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Monoterpenoid Indole Alkaloids from *Gardneria ovata*

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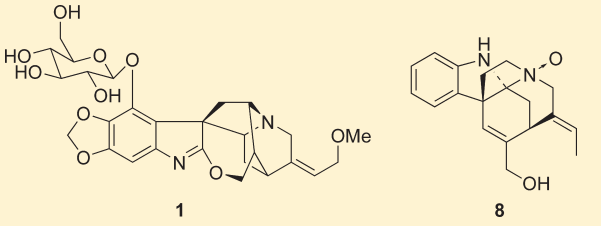
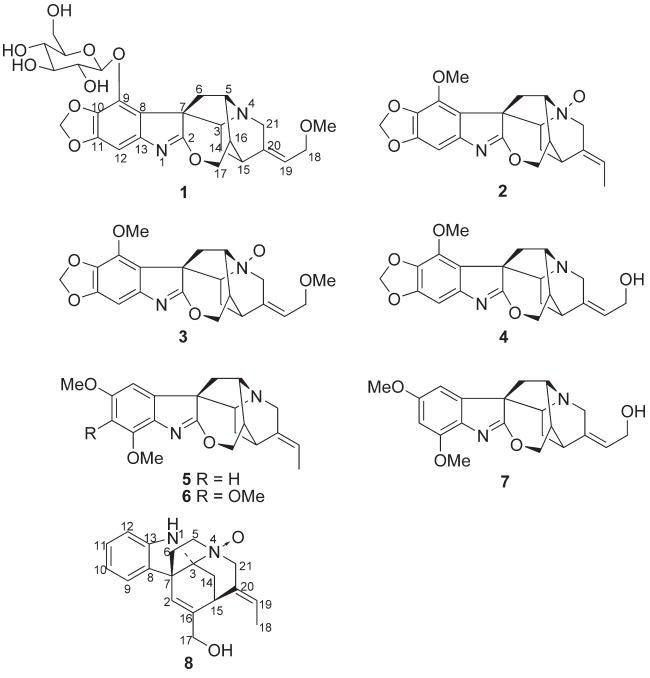
Supporting Information

ABSTRACT: Eight new monoterpenoid indole alkaloids, gardfloramine-9-*O*-β-D-glucopyranoside (**1**), 19(*E*)-18-demethoxygardfloramine-*N*(4)-oxide (**2**), gardfloramine-*N*(4)-oxide (**3**), 18-demethylgardfloramine (**4**), 19(*E*)-9,18-didemethoxygardneramine (**5**), 19(*E*)-11-methoxy-9,18-didemethoxygardneramine (**6**), 9-demethoxy-18-demethylgardneramine (**7**), and minfiensine-*N*(4)-oxide (**8**), along with six known alkaloids, were isolated from *Gardneria ovata*. The structures of the new alkaloids were established by means of spectroscopic methods. None of the compounds were cytotoxic to five human cancer cell lines.



Monoterpenoid indole alkaloids have attracted interest due to their complicated structures and potent biological activities.[1−3] Our previous studies have reported the alkaloids (19,20)-*E*/*Z*-alstoscholarine,[4] melohenines A and B,[5] melotenine A,[6] scholarisines A−G,[7,8] alstoyunines A−H,[9] and melodinines A−L.[10,11] In addition, these alkaloids have anti-inflammatory and cytotoxic activities.[6,9] Plants of the genus *Gardneria* in the Loganiaceae family are also sources of monoterpenoid indole alkaloids.[12] As part of our search for novel and bioactive alkaloids, phytochemical research on the alkaloids of *Gardneria ovata* Wall. resulted in the isolation of eight new alkaloids, gardfloramine-9-*O*-β-D-glucopyranoside (**1**), 19(*E*)-18-demethoxygardfloramine-*N*(4)-oxide (**2**), gardfloramine-*N*(4)-oxide (**3**), 18-demethylgardfloramine (**4**), 19(*E*)-9,18-didemethoxygardneramine (**5**), 19(*E*)-11-methoxy-9,18-didemethoxygardneramine (**6**), 9-demethoxy-18-demethylgardneramine (**7**), and minfiensine-*N*(4)-oxide (**8**), together with six known compounds. The six known alkaloids were identified as gardfloramine,[13,14] 19(*E*)-18-demethoxygardfloramine,[13,14] 18-demethylgardneramine,[15,16] gardneramine,[15−18] 19(*E*)-18-demethoxygardneramine,[18] and gardneramine-*N*(4)-oxide[19] by comparison with the literature. Compound **1** is the first example of *Gardneria* alkaloids containing a glucose unit. None of the compounds were cytotoxic to five human cancer cell lines.



