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Cytotoxic 7*S*-oxindole alkaloids from *Gardneria multiflora*

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ABSTRACT

Seven new oxindole alkaloids, gardmutines A–F (1–6) and 18-hydroxy-chitosenine (7), along with 15 known alkaloids, were isolated from the aerial parts of *Gardneria multiflora* Makino. The structures of the alkaloids were established by spectroscopic methods. Alkaloids 1–6 are the first *Gardneria* alkaloids possessing a 7*S* configuration. Gardmutines D and E were cytotoxic to HeLa, MCF-7 breast, and SW-480 colon cancer cell lines.

1. Introduction

Monoterpenoid indole alkaloids (MIAs) have been a focus of natural products research (Bonjoch and Sole, 2000; O'Connor and Maresh, 2006). Our previous studies have reported several novel alkaloids from plants of the family Apocynaceae, e.g., *E/Z*-alstoscholarine and scholarisine A. In addition, some of them exhibit cytotoxic activities (Bao et al., 2013; Liu et al., 2012). Plants of the genus *Gardneria* in the family Loganiaceae are also sources of MIAs (Haginiwa et al., 1970; Jiang et al., 2012). As part of our search for new and cytotoxic alkaloids, phytochemical research on this genus of plants was performed. In the literatures (Jiang et al., 2012), almost all reported oxindole alkaloids from the genus *Gardneria* were assigned to C-7*R* configuration. On the other hand, all of the MIAs from *Gardneria* shown no cytotoxicity against tumor cells to date (Bonjoch and Sole, 2000). However, oxindole alkaloids in nature usually possess C-7*R* and *S* configurations, such as in the genera *Uncaria* and *Gelsemium* (Kitajima et al., 2010; Wang et al., 2011). Therefore, although our previous research did not explore the cytotoxic MIAs from *G. ovate* (Li et al., 2011), we performed research on the congeneric species, *G. multiflora*. The present results showed the presence of oxindole alkaloids with the 7*S*-configuration, in addition to the 7*R*-isomers. This paper describes the isolation and structural elucidation of new alkaloids (**1**–**7**) and 15 known alkaloids. The cytotoxicity of these compounds against three human cancer cell lines was evaluated.

2. Results and discussion

The alkaloid fraction of *G. multiflora* was separated as described in Section 3 to yield a total of 22 MIAs, including 7 new ones (**1**–**7** in Fig. 1). All of the products probably belong to the class of alkaloid, as they were positive in a reaction with Dragendorff's reagent.

The molecular formula $C_{22}H_{28}N_2O_5$ of alkaloid **1** was determined by the molecular ion peak at *m/z* 400.1992 $[M]^+$ in the HREIMS in combination with the ^1H and ^{13}C NMR and DEPT spectra (Tables 1 and 2). Its UV and IR spectra showed absorption bands at 313 and 256 nm and bands at 3440, 1692, and 1633 cm^{-1}, respectively, which were consistent with those of monoterpenoid oxindole alkaloids (Li et al., 2011). The ^1H and ^{13}C NMR and DEPT spectra displayed a trisubstituted oxindole ring [δ_C 179.9 (s, C-2), 57.2 (s, C-7), 131.0 (s, C-8), 139.8 (s, C-9), 150.9 (s, C-10), 99.9 (d, C-11), 140.4 (s, C-12), 124.2 (s, C-13); δ_H 6.65 (s, H-11)] (Yang et al., 2008). In addition to the indole-ring signals, the ^{13}C NMR and DEPT spectra displayed 13 additional carbon signals, including a quaternary carbon (δ_C 139.8), five methines (δ_C 114.2, 67.7, 61.9, 47.9, 28.3), three methylenes (δ_C 66.3, 50.2, 28.0), three methoxyls (δ_C 62.0, 57.4, 57.2)

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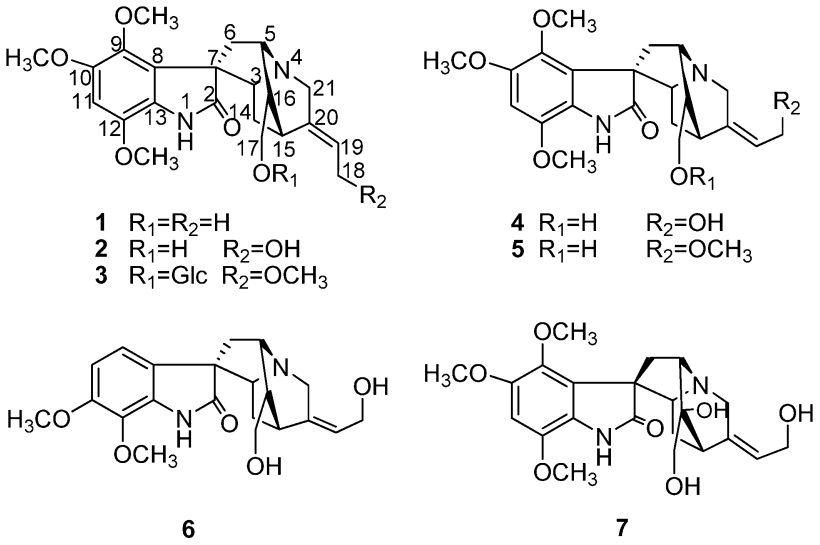

