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Koumine, Humantenine, and Yohimbane Alkaloids from *Gelsemium elegans*

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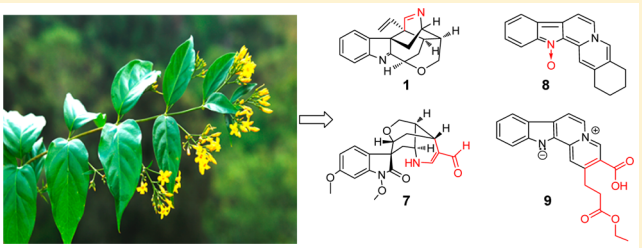
Supporting Information

ABSTRACT: Nine new alkaloids of the koumine (**1**−**4**), humantenine (**5**−**7**), and yohimbane (**8**, **9**) types as well as 12 known analogues were isolated from the leaves and vine stems of *Gelsemium elegans*. Compound **1** is the first *N*-4-demethyl alkaloid of the koumine type, compound **7** is the first *nor*-humantenine alkaloid, and compounds **8** and **9** are the first *N*-1-oxide and the first *seco*-E-ring alkaloids, respectively, of the yohimbane type. Compounds **1** and **7** exhibited moderate cytotoxicity against five human tumor cell lines with IC_{50} values in the range 4.6−9.3 μM.



The genus *Gelsemium* (Loganiaceae), which comprises three species in Asia (*G. elegans*) and North America (*G. sempervirens* and *G. rankinii*), has been recognized as a toxic plant and used as a folk medicine to treat migraines, neuralgia, sciatica, cancer, and various types of sores.[1] A large number of indole, bisindole, and monoterpenoid alkaloids have been isolated from the *Gelsemium* genus.[1−4] Pharmacological experiments showed that these *Gelsemium* alkaloids possessed cytotoxic, analgesic, anxiolytic, anti-inflammatory, and immunomodulating activities.[1,5] During an earlier investigation, five monoterpenoid indole alkaloids and a new monoterpenoid alkaloid that selectively inhibited the growth of A-549 tumor cell lines were isolated from *G. elegans*.[5,6] In the current investigation, nine new alkaloids, including four of the koumine type (**1**−**4**), three of the humantenine type (**5**−**7**), and two of the yohimbane type (**8**, **9**), as well as 12 known alkaloids were isolated from the leaves and perennial vine stems of *G. elegans*. Among the known compounds, 4*R*-koumine *N*-oxide, 11-methoxy-19-(*R*)-hydroxygelselegine, and *epi*-koumidine were isolated as natural products for the first time, and venoterpine was isolated from the Loganiaceae family for the first time. Evaluations of cytotoxicity and nitric oxide production inhibition revealed that compounds **1** and **7** exhibited moderate cytotoxic activity against five human tumor cell lines with IC_{50} values in the range 4.6−9.3 μM. This report describes the extraction, isolation, structure elucidation, cytotoxicity, and nitric oxide production inhibitory assays of these compounds.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white, amorphous powder and was assigned the molecular formula $C_{19}H_{18}N_2O$ on the basis of its ^{13}C NMR data and an HREIMS ion at m/z 290.1416 $[M]^+$ (calcd 290.1419), showing 16 mass units less than that of koumine (**10**),[7] a major alkaloid also isolated during the investigation. The UV (λ_{max} 221.0 and 261.6 nm) and NMR (Tables 1 and 2) data indicated the presence of an indolenine chromophore. The ^1H and ^{13}C NMR data (Tables 1 and 2) were similar to those of koumine,[7] including signals assignable to four aromatic protons [δ_H 7.54, dd, J = 7.5, 1.0 Hz (H-12); 7.38, td, J = 7.5, 1.0 Hz (H-11); 7.28, td, J = 7.5, 1.0 Hz (H-10); 7.09, dd, J = 7.5, 1.0 Hz (H-9)], one vinyl group [δ_H 5.28, d, J = 17.8 Hz (H-18); 5.09, d, J = 11.2 Hz (H-18); 4.84, dd, J = 17.8, 11.2 Hz (H-19)], and an oxymethine [δ_H 4.99, br s (H-3)], two oxymethylene [δ_H 4.31, dd, J = 12.0, 4.3 Hz (H-17a); 3.80, d, J = 12.0 Hz (H-17b)], and an aminomethine [δ_H 4.49, br s, (H-5)] proton. The exceptions involved the presence of additional signals for an imino group [δ_H 8.52 (s), δ_C 175.0] and the absence of signals for an *N*-4-methyl group. These observations suggested that **1** was likely an *N*-4-demethly-21-dehydro derivative of koumine. HMBC correlations between H-21 (δ_H 8.52, s) and C-5 (δ_C 61.0) and C-19 (δ_C 136.6) further validated the deduction (Figure 1). The relative and absolute configurations of **1** were established, on the basis of ROESY

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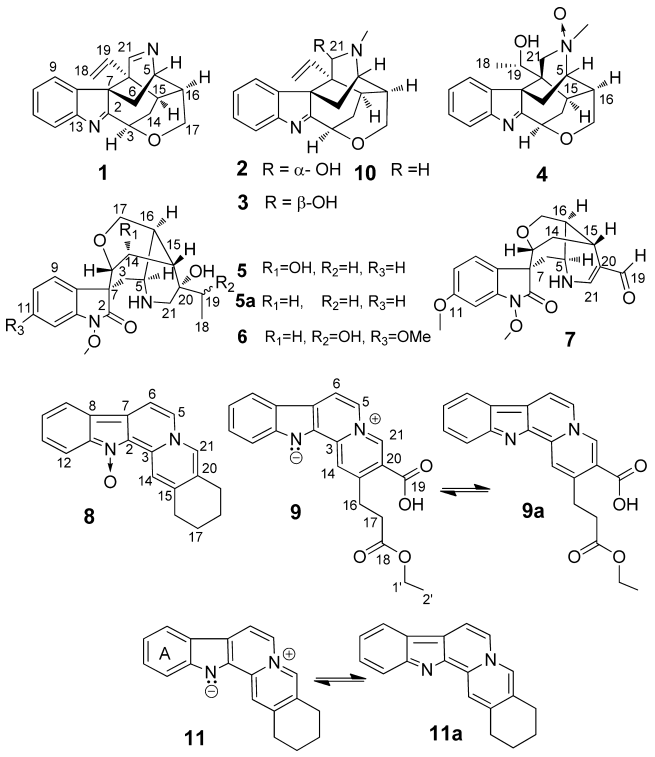

correlation analysis (Figure 1) and by comparing its electronic circular dichroism (ECD) spectrum to that of koumine (**10**), respectively (Figure 3). The structure of **1** was thus established as *N*-4-demethyl-21-dehydrokoumine. Compound **1** is the first koumine-type alkaloid lacking an *N*-4 methyl group.

