Five New *neo*-Clerodane Diterpenoid Alkaloids from *Scutellaria barbata* with Cytotoxic Activities

Sheng-Jun Dai,* Gong-Fu Wang, Meng Chen, Ke Liu, and Li Shen

School of Pharmaceutical Science, Yantai University; Yantai 264005, People's Republic of China.
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Five new *neo*-clerodane diterpenoid alkaloids, named scutebarbatine G (1), 6,7-di-*O*-nicotinoylscutebarbatine G (2), 6-*O*-nicotinoyl-7-*O*-acetylscutebarbatine G (3), scutebarbatine H (4) and 7-*O*-nicotinoylscutebarbatine H (5) were isolated from the whole plant of *Scutellaria barbata* D. Don. Their structures were elucidated by spectroscopic methods including extensive 1D and 2D NMR analyses. *In vitro*, compounds 1—5 showed significant cytotoxic activities against three human cancer lines, namely, HONE-1 nasopharyngeal, KB oral epidermoid carcinoma, and HT29 colorectal carcinoma cells, and gave IC_{50} values in the range 3.4—8.5 μM.

Key words *Scutellaria barbata*; Labiatae; *neo*-clerodane diterpenoid alkaloid; scutebarbatine G; scutebarbatine H; cytotoxic activity

In previous communications,[1,2] we have described the isolation of five *neo*-clerodane diterpenoid alkaloids from the aerial parts of *Scutellaria barbata*. As part of our continuing work to discover more novel *neo*-clerodane diterpenoids, we have re-investigated the aerial parts of this species and isolated the previously isolated scutebarbatine B (**6**), scutebarbatine D (**7**) and scutebarbatine F (**8**), together with five new *neo*-clerodane diterpenoid alkaloids scutebarbatine G (**1**), 6,7-di-*O*-nicotinoylscutebarbatine G (**2**), 6-*O*-nicotinoyl-7-*O*-acetylscutebarbatine G (**3**), scutebarbatine H (**4**) and 7-*O*-nicotinoylscutebarbatine H (**5**). By means of detailed spectral analyses, the structures of five new compounds (**1**—**5**) were established. In addition, five new compounds were screened for cytotoxity against three tumor cell lines (HONE-1 nasopharyngeal, KB oral epidermoid carcinoma, and HT29 colorectal carcinoma cells), with IC_{50} values being in the range 3.4—8.5 μM. We report here on the isolation, structure elucidation, as well as the evaluation of cytotoxic effects of these five new compounds.

Results and Discussion

Compound **1** was obtained as white needles, and showed positive response to alkaloid reagents. The molecular formula was established as $C_{26}H_{33}NO_7$ by HR-FAB mass spectrum, which displayed a quasi-molecular ion at *m/z* 472.2331 $[M+H]^+$. The IR spectrum showed absorption bands at 3450 (br), 1771, 1635, 1609, 1583, 1467, and 1361 cm^{-1}, which were assignable to hydroxyl, carbonyl, aromatic, and γ-lactone groups. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of four tertiary methyl groups (δ_H 1.05 s, 1.19 s, 1.42 s, 1.95 s, each 3H; δ_C 15.3 q, 20.9 q, 21.7 q, 21.8 q), a nicotinic acid ester moiety (δ_H 9.16, 1H, br s, H-3′; 8.83, 1H, br d, J=4.5 Hz, H-5′; 7.45, 1H, dd, J=4.5, 7.6 Hz, H-6′; 8.27, 1H, br d, J=7.6 Hz, H-7′; δ_C 164.6 s, C-1′; 126.5 s, C-2′; 151.1 d, C-3′; 153.8 d, C-5′; 123.8 d, C-6′; 137.7 d, C-7′), in addition to an 8,13-ether bridge (δ_H 1.42 s, H_3-17; δ_C 20.9 q, C-17; 81.5 s, C-8; 76.3 s, C-13) and a 13-spiro-15,16-γ-lactone moiety (δ_H 2.56, 1H, d, J=16.8 Hz, H_a-14; 2.78, 1H, d, J=16.8 Hz, H_b-14; 4.16, 1H, d, J=8.7 Hz, H_a-16; 4.20, 1H, d, J=8.7 Hz, H_b-16; δ_C 42.4 t, C-14; 174.7 s, C-15; 79.8 t, C-16), which several other *neo*-clerodane diterpenoids have this structural moiety.[3—5] Detailed examination of the ^{1}H–^{1}H COSY spectrum indicated the presence of another two spin systems. The first spin system included the signals of a methine (δ 2.51, 1H, dd, J=2.7, 12.0 Hz, H-10), two methylenes (δ 1.63, 1H, m, H_a-1; 1.92, 1H, m, H_b-1; 2.73, 2H, m, H-2) and a tri-substituted double bond (δ 5.31, br s, H-3). Thus, H_2-1 coupled with the signals of H-10 and H_2-2 which in turn was vicinally coupled with H-3. The latter, together with the crucial ^{1}H–^{13}C long-range correlations observed in the HMBC spectrum of **1** (Fig. 1) clearly indicated the presence of the double bond across C-3/C-4. The second spin system was comprised of two aliphatic protons on carbons bearing oxygen at δ 3.63 (1H, d, J=10.4 Hz, H-6) and 3.43 (1H, d, J=10.4 Hz, H-7). Observation of the cross peak in the HMBC spectrum from H-11 to C-1′ proved that the