RESULTS AND DISCUSSION

The alkaloid fraction of the aerial parts from *G. ovata* was separated as described in the Experimental Section to yield a total of 14 monoterpenoid indole alkaloids, including eight new ones (**1**−**8**).

The molecular formula $C_{27}H_{32}N_2O_{10}$ of compound **1** was determined by the molecular ion peak at *m*/*z* 544.2079 $[M]^+$ in the HREIMS in combination with ^{1}H and ^{13}C NMR and DEPT spectra. Its UV and IR spectra showed absorption bands at 295 and 233 nm and bands at 3415 and 1583 cm^{-1}, respectively, which were consistent with indole alkaloids.[14] The ^{1}H and ^{13}C NMR and DEPT spectra displayed a trisubstituted indolenine ring [δ_C 181.8 (s, C-2), 62.1 (s, C-7), 123.0 (s, C-8), 135.8 (s, C-9), 134.7 (s, C-10), 149.5 (s, C-11), 96.0 (d, C-12), 145.1 (s, C-13); δ_H 6.53 (s, H-12)].[13] Besides the signals of the indolenine ring, the ^{13}C NMR and DEPT spectra displayed 19 additional carbon signals, including a quaternary carbon (δ_C 146.2), 10

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Table 1. ^{1}H NMR Data of Compounds 1−4 (methanol-d_4, δ in ppm and J in Hz)

position	1	2	3	4
3	3.98, d (8.2)	3.98, overlap	4.03, overlap	3.70, d (8.0)
5	3.64, m	4.03, overlap	4.07, m	3.65, overlap
6a	2.58, overlap	3.82, dd (13.2, 3.6)	3.79, dd (13.2, 3.5)	2.53, overlap
6b	2.37, overlap	2.60, d (13.2)	2.61, d (13.2)	2.36, overlap
12	6.53, s	6.45, s	6.45, s	6.41, s
14a	2.04, dd (14.4, 5.3)	2.30, dd (15.0, 8.0)	2.37, dd (15.0, 7.9)	2.01, dd (14.5, 5.4)
14b	1.84, dd (14.4, 8.2)	2.24, dd (15.0, 5.9)	2.25, dd (15.0, 5.8)	1.84, dd (14.5, 8.0)
15	2.61, m	3.16, m	2.79, m	2.56, m
16	2.39, m	2.74, m	2.83, m	2.38, m
17a	4.58, d (12.5)	4.67, d (13.0)	4.68, d (13.0)	4.58, d (12.8)
17b	4.53, dd (12.5, 3.4)	4.55, overlap	4.53, dd (13.0, 4.1)	4.50, dd (12.8, 3.8)
18a	3.93, overlap	1.70, d (6.7)	3.94, d (5.8)	4.07, dd (12.5, 7.5)
18b	3.89, overlap			4.01, dd (12.5, 6.2)
19	5.41, t (6.2)	5.31, q (6.7)	5.51, t (5.8)	5.41, t (6.7)
21a	3.90, overlap	4.58, overlap	4.61, d (17.6)	3.90, d (17.5)
21b	3.70, d (17.0)	4.18, d (16.3)	4.49, d (17.6)	3.65, overlap
−OCH_2O−	5.91, 5.80	5.91, 5.89	5.92, 5.90	5.87, s
9-OMe		4.01, s	4.00, s	4.05, s
18-OMe	3.33, s		3.34, s	
1′	5.02, d (7.2)			
2′	3.48, overlap			
3′	3.45, overlap			
4′	3.37, m			
5′	3.34, overlap			
6′a	3.84, d (12.8)			
6′b	3.67, overlap			