Fig. 1. Alkaloids (**1**–**7**) isolated from *G. multiflora*.

and a methyl (δ_C 13.0). These NMR patterns indicated that **1** was similar to alkaloid M (Sakm et al., 1977) with the exception of a methyl group (C-18) instead of a hydroxymethyl in the latter. This presumption was supported by the HMBC correlations (Fig. 2) of the double bond proton δ_H 5.20 (H-19) with δ_C 28.3 (C-15), 50.2 (C-21) and δ_C 139.8 (C-20), and of δ_H 1.55 (H-18) with C-20.

To date, almost all oxindole alkaloids from *Gardneria* have been identified with the 7*R*-configuration (Jiang et al., 2012). However, a special methylene signal at δ_C 42.7 was present in the ^{13}C NMR spectrum of **1**, instead of at ab. δ_C 31.0 ± 2 (C-6), as in the 7*R*-isomers (Li et al., 2011). Further, the HMBC correlations from the methylene proton signals at δ_H 2.70 (dd, *J* = 11.6, 6.4 Hz) and 1.81 (d, *J* = 11.6 Hz) to δ_C 179.9 (C-2), 131.0 (C-8), 67.7 (C-3), and 47.9 (C-16) assigned the methylene to C-6. This chemical shift difference reminded us that **1** should be a 7*S* isomer, such as alstonisine and its derivatives with the methylene signal of C-6 at ab. δ_C 41.0 ± 2 (Yang et al., 2008). The ROESY correlations of δ_H 3.54 (H-21) with δ_H 5.20 (H-19) and of δ_H 2.74 (H-15) with δ_H 1.55 (H-18) established the *E*-orientation of the C-19/20 double bond (Fig. 2). Similarly, correlations of H-16 (δ_H 2.23) with H-5 (δ_H 3.05) placed H-16 in the β-orientation. All of the signals of ^1H and ^{13}C NMR spectroscopic data were assigned by the HSQC, HMBC, and ROESY spectra. Thus, **1** was named gardmutine A.

The ^{13}C NMR and DEPT data of **2** were similar to those of **1** except that a methylene signal at δ_C 56.2 in alkaloid **2** substituted the methyl one at δ_C 13.0 (C-18) in **1**. This indicated that alkaloid **2** and alkaloid M differ in the configuration of C-7. Additionally, the molecular formula $C_{22}H_{28}N_2O_6$ of **2** from HREIMS *m*/*z* at 416.1956, 16 mass units higher than that of **1**, further indicated that it was an 18-hydroxy derivative of gardmutine A. This presumption was supported by the HMBC correlations from δ_H 3.86 (2H, H-18) to δ_C 140.6 (C-20) and 119.6

Table 1
^1H NMR spectroscopic data for alkaloids **1**–**7** (δ in ppm and *J* in Hz)[a].