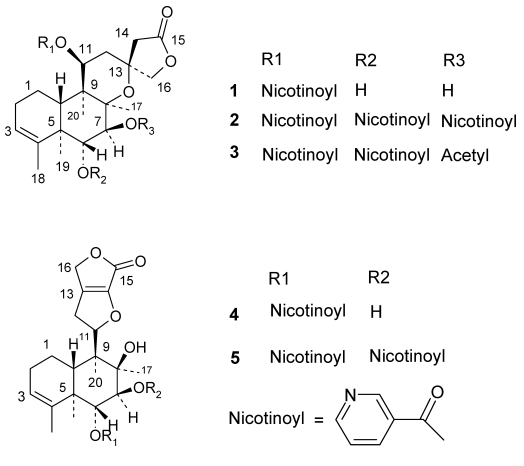

Chart 1. The Structures of New Compounds Isolated from *Scutellaria barbata*

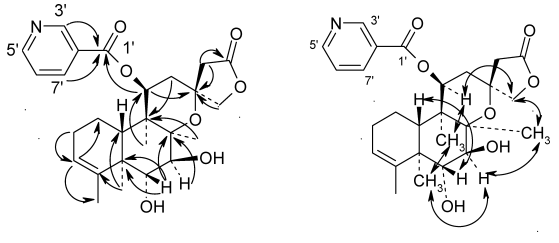

Fig. 1. Key HMBC and ROESY Correlations of **1**

* To whom correspondence should be addressed. e-mail: daishengjun_9@hotmail.com

Table 1. ^{1}H-NMR Data of Compounds **1**—**5** (400 MHz, in $CDCl_3$)[a)]