methine carbons (δ_C 36.2, 41.4, 59.8, 59.8, 70.2, 73.7, 76.1, 76.5, 102.8, 114.9), seven methylene carbons (δ_C 30.0, 31.8, 45.0, 61.2, 67.3, 72.2, 100.9), and a methoxy group (δ_C 56.6). These data indicated that **1** was similar to gardfloramine with an additional hexose unit. The connection between the hexose unit and C-9 was established by the HMBC correlation of the anomeric proton H-1′ at δ_H 5.02 with C-9 (δ_C 135.8). After acidic hydrolysis of **1**, D-glucose was identified by comparison with an authentic sample on TLC and a specific rotation of $[\alpha]^{24}_D$ +53.2 (*c* 0.24, H_2O). Analysis of the 2D NMR spectra established the structure of **1** as gardfloramine-9-*O*-glucopyranoside. The coupling constant of the anomeric proton at δ_H 5.02 (J = 7.2 Hz) suggested that the glucose was the β-glycoside. Correlations of δ_H 5.41 (1H, t, J = 6.2 Hz, H-19) and δ_H 2.61 (1H, m, H-15), δ_H 3.89 (1H, H-18b), and δ_H 3.70 (1H, d, J = 17.0 Hz, H-21b) in the ROESY spectrum indicated that the C-19−C-20 double bond had the *Z*-configuration. The relative configuration was the same as gardfloramine, from the ^{13}C NMR and the specific rotation data;[13,14] therefore, the structure of **1** was established as 19-*Z*-gardfloramine-9-*O*-β-D-glucopyranoside, which is a rare glucoside linked to an aromatic ring in monoterpene indole alkaloids.

The molecular formula of compound **2** was determined as $C_{21}H_{22}N_2O_5$ by HREIMS. The ^{13}C NMR and DEPT data were related to those of 19(*E*)-18-demethoxygardfloramine[13,14] with the exception of three downfield chemical shifts at δ_C 81.0 (C-3), 76.0 (C-5), and 67.1 (C-21) in compound **2**, indicating that it was an *N*(4)-oxide derivative of 19(*E*)-18-demethoxygardfloramine. Analysis of HMBC and HSQC data confirmed compound **2** was 19(*E*)-18-demethoxygardfloramine-*N*(4)-oxide. The ROESY spectrum showed correlations of H-19 to H-21b and of H-18 to H-15, which indicated that the configuration of the C-19−C-20 double bond was *E*.

Compound **3** was assigned the molecular formula $C_{22}H_{24}N_2O_6$, by HREIMS. The ^{13}C NMR and DEPT data were similar to those of gardfloramine,[13,14] except for three downfield signals of C-3 (δ_C 80.7), C-5 (δ_C 76.3), and C-21 (δ_C 64.9) due to the *N*(4)-oxide functional group, supported by the HMBC correlations of δ_H 4.49 (H-21b), 2.79 (H-15), 2.61 (H-6b), and 2.37 (H-14a) with δ_C 80.7 (C-3). The ROESY correlations of H-19 to H-15 and of H-18 to H-21 indicated that the configuration of the C-19−C-20 double bond was *Z*. Thus, compound **3** was named gardfloramine-*N*(4)-oxide.

