Rauvotetraphyllines A–E, new indole alkaloids from *Rauvolfia tetraphylla*

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Abstract: Five new indole alkaloids rauvotetraphyllines A–E (**1–5**), together with eight known analogues, were isolated from the aerial parts of *Rauvolfia tetraphylla*. The structures were established by means of spectroscopic methods.

Keywords: Rauvolfia tetraphylla, indole alkaloid, rauvotetraphylline

Introduction

Rauvolfia genus, family Apocynaceae, continues to be fascinating as it produces novel heterocyclic alkaloids with monoterpene indole skeletons, which have attracted great interests from biological and therapeutic aspects, due to their anticancer,[1] antimalarial,[2] antihypertensive,[3] and sedative[4] properties. The isolation and structure elucidation of three indole alkaloids rauvoyunines A, B, and C from the aerial parts of *Rauvolfia yunnanensis* have been reported earlier.[5] As one part of our ongoing research program exploring bioactive indole alkaloids from Chinese species of *Rauvolfia*, phytochemical analysis has been carried out on the aerial parts of *R. tetraphylla* collected from Yunnan Province, with particular attention to the monoterpene indole constituents, and has resulted in the isolation of five new alkaloids, rauvotetraphyllines A–E (**1–5**), as well as eight known analogues, alstonine (**6**),[6] nortetraphyllicine,[7] peraksine,[8] sarpagine,[9] 3-hydroxysarpagine,[10] dihydroperaksine,[11] 10-hydroxydihydroperaksine,[11] and raucaffricine.[12] The present paper reports the isolation, structure elucidation, and cytotoxic evaluation of the new compounds.

Results and Discussion

Compound **1**, obtained as amorphous powder, had a molecular formula of $C_{20}H_{26}N_2O_3$ based on HRESIMS (pos.) at *m/z* 343.2024 (calcd for $C_{20}H_{27}N_2O_3$, 343.2021). In the UV spectrum, two noticeable maxima absorption bands at 211 and 275 nm as well as two shoulders at 222 and 310 nm were detected, suggesting the existence of an *O*-substituted indole chromophore.[13] The IR spectrum showed band at 3407 cm^{-1},

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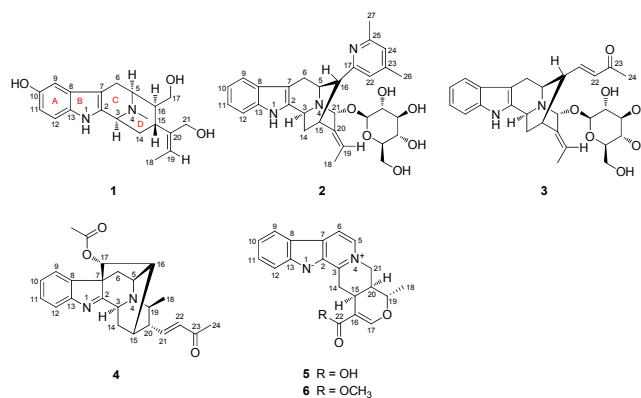
due to OH/NH functionalities. The ^1H NMR spectrum (Table 1) showed signals for an aromatic AMX spin system at δ_H 6.62 (dd, *J* = 8.5, 2.0 Hz), 6.82 (d, *J* = 2.0 Hz), and 7.11 (d, *J* = 8.5 Hz), typical of an indole moiety substituted by a hydroxy group at C-10 or C-11 position, signals characteristic of an ethylidene group at δ_H 1.16 (d, *J* = 6.3 Hz) and 5.51 (q, *J* = 6.3 Hz), protons for *N*-methyl group at δ_H 2.47 (s), and protons of two oxygenated methylene groups, one at δ_H 3.57 (dd, *J* = 11.1, 4.0 Hz) and 3.32 (dd, *J* = 11.1, 10.6 Hz), and another at δ_H 4.04 and 4.08 (each d, *J* = 13.4 Hz). In addition to resonances due to indole chromophore including an oxygen-bearing carbon at δ_C 151.2 (s), the ^{13}C NMR (DEPT) spectrum (Table 2) exhibited signals ascribed to one double bond at δ_C 123.6 (d) and 140.6 (s), two oxygenated carbons at δ_C 62.9 (t) and δ_C 63.2 (t), two methyl signals at δ_C 12.7 (q) and 41.7 (q), and the other six aliphatic carbons. These spectroscopic features resembled rauvoyunine A,[5] a macroline-type alkaloid recently isolated from *R. yunnanensis*, except for the absence of an *N*-methyl group in ring B, as well as discrepant coupling