H	1	2	3	4	5
1	1.63 (m, H_a-1)	1.71 (m, H_a-1)	1.72 (m, H_a-1)	1.76 (m, H_a-1)	1.83 (m, H_a-1)
	1.92 (m, H_b-1)	2.09 (m, H_b-1)	2.08 (m, H_b-1)	2.08 (m, H_b-1)	2.11 (m, H_b-1)
2	2.73 (m, 2H)	2.80 (m, 2H)	2.77 (m, 2H)	2.11 (m, 2H)	2.19 (m, 2H)
3	5.31 (br s)	5.31 (br s)	5.33 (br s)	5.28 (br s)	5.30 (br s)
6	3.63 (d, 10.4)	5.89 (d, 10.3)	5.71 (d, 10.4)	5.36 (d, 10.4)	5.87 (d, 10.3)
7	3.43 (d, 10.4)	5.68 (d, 10.3)	5.44 (d, 10.4)	3.85 (d, 10.4)	5.71 (d, 10.3)
10	2.51 (dd, 2.7, 12.0)	2.89 (dd, 2.6, 12.2)	2.83 (dd, 2.7, 12.3)	2.34 (dd, 1.8, 12.5)	2.54 (dd, 1.9, 12.0)
11	5.78 (dd, 3.7, 11.7)	5.85 (dd, 3.7, 12.0)	5.84 (dd, 3.8, 11.8)	5.57 (br d, 10.3)	5.59 (br d, 10.4)
12	1.58 (m, H_a-12)	1.78 (m, H_a-12)	1.76 (m, H_a-12)	2.58 (dd, 10.3, 13.9, H_a-12)	2.70 (dd, 10.4, 14.2, H_a-12)
	2.17 (m, H_b-12)	2.14 (m, H_b-12)	2.18 (m, H_b-12)	3.34 (br d, 13.9, H_b-12)	3.40 (br d, 14.2, H_b-12)
14	2.56 (d, 16.8, H_a-14)	2.68 (d, 16.6, H_a-14)	2.63 (d, 17.0, H_a-14)		
	2.78 (d, 16.8, H_b-14)	3.13 (d, 16.6, H_b-14)	3.17 (d, 17.0, H_b-14)		
16	4.16 (d, 8.7, H_a-16)	4.13 (d, 8.8, H_a-16)	4.15 (d, 8.5, H_a-16)	4.56 (d, 16.4, H_a-16)	4.56 (d, 16.4, H_a-16)
	4.20 (d, 8.7, H_b-16)	4.20 (d, 8.8, H_b-16)	4.22 (d, 8.5, H_b-16)	4.77 (d, 16.4, H_b-16)	4.75 (d, 16.4, H_b-16)
17	1.42 (s, 3H)	1.24 (s, 3H)	1.16 (s, 3H)	1.40 (s, 3H)	1.34 (s, 3H)
18	1.95 (s, 3H)	1.69 (s, 3H)	1.68 (s, 3H)	1.56 (s, 3H)	1.58 (s, 3H)
19	1.19 (s, 3H)	1.58 (s, 3H)	1.53 (s, 3H)	1.36 (s, 3H)	1.47 (s, 3H)
20	1.05 (s, 3H)	1.22 (s, 3H)	1.17 (s, 3H)	0.87 (s, 3H)	1.02 (s, 3H)
3′	9.16 (br s)	8.97 (br s)	9.18 (br s)	9.25 (br s)	9.09 (br s)
5′	8.83 (br d, 4.5)	8.67 (br d, 4.5)	8.79 (br d, 4.6)	8.79 (br d, 4.6)	8.69 (br d, 4.7)
6′	7.45 (dd, 4.5, 7.6)	7.23 (dd, 4.5, 7.8)	7.42 (dd, 4.6, 7.8)	7.46 (dd, 4.6, 7.8)	7.30 (dd, 4.7, 7.8)
7′	8.27 (br d, 7.6)	8.01 (br d, 7.8)	8.25 (br d, 7.8)	8.35 (br d, 7.8)	8.10 (br d, 7.8)
3″		9.19 (br s)	9.22 (br s)		8.96 (br s)
5″		8.83 (br d, 4.6)	8.82 (br d, 4.7)		8.68 (br d, 4.6)
6″		7.47 (dd, 4.6, 7.8)	7.45 (dd, 4.7, 7.8)		7.28 (dd, 4.6, 7.7)
7″		8.27 (br d, 7.8)	8.28 (br d, 7.8)		8.07 (br d, 7.7)
3‴		9.00 (br s)			
5‴		8.70 (br d, 4.6)			
6‴		7.30 (dd, 4.6, 7.7)			
7‴		8.12 (br d, 7.7)			
OAc			1.81		

a) Chemical shift values were in ppm and *J* values (in Hz) were presented in parentheses. The assignments were based on HMQC, HMBC, and ^{1}H–^{1}H COSY experiments.