The molecular formula of compound **4** was determined to be $C_{21}H_{22}N_2O_5$ by HREIMS. The ^{13}C NMR and DEPT data were related to those of gardfloramine,[13,14] except for one missing methoxy group and one upfield methylene carbon signal of δ_C 58.4 (C-18) in **4**. Instead, a C-18 hydroxy group in compound **4** was supported by the HMBC correlations of δ_H 4.07 (H-18a) and 4.01 (H-18b) with both δ_C 145.8 (C-20) and 119.1 (C-19). The ROESY correlations of H-19 with H-15 and of H-18a with H-21b indicated that the configuration of the C-19−C-20 double bond was *Z*. Thus, compound **4** was identified as 18-demethylgardfloramine.

The molecular formula of **5**, $C_{21}H_{24}N_2O_3$, was established by HREIMS, indicating 11 degrees of unsaturation. The UV absorption bands at 314 and 272 nm and an IR absorption band at 1579 cm^{-1} supported an indolenine fragment.[15] The ^{13}C NMR

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Table 2. ^{1}H NMR Data of Compounds 5−8 (methanol-d_4, δ in ppm and J in Hz)

position	5	6	7	8
2				6.33, s
3	3.56, d (7.8)	3.59, d (7.9)	3.57, d (7.8)	
5a	3.70, overlap	3.74, overlap	3.72, overlap	3.86, dd (20.2, 11.0)
5b				3.46, br, t
6a	2.57, d (12.6)	2.60, d (12.8)	2.56, d (12.6)	2.29, overlap
6b	2.16, dd (12.6, 3.4)	2.18, dd (12.8, 3.5)	2.17, dd (12.6, 3.6)	2.13, dd (13.9, 8.8)
9	6.77, d (2.2)	7.03, s	6.78, d (2.1)	7.16, d (7.4)
10				6.76, t (7.4)
11	6.45, d (2.2)		6.45, d (2.1)	7.05, t (7.7)
12				6.68, d (7.7)
14a	2.06, dd (14.4, 5.6)	2.08, dd (14.4, 5.6)	2.09, dd (14.3, 5.5)	2.34, dd (13.4, 2.5)
14b	1.81, dd (14.4, 7.8)	1.84, dd (14.4, 7.9)	1.84, dd (14.3, 7.8)	1.83, dd (13.4, 3.6)
15	3.02, m	3.04, m	2.60, m	3.57, s
16	2.32, m	2.34, m	2.42, m	
17a	4.59, d (12.9)	4.62, d (12.7)	4.59, d (12.7)	3.99, overlap
17b	4.52, dd (12.9, 3.7)	4.56, dd (12.7, 3.7)	4.50, dd (12.7, 3.7)	
18a	1.65, d (6.8)	1.65, d (6.6)	4.09, dd (12.7, 7.3)	1.79, d (6.4)
18b			4.00, dd (12.7, 6.3)	
19	5.23, q (6.8)	5.25, q (6.6)	5.44, t (6.3)	5.67, q (6.4)
21a	3.68, overlap	3.70, overlap	3.97, overlap	4.34, d (13.9)
21b			3.70, overlap	3.72, d (13.9)
10-OMe	3.77, s	3.83, s	3.78, s	
11-OMe		3.78, s		
12-OMe	3.83, s	3.93, s	3.82, s	

spectrum showed similar patterns to those of 19(*E*)-18-demethoxygardneramine,[18] the difference being a missing methoxy group at C-9 (δ_C 99.9) in **5**. The connectivity between H-9 and C-7 was established by a HMBC correlation at the proton of δ_H 6.77 (H-9) with C-7 (δ_C 63.7). The ROESY correlations of H-19 with H-21 and of H-18 with H-15 indicated that the configuration of the C-19−C-20 double bond was *E*. Analysis of the 2D NMR spectra established the structure of **5** as 19(*E*)-9,18-didemethoxygardneramine.

Compound **6** had the molecular formula $C_{22}H_{26}N_2O_4$, as suggested by HREIMS. The ^{13}C NMR spectrum showed similar patterns to those of **5** with an additional methoxy group (δ_H 3.78; δ_C 61.5) in **6**. The connectivity between the methoxy group and C-11 was established by the HMBC correlation of the protons of methoxy group with δ_C 142.9 (s, C-11). The ROESY correlations of H-19 to H-21 and of H-15 to H-18 indicated that the configuration of the C-19−C-20 double bond was *E*. Thus, the structure of **6** was established as 19(*E*)-11-methoxy-9,18-didemethoxygardneramine.