Position	δ_H (1)	δ_H (2)	δ_H (3)	δ_H (4)	δ_H (5)	δ_H (6)	δ_H (7)
NH	9.01 (1H, br.s)	10.17 (1H, br.s)	10.24 (1H, s)	10.15 (1H, br.s)	9.02 (1H, s)	9.47 (1H, br.s)	10.12 (1H, br.s)
3	3.19 (br. d, 8.4)	3.15 (1H, br. d, 8.4)	3.12 (1H, br.d, 8.0)	3.06 (1H, d, 8.4)	3.08 (1H, br.d, 8.0)	2.91 (1H, br.d, 8.0)	3.10 (1H, br.d, 8.0)
5	3.05 (1H, d, 6.4)	2.82 (1H, d, 6.6)	3.02 (1H, m)	2.85 (1H, d, 7.8)	2.87 (1H, d, 7.8)	3.63 (1H, d, 7.6)	2.83 (1H, d, 7.6)
6	2.70 (dd, 11.6, 6.4)	2.52 (1H, dd, 11.2, 6.6)	2.54 (1H, dd, 11.8, 6.6)	2.53 (1H, dd, 11.2, 7.8)	2.53 (1H, dd, 12.0, 7.8)	2.05 (1H, dd, 12.0, 7.6)	2.45 (1H, dd, 12.0, 7.6)
	1.81 (1H, d, 11.6)	1.66 (1H, d, 11.8)	1.69 (1H, d, 11.8)	1.66 (1H, d, 11.2)	1.66 (1H, d, 12.0)	2.31 (1H, d, 12.0)	1.98 (1H, d, 12.0)
9	/	/	/	/	/	7.27 (1H, d, 8.0)	/
10	/	/	/	/	/	6.55 (1H, d, 8.0)	/
11	6.65 (1H s	6.61 (1H, s)	6.63 (1H, s)	6.60 (1H, s)	6.61 (1H, s)	/	6.60 (1H, s)
14	2.52 (1H, br.d, 11.2)	2.36 (1H, d, 11.6)	2.37 (1H, d, 11.6)	2.35 (1H, d, 11.6)	2.37 (1H, d, 11.6)	2.90 (1H, d, 11.4)	2.48 (1H, d, 11.6)
	1.38 (1H, dd, 11.2, 8.4)	1.32 (1H, dd, 11.6, 8.4)	1.34 (1H, dd, 11.6, 8.0)	1.32 (1H, dd, 11.6, 8.4)	1.31 (1H, dd, 11.6, 8.0)	1.21 (1H, dd, 11.4, 8.0)	1.15 (1H, dd, 11.6, 8.0)
15	2.74 (1H, br.s)	2.65 (1H, s)	2.18 (1H, m)	2.12 (1H, s)	2.19 (1H, s)	2.20 (1H, s)	2.13 (1H, s)
16	2.23 (1H, m)	2.07 (1H, m)	2.18 (1H, m)	1.97 (1H, m)	2.00 (1H, m)	2.11 (1H, m)	/
17	3.43 (1H, m)	3.32 (1H, m)	3.73 (1H, m)	3.25 (1H, m)	3.26 (1H, m)	3.90 (1H, m)	3.62 (1H, m)
			3.24 (1H, m)	3.17 (1H, m)	3.19 (1H, m)	3.83 (1H, m)	3.83 (1H, m)
18	1.55 (3H, d, 5.0)	3.86 (2H, m)	3.80 (2H, m)	3.83 (2H, m)	3.76 (2H, m)	4.00 (1H, m)	3.86 (2H, m)
						3.64 (1H, m)	
19	5.20 (1H, q, 5.0)	5.30 (1H, t, 6.4)	5.15 (1H, t, 5.0)	5.11 (1H, t, 6.0)	5.10 (1H, t, 5.0)	5.25 (1H, t, 5.0)	5.10 (1H, t, 6.0)
21	3.54 (2H, m)	3.43 (2H, m)	3.50 (2H, m)	3.49 (2H, m)	3.53 (2H, m)	3.80 (2H, m)	3.52 (2H, m)
9-OCH_3	3.82 (3H, s)	3.75 (3H, s)	3.75 (3H, s)	3.75 (3H, s)	3.73 (3H, s)	/	3.75 (3H, s)
10/11-OCH_3	3.80 (3H, s)	3.74 (3H, s)	3.74 (3H, s)	3.74 (3H, s)	3.73 (3H, s)	3.81 (3H, s)	3.74 (3H, s)
12-OCH_3	3.69 (3H, s)	3.57 (3H, s)	3.59 (3H, s)	3.59 (3H, s)	3.59 (3H, s)	3.76 (3H, s)	3.62(3H, s)
18-OCH_3	/	/	3.18 (3H, s)		3.16 (3H, s)		

[a] Compounds **1** and **6** in acetone-d_6, **2**, **3**, **4**, **5** and **7** in DMSO.

Table 2
^{13}C NMR spectroscopic data for alkaloids **1**–**7**[a].