nicotinic acid ester moiety was connected to C-11. Based on the above data and comprehensive 2D NMR experiments (^{1}H–^{1}H COSY, HMQC, HMBC), the structure of **1** was established as shown in Fig. 1. The relative stereochemistry of the chiral centers in **1** was resolved by 2D ROESY data. In the ROESY spectrum (Fig. 1), the cross peaks were observed from H_3-20 to H-7, H-11, H_b-16, H_3-17, and H_3-19, from H-6 to H-10, from H_3-17 to H-7, H-11, H_a-16, H_b-16, and H_3-20, as well as from H-11 to H_b-12, H_a-16, H_b-16, H_3-17, and H_3-20. Thus, H_3-17, H_3-19, H_3-20, H-7, H-11, and H_2-16 were on the same molecular plane (α-configuration) while H-6 and H-10 were on the opposite side of the molecular plane (β-configuration).

Compound **2** was isolated as white needles and determined to have the molecular formula $C_{38}H_{39}N_3O_9$ by HR-FAB mass spectrum, which showed a quasi-molecular ion peak at *m/z* 682.2743 $[M+H]^+$. The IR spectrum gave absorption bands due to carbonyl, aromatic, and γ-lactone moieties at 1782, 1731, 1638, 1589, 1506, and 1474 cm^{-1}. Comparison of its ^{1}H- and ^{13}C-NMR data (Tables 1, 2) with those of **1** showed that **2** had many spectral features in common with **1**. The differences in their NMR spectra could be accounted for by the persence of the signals of two nicotinic acid ester moieties (δ_H 9.19, 1H, br s, H-3″; 8.83, 1H, br d, J=4.6 Hz, H-5″; 7.47, 1H, dd, J=4.6, 7.8 Hz, H-6″; 8.27, 1H, br d, J=7.8 Hz, H-7″; 9.00, 1H, br s, H-3‴; 8.70, 1H, br d, J=4.6 Hz, H-5‴; 7.30, 1H, dd, J=4.6, 7.7 Hz, H-6‴; 8.12, 1H, br d, J=7.7 Hz, H-7‴; δ_C 165.1 s, C-1″; 126.1 s, C-2″; 151.2 d, C-3″; 154.0 d, C-5″; 123.8 d, C-6″; 137.4 d, C-7″; 164.5 s, C-1‴; 125.8 s, C-2‴; 150.8 d, C-3‴; 153.9 d, C-5‴; 123.5 d, C-6‴; 137.2 d, C-7‴). The long-range correlations from H-6 to C-1″ and from H-7 to C-1‴ proved that two nicotinic acid ester moieties were connected to C-6 and C-7, respectively. The stereochemical assignments within **2** were accomplished in the similar manner as described for **1** (Fig. 2).

Compound **3** was isolated and purified as white needles. In the HR-FAB mass spectrum, **3** gave a quasi-molecular ion peak at *m/z* 619.2647 $[M+H]^+$, corresponding to a molecular formula $C_{34}H_{38}N_2O_9$. The IR spectrum exhibited absorption bands at 1781, 1733, 1642, 1592, 1500, and 1467 cm^{-1}, corresponding to carbonyl, aromatic, and γ-lactone groups. Comparison of the NMR spectra of **2** and **3** (Tables 1, 2) showed similarities except for the substitution of a nicotinic acid ester moiety at C-7 in **2** with one acetoxyl group at C-7 in **3**. The NOE experiments proved that **3** possessed the same relative configurations as **1** and **2**.

Compound **4** was obtained as white needles. The molecular formula was determined to be $C_{26}H_{31}NO_7$ by HR-FAB mass spectrum, which showed a quasi-molecular ion at *m/z* 470.2173 $[M+H]^+$. The IR spectrum exhibited absorption bands at 3447, 1783, 1740, 1641, 1588, 1512, and 1459 cm^{-1}, which were in agreement with hydroxy, carbonyl, aromatic, and α,β-unsaturated γ-lactone moieties. The ^{1}H- and ^{13}C-NMR spectra of **4** revealed the presence of the following fragments: Four tertiary methyl groups (δ_H 0.87 s, 1.36 s, 1.40 s, and 1.56 s, each 3H; δ_C 16.5 q, 17.5 q, 20.9 q, and 21.8 q), a nicotinic acid ester moiety (δ_H 9.25, 1H, br s, H-3′; 8.79, 1H, br d, J=4.6 Hz, H-5′; 7.46, 1H, dd, J=4.6,