Table 1. ^{1}H NMR data for compounds 1−5 (δ in ppm, J in Hz)

No.	1[a]	2[a]	3[a]	4[b]	5[c]
3	3.92 (br. s)	4.80 (br. d, 10.2)	4.76 (br. d, 10.2)	4.18 (d, 9.3)	
5	3.47 (dd, 6.7, 5.0)	3.67 (dd, 8.0, 5.1)	2.98 (dd, 7.1, 5.1)	3.66 (dd, 5.1, 4.9)	8.44 (d, 6.3)
6	2.92 (dd, 17.0, 6.7, H$_\alpha$)	3.07 (dd, 15.3, 5.1, H$_\alpha$)	3.02 (dd, 15.1, 5.1, H$_\alpha$)	1.63 (d, 12.0, H$_\alpha$)	8.51 (d, 6.3)
	2.69 (d, 17.0, H$_\beta$)	2.59 (d, 15.3, H$_\beta$)	2.63 (d, 15.1, H$_\beta$)	2.81 (dd, 12.0, 4.9, H$_\beta$)	
9	6.82 (d, 2.0)	7.35 (dd, 7.8, 0.8)	7.39 (dd, 7.8, 0.8)	7.47 (dd, 7.3, 0.7)	8.37 (d, 8.0)
10		6.95 (ddd, 7.8, 7.2, 0.6)	6.97 (ddd, 7.8, 7.1, 0.8)	7.22 (ddd, 7.6, 7.3, 0.7)	7.31 (dd, 8.0, 7.1)
11	6.62 (dd, 8.5, 2.0)	7.04 (ddd, 8.0, 7.2, 0.9)	7.05 (ddd, 8.0, 7.1, 1.0)	7.39 (ddd, 7.7, 7.6, 1.1)	7.63 (dd, 8.4, 7.1)
12	7.11 (d, 8.5)	7.29 (dd, 8.0, 0.9)	7.28 (dd, 8.0, 1.0)	7.61 (dd, 7.7, 1.1)	7.84 (d, 8.4)
14	2.19 (ddd, 12.9,12.0, 2.7, H$_\alpha$)	2.17 (m, H$_\alpha$)	2.13 (m, H$_\alpha$)	1.97 (dd, 15.3, 9.3, H$_\alpha$)	4.56 (dd, 17.3, 6.8)
	1.53 (br. d, 12.9, H$_\beta$)	1.89 (m, H$_\beta$)	1.71 (m, H$_\beta$)	1.58 (dd, 15.3, 3.8, H$_\beta$)	3.37 (m)
15	2.42 (ddd, 12.0, 11.8, 3.2)	2.84 (br. s)	2.76 (br. s)	2.40 (m, overlap)	3.12 (m)
16	2.32 (dddd, 11.8, 10.6, 5.0, 4.0)	3.11 (br. d, 8.0)	2.46 (dd, 9.1, 7.1)	2.40 (m, overlap)	
17	3.57 (dd, 11.1, 4.0)		6.73 (dd, 15.8, 9.1)	4.92 (s)	7.28 (s)
	3.32 (dd, 11.1, 10.6)				
18	1.16 (d, 6.3)	1.15 (d, 6.8)	1.70 (d, 6.9)	1.23 (d, 6.7)	1.33 (d, 6.0)
19	5.51 (q, 6.3)	5.88 (q, 6.8)	5.99 (q, 6.9)	2.77 (m)	3.64 (m)
20				2.06 (m)	2.38 (m)
21	4.04 (d, 13.4)	5.23 (s)	5.07 (s)	6.84 (dd, 15.9, 7.8)	4.61 (dd, 14.0, 4.7, H$_\alpha$)
	4.08 (d, 13.4)				4.96 (dd, 14.0, 5.7, H$_\beta$)
22		7.02 (s)	6.06 (d, 15.8)	6.18 (d, 15.9)	
24		6.96 (s)	2.22 (s)	2.28 (s)	
26		2.30 (s)			
27		2.39 (s)			
1′		4.70 (d, 7.8)	4.65 (d, 7.8)		
2′		3.32 (m)	3.30 (m)		
3′		3.39 (m)	3.38 (m)		
4′		3.31 (m, overlap)	3.29 (m, overlap)		
5′		3.31 (m, overlap)	3.29 (m, overlap)		
6′		3.63 (dd, 11.9, 5.0)	3.61 (dd, 11.8, 5.1)		
		3.80 (br. d, 11.9)	3.78 (br. d, 11.8)		
OAc				2.16 (s)	
NMe	2.47 (s)				