Position	δ_C (1)	δ_C (2)	δ_C (3)	δ_C (4)	δ_C (5)	δ_C (6)	δ_C (7)
2	179.9 s	179.5 s	179.7 s	179.5 s	179.5 s	180.3 s	179.1 s
3	67.7 d	65.7 d	65.3 d	65.5 d	65.4 d	66.8 d	67.3 d
5	61.9 d	59.7 d	60.3 d	60.2 d	60.2 d	58.4 d	65.1 d
6	42.7 t	41.2 t	41.3 t	41.3 t	41.3 t	38.0 t	32.6 t
7	57.2 s	58.4 s	58.5 s	58.5 s	58.4 s	55.3 s	57.7 s
8	131.0 s	130.2 s	130.3 s	130.3 s	130.2 s	133.3 s	129.7 s
9	139.8 s	138.8 s	138.9 s	138.8 s	138.8, s	120.1 d	138.8 s
10	150.9 s	148.9 s	149.0 s	148.9 s	148.9 s	106.5 d	149.1 s
11	99.9 d	98.7 d	98.7 d	98.7 d	98.7 d	153.3 s	98.8 d
12	140.4 s	140.3 s	140.2 s	140.3 s	140.3 s	133.3 s	140.8 s
13	124.2 s	122.9 s	122.9 s	122. s	122.9 s	134.5 s	122.9 s
14	28.0 t	25.9 t	27.1 t	27.1 t	27.0 t	23.7 t	23.2 t
15	28.3 d	26.9 d	33.9 t	33.7 d	33.8 d	34.5 d	39.6 d
16	47.9 d	45.6 s	42.9 d	45.9 d	45.7 d	44.4 d	74.9 s
17	66.3 t	63.8 t	72.7 t	64.5 t	64.5 t	61.5 t	63.7 t
18	13.0 q	56.2 t	67.4 t	56.8 t	67.2 t	58.4 t	57.0 t
19	114.2 d	119.6 d	116.4 d	120.1 d	116.0 d	118.3 d	120.1 d
20	139.8 s	140.6 s	142.7 s	139.6 s	143.1 s	146.3 s	140.8 s
21	50.2 t	48.3 t	46.0 t	45.9 t	46.0 t	47.1 t	46.3 t
9-OCH_3	57.2 q	56.5 q	56.5 q	56.4 q	56.4 q		56.4 q
10/11-OCH_3	57.4 q	56.5 q	56.5 q	56.4 q	56.4 q	56.3 q	56.4 q
12-OCH_3	62.0 CH_3	61.0 q	61.1 q	61.0 q	61.0 q	61.1 q	61.1 q
18-OCH_3			57.2 q		57.0 q		

[a] Compounds **1** and **6** in acetone-d_6, **2**, **3**, **4**, **5** and **7** in DMSO.

(C-19). Analysis of the HMBC and HSQC spectroscopic data of **2** further confirmed that its molecule skeleton was identical to that of **1**. The ROESY correlations of H-18/H-15 (δ_H 2.65) and H-16 (δ_H 2.07 m)/H-5 (δ_H 2.82) indicated the C-19/C-20 double bond *E*-configuration and the H-16 β-orientation. Therefore, **2** was named gardmutine B.

Alkaloid **3** was assigned the molecular formula $C_{29}H_{40}N_2O_{11}$ by HREIMS. The ^{1}H, ^{13}C NMR and DEPT spectra displayed a sugar unit (glucose) on the basis of an anomeric methine [δ_H 4.09 (d, J = 7.8 Hz) and δ_C 103.3 d], a methylene group (δ_C 61.1), and four other methine signals between δ_C 70 and 77. The J values of the anomeric proton of the sugar moieties revealed the configuration of the glucosyl residue. The identification of the sugar residue was continued by hydrolysis with 10% HCl to afford D-glucose, which was confirmed by comparison with authentic samples and the determination of their optical rotation values ($[\alpha]_D^{20}$ = +25.5°) (Eskander et al., 2005). Apart from the sugar unit, the ^{1}H, ^{13}C NMR and DEPT spectra of **3** showed 23 carbon signals with an additional methoxyl (δ_C 57.2 and δ_H 3.18) compared to **2**. The HMBC correlation from δ_H 5.15 (H-19) with this methoxy signal determined that it is connected with C-18. The glycosyl position was unambiguously determined to be at C-17 from the correlation of the anomeric proton with C-17 (δ_C 72.7) in the HMBC spectrum. The compound **3** was named gardmutine C.

Alkaloid **4** had the same molecular formula $C_{22}H_{28}N_2O_6$ as **2** as determined by HREIMS. The ^{1}H, ^{13}C NMR and DEPT spectra displayed were similar to those of **2** except for a downfield methine signal of δ_C 33.7 instead of δ_C 26.9 (C-15) in **2**. The HMBC and HSQC correlations supported that the alkaloids **4** and **2** (Supporting Information) possessed identical plane structures. The ^{1}H, ^{13}C NMR and DEPT spectra of **5** showed 23 carbon signals with an additional methoxy (δ_C 57.0 and δ_H 3.16) compared to **4**. The HMBC correlation of **5** between H-19 (δ_H 5.10) with this methoxy signal placed it connected with C-18. However, the ROESY correlations of H-19 to H-15 in **4** and **5** indicated the *Z*-configuration of the C-19/20 double bond. Thus, **4** and **5** were named gardmutines D and E, respectively.