Table 2. ^{13}C-NMR Data of Compounds **1**—**5** (100 MHz, in $CDCl_3$)[a]

Carbon	1	2	3	4	5
1	28.7 t	28.8 t	28.7 t	19.7 t	19.6 t
2	33.0 t	33.3 t	33.3 t	26.1 t	26.0 t
3	118.7 d	120.5 d	120.2 d	123.7 d	123.6 d
4	146.4 s	143.2 s	143.4 s	141.4 s	141.1 s
5	44.3 s	45.4 s	44.9 s	42.8 s	43.2 s
6	74.5 d	74.6 d	74.5 d	79.5 d	76.3 d
7	76.4 d	75.4 d	74.1 d	74.5 d	77.1 d
8	81.5 s	81.2 s	81.1 s	79.1 s	78.3 s
9	38.7 s	39.0 s	39.0 s	47.2 s	47.7 s
10	43.4 d	43.6 d	43.5 d	40.6 d	40.6 d
11	72.9 d	71.8 d	71.8 d	76.1 d	75.6 d
12	29.3 t	29.2 t	29.5 t	28.8 t	28.9 t
13	76.3 s	76.9 s	76.8 s	130.9 s	130.3 s
14	42.4 t	44.8 s	44.5 s	138.8 s	139.0 s
15	174.7 s	173.6 s	173.6 s	171.3 s	171.3 s
16	79.8 t	76.6 t	76.6 t	70.2 t	69.9 t
17	20.9 q	21.4 q	20.8 q	21.8 q	21.9 q
18	21.8 q	20.4 q	20.4 q	20.9 q	20.5 q
19	15.3 q	16.9 q	17.0 q	17.5 q	17.5 q
20	21.7 q	19.8 q	19.8 q	16.5 q	16.4 q
1′	164.6 s	164.3 s	164.4 s	166.3 s	164.6 s
2′	126.5 s	125.0 s	126.1 s	127.0 s	126.2 s
3′	151.1 d	150.7 d	150.9 d	150.5 d	150.5 d
5′	153.8 d	153.7 d	154.0 d	153.0 d	153.2 d
6′	123.8 d	123.6 d	123.7 d	124.0 d	123.7 d
7′	137.7 d	137.0 d	137.1 d	138.8 d	138.0 d
1″		165.1 s	164.6 s		164.5 s
2″		126.1 s	126.2 s		125.3 s
3″		151.2 d	151.2 d		150.2 d
5″		154.0 d	154.1 d		153.1 d
6″		123.8 d	123.8 d		123.6 d
7″		137.4 d	137.2 d		137.4 d
1‴		164.5 s			
2‴		125.8 s			
3‴		150.8 d			
5‴		153.9 d			
6‴		123.5 d			
7‴		137.2 d			
OAc			171.0 s		
			21.6 q		

a) The assignments were based on HMQC, HMBC, and ^{1}H–^{1}H COSY experiments.