Compound **7** had the molecular formula $C_{21}H_{24}N_2O_4$ on the basis of the HREIMS. The ^{13}C NMR spectrum was similar to that of 18-demethylgardneramine,[15,16] except for a missing methoxy group at C-9 (δ_C 99.7) in **7**, as established by a HMBC correlation at the proton of δ_H 6.78 (H-9) with C-7 (δ_C 63.8). The correlations of H-19 to H-15 and of H-18 to H-21b indicated that the configuration of the C-19−C-20 double bond was *Z*. Thus, compound **7** was determined to be 9-demethoxy-18-demethylgardneramine.

The molecular formula of compound **8** was determined to be $C_{19}H_{22}N_2O_2$ on the basis of its HREIMS, requiring 10 degrees of unsaturation. The IR absorption bands at 3407 and 1609 cm^{-1} suggested hydroxy and olefinic groups, and the UV absorption bands at 292, 236, and 215 nm were consistent with an indoline moiety.[20] The ^{1}H and ^{13}C NMR and DEPT spectra displayed a substituted indoline ring [δ_C 99.6 (C-3), 52.9 (C-7), 135.6 (C-8), 123.0 (C-9), 121.0 (C-10), 129.5 (C-11), 111.4 (C-12), 147.6 (C-13); δ_H 7.16 (H-9), 6.76 (H-10), 7.05 (H-11), 6.68 (H-12)].[13] Besides the signals of the indoline ring, the ^{13}C NMR and DEPT spectra displayed 11 additional carbon signals, including two quaternary carbons (δ_C 140.0, 129.9), three methine carbons (δ_C 125.2, 33.1, 126.7), five methylene carbons (δ_C 66.1, 39.0, 31.1, 64.7, 69.9), and one methyl group (δ_C 13.2). The carbons at δ_C 125.2 and 140.0 were ascribed to two olefinic carbons located at C-2 and C-16, respectively, according to the HMBC correlations of δ_H 3.99 (H-17) and δ_H 2.29 (H-6a) with δ_C 125.2 (C-2) and of δ_H 3.99 (H-17), δ_H 3.57 (H-15), and δ_H 2.34 (H-14a) with δ_C 140.0 (C-16). These data suggested that **8** possessed a similar carbon skeleton to that of minfiensine,[20] except for three downfield signals of δ_C 99.6 (C-3), δ_C 66.1 (C-5), and δ_C 69.9 (C-21) due to the *N*(4)-oxide functionality. Compound **8** displayed $[\alpha]^{25}_D$ +183.4 (*c* 0.10, MeOH), whereas an $[\alpha]^{25}_D$ +134 (*c* 0.82, $CHCl_3$) was reported for minfiensine,[20] which suggested the same configuration for both compounds. The ROESY correlations of H-19 with H-21 and of H-18 with H-15 indicated that the configuration of the C-19−C-20 double bond was *E*; therefore, the structure of compound **8** was established as minfiensine-*N*(4)-oxide.

The NMR data of compounds **1**−**8**, as shown in Tables 1,2, and 3, were assigned on the basis of 2D NMR spectra (HSQC, HMBC, ROESY).