Compounds **2** and **3** were obtained as an inseparable 1:2 mixture as determined by ^{1}H NMR peak integration. The compounds shared the same molecular formula of $C_{20}H_{22}N_2O_2$ as determined by their ^{13}C NMR data and HRESIMS ions at *m/z* 323.1749 ($[M + H]^+$) These structurally similar koumine alkaloids were readily identified by two sets of peaks in the ^{1}H NMR data (Table 1) and by an HSQC experiment, in which the 1,2-disubstituted aromatic groups [δ_H 7.65/7.94 (H-9, dd, *J* = 7.5/7.5, 1.1/1.1 Hz), 7.54/7.44 (H-12, dd, *J* = 7.5/7.5, 1.1/1.1 Hz), 7.42/7.32 (H-11, td, *J* = 7.5/7.5, 1.1/1.1 Hz), and δ_H 7.37/7.25 (H-10, td, *J* = 7.5/7.5, 1.1/1.1 Hz)], two vinylic groups [δ_H 4.96/4.90 (H_a-18), 4.73/4.83(H_b-18), and 4.79/4.84 (H-19)], and six oxymethine/methylene [δ_H 4.84/4.84 (H-3), δ_H 3.58/3.58 (H-17α, d, *J* = 11.9/11.9 Hz), δ_H 4.25/4.28 (H-17β, dd, *J* = 11.9/11.9, 4.5/4.5 Hz)], two aminomethine [δ_H 2.97/2.98 (H-5, br s/br s)], and two methylene [δ_H 1.93/1.93 (H-14α, dd, br d, *J* = 14.8/14.8 Hz) and δ_H 2.68/2.63 (H-14β, dt, *J* = 14.8/14.8, 3.7/3.7 Hz)] protons were identified. The ^{1}H and ^{13}C NMR data of **2**/**3** (Tables 1 and 2) are similar to those of koumine (**10**),[7] with the distinct difference being the replacement of signals from a methylene (δ_H 3.11, br d, *J* = 14.2 Hz; δ_H 3.17, br d, *J* = 14.2 Hz, δ_C 57.2) in koumine by those from an oxymethine (δ_H 4.24/3.92, s; δ_C 98.6/103.3) group. These observations suggested that **2** and **3** were likely two 21-hydroxy derivatives of koumine. HMBC and ROESY correlation analyses (Figure 1) as well as ECD spectroscopic comparison [Figure 3, with koumine (**10**)] further confirmed this conclusion. In particular, the presence of a 21-hydroxy group was confirmed by HMBC correlations (Figure 1) between H-21 and C-7 and C-15 and between H_3-*N*-Me and C-21 in both compounds. Moreover, the ROESY correlation of H-21/H-6β indicated that the 21-OH group was

Table 1. ^{1}H NMR Spectroscopic Data for 1−4 (δ in ppm, *J* in Hz)

position	1[a]	2[b]	3[b]	4[c]
3	4.99, br s	4.84,[d]	4.84,[d]	4.97, br s
5	4.49, br s	2.97, br s	2.98, br s	4.28, d (5.2)
6	1.67, dt (13.6, 1.9)	2.23, dd (14.3, 3.2)	2.34, dt (13.7, 1.9)	2.43, d (17.1)
	2.63, dd (13.6, 3.0)	2.40, br d (14.3)	2.45, dd (13.7, 4.3)	2.86, dd (17.1, 3.7)
9	7.09, dd (7.5, 1.0)	7.65, dd (7.5, 1.1)	7.94, dd (7.5, 1.1)	7.40, d (7.4)
10	7.28, td (7.5, 1.0)	7.37, td (7.5, 1.1)	7.25, td (7.5, 1.1)	7.26, t (7.4)
11	7.38, td (7.5, 1.0)	7.42, td (7.5, 1.1)	7.32, td (7.5, 1.1)	7.36, t (7.4)
12	7.54, dd (7.5, 1.0)	7.54, dd (7.5, 1.1)	7.44, dd (7.5, 1.1)	7.57, d (7.4)
14	1.83, dt (15.0, 2.6)	1.93, br d (14.8)	1.93, br d (14.8)	1.57, d (15.0)
	2.72, dt (15.0, 3.9)	2.68, dt (14.8, 3.7)	2.63, dt (14.8, 3.7)	2.65, dt (15.0, 3.7)
15	2.02, br d (12.2)	2.81, br d (12.2)	2.31, br d (12.1)	2.49, br d (12.5)
16	2.28, br d (12.2)	2.73,[d]	2.89, br d (12.1)	3.50,[d]
17	3.80, d (12.0)	3.58, d (11.9)	3.58, d (11.9)	3.69, d (12.9)
	4.31, dd (12.0, 4.3)	4.25, dd (11.9, 4.5)	4.28, dd (11.9, 4.5)	4.25, dd (12.9, 5.2)
18	5.09, d (11.2)	4.73, dd (11.0, 7.1)	4.83,[d]	0.92, d (6.4)
	5.28, d (17.8)	4.96, dd (11.0, 1.9)	4.90,[e]	
19	4.84, dd (17.8, 11.2)	4.79,[d]	4.84,[d]	2.91, q (6.4)
21	8.52, s	4.24, s	3.92, s	3.87, d (14.8)
				4.16, d (14.8)
N_4-Me		2.78, s	2.75, s	3.82, s

[a]Recorded at 500 MHz in methanol-d_4. [b]Recorded at 600 MHz in methanol-d_4. [c]Recorded at 600 MHz in $CDCl_3$ (80%) + methanol-d_4 (20%) (v/v). [d]Overlapped by other signals. [e]Overlapped by solvent signals.

