Cytotoxic *neo*-Clerodane Diterpenoid Alkaloids from *Scutellaria barbata*

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Six new *neo*-clerodane diterpenoid alkaloids, named scutehenanines A–D (**1**, **4**, **5**, **6**), 6-*O*-acetylscutehenanine A (**2**), and 6-*O*-(2-carbonyl-3-methylbutanoyl)scutehenanine A (**3**), were isolated from the whole plant of *Scutellaria barbata*. Their structures were established on the basis of detailed physical data analyses. In vitro, the six new isolated compounds showed cytotoxic activities against three human cancer lines (HONE-1 nasopharyngeal, KB oral epidermoid carcinoma, and HT29 colorectal carcinoma cells) and gave IC_{50} values in the range 2.8–6.4 μM.

Scutellaria L. (Labiatae) is a large subcosmopolitan genus with 360 currently recognized species.[1] Recently, plants belonging to this genus have attracted attention owing to interesting biological activities observed for some constituent *neo*-clerodane diterpenoids. *Scutellaria barbata* D. Don is a perennial herb that is natively distributed throughout Korea and southern China. This herb is known in traditional Chinese medicine as Ban-Zhi-Lian and traditional Korean medicine as Banjiryun, respectively, and has been used as an anti-inflammatory and antitumor agent.[2–5] In previous phytochemical studies on *S. barbata*, we reported the isolation of a series of *neo*-clerodane diterpenoid alkaloids.[6–9] From a chemotaxonomic point of view, it is of interest to note that these *neo*-clerodane diterpenoid alkaloids lack an oxygenated substituent at C-19 found in almost all of the *neo*-clerodane diterpenoids from European *Scutellaria* species.[10]

As part of our ongoing search for new *neo*-clerodane diterpenoid alkaloids, we investigated the aerial parts of *S. barbata* collected from the Zhumadian district, Henan Province, China. The EtOH extract of this species was successively partitioned with $CHCl_3$ and EtOAc. The $CHCl_3$ fraction was subjected to extraction with 3% HCl, and the aqueous layer was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ fraction was concentrated in vacuo and sequentially subjected to column chromatography over silica gel, silica gel RP-18, and Sephadex LH-20 to give six new *neo*-clerodane diterpenoids, named scutehenanines A–D (**1**, **4**, **5**, **6**), 6-*O*-acetylscutehenanine A (**2**), and 6-*O*-(2-carbonyl-3-methylbutanoyl)scutehenanine A (**3**), the structures of which were elucidated by means of extensive spectroscopic analyses. In addition, the compounds were screened for cytotoxicity against three tumor cell lines (HONE-1 nasopharyngeal, KB oral epidermoid carcinoma, and HT29 colorectal carcinoma cells), with IC_{50} values in the range 2.8–6.4 μM. Herein we report the isolation, structure elucidation, and cytotoxic effects of these six new *neo*-clerodane diterpenoid alkaloids.

Results and Discussion

Compound **1** was obtained as white needles and showed a positive response to Dragendorff's reagent. The molecular formula was established as $C_{26}H_{31}NO_6$ by HRFABMS, which displayed a quasi-molecular ion at *m/z* 454.2233 [M + H]$^+$. The IR spectrum showed absorption bands at 3348, 1729, 1635, 1590, 1493, 1470, and 1412 cm^{-1}, which were assignable to hydroxy, conjugated carbonyl, α,β-unsaturated γ-lactone, and aromatic groups. The ^{1}H NMR spectrum of **1** revealed the presence of the following fragments: four tertiary methyl groups at δ 1.09 (3H, s, H-17), 1.21 (3H, s, H-20), 1.23 (3H, s, H-19), and 1.84 (3H, s, H-18); an α,β-unsaturated γ-lactone moiety at δ 5.92 (1H, br s, H-14), 4.98 (1H, dd, J = 1.3, 16.4 Hz, H_a-16), and 5.01 (1H, dd, J = 1.3, 16.4, H_b-16); a double bond with *E* configuration at δ 6.42 (1H, d, J = 16.9 Hz, H-11) and 6.36 (1H, d, J = 16.9 Hz, H-12); and a nicotinic acid ester moiety at δ 9.30 (1H, br s, H-3′), 8.78 (1H, br d, J = 4.6 Hz, H-5δ), 7.45 (1H, dd, J = 4.6, 7.8 Hz, H-6′), and 8.37 (1H, br d, J = 7.8 Hz, H-7′). In addition, the ^{1}H–^{1}H COSY experiment revealed two spin systems. The first spin system included the signals of a methine (δ 2.23, 1H, dd, J = 2.0, 12.8 Hz, H-10), two methylenes (δ 1.35, 1H, m, H_a-1; 1.63, 1H, m, H_b-1; 2.06, 2H, m, H-2), and a trisubstituted double bond (δ 5.19, 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 **1** (Figure 1), indicated the presence of the double bond across C-3/C-4. The second spin system was traced from two aliphatic protons on oxygenated carbons at δ 4.18 (1H, d, J = 10.2 Hz, H-6) and 5.47 (1H, d, J = 10.2 Hz, H-7). Observation of the cross-peak in the HMBC spectrum from H-7 to C-1′ proved that the nicotinic acid ester moiety was connected to C-7. On the basis of the above data and comprehensive 2D NMR experiments (^{1}H–^{1}H COSY, HMQC, HMBC), the structure of **1** was established as shown in Figure 1. The relative configuration of **1** was determined from ROESY data. In the ROESY spectrum (Figure 2), cross-peaks were observed from H-6 to H-10, from H_3-20 to H-7, H_3-17, and H_3-19, and from H-7 to H_3-17, H_3-19, and H_3-20. Therefore, H_3-17, H_3-19, H_3-20, and H-7 were on the same side of the octalin ring and α-oriented, while H-6 and H-10 were on the opposite side of the octalin ring and thus β-oriented.