[a]Measured in CD$_3$OD (3.30 ppm); [b]Measured in CDCl$_3$ (7.26 ppm); [c]Measured in DMSO-d_6 (2.50 ppm).

constants in ring D. The position of the *N*-methyl group was confirmed by HMBC correlations from N(4)-Me to C-3 and C-5, while the location of the hydroxy group was confirmed by HMBC correlation from an *m*-coupling doublet of H-9 at δ_H 6.82 (d, J = 2.0 Hz) to C-7 and ROESY correlation between H-9 and H-6β (Figure 1). Since the $J_{5,6}$, $J_{3,14}$, and $J_{14,15}$ values corresponding to *vicinal* interaction ($^3J_{H,H}$) were essentially unchanged compared to those of rauvoyunine A, it is safe to deduce that the C/D ring junction stereochemistry remained intact, whereas revealing as the major difference from rauvoyunine A, the J values between H-15 and H-16 (**1**: J = 11.8 Hz; rauvoyunine A: J = 5.6 Hz), H-5 and H-16 (**1**: J = 5.0 Hz; rauvoyunine A: $J \approx 0$ Hz), and H-16 and H$_2$-17 (**1**: J = 10.6 and 4.0 Hz; rauvoyunine A: J = 5.5 and 3.7 Hz) strongly suggested that this new compound had a macroline-type skeleton with the unique feature of a 16-epi (16αH) form. Consistent with this deduction, the ROESY spectrum of **1**

HMBC: ⌒   ROESY: ⌒
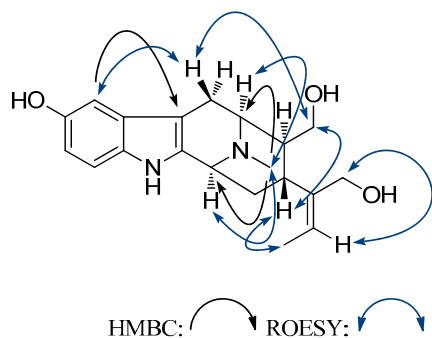
Figure 1. Key HMBC and ROESY correlations of **1**

showed cross-peaks of H-15↔H$_2$-17 and H$_2$-17↔H-6β. The C-19–C-20 double bond had the *E*-configuration since the ROESY correlations of Me-18↔H-15 and H-19↔H$_2$-21 were observed. Thus, the structure of the new alkaloid was established as shown, named rauvotetraphylline A.