α-oriented in **2** (Figure 1), whereas the ROESY correlation H-21/H-19 was consistent with a β-orientation of the 21-OH group in **3** (Figure 1). Compounds **2** and **3** were therefore determined to be 21α- and 21β-hydroxykoumine, respectively. Failure to separate the two compounds is attributed to the presence of a 21-aza-hemiacetal group in both compounds, which allows interconversion of the two epimers. Detection of a relatively weaker $[M + H - H_2O]^+$ ion (*m/z* 305) but a stronger total ion current of its product ions in the LC-(+)-ESIMS chromatogram of the ethanolic extract suggested that compounds **2** and **3** were natural products (Supporting Information S2/3−9).

Compound **4** was obtained as a white, amorphous powder and was assigned the molecular formula $C_{20}H_{24}N_2O_3$ on the basis of ^{13}C NMR data and the HREIMS ion at *m/z* 340.1792 $[M]^+$ (calcd 340.1787), showing 16 mass units more than that of (19*S*)-hydroxydihydrokoumine,[7] a known alkaloid that was also isolated during this investigation. Comparison of the NMR data (Tables 1 and 2) of **4** with those of (19*S*)-hydroxydihydrokoumine[7] revealed that their NMR signals were similar, the major differences being the deshielding of peaks for *N*-4-CH_3 (δ_H 3.82, s; δ_C 56.9), CH-5 (δ_H 4.28, d, *J* = 5.2 Hz; δ_C 70.1), and CH_2-21 (δ_H 4.16, d, *J* = 14.8 Hz; 3.87, d, *J* = 14.8 Hz; δ_C 59.6) in **4**. These observations suggested that the

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Table 2. ^{13}C NMR Spectroscopic Data for 1−4 (δ in ppm)

position	1[a]	2[b]	3[b]	4[c]
2	186.2, C	188.2, C	188.2, C	183.2, C
3	71.9, CH	72.2, CH	72.1, CH	70.4, CH
5	61.0, CH	59.2, CH	57.5, CH	70.1, CH
6	36.1, CH	29.4, CH	37.6, CH	30.4, CH
7	58.2, C	58.0, C	56.5, CH	55.7, CH
8	144.4, C	145.1, C	145.1, C	141.9, C
9	123.9, CH	124.7, CH	129.0, CH	124.2, CH
10	128.2, CH	127.9, CH	127.2, CH	126.7, CH
11	129.9, CH	129.6, CH	129.0, CH	129.2, CH
12	121.7, CH	121.7, CH	120.7, CH	121.6, CH
13	154.9, C	155.2, C	155.2, C	153.5, C
14	25.8, CH_2	25.7, CH_2	24.9, CH_2	23.8, CH_2
15	34.9, CH	25.4, CH	31.8, CH	26.3, CH
16	36.1, CH	41.1, CH	33.9, CH	32.3, CH
17	61.7, CH_2	62.4, CH_2	61.9, CH_2	67.9, CH_2
18	119.1, CH_2	120.8, CH_2	116.8, CH_2	17.2, CH_3
19	136.6, CH	135.4, CH	139.1, CH	65.7, CH
20	51.1, C	51.7, C	51.7, C	48.2, C
21	175.0, CH	98.6, CH	103.3, CH	59.6, CH_2
N_4-Me		41.3, CH_3	43.8, CH_3	56.9, CH_3

[a]Recorded at 100 MHz in methanol-d_4. [b]Recorded at 150 MHz in methanol-d_4. [c]Recorded at 150 MHz in $CDCl_3$ (80%) + methanol-d_4 (20%) (v/v).

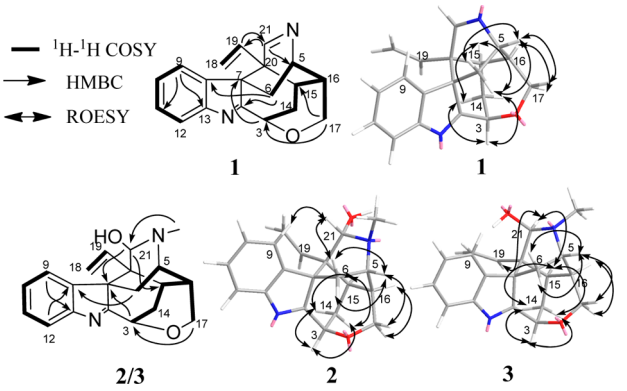

Figure 1. COSY (bold bond) and key HMBC and ROESY correlations of 1 and 2/3.

structural variation of this compound occurs around *N*-4. Considering **4** had an additional oxygen atom relative to (19*S*)-hydroxydihydrokoumine, compound **4** was proposed to be the *N*-4-oxide derivative of (19*S*)-hydroxydihydrokoumine. The deshielding of peaks for H-15 and H-16 was also consistent with the anisotropic effect of the *N*-4-oxide.[8] Using ^{1}H−^{1}H COSY, HSQC, HMBC, ROESY (Supporting Information S4), and ECD (Figure 3) data, the structure of **4** was confirmed and the ^{1}H and ^{13}C NMR data were assigned (Supporting Information S4). Thus, the structure of compound **4** was assigned as (19*S*)-hydroxydihydrokoumine *N*-4-oxide.

Compound **5** was obtained as a white, amorphous powder and was assigned the molecular formula $C_{20}H_{26}N_2O_5$ on the basis of its ^{13}C NMR data and the HRESIMS ion at *m*/*z* 375.1918 $[M + H]^+$ (calcd 375.1920), i.e., 16 mass units higher than that of the known 20-hydroxydihydrorankinidine (**5a**).[8] The ^{1}H and ^{13}C NMR data of **5** (Tables 3 and 4) were similar to those reported for 20-hydroxydihydrorankinidine (**5a**). However, signals from the CH_2-14 (δ_H 2.13, 2.20; δ_C 24.2) have been replaced by those from an oxymethine (δ_H 4.46; δ_C 66.1), which suggested that **5** was the 14-hydroxy derivative of 20-hydroxydihydrorankinidine (**5a**). ^{1}H−^{1}H COSY correlations as well as HMBC correlations between H-3 (δ_H 3.34) and the oxymethine carbon (δ_C 66.1) and between the protons at δ_H 4.46 and C-16 (δ_C 39.3) and C-20 (δ_C 69.3) (Figure 2) indicated that the hydroxy group was located at C-14. The ROESY correlations for H-14/H_2-19 suggested that H-14 and the 20-ethyl group occupied the same side of the two fused rings (Figure 2). H-14 was thus assigned to be α-oriented and the hydroxy group was β-oriented. H-14 attains a 90° dihedral angle with both H-3 and H-15, which is consistent with its ^{1}H NMR singlet resonance. The configuration of the C-7 spiro center was deduced to be *S* as in other *Gelsemium* alkaloids from the negative Cotton effect at ca. 260 nm[9] in its ECD data