All compounds were evaluated for their cytotoxicity against five human cancer cell lines using the MTT method as reported

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Table 3. ^{13}C NMR Data of Compounds 1−8 (methanol-d_4, δ in ppm)

position	1	2	3	4	5	6	7	8
2	181.8, qC	181.1, qC	180.9, qC	183.4, qC	180.9, qC	181.8, qC	180.7, qC	125.2, CH
3	59.8, CH	81.0, CH	80.7, CH	62.9, CH	65.6, CH	65.6, CH	65.0, CH	99.6, qC
5	59.8, CH	76.0, CH	76.3, CH	61.9, CH	61.5, CH	62.0, CH	61.9, CH	66.1, CH_2
6	31.8, CH_2	28.6, CH_2	28.4, CH_2	32.0, CH_2	35.5, CH_2	35.4, CH_2	35.4, CH_2	39.0, CH_2
7	62.1, qC	63.6, qC	63.6, qC	64.1, qC	63.7, qC	63.4, qC	63.8, qC	52.9, qC
8	123.0, qC	120.3, qC	120.1, qC	122.3, qC	144.0, qC	137.8, qC	143.8, qC	135.6, qC
9	135.8, qC	142.5, qC	142.1, qC	140.9, qC	99.9, CH	102.5, CH	99.7, CH	123.0, CH
10	134.7, qC	136.8, qC	136.1, qC	134.5, qC	160.7, qC	153.0, qC	160.7, qC	121.0, CH
11	149.5, qC	151.5, qC	151.5, qC	151.1, qC	100.0, CH	142.9, qC	99.9, CH	129.5, CH
12	96.0, CH	96.1, CH	96.1, CH	95.6, CH	151.5, qC	145.1, qC	151.5, qC	111.4, CH
13	145.1, qC	147.1, qC	147.0, qC	146.7, qC	133.1, qC	137.0, qC	133.1, qC	147.6, qC
14	30.0, CH_2	33.3, CH_2	33.9, CH_2	32.1, CH_2	30.6, CH_2	30.5, CH_2	31.3, CH_2	31.1, CH_2
15	36.2, CH	29.5, CH	35.9, CH	37.7, CH	30.5, CH	30.4, CH	37.5, CH	33.1, CH
16	41.4, CH	42.7, CH	42.7, CH	43.0, CH	42.8, CH	42.7, CH	42.9, CH	140.0, qC
17	72.2, CH_2	71.5, CH_2	71.3, CH_2	73.4, CH_2	73.6, CH_2	73.7, CH_2	73.4, CH_2	64.7, CH_2
18	67.3, CH_2	12.5, CH_3	69.0, CH_2	58.4, CH_2	12.7, CH_3	12.7, CH_3	58.4, CH_2	13.2, CH_3
19	114.9, CH	116.2, CH	117.8, CH	119.1,CH	114.4, CH	114.6, CH	119.8, CH	126.7, CH
20	146.2, qC	134.7, qC	138.4, qC	145.8, qC	141.3, qC	140.8, qC	144.7, qC	129.9, qC
21	45.0, CH_2	67.1, CH_2	64.9, CH_2	46.7, CH_2	49.7, CH_2	49.4, CH_2	46.5, CH_2	69.9, CH_2
−OCH_2O−	100.9, CH_2	102.5,CH_2	102.5, CH_2	102.2,CH_2				
9-OMe		60.2, CH_3	60.2, CH_3	59.9, CH_3				
10-OMe					56.2, CH_3	57.0, CH_3	56.2, CH_3	
11-OMe						61.5, CH_3		
12-OMe					56.4, CH_3	61.6, CH_3	56.3, CH_3	
18-OMe	56.6, CH_3		58.6, CH_3					
1′	102.8, CH							
2′	73.7, CH							
3′	76.1, CH							
4′	70.2, CH							
5′	76.5, CH							
6′	61.2, CH_2							

previously;[21] however, all were inactive and showed IC_{50} values of >40 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrometer. IR spectra were recorded on a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D NMR spectra were recorded on Bruker Avance III 600 MHz, Bruker DRX-500 MHz, or AV-400 MHz spectrometers with TMS as the internal standard. Chemical shifts (δ) are expressed in ppm relative to TMS. HREIMS was recorded on a Waters Auto Premier P776 spectrometer. Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China), RP-18 gel (20−45 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Fractions were monitored by TLC (GF_{254}, Qingdao Haiyang Chemical Co., Ltd. Qingdao), and spots were visualized with Dragendorff's reagent. HPLC was performed using Waters 600 pumps coupled with analytical and semipreparative Sunfire C18 columns (150 × 4.6 and 150 × 10 mm, respectively). The HPLC system employed a Waters 2996 photodiode array detector and a Waters fraction collector II.