The ^{1}H NMR spectral data of **6** showed a bis-substituted indole ring A [δ_H 7.27 (d, J = 8.0 Hz) and 6.55 (d, J = 8.0 Hz)]. Additionally, its molecular formula $C_{21}H_{26}N_2O_5$ determined by HREIMS indicated the absence of a methoxyl group compared with **4**. The ^{13}C NMR and DEPT spectral data of **6** were similar to those of **4** with the exception of the indole-ring signals (Table 2). The HMBC correlation of δ_H 7.27 with δ_C 55.3 (C-7) placed the two neighboring methoxyls at C-11 and C-12. Correlations of δ_H 5.25 (t, J = 5.0 Hz, H-19) with δ_H 2.20 (1H, m, H-15) and of δ_H 4.00 (1H, H-18b) with δ_H 3.80 (1H, d, J = 17.0 Hz, H-21b) in the ROESY spectrum indicated

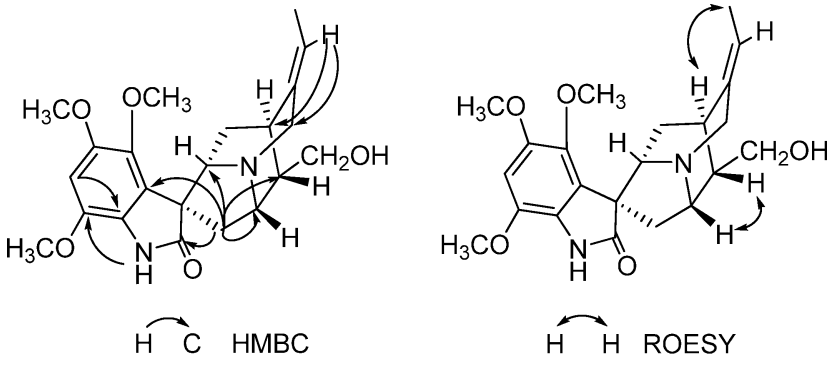

Fig. 2. Key HMBC and ROESY correlations of **1**.

Table 3
Cytotoxicity data of the alkaloids (IC_{50}, μM).

Compd.	HeLa	MCF-7	SW480
4	4.52 ± 0.42	8.10 ± 0.36	1.37 ± 0.10
5	2.52 ± 0.12	1.67 ± 0.21	3.01 ± 0.14
Cisplatin	11.84 ± 1.04	13.63 ± 0.45	19.63 ± 1.59

that the C-19/C-20 double bond had the *Z*-configuration. Thus, **6** was named gardmutine F.

The ^{13}C NMR and DEPT data of **7** were closed to those of chitosenine with the exception of an additional downfield methylene at δ_C 57.0 and the absence of a methyl signal at δ_C 13.0 (C-18) in chitosenine (Aimi et al., 1978). The ROESY correlations of H-19 (δ_H 5.10) to H-15 (δ_H 2.13) in **7** indicated the *Z*-configuration of the C-19/20 double bond. Thus, **7** was named 18-hydroxy-chitosenine.

Other known alkaloids were identified, such as vallesiachotamine (Djerassi et al., 1966), cantleyine (Massiot et al., 1992), chitosenine (Sakai et al., 1975), 18-demethoxygardneramine (Aimi et al., 1978), 18-demethoxygardfloramine (Sakai et al., 1987), gardfloramine (Sakai et al., 1987), 18-demethylgardneramine (Aimi et al., 1978), gardneramine (Aimi et al., 1978), N^4-oxide gardneramine (Sakm et al., 1977), Alkaloid M (Sakm et al., 1977), Alkaloid N (Sakm et al., 1977), Alkaloid I (Sakm et al., 1977), Alkaloid J (Sakm et al., 1977), bemethoxygardmultine (Sakai et al., 1982), and gardmultine (Sakm et al., 1977) by comparison of their NMR spectroscopic data with that in the literature. Alkaloids **1**–**6** are the first *Gardneria* alkaloids possessing the 7*S*-configuration. The cytotoxicities of all of the alkaloids against three human cancer cell lines were evaluated by using the MTT method. Only alkaloids **4** and **5** exhibited inhibitory effects against these three cell lines. These are the first *Gardneria* alkaloids discovered with moderate cytotoxicity (Table 3).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with an Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrometer. IR spectra were obtained with a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D NMR spectroscopic data were run on a Bruker AVANCE III-600, DRX-500, and AM-400 MHz spectrometer with TMS as an internal standard. ESI–MS spectra were measured on a Bruker HTC/Esquire spectrometer, and 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). Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd., Qingdao), and spots were visualized by Dragendorff's reagent. Medium-pressure liquid chromatography was employed using a *Buchi* pump system coupled with a C_{18}-silica gel-packed glass column (15 mm × 230 mm and 26 mm × 460 mm, respectively). HPLC was performed using a Waters 1525EF pumps coupled with an Xbridge/Sunfire analytical, semipreparative, or preparative C_{18} column (150 mm × 4.6 mm, 150 mm × 10 mm, and 250 mm × 19 mm, respectively). The HPLC system employed a Waters 2998 photodiode array detector and a Waters fraction collector III.