Compound **2**, obtained as amorphous powder, possessed a molecular formula of $C_{31}H_{37}N_3O_6$, as evidenced by HRESIMS (pos.) at *m/z* 548.2768, in combination with NMR spectra (Tables 1 and 2), requiring 15 degrees of unsaturation. The NMR spectra exhibited a β-glucopyranosyl unit (δ_C 102.8, 75.3, 78.1, 71.5, 78.0, 62.8), and a set of resonances [δ_C 121.0 (d), 150.1 (s), 123.6 (d), 158.4 (s), 21.0 (q), 23.6 (q); δ_H 7.02 (s), 6.96 (s), 2.30 (s), 2.39 (s)] which can be easily determined as a 4,6-dimethyl-2-pyridyl moiety.[14] The remainder were closely related to those of vellosimine[15]—a sarpagine-type alkaloid, except for the absence of an aldehyde group, as well as an unusual low-field methine signal at δ_C 91.8 instead of a methylene signal for C-21. The significant downfield shift for C-21 suggested that the sugar unit was adjacent to C-21, as supported by HMBC correlation from the anomeric proton of β-glucose at δ_H 4.70 (d, J = 7.8 Hz) to C-21. Considering that it lacks an aldehyde group at C-16, which is usual for the sarpagine series such as 12-methoxy-vellosimine[16] or dihydroperaksine-17-al[11] from species of *Rauvolfia*, the 4,6-dimethyl-2-pyridyl moiety was assumed to be an artifact (produced by aldehyde-ammonia condensation reaction) linked to C-16 since acetone and NH$_3$·H$_2$O were used as eluent during the isolation process. The above deduction was confirmed by HMBC correlations from H-5 and H-15 to C-17, and H-16 to C-17 and C-22. ROESY correlations of H-5↔H-6α (strong), H-5↔H6β (slight), H-3↔H-5 (slight), H-6β↔H-16, and H-16↔H-14β suggested that the new alkaloid had the same ring junction stereochemistry as that of vellosimine,

Table 2. ^{13}C NMR data for compounds 1−5 (δ in ppm)

No.	1[a]	2[a]	3[a]	4[b]	5[c]
2	134.1 (s)	139.3 (s)	139.3 (s)	183.0 (s)	135.1 (s)
3	56.0 (d)	44.9 (d)	44.7 (d)	57.2 (d)	142.2 (s)
5	55.4 (d)	54.2 (d)	54.9 (d)	50.9 (d)	131.1 (d)
6	16.6 (t)	27.0 (t)	26.3 (t)	37.5 (t)	115.1 (d)
7	106.6 (s)	104.6 (s)	104.4 (s)	64.9 (s)	130.1 (s)
8	128.5 (s)	128.9 (s)	128.8 (s)	136.2 (s)	119.8 (s)
9	103.1 (d)	118.5 (d)	118.6 (d)	123.8 (d)	122.9 (d)
10	151.2 (s)	119.8 (d)	119.9 (d)	125.5 (d)	120.4 (d)
11	111.5 (d)	122.0 (d)	122.1 (d)	128.7 (d)	130.3 (d)
12	112.3 (d)	112.0 (d)	112.0 (d)	121.0 (d)	114.3 (d)
13	132.6 (s)	138.2 (s)	138.2 (s)	156.4 (s)	145.7 (s)
14	35.9 (t)	33.8 (t)	32.7 (t)	22.0 (t)	29.5 (t)
15	31.3 (d)	34.7 (d)	32.5 (d)	30.0 (d)	27.5 (d)
16	45.7 (d)	50.3 (d)	46.8 (d)	48.6 (d)	114.7 (s)
17	62.9 (t)	162.8 (s)	151.7 (d)	78.0 (d)	149.8 (d)
18	12.7 (q)	12.9 (q)	13.4 (q)	17.3 (q)	18.4 (q)
19	123.6 (d)	126.0 (d)	126.1 (d)	55.0 (d)	69.3 (d)
20	140.6 (s)	135.6 (s)	136.0 (s)	46.7 (d)	35.7 (d)
21	63.2 (t)	91.8 (d)	91.7 (d)	146.0 (d)	54.1 (t)
22		121.0 (d)	131.6 (d)	131.7 (d)	170.3 (s)
23		150.1 (s)	201.1 (s)	198.0 (s)	
24		123.6 (d)	27.0 (q)	27.4 (q)	
25		158.4 (s)			
26		21.0 (q)			
27		23.6 (q)			
1′		102.8 (d)	102.7 (d)		
2′		75.3 (d)	75.4 (d)		
3′		78.1 (d)	78.1 (d)		
4′		71.5 (d)	71.5 (d)		
5′		78.0 (d)	78.0 (d)		
6′		62.8 (t)	62.8 (t)		
*CH₃*COO				21.1 (q)	
CH₃*COO*				170.0 (s)	
N*CH₃*	41.7 (q)				