Compound **2** was obtained as white needles, and the molecular formula was determined as $C_{28}H_{33}NO_7$ by HRFABMS, which showed a quasi-molecular ion at *m/z* 496.2340 [M + H]$^+$. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) indicated a substitution pattern in the octalin moiety similar to **1**. The observed difference was consistent with the presence of an *O*-acetyl group (δ_H 1.79, 3H, s; δ_C 170.6 s; 21.4 q). The location of the acetoxy substituent in **2** was established from the HMBC spectrum, showing a correlation between the signal at δ 170.6 (acetoxy carbonyl) and the proton at δ 5.66 (1H, d, J = 10.3 Hz, H-6). This proton correlated with the singlet attributed to C-4 (δ 140.7), which in turn showed a cross-peak with H-10 (1H, dd, J = 1.9, 12.7 Hz). These facts established the acetoxy group at C-6 and thus the structure of compound **2** as 6-*O*-acetylscutehenanine A. Compound **3** was isolated and purified as white needles and showed a molecular formula of $C_{31}H_{37}NO_8$, established by a pseudomolecular ion at *m/z* 552.2593 [M + H]$^+$ in the HRFABMS. In contrast to compound **1** (Table 1), the ^{1}H NMR spectrum of **3** contained a 2-carbonyl-3-methylbutanoyloxy

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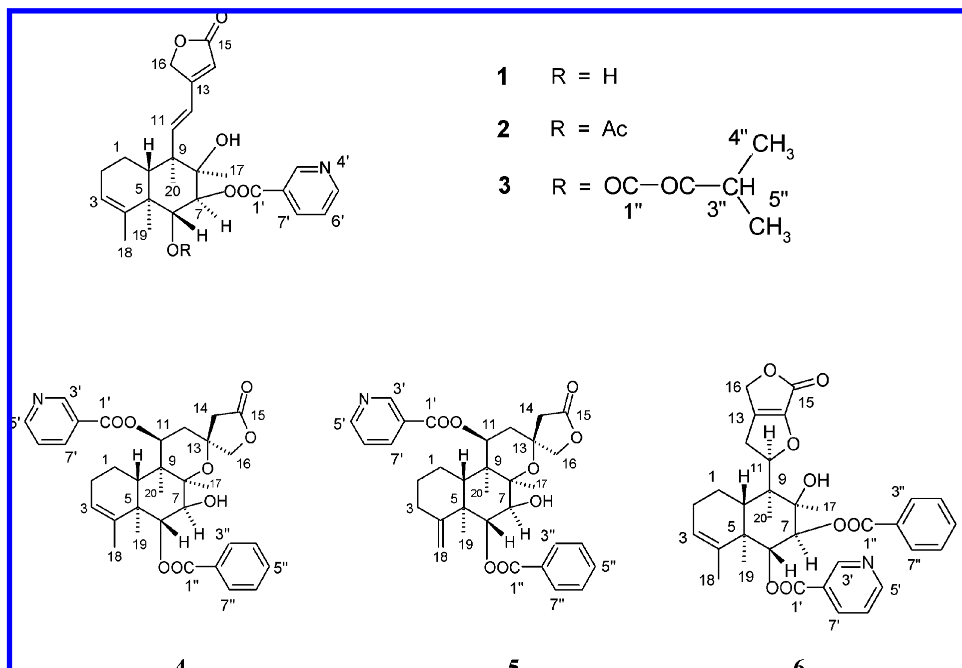
Chart 1. The Structures of Compounds **1**−**6** Isolated from *Scutellaria barbata*



group (δ_H 2.92, 1H, m, H-3″; 0.95, 3H, d, J = 5.5 Hz, H-4″; 0.79, 3H, d, J = 5.5 Hz, H-5″; δ_C 161.9 s, C-1″; 197.4 s, C-2″; 36.7 d, C-3″; 16.7 q, C-4″; 16.9 q, C-5″). In the HMBC experiment, a correlation from H-6 to C-1″ indicated that the 2-carbonyl-3-methylbutanoyloxy group was attached to C-6. The stereochemical assignments of the stereogenic centers in **2** and **3** were accomplished in a similar manner to that described for **1**, with H_3-17, H_3-19, H_3-20, and H-7 being on the same face and α-oriented, while H-6 and H-10 were on the opposite face and thus β-configured. Thus, the structure of compound **3** was identified as 6-*O*-(2-carbonyl-3-methylbutanoyl)scutehenanine A.