Plant Material. The aerial parts of *Gardneria ovata* were collected from Mengna County, Yunnan Province, P. R. China, and authenticated by Mr. Jing-Yun Cui, Xishuangbanna Tropical Plant Garden. A voucher specimen (No. Cui20081129) has been deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. An air-dried, powdered sample (7 kg) was extracted with 90% MeOH (25 L × 4). The extract was partitioned between EtOAc and 0.5% HCl solution. The acidic water-soluble materials, adjusted to pH 9−10 with 10% ammonia solution, were extracted with EtOAc to give an alkaloidal extract (11 g). The extract was subjected to silica gel column chromatography ($CHCl_3$/MeOH, 1:0, 30:1, 20:1, 10:1, 5:1, 0:1) to afford fractions A−F. Fraction A (2.4 g) was separated by RP-18 CC (MeOH/H_2O, 1:1−1:0) to afford gardfloramine (712 mg), gardneramine (760 mg), 19(*E*)-18-demethoxygardfloramine (110 mg), and subfraction A_1. Subfraction A_1 was chromatographed over silica gel CC ($CHCl_3$/MeOH, 50:1−10:1) and then chromatographed over Sephadex LH-20 CC (MeOH) to afford 19(*E*)-18-demethoxygardneramine (35 mg), **5** (13 mg), and **6** (5 mg). Fraction C (745 mg) was subjected to RP-18 CC (MeOH/H_2O, 4:6−4:1) to yield 18-demethylgardneramine (76 mg) and **7** (5 mg). Fraction D (590 mg) was separated by RP-18 CC (MeOH/H_2O, 3:7−8:2) and purified by silica gel CC ($CHCl_3$/MeOH, 40:1−10:1) to give **8** (11 mg) and **4** (40 mg). Fraction E (700 mg) was chromatographed over RP-18 (MeOH/H_2O, 6:4−8:2) and further

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resolved by HPLC to yield **2** (3 mg) and **3** (9 mg). Fraction F (1.2 g) was separated by RP-18 CC (MeOH/H_2O, 3:7−9:1) and further purified by silica gel CC ($CHCl_3$/MeOH, 40:1−5:1) to give **1** (31 mg) and gardneramine-*N*(4)-oxide (46 mg).

Gardfloramine-9-*O*-*β*-D-glucopyranoside (1): white needles (MeOH); mp 207−208 °C; $[\alpha]^{24}_D$ −252.8 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 295 (0.91), 233 (3.43) nm; IR (KBr) ν_{max} 3415, 2897, 1583, 1459, 1346, 1073 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (100 MHz) data (MeOH-d_4), see Tables 1 and 3, respectively; HREIMS *m*/*z* 544.2079 (calcd for $C_{27}H_{32}N_2O_{10}$ $[M]^+$, 544.2057).

19(*E*)-18-Demethoxygardfloramine-*N*(4)-oxide (2): yellow oil; $[\alpha]^{24}_D$ −125.9 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 296 (0.07), 240 (0.42), 203 (0.43), 196 (0.31) nm; IR (KBr) ν_{max} 3424, 2920, 1622, 1587, 1449, 1349, 1050 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data (MeOH-d_4), see Tables 1 and 3, respectively; HREIMS *m*/*z* 382.1530 (calcd for $C_{21}H_{22}N_2O_5$ $[M]^+$, 382.1529).