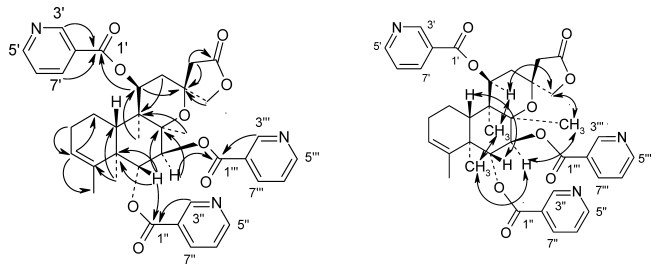

Fig. 2. Key HMBC and ROESY Correlations of **2**

7.8 Hz, H-6′; 8.35, 1H, br d, J=7.8 Hz, H-7′; δ_C 166.3 s, C-1′; 127.0 s, C-2′; 150.5 d, C-3′; 153.0 d, C-5′; 124.0 d, C-6′; 138.8 d, C-7′), an α,β-unsaturated γ-lactone moiety which was fused with a furan ring (δ_H 2.58, 1H, dd, J=10.3, 13.9 Hz, H_a-12; 3.34, 1H, br d, J=13.9 Hz, H_b-12; 4.56, 1H, d, J=16.4 Hz, H_a-16; 4.77, 1H, d, J=16.4 Hz, H_b-16; δ_C 76.1 d, C-11; 28.8 t, C-12; 130.9 s, C-13; 138.8 s, C-14; 171.3 s, C-15; 70.2 t, C-16). In addition, the ^{1}H–^{1}H COSY experiment revealed two spin systems. The first spin system was traced from the signals of a methine (δ 2.34, 1H, dd, J=1.8, 12.5 Hz, H-10), two methylenes (δ 1.76, 1H, m, H_a-1; 2.08, 1H, m, H_b-1; 2.11, 2H, m, H-2) and a tri-substituted double bond (δ 5.28, br s, H-3). Thus, H_2-1 coupled with the signals of H-10 and H_2-2 which in turn was vicinally coupled with H-3. The latter, together with the crucial long-range correlations observed in the HMBC spectrum of **4** (Fig. 3) indicated the presence of the double bond across C-3/C-4. The second spin system included two aliphatic protons on oxygenated carbons at δ 5.36 (1H, d, J=10.4 Hz, H-6) and 3.85 (1H, d, J=10.4 Hz, H-7). Based on all above data and HMBC experiment (Fig. 3), the structure of **4** was established as shown in Fig. 3. The relative stereochemistry of all the asymmetric centers of **4** was firmly established from the ROESY spectrum (Fig. 3). H-6 showed NOEs cross peaks with H-10, and H_3-20 showed NOEs with H-7, H-11, H_3-17, and H_3-19. Therefore, H_3-17, H_3-19, H_3-20, H-7 and H-11 were on the same molecular plane (α-configuration) while H-6 and H-10 were on the opposite side of the molecular plane (β-configuration).

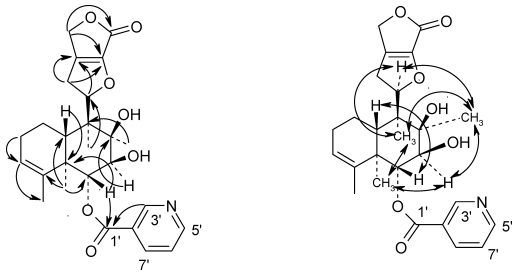

Fig. 3. Key HMBC and ROESY Correlations of **4**

Table 3. Cytotocity of Compounds **1**—**5** against Cultured HONE-1, KB and HT29 Cancer Cell Lines

Compounds	Growth inhibition constant (IC_{50})[a] [μM]		
	HONE-1	KB	HT29
Etoposide[b]	0.5±0.4	0.8±0.3	2.0±0.7
Cisplatin[b]	3.6±0.3	4.1±0.7	5.2±1.5
1	3.7±2.0	7.1±2.2	6.9±1.3
2	5.0±3.2	8.5±1.7	6.6±2.4
3	4.5±1.5	6.1±2.6	5.3±2.0
4	4.4±2.7	6.1±2.2	3.5±3.4
5	3.7±2.0	6.7±2.6	3.4±2.1

a) IC_{50} is defined as the concentration that resulted in a 50% decrease in cell number and the results are means±standard deviation of three independent replicates. The IC_{50} greater than 10 μM was considered to be no cytotocity. *b*) Positive control substance.