[a]Measured in CD$_3$OD (49.0 ppm); [b]Measured in CDCl$_3$ (77.0 ppm); [c]Measured in DMSO-d_6 (39.5 ppm).

while the proton at C-22 showing cross-peaks with H-5, H-19, and H-21 supported *exo* position of the pyridyl moiety and β-orientation of H-21. The Me-18 at δ_H 1.15 (noticeable upfield shift being due to shielding by the pyridyl ring current) showed ROESY correlation with H-15 while H-19 exhibited correlation with H-21, revealing an *E*-geometry of the ethylidene. Therefore, the structure of **2** was unambiguously elucidated as shown, named rauvotetraphylline B.

Compound **3** was obtained as amorphous powder. High resolution mass spectrometry revealed the [M + H]$^+$ peak at *m/z* 511.2431, suggesting the molecular formula C$_{28}$H$_{34}$N$_2$O$_7$. The NMR signals (Tables 1 and 2) were very similar to those of rauvotetraphylline B (**2**), which revealed that **3** was also a sarpagine alkaloid. However, there was a prominent difference as follows: the signals assigned to 4,6-dimethyl-2-pyridyl moiety in **2** were absent, and there was a set of newly risen resonances: δ_C 151.7 (d), 131.6 (d), 201.1 (s), 27.0 (q); δ_H 6.73 (dd, *J* = 15.8, 9.1 Hz), 6.06 (d, *J* = 15.8 Hz), and 2.22 (s), which was easily assigned as an *E*-3-oxo-1-butenyl unit. In the HMBC spectrum, significant correlations from olefinic proton at δ_H 6.73 to carbons at δ_C 54.9 (d, C-5), 32.5 (d, C-15), and 46.8 (d, C-16) were observed, which indicated that the butenyl group was also attached to C-16. Other structural parts of **3** were identical to those of **2**, as indicated by the HMBC, HSQC, and ROESY spectra. Consequently, the structure of **3** was determined as shown, named rauvotetraphylline C.

Compound **4** was isolated as amorphous powder. Its molecular formula was determined as C$_{24}$H$_{26}$N$_2$O$_3$ by the positive HRESIMS ([M + H]$^+$ at *m/z* 391.2019). The NMR data (Tables 1 and 2) were analogous to those of perakine[17]—a ajmaline-type alkaloid. The principal difference between them

was the aldehyde group in perakine changing into an *E*-3-oxo-1-butenyl unit [δ_C 146.0 (d), 131.7 (d), 198.0 (s), 27.4 (q); δ_H 6.84 (dd, *J* = 15.9, 7.8 Hz), 6.18 (d, *J* = 15.9 Hz), 2.28 (s)] on the basis of HMBC correlations from H-21 at δ_H 6.84 to C-15, C-19, and C-20. The ROESY correlations of H-3↔H-19↔H-14α, H-14β↔H-17, and Me-18↔H-20 indicated that **4** possessed the same stereochemical characteristics as that of perakine. Hence, the structure of **4** was assigned as shown, named rauvotetraphylline D.