3.2. Plant material

The whole herbs of *G. multiflora* were collected from Yellow Mount, Anhui Province, P.R. China and authenticated by Prof. Shou-Jin Liu, Anhui University of Traditional Medicine. A voucher specimen (No. Liu20120720) has been deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The dried whole-plant of *G. multiflora* (10 kg) was extracted three times with 90% MeOH (25 L each). The extract was partitioned between EtOAc and a 0.5% HCl solution three times. The acidic water layer, adjusted to pH 9–10 with a 10% ammonia solution, was extracted with EtOAc to give an alkaloidal extract (34 g). The total alkaloid was subjected to a silica gel column ($CHCl_3$/acetone, 10:1 to 1:1) to give the five fractions I–V. Fraction I (0.5 g) was separated by silica gel CC (petroleum ether/Me_2CO, 4:1–1:1), then by HPLC, eluted with MeOH/H_2O (60–75%), to give vallesiachotamine (35.7 mg). Fraction II (1.2 g) was separated by silica gel CC (petroleum ether/Me_2CO, 2:1 to 1:1), to give a mixture A. Fraction III (4.7 g) was subjected to a C18 silica gel column and eluted with MeOH/H_2O (2:5–4:5, v/v) to yield fraction III-1, fraction III-2 and the mixture of compounds B. A and B were further purified by RP-18 CC (MeOH/H2O, from 3:20 to 3:10, v/v) to yield cantleyine (260.1 mg). Chitosenine (222.1 mg) was crystallized in methanol from fraction III-1. Fraction III-1 (107.5 mg) was further purified by HPLC (MeOH/H_2O, from 50% to 60%). Fraction III-2 (1.2 g) was separated by a C18 column (MeOH/H_2O, 2:5–13:20, v/v) to afford fraction III-2-1 (19 mg) and fraction III-2-2 (27 mg). Fraction III-2-1 and fraction III-2-2 were further purified by HPLC (MeOH/H_2O, from 52% to 63%) to yield 18-demethoxygardneramine (6.4 mg) and 18-demethoxygardfloramine (8.7 mg), respectively. Fraction IV (19 g) was subjected to RP-18 (MeOH/H2O, from 1:5 to 4:5, v/v), yielding three subfractions (IV-1–IV-3). Fraction IV-1 (5.4 g) was applied to MPLC (MeOH/H_2O, 1:9-7:20, v/v) to offer fraction IV-1-1 and fraction IV-1-2. Fraction IV-1-1 was further purified by HPLC to yield **7** (22.4 mg), **4** (8.2 mg), Alkaloid M (10.5 mg), and **2** (6.6 mg). Fractions IV-1-2 and IV-2 were combined and purified by HPLC (MeOH/H_2O: 50–70%) to yield 18-demethylgardneramine (5.8 mg), Alkaloid N (3.7 mg), **3** (11.5 mg), **5** (21.7 mg), Alkaloid J (3.9 mg), Alkaloid I (39.6 mg), and **1** (6.4 mg). Fraction IV-3 (6.2 g) was subjected to a C18 silica gel column and eluted with MeOH/H_2O (1:4–3:5, v/v) to give fraction IV-3-1 (1.37 g) and fraction IV-3-2 (0.11 g). Fraction IV-3-1 was further purified by HPLC (MeOH/H_2O: 55–75%) to yield gardneramine (8.6 mg) and **6** (11.5 mg). Fraction IV-3-2 was purified by HPLC (MeOH/H_2O: 60–80%) to yield gardfloramine (27.9 mg) and gardneramine (6.5 mg). Fraction V (3.9 g) was subjected to RP-18 (MeOH/H_2O, from 1:5 to 13:20, v/v), affording to 2 subfractions (V-1–V-2). Subfraction V-1 and fraction V-2 were further purified by HPLC (MeOH/H_2O, from 70% to 80%) to yield bemethoxygardmultine (2.5 mg) and gardmultine (3.3 mg), respectively.

Gardmutine A (1): White powder; $[\alpha]_D^{17}$ −18.2 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 313 (2.73), 256 (2.94), 203 (3.56) nm; IR (KBr) ν_{max} 3440, 2925, 1692, 1633, 1497, 1245 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (acetone-d_6), see Tables 1 and 2, respectively; HREIMS *m*/*z* 400.1992 (calcd for $C_{22}H_{28}N_2O_5$ $[M]^{+\cdot}$, 400.1998, error: 1.5 ppm).