Compound **5** was isolated and purified as white needles. In the HR-FAB mass spectrum, **5** gave a quasi-molecular ion peak at m/z 575.2387 $[M+H]^+$, consistent with a molecular formula $C_{32}H_{34}N_2O_8$. The IR spectrum exhibited absorption bands at 3341, 1770, 1736, 1639, 1601, 1513, and 1448 cm^{-1}, assignable to hydroxyl, carbonyl, aromatic, and α,β-unsaturated γ-lactone groups. Compared to **4**, the ^{1}H- and ^{13}C-NMR data of **5** (Tables 1, 2) revealed the absence of one hydroxyl group and the presence of a nicotinic acid ester moiety (δ_H 8.96, 1H, br s, H-3″; 8.68, 1H, br d, J=4.6 Hz, H-5″; 7.28, 1H, dd, J=4.6, 7.7 Hz, H-6″; 8.07, 1H, br d, J=7.7 Hz, H-7″; δ_C 164.5 s, C-1″; 125.3 s, C-2″; 150.2 d, C-3″; 153.1 d, C-5″; 123.6 d, C-6″; 137.4 d, C-7″) at C-7. The NOE experiments proved that **5** possessed the same relative

configurations as **4**.

The five isolated compounds (**1**—**5**) were evaluated for their cytotoxic activities against HONE-1, KB, and HT29 cancer cell lines by using methylene blue dye assay and anticancer drugs, etoposide and cisplatin,[6,7] as positive controls. These new *neo*-clerodane diterpenoids exhibited significant cytotoxicity as shown in Table 3.

Experimental

General Experimental Procedures Melting points were measured on an XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Shimadzu UV-160 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 683 infrared spectrometer with KBr disks. FAB-MS and HR-FAB-MS were recorded on an Autospec-Ultima ETOF MS spectrometer. NMR spectra were recorded on a Varian Unity BRUKER 400 at 400 MHz (^{1}H) and 100 MHz (^{13}C), with TMS as the internal standard. Silica gel (200—300 mesh) for column chromatography and silica gel GF254 for preparative TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, People's Republic of China.

Plant Material *Scutellaria barbata* D. Don was collected in Linyi district, Shandong Province, People's Republic of China, in September 2006, and identified by Professor Yan-yan Zhao, School of Pharmaceutical Science, Yantai University. The whole plants of *S. barbata* was harvested and air-dried at room temperature in the dark. A voucher specimen (YP03063) has been deposited at the Herbarium of School of Pharmaceutical Science, Yantai University.

Extraction and Isolation The air-dried whole plant (30.0 kg) of *Scutellaria barbata* was finely cut and extracted three times (1 h×3) with refluxing EtOH (100×3 l). Evaporation of the solvent under reduced pressure provided the ethanolic extract (1.2 kg). The extract was dissolved and suspended in H_2O (5.0 l), and partitioned with $CHCl_3$ (3×10 l) and EtOAc (3×10 l). The $CHCl_3$ fraction (179.8 g) was subjected to extraction with 3% HCl (3×500 ml). Following this, the aqueous solution was adjusted with NH_4OH to pH 10 and extracted with $CHCl_3$ (4×500 ml). The organic fractions were combined, and the solvent was evaporated under vacuum to yield the $CHCl_3$ alkaloidal fraction (48.3 g).

The alkaloidal fraction was initially subjected to column (10×80 cm) chromatography on silica gel (200—300 mesh, 1.0 kg), eluted with cyclohexane–acetone [95 : 5 (3.0 l), 90 : 10 (5.0 l), 85 : 15 (4.0 l), 80 : 20 (5.0 l), 75 : 25 (4.0 l), 70 : 30 (3.0 l), 60 : 40 (2.0 l), and 50 : 50 (2.0 l)] to give eight fractions. Fraction 4 (5.7 g) was separated by reversed-phase silica gel (150 g, 40—50 μ) CC [eluted by MeOH–H_2O, 55 : 45, v/v], giving **5** (33 mg), **6** (230 mg) and a mixture (339 mg). The mixture was further separated by preparative TLC [$CHCl_3$–MeOH–CH_3COCH_3, 8 : 0.5 : 0.5, v/v] to afford **2** (37 mg), **3** (30 mg) and **8** (183 mg), and subsequently purified on Sephadex LH-20 [100 g, eluting with $CHCl_3$–CH_3OH, 10 : 40, v/v] to give **2** (31 mg), and **3** (26 mg). Fraction 6 (4.3 g) was separated by reversed-phase silica gel (150 g, 40—50 μ) CC [eluted by MeOH–H_2O, 45 : 55, v/v] and purified on Sephadex LH-20 [100 g, eluting with $CHCl_3$–CH_3OH, 10 : 40, v/v] to give **1** (76 mg), **4** (37 mg) and **7** (93 mg).