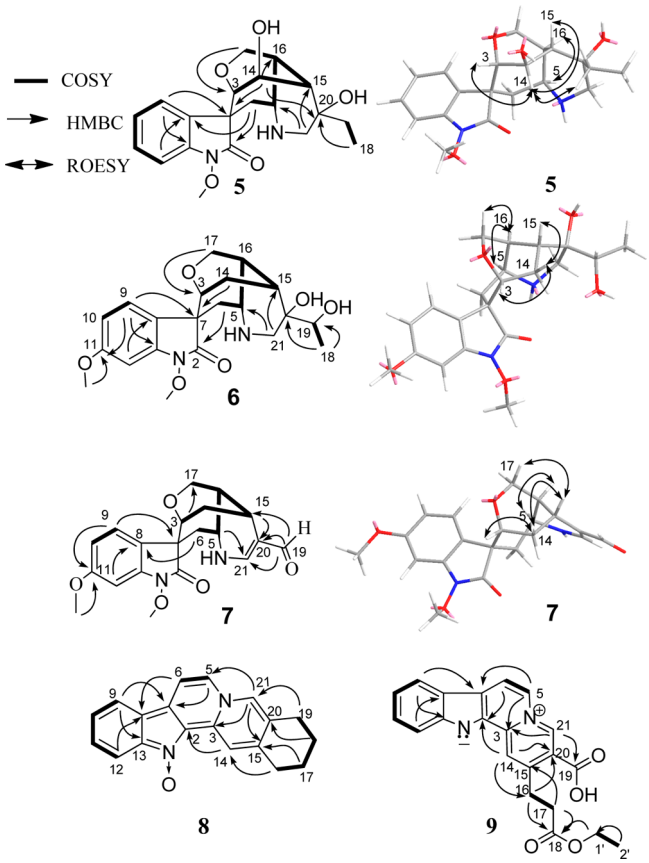

Figure 2. COSY (bold bond) and key HMBC and ROESY (5−7) correlations of compounds 5−9.

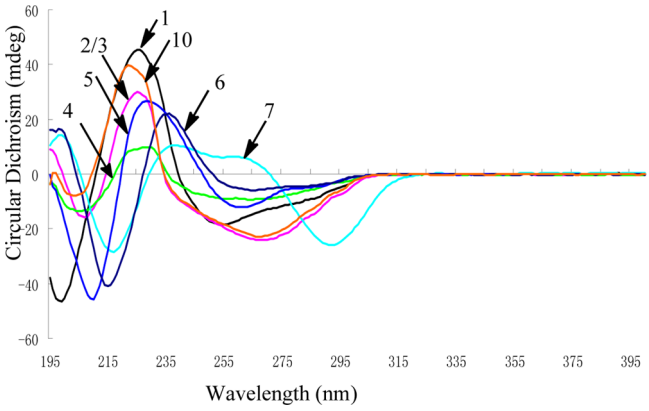

Figure 3. ECD spectra of compounds 1−7 and 10.

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Table 3. ^{1}H NMR Spectroscopic Data for 5−9 and 11 (δ in ppm, J in Hz)

position	5[a]	6[a]	7[a]	8[b]	9[a]	11[a]
3	3.34,[c]	3.57,[d]	3.62, br d (6.6)			
5	3.55, br d (9.4, 2.9)	3.56,[d]	4.12, m	8.04, d (4.0)	8.98, d (6.9)	8.57, d (6.1)
6	1.96, br d (15.7, 2.3)	2.22,[d]	2.03, dd (15.1, 6.9)	8.09, d (4.0)	8.81, d (6.9)	8.35, d (6.6)
	2.34,[d]	2.32,[d]	2.18, dd (15.1, 4.3)			
9	7.47, d (7.6)	7.40, d (8.3)	7.38, d (8.3)	8.06, d (8.2)	8.43, d (8.0)	8.17, d (7.7)
10	7.15, t (7.6)	6.68, dd (8.3, 2.4)	6.67, dd (8.3, 2.4)	7.29, br t (8.2)	7.53, br t (8.0)	7.38, t (7.7)
11	7.35, t (7.6)			7.62, br t (8.26)	7.78, br t (8.0)	7.62, t (7.7)
12	7.05, d (7.6)	6.65, d (2.4)	6.62, d (2.4)	7.91, d (8.2)	7.85, d (8.0)	7.67, d (7.7)
14	4.46, s	1.87, dd (15.9, 8.3)	2.17, dd (15.1, 7.2)	9.75, s	9.23, s	8.38, s
		2.33, dd (15.9, 7.9)	2.50, ddd (15.1, 11.8, 6.7)			
15	2.18, br d (5.9)	2.22,[d]	3.01, m			
16	2.34,[d]	2.55, m	2.23, dd, (8.6, 4.1)	3.11 (2H), m	3.54 (2H), t (7.5)	3.18 (2H), br s
17	4.39 (2H), br s	3.92, dd (10.8, 5.5)	4.20, dd (11.0, 4.1)	1.93 (2H), m	2.88 (2H), t (7.5)	1.98 (2H), br s
		4.28, d (10.8)	4.35, d (11.0)			
18	1.12, t (7.4)	1.20, d (6.5)		1.90 (2H), m		1.98 (2H) br s
19	2.00, dq (14.2, 7.4)	3.77, q (6.5)	8.78, s	2.83 (2H), m		3.01 (2H), br s
	2.05, dq (14.2, 7.4)					
21	3.44, d (11.5)	2.50, d (14.7)	7.36, s	8.55, s	9.42, s	8.86, s
	3.50, d (11.5)	3.22, d (14.7)				
1′					4.14 (2H), q (7.1)	
2′					1.24 (3H), t (7.1)	
N_1-OMe	4.01 (3H), s	3.96 (3H), s	3.93 (3H), s			
C-11-OMe		3.83 (3H), s	3.83 (3H), s			

[a]Recorded at 600 MHz in methanol-d_4. [b]Recorded at 500 MHz in methanol-d_4. [c]Overlapped by solvent signals. [d]Overlapped by other signals.

