and lung cancer (A-549), were used for cytotoxic assays. Cells were cultured in RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA) or in a DMEM (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37°C. Cytotoxicity assays were performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 µl of adherent cells was seeded onto each well of 96-well cell culture plates and allowed to adhere for 12 h before the addition of test compounds. Suspended cells were seeded with an initial density of 1×10^5 cells/ml just before drug addition. Each tumor cell line was exposed to a test compound at concentrations of 0.039, 0.201, 1.005, 5.024, and 25.120 µg/ml in triplicate for 48 h, with cisplatin (Sigma-Aldrich) as the positive control. After the treatment, cell viability was assessed, cell growth was graphed, and IC_{50} values were calculated by Reed and Muench's method.

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