Compound **5** and alstonine (**6**) exhibited nearly the same UV spectrum, with strong absorption maxima at 252, 308, and 370 nm. TLC analysis displayed spots with a clear blue fluorescence at 365 nm, between which **5** exhibited higher polarity. The molecular formula of **5** was determined as C$_{20}$H$_{18}$N$_2$O$_3$ by HRESIMS, 14 mass units lower than that of **6**. The 1D NMR data of **5** (Tables 1 and 2) have similar chemical shifts and the same multiplicity as given for **6**, except for a carboxyl group (δ_C 170.3) in **5** instead of the methoxycarbonyl group in **6**, as supported by the lack of the methyl ester signals in the ^{1}H and ^{13}C NMR spectra and the presence of a hydroxy group as IR absorption band at 3423 cm^{-1} revealed. Other structural parts of **5** were identical to those of **6**, as indicated by the HMBC, HSQC, and ROESY spectra. Thus, the structure of the alstonine derivative was established as shown, named rauvotetraphylline E.

All of the new alkaloids were evaluated for their cytotoxicity *in vitro* against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480) using the MTT method as reported previously.[18] However, all tested compounds were inactive, and they showed IC$_{50}$ values > 40 μM. Considering

the structural characteristics of the new compounds, we thought that **2–4** might have been formed as artifacts during isolation process.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco P-1020 automatic digital polarimeter. IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. UV data were obtained from online HPLC analysis. NMR spectra were acquired with a Bruker DRX-500 instrument at room temperature. ESIMS (including HRESIMS) were measured on API QSTAR Pulsar i mass spectrometers. Silica gel (200−300 mesh, Qing-dao Marine Chemical Inc., China), Sephadex LH-20 (Amersham Biosciences, Sweden) and MCI gel CHP 20P (75−150 μm, Mitsubishi Chemical Corp., Tokyo, Japan) were used for column chromatography. Medium pressure liquid chromatography (MPLC) was performed on a Büchi Sepacore System equipping 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 (Qingdao Marine Chemical Inc., China) in combination with reversed-phase HPLC (Agilent 1200, Extend-C18 column, 5 μm, 4.6 × 150 mm).

Plant Material. The aerial parts of *R. tetraphylla* were collected in Xiaomenglun of Yunnan Province, China, in June 2010 and were identified by Prof. Yu Chen of Kunming Institute of Botany, Chinese Academy of Sciences. The voucher specimen was deposited at BioBioPha Co., Ltd.

Extraction and Isolation. The air-dried and powdered aerial parts of *R. tetraphylla* (7.5 kg) were extracted three times with 95% ethanol (3 × 50 L, each 3 days) at room temperature and filtered. The filtrate was evaporated under reduced pressure to get a residue (*ca.* 400 g), which was fractionized by silica gel column chromatography, eluted with a gradient solvent system of petroleum ether-acetone and then MeOH to yield six fractions A–F. Fraction F, eluted by MeOH, was separated on silica gel CC (CHCl$_3$-MeOH, 50:1 → 0:1, v/v) to give five subfractions (F1–F5), Compounds **4** (6 mg) and **7** (7 mg) were obtained from the subfraction F1 by silica gel (CHCl$_3$-MeOH-NH$_3$·H$_2$O, 50:1:0.1) and Sephadex LH-20 (MeOH) columns. Fraction F2 was separated by silica gel (CHCl$_3$-MeOH-NH$_3$·H$_2$O, 40:1:0.1 → 5:1:0.1), MCI (50% → 100% MeOH in water), and Sephadex LH-20 (MeOH) columns to afford **8** (230 mg), **9** (94 mg), and **10** (84 mg). In the same way, **1** (119 mg) and **11** (29 mg) were isolated from fraction F3. Also, **12** (83 mg) and **6** (3 mg) were obtained from fractions F4. After repeated silica gel (CHCl$_3$-MeOH-NH$_3$·H$_2$O, 10:1:0.1 → 1:1:0.05), Sephadex LH-20 (CHCl$_3$-MeOH, 1:1), and MPLC (30% → 50% MeOH in H$_2$O), fraction F5 afforded **13** (135 mg), **3** (26 mg), **2** (21 mg), and **5** (272 mg). The retention times (t_R) of new compounds **1–5** from analysis-type HPLC (20% → 80% MeOH in H$_2$O over 8.0 min followed by 100% MeOH to 10 min, 1.0 ml/min, 20 °C) were 7.8, 8.8, 7.9, 8.4, and 6.7 min, respectively.