Table 4. ^{13}C NMR Spectroscopic Data for 5−9 and 11 (in Methanol-d_4, δ in ppm)

position	5[a]	6[a]	7[a]	8[b]	9[a]	11[a]
2	176.4, C	177.0, C	175.1, C	126.2, C	133.1, C	131.1, C
3	83.1, CH	74.2, CH	74.6, CH	132.7, C	132.2, C	132.3, C
5	59.5, CH	55.0, CH	55.1, CH	122.5, CH	128.2, CH	127.3, CH
6	35.0, CH	25.4, CH	36.1, CH	116.4, CH	119.3, CH	117.1, CH
7	56.9, C	56.5, C	56.3, C	114.3, C	126.1, C	123.2, C
8	132.7, C	122.5, C	122.6, C	116.6, C	122.3, C	122.4, C
9	126.9, CH	128.2, CH	127.8, CH	121.6, CH	123.2, CH	122.6, CH
10	125.1, CH	109.1, CH	109.1, CH	121.4, CH	123.5, CH	123.1, CH
11	129.8, CH	162.0, C	162.1, C	128.2, CH	131.5, CH	130.4, CH
12	108.7, CH	95.8, CH	95.7, CH	111.8, CH	113.9, CH	113.8, CH
13	139.3, C	141.3, C	141.1, C	141.5, C	143.7, C	142.6, C
14	66.1, CH	31.8, CH_2	30.5, CH_2	122.0, CH	138.4, CH	120.7, CH
15	47.6, CH	35.0, CH	24.7, CH	147.7, C	135.3, C	151.5, C
16	39.3, CH	35.5, CH	35.7, CH	30.4, CH_2	27.8, CH_2	30.6, CH_2
17	64.1, CH_2	68.5, CH_2	66.8, CH_2	23.0, CH_2	35.3, CH_2	22.9, CH_2
18	9.5, CH_3	17.0, CH_3		23.0, CH_2	173.9, C	22.9, CH_2
19	24.6, CH_2	69.2, CH	188.7, CH	27.3, CH_2	167.1, C	27.5, CH_2
20	69.3, C	74.7, C	118.7, C	133.6, C	135.3, C	135.2, C
21	64.1, CH_2	43.3, CH_2	155.9, CH	134.6, CH	124.8, CH	136.0, CH
1′					61.8, CH_2	
2′					14.5, CH_3	
N_1-OMe	64.3, CH_3	64.2, CH_3	64.2, CH_3			
C-11OMe		56.2, CH_3	56.2, CH_3			

[a]Recorded at 150 MHz. [b]Recorded at 100 MHz.

(Figure 3). Compound **5** was therefore defined as 14β,20α-dihydroxydihydrorankinidine.

Compound **6** was obtained as an amorphous powder and was assigned the molecular formula $C_{21}H_{28}N_2O_6$ on the basis of its ^{13}C NMR data and the HRESIMS ion at m/z 405.2025 [M + H]$^+$ (calcd 405.2026), i.e., 30 mass units higher than that of compound **5**. The ^{1}H and ^{13}C NMR spectra (Tables 3 and 4) of **6** bore a resemblance to those of **5**. The spin pattern of the aromatic protons indicated the presence of the aromatic methoxy group (δ_H 7.40, d, J = 8.3 Hz; δ_H 6.68, dd, J = 8.3, 2.4 Hz; δ_H 6.65, d, J = 2.4 Hz) at either C-10 or C-11. Moreover, distinctive coupling patterns and chemical shifts for the oxymethine proton (δ_H 4.46, s, in **5** and δ_H 3.77, q, in **6**) suggested that the two compounds had different oxygenation

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sites. The coupling patterns of the oxymethine proton (δ_H 3.77, q, J = 6.5 Hz) and the methyl group (δ_H 1.20, d, J = 6.5 Hz) indicated that the hydroxy group was at C-19, and HMBC correlations between H_3-18/C-19, H_3-18/C-20, H-19/C-20, and H-19/C-15 verified the deduction. The relative configuration of **6** was assigned by a ROESY experiment (Figure 2), in which the ROESY correlations of H-5/H-6α, H-5/H-16, and H-16/H-17α showed their α-orientations. Compound **6** had an ECD curve similar to that of compound **5** (Figure 3), indicating that they have the same absolute configuration. Compound **6** was therefore defined as 11-methoxy-19,20α-dihydroxydihydrorankinidin. However, the configuration of C-19 remains undetermined.

The molecular formula of compound **7** was established by its ^{13}C NMR data and the HREIMS ion at m/z 370.1542 $[M]^+$ (calcd for $C_{20}H_{22}N_2O_5$, 370.1529). The ^{1}H and ^{13}C NMR data (Tables 3 and 4) of **7** resembled those of **6**. The distinct difference was the replacement of signals from the C-18−C-21 subunits in **6** by those from an α,β-unsaturated formyl functionality (CHO: δ_H 8.78, s, δ_C 188.7; C: δ_C 118.7; CH: δ_H 7.36, s, δ_C 155.9). ^{1}H−^{1}H COSY, HSQC, and HMBC (Figure 2) correlation analyses facilitated the structure elucidation of compound **7**. In particular, HMBC correlations between H-19 and C-20 and C-21 were consistent with an α,β-unsaturated formyl moiety, and HMBC correlations between H-5/C-21 and H-15/C-20 suggested the connection of this group to C-5 (via N) and C-15, respectively. The ROESY correlations (Figure 2) and similar ECD spectra in the 195−240 nm range indicated that **7** and **6** shared the same configuration in their common structural units. The structure of **7** was thus established as depicted and named norhumantenine A. This is the first report of a 19-*nor*-humantenine-type alkaloid.