Compound **4** was isolated and purified as white needles and exhibited a positive response to Dragendorff's reagent. HRFABMS gave a quasi-molecular ion peak at *m/z* 576.2592 [M + H]$^+$, corresponding to a molecular formula of $C_{33}H_{37}NO_8$. The IR spectrum showed absorption bands at 3387, 1740, 1631, 1597, 1500, and 1459 cm^{-1}, which were in agreement with hydroxy, carbonyl, aromatic, and γ-lactone groups. The ^{1}H and ^{13}C NMR spectra indicated the presence of four tertiary methyl groups (δ_H 1.10 s, 1.69 s, 1.45 s, and 1.41 s, each 3H; δ_C 21.4 q, 20.5 q, 16.4 q, and 20.3 q), a nicotinic acid ester moiety (δ_H 9.28, 1H, br s, H-3′; 8.81, 1H, br d, J = 4.6 Hz, H-5′; 7.43, 1H, dd, J = 4.6, 7.8 Hz, H-6′; 8.34, 1H, br d, J = 7.8 Hz, H-7′; δ_C 165.6 s, C-1′; 126.5 s, C-2′; 150.8 d, C-3′; 153.4 d, C-5′; 123.5 d, C-6′; 137.4 d, C-7′), a benzoyloxy moiety (δ_H 7.97, 2H, m, H-3″ and H-7″; 7.47, 2H, m, H-4″ and H-6″; 7.60, br t, J = 7.9 Hz, H-5″; δ_C 165.9 s, C-1″; 130.0 s, C-2″; 129.4 d, C-3″ and C-7″; 128.6 d, C-4″ and C-6″; 133.4 d, C-5″), an 8,13-ether bridge (δ_H 1.10 s, H_3-17; δ_C 21.4 q, C-17; 82.2 s, C-8; 76.2 s, C-13), and a 13-spiro-15,16-γ-lactone moiety (δ_H 2.59, 1H, d, J = 17.0 Hz, H_a-14; 2.75, 1H, d, J = 17.0 Hz, H_b-14; 4.25, 1H, d, J = 8.8 Hz, H_a-16; 4.34, 1H, d, J = 8.8 Hz, H_b-16; δ_C 42.2 t, C-14; 174.5 s, C-15; 79.7 t, C-16), which is a structural moiety present in several other *neo*-clerodane diterpenoids.[11−13] Detailed examination of the ^{1}H−^{1}H COSY spectrum indicated the presence of another two spin systems. The first spin system was due to the signals of a methine (δ 2.71, 1H, dd, J = 2.3, 12.2 Hz, H-10), two methylenes (δ 1.63, 1H, m, H_a-1; 2.06, 1H, m, H_b-1; 2.74, 2H, m, H-2), and a trisubstituted double bond (δ 5.38, br s, H-3). Interpretation of the above correlations, together with the crucial ^{1}H−^{13}C long-range correlations observed in the HMBC spectrum of **4** clearly elucidated the position of the double bond as being between C-3 and C-4. The second spin system comprised two protons on different oxymethine carbons at δ 5.44 (1H, d, J = 9.9 Hz, H-6) and 3.70 (1H, d, J = 9.9 Hz, H-7), and the coupling constant suggested that H-6 and H-7 were *trans*-diaxially oriented. In the HMBC spectrum, the cross-peaks from H-11 to C-1′ and H-6 to C-1″ confirmed that the nicotinic acid ester and benzoyloxy moieties were attached to C-11 and C-6, respectively. On the basis of the evidence above and comprehensive 2D NMR experiments (^{1}H−^{1}H COSY, HMQC, HMBC), the structure of **4** was determined to be as shown in Figure 1. The relative configurations at C-5, C-6, C-7, C-8, C-9, C-10, C-11, and C-13 were elucidated on the basis of NOE results (Figure 2). NOEs 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, and from H-11 to H_b-12, H_a-16, H_b-16, H_3-17, and H_3-20 indicated that H_3-17, H_3-19, H_3-20, H-7, H-11, and H_2-16 were cofacial and α-oriented, while H-6 and H-10 were on the opposite side of the molecular plane and thus β-oriented.

Compound **5** was obtained as white needles. HRFABMS indicated a quasi-molecular ion peak at *m/z* 576.2597 [M + H]$^+$, consistent with a molecular formula of $C_{33}H_{37}NO_8$. Comparison of its ^{1}H and ^{13}C NMR data (Table 1) with those of **4** showed that **5** had many features in common with **4**. The differences in their NMR spectra could be accounted for by the absence of the signals of the C_3−C_4 double bond in **4**. Instead, an exomethylene (δ_H 4.60, 2H, br s, H-18; δ_C 104.7 t, C-18; 154.2 s, C-4) was introduced in **5**. With the aid of the ROESY data, it was readily confirmed that **5** had the same relative configuration at C-5, C-6, C-7, C-8, C-9, C-10, C-11, and C-13 as **4**.