Gardmutine B (2): White powder; $[\alpha]_D^{17}$ −23.4 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 313 (2.88), 253 (3.11), 203 (3.75) nm; IR (KBr) ν_{max} 3435, 2930, 1690, 1633, 1498, 1247 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (DMSO), see Tables 1 and 2, respectively; positive ESIMS *m*/*z* 417 $[M+H]^+$, HREIMS *m*/*z* 416.1956 (calcd for $C_{22}H_{28}N_2O_6$ $[M]^{+\cdot}$, 416.1947, error: 2.2 ppm).

Gardmutine C (3): Colorless oil; $[\alpha]_D^{17}$ −32.4 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 313 (2.91), 252 (3.10), 203 (3.78) nm; IR (KBr) ν_{max} 3440, 2925, 1703, 1640, 1489, 1244 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (DMSO), δ_H 4.09 (1H, d, *J* = 7.8 Hz, H-1′),

3.70 (2H, overlap, H-6′), 3.11 (1H, overlap, H-5′) 3.08 (1H, overlap, H-3′), 3.06 (1H, overlap, H-4′), 2.96 (1H, m, H-2′), δ_C 103.3 (d, C-1′), 76.9 (d, C-5′), 76.8 (d, C-3′), 73.5 (d, C-2′), 70.0 (d, C-4′), 61.1 (t, C-6′), others see Tables 1 and 2, respectively; positive ESIMS *m/z* 593 $[M+H]^+$, HREIMS *m/z* 592.2617 (calcd for $C_{29}H_{40}N_2O_{11}$ $[M]^{+\cdot}$, 592.2632, error: 2.5 ppm).

Gardmutine D (4): Colorless oil; $[\alpha]_D^{17}$ −24.7 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 313 (3.03), 253 (3.24), 203 (3.89) nm; IR (KBr) ν_{max} 3440, 2925, 1688, 1639, 1498, 1247 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (DMSO), see Tables 1 and 2, respectively; positive ESIMS *m/z* 417 $[M+H]^+$, HREIMS *m/z* 416.1939 (calcd for $C_{22}H_{28}N_2O_6$ $[M]^{+\cdot}$, 416.1947, error: 1.9 ppm).

Gardmutine E (5): White powder; $[\alpha]_D^{17}$ −17.5 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 312 (2.88), 252 (3.14), 204 (3.74) nm; IR (KBr) ν_{max} 3425, 2927, 1711, 1699, 1642, 1498, 1245 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (DMSO), see Tables 1 and 2, respectively; positive ESIMS *m/z* 431 $[M+H]^+$, HREIMS *m/z* 430.2115 (calcd for $C_{23}H_{30}N_2O_6$ $[M]^{+\cdot}$, 430.2104, error: 2.6 ppm).

Gardmutine F (6): White powder; $[\alpha]_D^{17}$ −53.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 221 (3.79), 203 (3.72) nm; IR (KBr) ν_{max} 3432, 2922, 1701, 1636, 1505, 1462, 1219 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (125 MHz) data (acetone-d_6), see Tables 1 and 2, respectively; positive ESIMS *m/z* 387 $[M+H]^+$, HREIMS *m/z* 386.1831 (calcd for $C_{21}H_{26}N_2O_5$ $[M]^{+\cdot}$, 386.1842, error: 2.8 ppm).

18-Hydroxy-chitosenine (7): White powder; $[\alpha]_D^{17}$ −61.9 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 312 (2.90), 252 (3.21), 203 (3.75) nm; IR (KBr) ν_{max} 3424, 2933, 1704, 1679, 1496, 1226 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data (DMSO), see Tables 1 and 2, respectively; positive ESIMS *m/z* 433 $[M+H]^+$, HREIMS *m/z* 432.1908 (calcd for $C_{22}H_{28}N_2O_7$ $[M]^{+\cdot}$, 432.1897, error: 2.5 ppm).

3.4. Acid hydrolysis of **3**

Alkaloid **3** (5 mg) was refluxed with 10% HCl–MeOH (10 ml) at 80 °C for 4 h. After cooling, the reaction mixture was evaporated to dryness and partitioned with EtOAc. The sugar was identified as glucose by TLC comparison using MeCOEt-isoPrOH-Me_2CO-H_2O (20:10:7:6). Purification of the H_2O layer was performed by preparative TLC eluted four times with $CHCl_3$-MeOH-H_2O (70:30:1) to afford D-glucose (R_f 0.50) with positive values of the specific rotation [20°].

3.5. Cytotoxicity assay

Three human cancer cell lines, breast cancer (MCF-7), colon cancer (SW480), and HeLa cells, were used in the cytotoxic assay. All of the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 μL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, and the suspended cells were seeded just before drug addition with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0624, 0.32, 1.6, 8, and 40 μM in triplicate for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, the cell viability was detected (Table 3), and the cell-growth curve was graphed. The IC_{50} value was calculated by Reed and Muench's method.