Compound **8** was obtained as a yellow powder and was assigned the molecular formula $C_{19}H_{16}N_2O$ on the basis of its ^{13}C NMR data and the HREIMS ion at m/z 288.1280 $[M]^+$ (calcd for 288.1263), showing an extra oxygen atom relative to sempervirine (**11**),[10] which was also isolated during this investigation (Tables 3 and 4 and Supporting Information S11). The major differences in the ^{1}H NMR data of **8** and **11** were the shielded signals for H-5 ($\Delta\delta_H$ −0.53), H-6 ($\Delta\delta_H$ −0.26), H-9 ($\Delta\delta_H$ −0.11), H-10 ($\Delta\delta_H$ −0.09), H_2-19 ($\Delta\delta_H$ −0.18), and H-21 ($\Delta\delta_H$ −0.31) and deshielded signals for H-12 ($\Delta\delta_H$ 0.24) and H-14 ($\Delta\delta_H$ 1.37), with the most significant shifts occurring at H-5, H-6, H-21, and H-14. These observations suggested that the shielding-related structural variation occurs around N-4 and the deshielding-related one occurs near C-12 and C-14. Considering the presence of a conjugated π-electron system between N_4 and N_1 in **11**, it is likely that such a resonance system disappears in **8** and that the additional O is located at N_1 to form an N→O dative covalent bond, the presence of which disfavors formation of a conjugated system between N_4 and N_1 and is consistent with the above NMR data. Reduction of compound **8** to **11** suggested it to be the *N*-1-oxide of sempervirine (**11**), and it was named sempervirinoxide. This is the first *N*-1-oxide alkaloid of the yohimbane type.

Compound **9** was assigned the molecular formula $C_{21}H_{18}N_2O_4$ on the basis of its ^{13}C NMR data and the HREIMS ion at m/z 362.1262 $[M]^+$ (calcd 362.1267). The ^{1}H and ^{13}C NMR data (Tables 3 and 4) resembled those of sempervirine (**11**); differences included replacement of the signals from ring E [δ_H 3.18 (2H), br s, δ_C 30.6 (CH_2-16); δ_H 1.98 (4H), br s, δ_C 22.9 (CH_2-17 and CH_2-18); δ_H 3.01 (2H), br s, δ_C 27.5 (CH_2-19)] in **11** (Tables 3 and 4, S11) by those from two carboxylic/ester carbons (δ_C 167.1, C-19; δ_C 173.9, C-18), an ethoxy group [δ_H 1.24 (3H), t, J = 7.1 Hz, δ_C 14.5, (CH_3-2′); δ_H 4.14 (2H), q, J = 7.1 Hz, δ_C 61.8, (CH_2-1′)], and two contiguous methylene groups [δ_H 3.54 (2H), t, J = 7.5 Hz, δ_C 27.8 (CH_2-16); δ_H 2.88 (2H), t, J = 7.5 Hz, δ_C 35.3 (CH_2-17)]. These observations suggested that ring E in **11** was opened in **9**/**9a**. 2D NMR spectroscopic analysis (Figure 2) not only confirmed the above inference but also established the remaining substructure for **9**. In particular, the ^{1}H−^{1}H COSY correlations between H-16/H-17 and H-1′/H-2′ and HMBC correlations between H-16 and both C-18 and the carbon at δ_C 173.9 suggested the connection of −CH_2(16)−CH_2(17)− and −O−CH_2(1′)−CH_3(2′) via ester carbonyl carbon C-18. HMBC correlations between H-16 and C-14 and C-20 located the C-16−C-17−C-18−O−C-1′−C-2′ substructure at C-15. Moreover, HMBC correlation between H-21 and C-19 indicated the remaining carbon (δ_C 167.1) as C-20 and suggested this carbon to be part of a carboxylic group on the basis of elemental constitution analysis. Thus, the structure of **9** was elucidated as depicted, and this compound was named *seco*-semperviroic acid. Compound **9** is the first known yohimbane-type E-*seco* alkaloid.

Although the structure of *seco*-sempervirinic acid (**9**) is of great interest, the presence of an ethoxy group in **9** also emphasizes whether **9** is a natural product or an artifact. Thus, the methanol crude extract was chemically analyzed by LC-ESIMS, and the absence of **9** in the extract suggested that it was an artifact.

Eleven known indole monoterpenoid alkaloids, koumine (**10**),[7] (19*R*)-hydroxydihydrokoumine,[7] (19*S*)-hydroxydihydrokoumine,[7] (4*R*)-koumine *N*-oxide,[11] (4*S*)-koumine *N*-oxide,[11] *N*-demethoxyhumantenine,[8] 11-methoxy-(19*R*)-hydroxygelselegine,[12] sempervirine (**11**),[10] koumidine,[13] *epi*-koumidine,[14] and gelsemine,[15] and a monoterpenoid alkaloid, venoterpine,[16] were also isolated. Their structures were determined by comparison of their experimental and reported NMR and MS data. Among the known compounds, (4*R*)-koumine *N*-oxide, 11-methoxy-(19*R*)-hydroxygelselegine, and *epi*-koumidine were obtained as natural products for the first time, and venoterpine was isolated from the Loganiaceae family for the first time.

The new compounds **1**−**7** and **9** (**8** was not tested due to its poor solubility) were evaluated for their cytotoxic activity against human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, colon cancer SW480, and human bronchial epithelial BEAS-2B cell lines. Compounds **1** and **7** exhibited moderate cytotoxicity against the five human tumor cell lines, with IC_{50} values in the range 4.6−9.3 μM (Table 5). Compounds **1**−**7** and **9** were also tested against nitric oxide production in LPS-activated RAW264.7 macrophages, but none of the tested compounds exhibited significant inhibitory activity (IC_{50} >25 μM).