Compound **6** was obtained as white needles and showed a positive response to Dragendorff's reagent. The molecular formula was determined to be $C_{33}H_{35}NO_8$ by HRFABMS, which showed a quasi-molecular ion at *m/z* 574.2438 [M + H]$^+$. The IR spectrum exhibited absorption bands at 3440, 1771, 1729, 1635, 1600, 1514, and 1455 cm^{-1}, which corresponded to hydroxy, carbonyl, aromatic, and α,β-unsaturated γ-lactone moieties. The ^{1}H and ^{13}C NMR spectra of **6** revealed the presence of the following fragments: four tertiary methyl groups (δ_H 1.02 s, 1.34 s, 1.46 s, and 1.59 s, each 3H; δ_C 16.3 q, 17.3 q, 20.6 q, and 22.0 q), a benzoyloxy moiety (δ_H 7.37, 2H, m, H-3″ and H-7″; 7.24, 2H, m, H-4″ and H-6″; 7.41, br t, J = 7.9 Hz, H-5″; δ_C 166.0 s, C-1″; 129.8 s, C-2″;

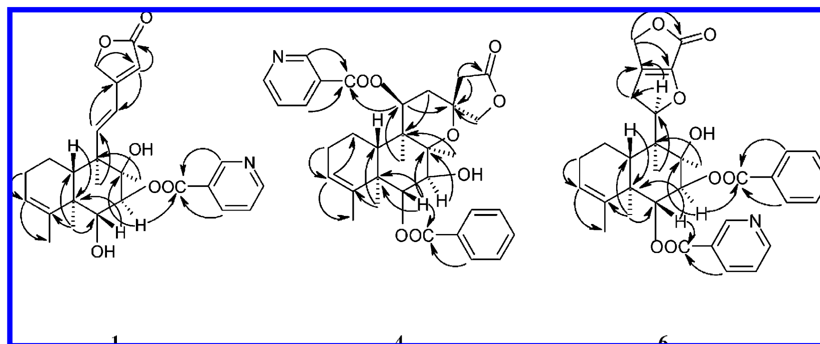


Figure 1. Key HMBC correlations of compounds **1**, **4**, and **6**.

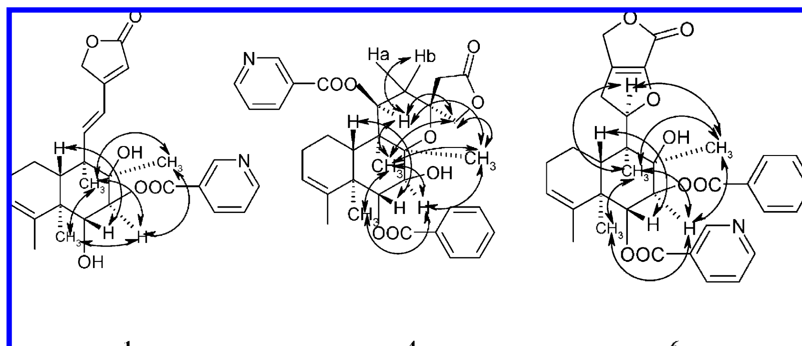


Figure 2. Key ROESY correlations of compounds **1**, **4**, and **6**.

129.4 d, C-3″ and C-7″; 128.3 d, C-4″ and C-6″; 133.1 d, C-5″), a nicotinic acid ester moiety (δ_H 9.05, 1H, br s, H-3′; 8.67, 1H, br d, $J = 4.7$ Hz, H-5′; 7.22, 1H, dd, $J = 4.7$, 7.7 Hz, H-6′; 8.06, 1H, br d, $J = 7.7$ Hz, H-7′; δ_C 164.1 s, C-1′; 125.7 s, C-2′; 150.3 d, C-3′; 152.1 d, C-5′; 123.3 d, C-6′; 138.5 d, C-7′), and an α,β-unsaturated γ-lactone moiety, which was fused with a furan ring (δ_H 2.71, 1H, dd, $J = 10.4$, 13.7 Hz, H_a-12; 3.39, 1H, br d, $J = 13.7$ Hz, H_b-12; 4.51, 1H, d, $J = 16.5$ Hz, H_a-16; 4.75, 1H, d, $J = 16.5$ Hz, H_b-16; δ_C 75.4 d, C-11; 28.8 t, C-12; 130.1 s, C-13; 138.7 s, C-14; 171.0 s, C-15; 69.9 t, C-16). In addition, the ^1H and ^{13}C NMR spectra of **6** indicated an octalin moiety with a similar substitution pattern to that found for **1**. The locations of the nicotinic acid ester and benzoyloxy moieties were established from the HMBC spectrum. The HMBC showed correlations from H-6 to C-1′ and H-7 to C-1″. This proved that the nicotinic acid ester moiety and benzoyloxy group were connected to C-6 and C-7, respectively. On the basis of the above data and HMBC experiment (Figure 1), the structure of **6** was established as shown in Figure 1. The relative configuration of the stereogenic centers of **6** was the same as those in **1**, as determined by the ROESY spectrum (Figure 2).

Although the absolute configuration of compounds **1**−**6** was not ascertained, we can assume that compounds **1**−**6** belong to the same *neo*-cleordane series as other diterpenoids isolated from *Scutellaria* spp., whose absolute configuration was established by X-ray diffraction analysis or CD exciton chirality methods.[14]

Compounds (**1**−**6**) were evaluated for their cytotoxic activities against HONE-1, KB, and HT29 cancer cell lines by using the methylene blue dye assay and anticancer drugs, etoposide and cisplatin,[15,16] as positive controls. These new *neo*-clerodane diterpenoids exhibited significant cytotoxicity, as shown in Table 2.

Experimental Section

General Experimental Procedures. Melting points were measured on an XT-4 micromelting 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. FABMS and HRFABMS were recorded on an Autospec-Ultima ETOF MS spectrometer. NMR spectra were recorded on a Varian Unity Bruker 400 at 400 MHz (^1H) and 100 MHz (^{13}C), with TMS as the internal standard. HPLC separation was performed on a CONSTA METRIC 3200 and a UV detector at 254 nm. 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. Precoated plates of silica gel GF254 and silica gel RP-18 F254s (Merck) were used for TLC, with detection under UV light.