Scutebarbatine G (**1**): White needles, mp 156—158 °C, $[\alpha]_D^{29}$ −55.7° (c=0.14, MeOH). UV ($CDCl_3$) λ_{max} (log ε): 220 (4.21), 256 (4.37) nm. IR (KBr) ν_{max}: 3450 (br), 1771, 1635, 1609, 1583, 1467, and 1361 cm^{-1}. FAB-MS m/z: 472.4 $[M+H]^+$. HR-FAB-MS m/z: 472.2331 $[M+H]^+$ (Calcd for $C_{26}H_{33}NO_7$, 472.2335). ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2.

6,7-Di-*O*-nicotinoylscutebarbatine G (**2**): White needles, mp 150—152 °C, $[\alpha]_D^{29}$ −57.9° (c=0.13, MeOH). UV ($CDCl_3$) λ_{max} (log ε): 222 (3.86), 261 (4.14) nm. IR (KBr) ν_{max}: 1782, 1731, 1638, 1589, 1506, and 1474 cm^{-1}. FAB-MS m/z: 682.2 $[M+H]^+$. HR-FAB-MS m/z: 682.2743 $[M+H]^+$ (Calcd for $C_{38}H_{39}N_3O_9$, 682.2765). ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2.

6-*O*-Nicotinoyl-7-*O*-acetylscutebarbatine G (**3**): White needles, mp 149—151 °C, $[\alpha]_D^{29}$ −60.3° (c=0.12, MeOH). UV ($CDCl_3$) λ_{max} (log ε): 221 (4.08), 258 (4.45) nm. IR (KBr) ν_{max}: 1781, 1733, 1642, 1592, 1500, and 1467 cm^{-1}. FAB-MS m/z: 619.3 $[M+H]^+$. HR-FAB-MS m/z: 619.2647 $[M+H]^+$ (Calcd for $C_{34}H_{38}N_2O_9$, 619.2656). ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2.

Scutebarbatine H (**4**): White needles, mp 157—159 °C, $[\alpha]_D^{29}$ −69.8° (c=0.14, MeOH). UV ($CDCl_3$) λ_{max} (log ε): 217 (1.39), 220 (3.97), 251 (4.22) nm. IR (KBr) ν_{max}: 3447 (br), 1783, 1740, 1641, 1588, 1512, and 1459 cm^{-1}. FAB-MS m/z: 470.4 $[M+H]^+$. HR-FAB-MS m/z: 470.2173 $[M+H]^+$ (Calcd for $C_{26}H_{31}NO_7$: 470.2179). ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2.

7-*O*-Nicotinoylscutebarbatine H (**5**): White needles, mp 153—154 °C, $[\alpha]_D^{29}$ −73.5° (c=0.13, MeOH). UV ($CDCl_3$) λ_{max} (log ε): 218 (1.67), 223 (4.01), 260 (4.39) nm. IR (KBr) ν_{max}: 3341, 1770, 1736, 1639, 1601, 1513, and 1448 cm^{-1}. FAB-MS m/z: 575.3 $[M+H]^+$. HR-FAB-MS m/z: 575.2387 $[M+H]^+$ (Calcd for $C_{32}H_{34}N_2O_8$, 575.2392). ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2.

Antitumoral Cytotoxic Bioassays Human nasopharyngeal carcinoma HONE-1, oral epidermoid carcinoma KB, and colorectal carcinoma HT29 cells were maintained in RPMI-1640 medium supplied with 5% fetal bovine serum. Cells in logarithmic phase were cultured at a density of 5000 cells/ml/well in a 24-well plate. The cells were exposed to various concentrations of the tested drugs for 72 h. The methylene blue dye assay was used to evaluate the effects of the tested drugs on cell growth, as described previously.[8] The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically as a comparison with the control.

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