Table 5. Cytotoxic Activity (IC_{50} μM) of Selected Compounds against Cancer Cell Lines

compound	HL-60	SMMC-7721	A-549	MCF-7	SW480	BEAS-2B
1	4.6	5.3	4.9	9.3	7.3	8.6
7	8.5	7.3	9.3	>10	>10	>10
cisplatin	2.0	5.2	8.6	>10	>10	>10

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EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained with a JASCO P-1020 polarimeter equipped with a 1 dm path length cell. UV spectra were measured with a Shimadzu UV-2401A equipped with a 1 cm path length cell. ECD spectra were recorded on a JASCO 810 spectrometer. IR spectra (KBr) were determined on a Bruker Tensor-27 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on Bruker AM-400, Bruker DRX-500, and Bruker Avance III 600 spectrometers with TMS as an internal standard. EIMS and HREIMS spectra were recorded on an AutoSpec Premier P776 instrument; ESIMS and HRESIMS were measured with a Finnigan MAT 90 mass spectrometer; LC-ESIMS was done on an ACQUITY UPLC SYNAPT G2MS (Waters Corp., USA), equipped with a BEH C_{18} column (1.7 μm, 2.1 × 50 mm; Waters Corporation). The mobile phase included (A) pure H_2O and (B) MeOH. The concentration of eluent B was changed from 20% to 95% within 10 min (linear gradient). The flow rate of the eluent was 0.4 mL min^{-1}, the injection volume of the extract was 1 μL, and the column oven was set at 30 °C. Identification of compounds **2** and **3** as natural products in *G. elegans* was analyzed by LC-PDA-(+)-ESIMS/MS (Supporting Information S2/3−9). Semipreparative HPLC was carried out using a Waters system consisting of a 600 pump and a 2996 photodiode array detector. Silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 gel (40−70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and MCI gel (CHP20/P120, 75−150 μm, Mitsubishi Chemical Corporation, Japan) were used for column chromatography.

Plant Material. The leaves and perennial vine stems of *G. elegans* were collected from Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Science (CAS), Mengla County, Yunnan Province, People's Republic of China, in October 2009 and were identified by one of the authors (Y.-K.X.). A voucher specimen (No. GE-2009-1012) was deposited in the herbarium of XTBG.

Extraction and Isolation. The air-dried, powdered plant material (6.0 kg) was extracted three times (5 days each) with EtOH/H_2O (90/10, v/v, 50 L) at room temperature. Removal of the solvent from the combined extracts in vacuum afforded a crude residue (600 g). The aqueous EtOH extract was dissolved in H_2O (3.0 L) to form a suspension, which was acidified with 10% H_2SO_4 to a pH ca. 3. The acidic suspension was partitioned with EtOAc to remove the neutral compounds, and the aqueous phase was basified with Na_2CO_3 to a pH ca. 10 and extracted with $CHCl_3$ to yield a crude alkaloid extract (105 g). The crude alkaloid mixture was applied to an MCI gel column (MeOH/H_2O from 2/8 to 5/1, v/v) to yield five major fractions (Frs. 1−5). Fr. 1 (15 g) was subjected to column chromatography (CC) over silica gel ($CHCl_3$/MeOH, 5/1 to 1/1, v/v) to yield four major fractions (1a−1d). Frs. 1a−1d were purified first by Sephadex LH-20 CC (MeOH/H_2O, 2/3 to 4/1, v/v) and then by semipreparative HPLC (MeOH/H_2O from 3/7 to 6/1, v/v) to yield **2**/**3** (10 mg), 11-methoxy-(19*R*)-hydroxygelselegine (5 mg), **5** (5 mg), and (19*S*)-hydroxydihydrokoumine (50 mg), respectively. Fr. 2 (10 g) was enriched by Sephadex LH-20 CC (MeOH/H_2O, from 1/1 to 4/1, v/v) and separated on silica gel CC ($CHCl_3$/MeOH, from 10/1 to 3/1, v/v) to give four subfractions, purification of which by semipreparative HPLC (MeOH/H_2O, from 1/1 to 5/1, v/v) yielded (4*S*)-koumine *N*-oxide (15 mg), (4*R*)-koumine *N*-oxide (3 mg), **7** (5 mg), and (19*R*)-hydroxydihydrokoumine (8 mg). Fr. 3 (12 g) was applied to a silica gel column eluted with petroleum ether/EtOAC (from 3/1 to 1/5, v/v) to give seven fractions (3a−3g). Purification of Frs. 3a−3g by HPLC (MeOH/H_2OH, from 3/2 to 5/1, v/v) yielded *N*-demethoxyhumantenine (5 mg), sempervirine (**11**) (100 mg), gelsemine (50 mg), **8** (10 mg), **9** (5 mg), koumidine (50 mg), and venoterpine (10 mg). Fr. 4 (17 g) was applied to a silica gel column eluted with petroleum ether/EtOAC (from 4/1 to 1/5, v/v) to yield three major fractions (4a−4c). Fr. 4a (6 g) was subjected to silica gel CC ($CHCl_3$/MeOH, from 1/0 to 4/2, v/v) to yield koumine (**10**) (1500 mg), **4** (4 mg), and *epi*-koumidine (15 mg). Fr. 4b (3 g) was enriched by MCI CC (MeOH/H_2O, from 3/2 to 5/1, v/v) and separated by semipreparative HPLC (MeOH/H_2O, 3/2 to 5/1, v/v) to afford **1** (12 mg) and **6** (15 mg).

*N-4-Demethyl-21-dehydrokoumine (**1**):* amorphous powder; $[\alpha]^{25}_D$ −203 (*c* 0.01, MeOH); UV (MeOH), λ_{max} (log ε) 221 (4.12), 261 (3.59) nm; ECD (*c* 4.13 × 10^{-1} mol/L, MeOH, 20 °C) λ_{max} ($\Delta\varepsilon$) 255 (−18.55), 225 (45.56), 199 (46.5); IR (KBr) ν_{max} 3442, 2923, 2852, 1630, 1384, 1075 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 290.1416 $[M]^+$ (calcd for $C_{19}H_{18}N_2O$, 290.1419).

*21α-Hydroxylkoumine (**2**) and 21β-hydroxylkoumine (**3**):* amorphous powder; $[\alpha]^{21}_D$ −232 (*c* 0.1, MeOH); UV (MeOH), λ_{max} (log ε) 220.6 (4.27), 263.4 (3.69) nm; ECD (*c* 5.65 × 10^{-1} mol/L, MeOH, 20 °C) λ_{max} ($\Delta\varepsilon$) 267 (−24.01), 225 (29.85), 207 (−15.75); IR (KBr) ν_{max} 3422, 2927, 2866, 1636, 1447, 1085 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 323.1749 $[M + H]^+$ (calcd for $C_{20}H_{23}N_2O_2$, 323.1760).