Plant Material. *S. barbata* was collected in the Zhumadian district, Henan Province, People's Republic of China, in September 2008, and identified by Professor Yan-yan Zhao, School of Pharmaceutical Science, Yantai University. The whole plant of *S. barbata* was harvested and air-dried at room temperature in the dark. A voucher specimen (YP08077) has been deposited at the Herbarium of School of Pharmaceutical Science, Yantai University.

Extraction and Isolation. The air-dried whole plant (49.6 kg) was finely cut and extracted three times with refluxing EtOH. The solvent was removed under reduced pressure, and the resulting extract was suspended in H_2O and partitioned with $CHCl_3$ and EtOAc. The $CHCl_3$ fraction (483.7 g) was subjected to extraction with 3% HCl. Following this, the aqueous solution was adjusted with NH_4OH to pH 10 and extracted with $CHCl_3$. The organic fractions were combined, and the solvent was evaporated under vacuum to yield an alkaloidal fraction (90.2 g). This fraction was initially subjected to column chromatography on silica gel, eluted with cyclohexane−acetone (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, and 50:50) to give eight fractions. Fraction 5 (4.9 g) was separated by reversed-phase silica gel (150 g, 40−50 μm) CC [eluted by $MeOH-H_2O$, 55:45, v/v], giving **6** (83 mg) and a mixture (253 mg). The mixture was further separated by preparative TLC [$CHCl_3-MeOH-CH_3COCH_3$, 8:0.5:0.5, v/v] to afford **4** (116 mg) and **5** (82 mg) and subsequently purified on Sephadex LH-20 [100 g, eluting with $CHCl_3-CH_3OH$, 10:40, v/v] to give **4** (108 mg) and **5** (79 mg). Fraction 6 (5.1 g) was separated by reversed-phase silica gel (150 g, 40−50 μm) CC [eluted by $MeOH-H_2O$, 55:45, v/v], giving **1** (167 mg) and a mixture (158 mg). The mixture was further separated by semipreparative HPLC (Alltech C-18, 250 × 10 mm, eluted with $MeOH-CH_3CN-H_2O$, 20:20:60) to give **3** (54 mg) and **2** (91 mg).

Scutehenanine A (1): white needles (acetone), mp 148−149 °C, $[\alpha]^{29}_D$ −110.7 (*c* 0.14, in MeOH); UV ($CHCl_3$) λ_{max} 222, 258 nm; IR

Table 1. ^{13}C NMR Data of Compounds **1−6** (100 MHz, in $CDCl_3$)[a]

position	1	2	3	4	5	6
1	19.4 t	19.3 t	19.2 t	28.5 t	22.4 t	19.5 t
2	26.2 t	26.2 t	26.0 t	32.8 t	28.5 t	25.8 t
3	122.4 d	123.3 d	123.4 d	120.4 d	32.6 t	123.7 d
4	142.4 s	140.7 s	140.4 s	143.5 s	154.2 s	141.4 s
5	44.0 s	43.1 s	43.3 s	43.9 s	45.1 s	43.2 s
6	74.1 d	74.9 d	77.4 d	77.2 d	76.1 d	75.3 d
7	78.9 d	76.8 d	76.9 d	74.7 d	74.5 d	77.2 d
8	76.7 s	77.2 s	76.4 s	82.2 s	84.9 s	78.4 s
9	48.4 s	48.4 s	48.4 s	38.4 s	44.3 s	47.6 s
10	42.3 d	42.6 d	42.5 d	43.5 d	43.3 d	40.8 d
11	147.2 d	147.0 d	146.7 d	71.1 d	74.7 d	75.4 d
12	121.7 d	121.9 d	122.0 d	29.5 t	29.2 t	28.8 t
13	162.2 s	162.2 s	162.1 s	76.2 s	77.7 s	130.1 s
14	114.8 d	114.9 d	115.0 d	42.2 t	42.7 t	138.7 s
15	174.1 s	174.0 s	174.0 s	174.5 s	173.9 s	171.0 s
16	70.7 t	70.7 t	70.7 t	79.7 t	78.9 t	69.9 t
17	22.6 q	22.3 q	22.2 q	21.4 q	21.1 q	22.0 q
18	22.0 q	20.0 q	20.2 q	20.5 q	104.7 t	20.6 q
19	16.1 q	17.3 q	17.3 q	16.4 q	17.0 q	17.3 q
20	15.4 q	15.4 q	15.3 q	20.3 q	19.9 q	16.3 q
1′	165.4 s	164.5 s	164.2 s	165.6 s	165.0 s	164.1 s
2′	126.0 s	125.3 s	125.4 s	126.5 s	125.6 s	125.7 s
3′	150.6 d	150.8 d	150.9 d	150.8 d	150.6 d	150.3 d
5′	153.4 d	153.4 d	153.6 d	153.4 d	153.7 d	152.1 d
6′	123.7 d	123.3 d	123.7 d	123.5 d	123.5 d	123.3 d
7′	137.8 d	137.6 d	138.1 d	137.4 d	137.3 d	138.5 d
1″			161.9 s	165.9 s	167.6 s	166.0 s
2″			197.4 s	130.0 s	130.3 s	129.8 s
3″			36.7 d	129.4 d	129.7 d	129.4 d
4″			16.7 q	128.6 d	128.3 d	128.3 d
5″			16.9 q	133.4 d	133.0 d	133.1 d
6″				128.6 d	128.3 d	128.3 d
7″				129.4 d	129.7 d	129.4 d
OAc		170.6 s 21.4 q				

[a] The assignments were based on DEPT, HMQC, HMBC, and $^1H-^1H$ COSY experiments.