Rauvotetraphylline A (1): yellowish, amorphous powder;

$[\alpha]_D^{16}$ +16.2 (*c* 0.20, MeOH); UV (MeOH) λ_{max}: 211, 222 (sh), 275, 310 (sh) nm; IR (KBr) ν_{max} 3407, 2923, 2892, 1630, 1596, 1454, 1382, 1364, 1236, 1190, 1144, 1114, 1059, 997, 798, 743 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; ESIMS (pos.): *m/z* 343 [M + H]$^+$; HRESIMS (pos.): *m/z* 343.2024 (calcd for C$_{20}$H$_{27}$N$_2$O$_3$, 343.2021).

Rauvotetraphylline B (2): yellowish, amorphous powder; $[\alpha]_D^{16}$ +52.7 (*c* 0.21, MeOH); UV (MeOH) λ_{max}: 225, 265 (sh), 272, 282 (sh), 290 (sh) nm; IR (KBr) ν_{max} 3406, 2921, 2856, 1608, 1568, 1452, 1411, 1384, 1335, 1238, 1170, 1075, 1026, 743 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; ESIMS (pos.): *m/z* 548 [M + H]$^+$; HRESIMS (pos.): *m/z* 548.2768 (calcd for C$_{31}$H$_{38}$N$_3$O$_6$, 548.2760).

Rauvotetraphylline C (3): yellowish, amorphous powder; $[\alpha]_D^{16}$ +31.4 (*c* 0.19, MeOH); UV (MeOH) λ_{max}: 225, 270, 284 (sh), 293 (sh) nm; IR (KBr) ν_{max} 3405, 2918, 1669, 1622, 1572, 1452, 1419, 1384, 1362, 1335, 1259, 1238, 1170, 1075, 1025, 746 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; ESIMS (pos.): *m/z* 511 [M + H]$^+$; HRESIMS (pos.): *m/z* 511.2431 (calcd for C$_{28}$H$_{35}$N$_2$O$_7$, 511.2444).

Rauvotetraphylline D (4): yellowish, amorphous powder; $[\alpha]_D^{14}$ +26.5 (*c* 0.20, CHCl$_3$); UV (MeOH) λ_{max}: 221, 226 (sh), 253 (sh) nm; IR (KBr) ν_{max} 3431, 2964, 2931, 1741, 1695, 1673, 1620, 1592, 1468, 1453, 1363, 1231, 1177, 1138, 1034, 980, 952, 774, 753 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; ESIMS (pos.): *m/z* 391 [M + H]$^+$; HRESIMS (pos.): *m/z* 391.2019 (calcd for C$_{24}$H$_{27}$N$_2$O$_3$, 391.2021).

Rauvotetraphylline E (5): yellowish, amorphous powder; $[\alpha]_D^{15}$ +136.8 (*c* 0.20, MeOH); UV (MeOH) λ_{max}: 218 (sh), 234 (sh), 252, 260 (sh), 308, 370 nm; IR (KBr) ν_{max} 3423, 3066, 2976, 2907, 1639, 1550, 1529, 1504, 1448, 1384, 1364, 1332, 1250, 1180, 1129, 790, 754 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; ESIMS (pos.): *m/z* 335 [M + H]$^+$; HRESIMS (pos.): *m/z* 335.1388 (calcd for C$_{20}$H$_{19}$N$_2$O$_3$, 335.1395).

Cytotoxicity Assay. The cytotoxicity assay was performed according to the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method,[19] by use of the following 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 SW-480. The IC50 values were calculated by Reed and Muench's method.[20]

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s13659-012-0012-5 and is accessible for authorized users.

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