Gardfloramine-*N*(4)-oxide (3): yellow oil; $[\alpha]^{24}_D$ −232.3 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 307 (0.88), 298 (0.87), 229 (3.97) nm; IR (KBr) ν_{max} 3425, 2927, 1624, 1588, 1451, 1348, 1045 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (MeOH-d_4), see Tables 1 and 3, respectively; HREIMS *m*/*z* 412.1642 (calcd for $C_{22}H_{24}N_2O_6$ $[M]^+$, 412.1634).

18-Demethylgardfloramine (4): yellow oil; $[\alpha]^{25}_D$ −270.4 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 295 (0.81), 237 (3.64) nm; IR (KBr) ν_{max} 3422, 2926, 1622, 1575, 1449, 1344, 1052, 751 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data (MeOH-d_4), see Tables 1 and 3, respectively; HREIMS *m*/*z* 382.1535 (calcd for $C_{21}H_{22}N_2O_5$ $[M]^+$, 382.1529).

19(*E*)-9,18-Didemethoxygardneramine (5): yellow oil; $[\alpha]^{25}_D$ −333.2 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 375 (0.01), 314 (0.72), 272 (1.32), 228 (3.44) nm; IR (KBr) ν_{max} 3432, 2933, 1579, 1474, 1280, 1080, 822 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (125 MHz) data (MeOH-d_4), see Tables 2 and 3, respectively; HREIMS *m*/*z* 352.1797 (calcd for $C_{21}H_{24}N_2O_3$ $[M]^+$, 352.1787).

19(*E*)-11-Methoxy-9,18-didemethoxygardneramine (6): colorless needles (MeOH); mp 102−104 °C; $[\alpha]^{24}_D$ −135.7 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 279 (0.82), 233 (3.50), 206 (2.25) nm; IR (KBr) ν_{max} 3426, 2942, 1576, 1472, 1344, 1119, 1049 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (100 MHz) data (MeOH-d_4), see Tables 2 and 3, respectively; HREIMS *m*/*z* 382.1902 (calcd for $C_{22}H_{26}N_2O_4$ $[M]^+$, 382.1893).

9-Demethoxy-18-demethylgardneramine (7): yellow oil; $[\alpha]^{24}_D$ −349.1 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 312 (0.41), 272 (0.81) 227 (2.40), 207 (2.20) nm; IR (KBr) ν_{max} 3424, 2928, 1577, 1467, 1296, 1074 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (MeOH-d_4), see Tables 2 and 3, respectively; HREIMS *m*/*z* 368.1737 (calcd for $C_{21}H_{24}N_2O_4$ $[M]^+$, 368.1736).

Minfiensine-*N*(4)-oxide (8): yellow oil; $[\alpha]^{25}_D$ +183.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 360 (0.04), 292 (1.02), 236 (2.09), 215 (2.99), 193 (0.87) nm; IR (KBr) ν_{max} 3407, 2920, 1609, 1468, 1127, 750 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (MeOH-d_4), see Tables 2 and 3, respectively; HREIMS *m*/*z* 310.1675 (calcd for $C_{19}H_{22}N_2O_2$ $[M]^+$, 310.1681).

Acid Hydrolysis of 1. Compound **1** (5 mg) was refluxed with 2 M HCl/MeOH (1:1, 10 mL) for 6 h at 60 °C. The reaction mixture was evaporated to dryness and diluted with H_2O (5 mL). After extraction with EtOAc (3 × 5 mL), the aqueous layer was concentrated. The residue was identified as glucose by comparison with an authentic sample using TLC ($CHCl_3$/MeOH/H_2O, 6:4:1; R_f = 0.3). Purification of the aqueous layer was performed by Sephadex LH-20 CC (MeOH) followed by silica gel CC ($CHCl_3$/MeOH/H_2O, 3:1:0.1) to afford D-glucose with a specific rotation $[\alpha]^{24}_D$ +53.2 (*c* 0.24, H_2O).

Cytotoxicity Assay. The cytotoxicity assay was performed according to the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method,[21] by use of the following 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 cells, and IC_{50} values were calculated by Reed and Muench's method.[22]

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR and HREIMS spectra of compounds **1**−**8**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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