(KBr) ν_{max} 3348, 1729, 1635, 1590, 1493, 1470, and 1412 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.35 (1H, m, H_a-1), 1.63 (1H, m, H_b-1), 2.06 (2H, m, H-2), 5.19 (1H, br s, H-3), 4.18 (1H, d, J = 10.2 Hz, H-6), 5.47 (1H, d, J = 10.2 Hz, H-7), 2.23 (1H, dd, J = 2.0, 12.8 Hz, H-10), 6.42 (1H, d, J = 16.9 Hz, H-11), 6.36 (1H, d, J = 16.9 Hz, H-12), 5.92 (1H, br s, H-14), 4.98 (1H, dd, J = 1.3, 16.4 Hz, H_a-16), 5.01 (1H, dd, J = 1.3, 16.4, H_b-16), 1.09 (3H, s, H-17), 1.84 (3H, s, H-18), 1.23 (3H, s, H-19), 1.21 (3H, s, H-20), 9.30 (1H, br s, H-3′), 8.78 (1H, br d, J = 4.6 Hz, H-5′), 7.45 (1H, dd, J = 4.6, 7.8 Hz, H-6′), 8.37 (1H, br d, J = 7.8 Hz, H-7′); ^{13}C NMR data, see Table 1; FABMS m/z 454.4 $[M + H]^+$; HRFABMS m/z 454.2233 $[M + H]^+$ (calcd for $C_{26}H_{32}NO_6$ 454.2230).

6-*O*-Acetylscutehenanine A (2): white needles (acetone), mp 148−150 °C, $[\alpha]^{29}_D$ −113.2 (c 0.13, in MeOH); UV ($CHCl_3$) λ_{max} 221, 257 nm; IR (KBr) ν_{max} 3341, 1777, 1728, 1630, 1590, 1498, 1455, and 1412 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.38 (1H, m, H_a-1), 1.67 (1H, m, H_b-1), 2.07 (2H, m, H-2), 5.23 (1H, br s, H-3), 5.66 (1H, d, J = 10.3 Hz, H-6), 5.58 (1H, d, J = 10.3 Hz, H-7), 2.33 (1H, dd, J = 1.9, 12.7 Hz, H-10), 6.43 (1H, d, J = 16.8 Hz, H-11), 6.39 (1H, d, J = 16.8 Hz, H-12), 5.94 (1H, br s, H-14), 4.98 (1H, dd, J = 1.2, 16.9 Hz, H_a-16), 5.02 (1H, dd, J = 1.2, 16.9, H_b-16), 1.05 (3H, s, H-17), 1.59 (3H, s, H-18), 1.46 (3H, s, H-19), 1.24 (3H, s, H-20), 9.07 (1H, br s, H-3′), 8.69 (1H, br d, J = 4.7 Hz, H-5′), 7.27 (1H, dd, J = 4.7, 7.8 Hz, H-6′), 8.11 (1H, br d, J = 7.8 Hz, H-7′), 1.79 (3H, s, −OAc); ^{13}C NMR data, see Table 1; FABMS m/z 496.3 $[M + H]^+$; HRFABMS m/z 496.2340 $[M + H]^+$ (calcd for $C_{28}H_{34}NO_7$, 496.2335).

6-*O*-(2-Carbonyl-3-methylbutanoyl)scutehenanine A (3): white needles (acetone), mp 150−152 °C, $[\alpha]^{29}_D$ −111.6 (c 0.13, in MeOH); UV ($CHCl_3$) λ_{max} 220, 258 nm; IR (KBr) ν_{max} 3340, 1769, 1735, 1628, 1500, and 1405 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.37 (1H, m, H_a-1), 1.68 (1H, m, H_b-1), 2.06 (2H, m, H-2), 5.26 (1H, br s, H-3), 5.79 (1H, d, J = 10.0 Hz, H-6), 5.67 (1H, d, J = 10.0 Hz, H-7), 2.36 (1H, dd, J = 2.0, 12.8 Hz, H-10), 6.45 (1H, d, J = 16.8 Hz, H-11), 6.39 (1H, d, J = 16.8 Hz, H-12), 5.94 (1H, br s, H-14), 4.98 (1H, dd, J = 1.4, 16.6 Hz, H_a-16), 5.01 (1H, dd, J = 1.4, 16.6, H_b-16), 1.06 (3H, s, H-17), 1.60 (3H, s, H-18), 1.37 (3H, s, H-19), 1.25 (3H, s, H-20), 9.21 (1H, br s, H-3′), 8.80 (1H, br d, J = 4.7 Hz, H-5′), 7.42 (1H, dd, J = 4.7, 7.7 Hz, H-6′), 8.29 (1H, br d, J = 7.7 Hz, H-7′), 2.92 (1H, m, H-3″), 0.95 (3H, d, J = 5.5 Hz, H-4″), 0.79 (3H, d, J = 5.5 Hz, H-5″); ^{13}C NMR data, see Table 1; FABMS m/z 552.5 $[M + H]^+$; HRFABMS m/z 552.2593 $[M + H]^+$ (calcd for $C_{31}H_{38}NO_8$ 552.2597).