*(19S)-Hydroxydihydrokoumine N-4-oxide (**4**):* amorphous powder; $[\alpha]^{25}_D$ −73 (*c* 0.1, MeOH); UV (MeOH), λ_{max} (log ε) 222.2 (3.70), 262.2 (3.11) nm; ECD (*c* 2.45 × 10^{-1} mol/L, MeOH, 20 °C) λ_{max} ($\Delta\varepsilon$) 263 (−9.29), 228 (9.92), 205 (−13.56), 195 (−3.53); IR (KBr) ν_{max} 3441, 2957, 2923, 1630, 1454, 1040 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 340.1792 $[M]^+$ (calcd for $C_{20}H_{24}N_2O_3$, 340.1787).

*1β,20α-Dihydroxydihydrorankinidine (**5**):* white, amorphous powder, $[\alpha]^{26.5}_D$ −211 (*c* 0.1, MeOH); UV (MeOH), λ_{max} (log ε) 201.6 (2.81), 251.4 (1.69) nm; ECD (*c* 3.21 × 10^{-1} mol/L, MeOH, 20 °C) λ_{max} ($\Delta\varepsilon$) 261 (−12.08), 228 (26.52), 210 (−45.63); IR (KBr) ν_{max} 3422, 2926, 1704, 1618, 65, 1042, 750 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS *m*/*z* 375.1918 $[M + H]^+$ (calcd for $C_{20}H_{27}N_2O_5$, 375.1920).

*11-Methoxy-19,20α-dihydroxydihydrorankinidin (**6**):* white, amorphous powder; $[\alpha]^{24}_D$ −93 (*c* 0.1, MeOH); UV (MeOH), λ_{max} nm (log ε) 258 (3.44), 213 (4.31) nm; ECD (*c* 5.98 × 10^{-1} mol/L, MeOH, 20 °C) λ_{max} ($\Delta\varepsilon$) 266 (−5.89), 236 (22.02), 215 (−40.93), 196 (16.22); IR (KBr) ν_{max} 3427, 2934, 1719, 1630, 1499, 1456, 1384, 1217, 1119, 1063, 963, 804 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS *m*/*z* 405.2025 $[M + H]^+$ (calcd for $C_{21}H_{29}N_2O_6$, 405.2026).

*Norhumantenine A (**7**):* white, amorphous powder; $[\alpha]^{21}_D$ −307 (*c* 0.03, MeOH); UV (MeOH), λ_{max} (log ε) 212 (3.31), and 291 (3.02) nm; ECD (*c* 3.53 × 10^{-1} mol/L, MeOH, 20 °C) λ_{max} ($\Delta\varepsilon$) 290 (−25.6), 237 (10.5), 217 (−28.3), 199 (14.3); IR (KBr) ν_{max} 3448, 2921, 1716, 1631, 1583, 1499, 1384 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HREIMS *m*/*z* 370.1542 $[M]^+$ (calcd for $C_{20}H_{22}N_2O_5$, 370.1529).

*Sempervirinoxide (**8**):* yellow powder; UV (MeOH) λ_{max} nm (log ε) 471 (3.35), 355 (4.25), 300 (4.10), 245.5 (4.33), 197.5 (4.11) nm; IR (KBr) ν_{max} 3440, 2924, 2853, 1640, 1469, 1403, 1347, 1037, 745 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HREIMS *m*/*z* 288.1280 $[M]^+$ (calcd for $C_{19}H_{16}N_2O$, 288.1263).

*seco-Sempervirоic acid (**9**):* white, amorphous powder; UV (MeOH), λ_{max} nm (log ε) 390 (3.52), 353 (3.55), 282 (3.66), 248 (3.92), 218 (4.12), 204 (4.12) nm; IR (KBr) ν_{max} 3440, 2920, 1725, 1633, 1114 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HREIMS *m*/*z* 362.1262 $[M]^+$ (calcd for $C_{21}H_{18}N_2O_4$, 362.1267).

Chemical Transformation of Compound 8 to Compound 11. To a stirred suspension of compound **8** (1 mg, 0.0035 mmol) and an equal weight of 5% Pd/C (1 mg, 0.00047 mmol Pd) in dry MeOH (0.5 mL) was added anhydrous ammonium formate (31.5 mg, 0.5 mmol) in a single portion under a nitrogen atmosphere. The mixture was stirred at room temperature, and the progress was monitored by TLC. After 48 h, the catalyst and the ammonium salt were removed by filtration through a Celite pad, using a $CHCl_3$/MeOH (15/1) solvent mixture (ca. 3 mL) as the eluent. The combined organic filtrate upon evaporation under reduced pressure afforded the target compound **11**.

Analysis of Synthetic and Natural Sempervirine (11). Components **8** and **11** were indistinguishable by R_f values on silica gel 60 F254 precoated plates. However, when the samples were dissolved in MeOH in identical concentrations (approximately 1 mg/mL), **8** appeared dark brown on TLC under visible light, whereas **11** was only pale yellow or nearly colorless. The ^1H NMR (methanol-d_4,

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500 MHz) spectroscopic data of synthetic **11** are consistent with those of the natural sample previously isolated (S11-6).

Cytotoxicity Assays.[17] *Inhibition of NO Production Assays.* The assay was performed according to a published method.[18] Each compound was dissolved in DMSO and further diluted in the medium to produce different concentrations with a maximum concentration of 25 μM. The absorbance was measured at 570 nm with a 2104 Envision multilabel plate reader (PerkinElmer Life Sciences, Inc., Boston, MA, USA). Cytotoxicity was determined with the MTT assay. MG-132 (Sigma-Aldrich, Foster City, CA, USA) was used as the positive control.

None of the eight (**1**−**7**, **9**) compounds exhibited significant inhibitory activities against nitric oxide production in LPS-activated RAW264.7 macrophages.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR, HRESIMS/HREIMS, IR, and UV spectra of compounds **1**−**9** and **11** (1D and 2D), LC-ESIMS/MS analysis of compounds **2** and **3** in the EtOH crude extracts of *G. elegans*, and the ^{1}H NMR spectrum of sempervirine (**11**). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/np5009619.

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Notes

The authors declare no competing financial interest.

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