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References

Aimi, N., Yamaguchi, K., Sakai, S., Haginiwa, J., Kubo, A., 1978. Gardneria alkaloids XII. Carbon magnetic resonance spectra of Gardneria alkaloids. A study on the configuration of the side chain double bonds of indole alkaloid. Chem Pharm Bull 26, 3444–3449.

Bonjoch, J., Sole, D., 2000. Synthesis of strychnine. Chem Rev 100, 3455–3482.

Bao, M.F., Yan, J.M., Cheng, G.G., Li, X.Y., Liu, Y.P., Li, Y., Cai, X.H., Luo, X.D., 2013. Cytotoxic indole alkaloids from *Tabernaemontana divaricate*. J Nat Prod 76, 1406–1412.

Djerassi, J., Monteiro, H.J., Walser, A., Durham, L.J., 1966. Alkaloid studies. LVI. The constitution of vallesiachotamine. J Am Chem Soc 88, 1792–1798.

Eskander, J., Lavaud, C., Abdel-khalik, S.M., Soliman, H.S.M., Mahmoud, I.I., Long, C., 2005. Saponins from the leaves of *Mimusops laurifolia*. J Nat Prod 68, 832–841.

Haginiwa, J., Sakai, S., Kubo, A., Takahashi, K., Taguchi, M., 1970. Gardneria alkaloids IV. Comparative study of alkaloids on *Gardneria nutans* Sieb et Zucc., *G. multiforia* Makino, *G. shimadai* Hayata and so-called *G. insularis* Nakai. Yakugaku Zasshi 90, 219–223.

Jiang, J.H., Zhang, Y.M., Zhang, Y., Yang, G.M., Chen, Y.G., 2012. Chemical constituents of genus *Gardneria*, vol. 39. Yunnan Chem. Technology, , pp. 32–35.

Kitajima, M., Kobayashi, H., Kogure, N., Takayama, H., 2010. New oxindole and indole alkaloids from *Gelsemium rankinii*. Tetrahedron 66, 5987–5992.

Li, X.N., Cai, X.H., Feng, T., Li, Y., Liu, Y.P., Luo, X.D., 2011. Monoterpenoid indole alkaloids from *Gardneria ovate*. J Nat Prod 74, 1073–1078.

Liu, Y.P., Li, Y., Cai, X.H., Li, X.Y., Kong, L.M., Cheng, G.G., Luo, X.D., 2012. Melodinines M–U, cytotoxic alkaloids from *Melodinus suaveolens*. J Nat Prod 75, 220–224.

Massiot, G., Boumendjel, A., Nuzillard, J., Richard, B., Le Men-Oliver, L., David, B., HadiH., A., 1992. Alkaloids from *Alstonia undulifolia*. Phytochemistry 31, 1078–1079.

O'Connor, S.E., Maresh, J., 2006. Chemistry and biology of monoterpene indole alkaloid biosynthesis. Nat Prod Rep 23, 532–547.

Sakai, S., Aimi, N., Yamaguchi, K., Ohhira, H., HORI, K., Haginiwa, J., 1975. Gardneria alkaloids IX. Structures of chitosenine and three other minor bases: from *Gardneria multiflora* Makino. Tetrahedron Lett 16, 715–718.

Sakm, S., Aimi, N., Yamaguchi, K., Hori, K., Haginiwa, J., 1977. Gardneria alkaloids XI. Several minor base of *Gardneria multiflora* Makino. Yakugaku Zasshi 97, 399–409.

Sakai, S., Aimi, N., Yameguchi, K., Yamanaka, E., Haginiwa, J., 1982. Gardneria alkaloids Part 13. Structure of gardmultine and demethoxygardmultine; bis-type indole alkaloids of *Gardneria multiflora* Makino. J Chem Soc Perkin Trans I 1257–1262.

Sakai, S., Aimi, N., Yamaguchi, K., Ogata, K., Haginiwa, J., 1987. Gradneria alkaloids Part 14. The structure of gardflopramine and 18-demethoxygardfloramine. Chem Pharm Bull 35, 453–455.

Wang, K., Zhou, X.Y., Wang, Y.Y., Li, M.M., Li, Y.S., Peng, L.Y., Cheng, X.A., Li, Y., Wang, Y.P., Zhao, Q.S., 2011. Macrophyllionium and macrophyllines A and B, oxindole alkaloids from *Uncaria macrophylla*. J Nat Prod 74, 12–15.

Yang, J., Wearing, X.Z., Le Quesne, P.W., Deschamps, J.R., Cook, J.M., 2008. Enantiospecific synthesis of (+)-alstonisine via a stereospecific osmylation process. J Nat Prod 71, 1431–1440.