Table 2. Cytotoxicity of Compounds **1−6** against Cultured HONE-1, KB, and HT29 Cancer Cell Lines

	growth inhibition constant (IC_{50})[a] [μM]		
compound	HONE-1	KB	HT29
etoposide[b]	1.2 ±0.8	1.3 ±0.7	2.3 ±0.8
cisplatin[b]	2.4 ±0.9	2.7 ±1.1	3.1 ±0.9
1	3.1 ±2.2	4.5 ±2.0	5.8 ±1.3
2	3.7 ±1.9	3.2 ±2.8	5.2 ±1.6
3	4.0 ±1.6	4.2 ±2.0	5.5 ±2.1
4	3.5 ±2.0	4.7 ±1.3	5.0 ±1.1
5	3.4 ±1.4	2.9 ±2.0	5.3 ±2.7
6	4.1 ±2.2	2.8 ±1.3	6.4 ±2.7

[a] IC_{50} is mean ± standard deviation of three independent replicates. An IC_{50} greater than 10 μM was considered to indicate no cytotoxicity. [b] Positive control substance.

Scutehenanine B (4): white needles (acetone), mp 150−151 °C, $[\alpha]^{29}_D$ −68.7 (c 0.14, in MeOH); UV ($CHCl_3$) λ_{max} 222, 258 nm; IR (KBr) ν_{max} 3387, 1740, 1631, 1597, 1500, and 1459 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.63 (1H, m, H_a-1), 2.06 (1H, m, H_b-1), 2.74 (2H, m, H-2), 5.38 (1H, br s, H-3), 5.44 (1H, d, J = 9.9 Hz, H-6), 3.70 (1H, d, J = 9.9 Hz, H-7), 2.71 (1H, dd, J = 2.3, 12.2 Hz, H-10), 5.78 (1H, dd, J = 3.5, 12.2, H-11), 1.61 (1H, m, H_a-12), 2.15 (1H, m, H_b-12), 2.59 (1H, d, J = 17.0 Hz, H_a-14), 2.75 (1H, d, J = 17.0 Hz, H_b-14), 4.25 (1H, d, J = 8.8 Hz, H_a-16), 4.34 (1H, d, J = 8.8 Hz, H_b-16), 1.10 (3H, s, H-17), 1.69 (3H, s, H-18), 1.45 (3H, s, H-19), 1.41 (3H, s, H-20), 9.28 (1H, br s, H-3′), 8.81 (1H, br d, J = 4.6 Hz, H-5′), 7.43 (1H, dd, J = 4.6, 7.8 Hz, H-6′), 8.34 (1H, br d, J = 7.8 Hz, H-7′), 7.97 (2H, m, H-3″ and H-7″), 7.47 (2H, m, H-4″ and H-6″), 7.60 (1H, br t, J = 7.9 Hz, H-5″); ^{13}C NMR data, see Table 1; FABMS m/z 576.3 $[M + H]^+$; HRFABMS m/z 576.2592 $[M + H]^+$ (calcd for $C_{33}H_{38}NO_8$ 576.2597).

Scutehenanine C (5): white needles (acetone), mp 150−152 °C, $[\alpha]^{29}_D$ −65.7 (c 0.13, in MeOH); UV ($CHCl_3$) λ_{max} 222, 257 nm; IR (KBr) ν_{max} 3399, 3090, 1738, 1630, 1600, 1504, 1466, and 1002 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.84 (1H, m, H_a-1), 2.43 (1H, m, H_b-1), 1.45 (1H, m, H_a-2), 2.06 (1H, m, H_b-2), 2.12 (1H, m, H_a-3), 2.28 (1H, m, H_b-3), 5.65 (1H, d, J = 10.3 Hz, H-6), 3.75 (1H, d, J = 10.3 Hz, H-7), 2.25 (1H, dd, J = 2.1, 12.2 Hz, H-10), 5.69 (1H, dd, J = 3.6, 12.4, H-11), 1.73 (1H, m, H_a-12), 2.09 (1H, m, H_b-12), 2.85 (1H, d, J = 17.0 Hz, H_a-14), 2.89 (1H, d, J = 17.0 Hz, H_b-14), 4.23 (1H, d, J = 9.0 Hz, H_a-16), 4.38 (1H, d, J = 9.0 Hz, H_b-16), 1.13 (3H, s, H-17), 4.60 (2H, br s, H-18), 1.42 (3H, s, H-19), 1.58 (3H, s, H-20), 9.21 (1H, br s, H-3′), 8.83 (1H, br d, J = 4.8 Hz, H-5′), 7.48 (1H, dd, J = 4.8, 7.8 Hz, H-6′), 8.30 (1H, br d, J = 7.8 Hz, H-7′), 8.01 (2H, m, H-3″ and H-7″), 7.44 (2H, m, H-4″ and H-6″), 7.55 (1H, br t, J = 7.8 Hz, H-5″); ^{13}C NMR data, see Table 1; FABMS m/z 576.2 $[M + H]^+$; HRFABMS m/z 576.2597 $[M + H]^+$ (calcd for $C_{33}H_{38}NO_8$ 576.2592).

Scutehenanine D (6): white needles (acetone), mp 154−155 °C, $[\alpha]^{29}_D$ −79.6 (c 0.13, in MeOH); UV ($CHCl_3$) λ_{max} 223, 259 nm; IR (KBr) ν_{max} 3440, 1771, 1729, 1635, 1600, 1514, and 1455 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.83 (1H, m, H_a-1), 2.09 (1H, m, H_b-1), 2.18 (2H, m, H-2), 5.29 (1H, br s, H-3), 5.82 (1H, d, J = 10.4 Hz, H-6), 5.70 (1H, d, J = 10.4 Hz, H-7), 2.52 (1H, dd, J = 1.8, 12.6 Hz, H-10), 5.58 (1H, br d, J = 10.4 Hz, H-11), 2.71 (1H, dd, J = 10.4, 13.7 Hz, H_a-12), 3.39 (1H, br d, J = 13.7 Hz, H_b-12), 4.51 (1H, d, J = 16.5 Hz, H_a-16), 4.75 (1H, d, J = 16.5 Hz, H_b-16), 1.34 (3H, s, H-17), 1.59 (3H, s, H-18), 1.46 (3H, s, H-19), 1.02 (3H, s, H-20), 9.05 (1H, br s, H-3′), 8.67 (1H, br d, J = 4.7 Hz, H-5′), 7.22 (1H, dd, J = 4.7, 7.7 Hz, H-6′), 8.06 (1H, br d, J = 7.7 Hz, H-7′), 7.37 (2H, m, H-3″ and H-7″), 7.24 (2H, m, H-4″ and H-6″), 7.41 (1H, br t, J = 7.9 Hz, H-5″); ^{13}C NMR data, see Table 1; FABMS m/z 574.4 $[M + H]^+$; HRFABMS m/z 574.2438 $[M + H]^+$ (calcd for $C_{33}H_{36}NO_8$ 574.2441).

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 compounds for 72 h. The methylene blue dye assay was used to evaluate the effects of the compounds on cell growth, as described previously.[17] The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically as a comparison with the control.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Paton, A. In *Advances in Labiatae Science*; Harley, R. M., Reynolds, T., Eds.; Royal Botanic Gardens: Kew, 1992; pp 203−210.
(2) Wong, B. Y.; Lau, B. H.; Yamasaki, T.; Teel, R. W. *Cancer Lett.* **1993**, *68*, 75–82.
(3) Rugo, H.; Shtivelman, E.; Perez, A.; Vogel, C.; Franco, S.; Melisko, M.; Tagliaferri, M.; Cohen, I.; Shoemaker, M.; Tran, Z.; Tripathy, D. *Breast Cancer Res. Treat* **2007**, *105*, 17–28.
(4) Suh, S. J.; Yoon, J. W.; Lee, T. K.; Jin, U. H.; Kim, S. L.; Kim, M. S.; Kwon, D. Y.; Lee, Y. C.; Kim, C. H. *Phytother. Res.* **2007**, *21*, 135–141.
(5) Wang, Z. Q.; Xu, F. M.; Yan, X. Z.; Zhu, Y. *Chin. Chem. Lett.* **1996**, *4*, 333–334.
(6) Dai, S. J.; Tao, J. Y.; Liu, K.; Jiang, Y. T.; Shen, L. *Phytochemistry* **2006**, *67*, 1326–1330.
(7) Dai, S. J.; Wang, G. F.; Chen, M.; Liu, K.; Shen, L. *Chem. Pharm. Bull.* **2007**, *55*, 1218–1221.
(8) Dai, S. J.; Sun, J. Y.; Ren, Y.; Liu, K.; Shen, L. *Planta Med.* **2007**, *73*, 1217–1220.
(9) Dai, S. J.; Liang, D. D.; Ren, Y.; Liu, K.; Shen, L. *Chem. Pharm. Bull.* **2008**, *56*, 207–209.
(10) Rodriguez-Hahn, L.; Esquivel, B.; Cardenas, J. *Prog. Chem. Org. Nat. Prod.* **1994**, *63*, 107–196.
(11) Malakov, P. Y.; Papanov, G. Y. *Phytochemistry* **1996**, *43*, 173–178.
(12) Malakov, P. Y.; Papanov, G. Y.; Spassov, S. L. *Phytochemistry* **1997**, *44*, 121–124.
(13) Bruno, M.; Piozzi, F.; Rosselli, S. *Nat. Prod. Rep.* **2002**, *19*, 357–378.
(14) Esquivel, B.; Dominguez, R. M.; Martinez, M. A.; Espinosa-Perez, G. *Heterocycles* **1997**, *45*, 2247–2252.
(15) Chang, J. Y.; Liu, J. F.; Juang, S. H.; Liu, T. W.; Chen, L. T. *Cancer Res.* **2002**, *62*, 3716–3721.
(16) Chang, C. I.; Kuo, C. C.; Chang, J. Y.; Kuo, Y. H. *J. Nat. Prod.* **2004**, *67*, 91–93.
(17) Chang, J. Y.; Chang, C. Y.; Kuo, C. C.; Chen, L. T.; Wei, Y. S.; Kuo, Y. H. *Mol. Pharmacol.* **2004**, *65*, 77–84.

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