

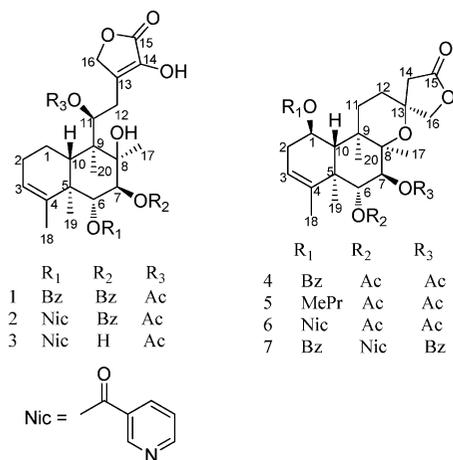
Cytotoxic Neoclerodane Diterpenoids from *Scutellaria barbata*Feng Zhu,<sup>†,‡</sup> Ying-Tong Di,<sup>†</sup> Ling-Li Liu,<sup>†</sup> Qiang Zhang,<sup>†</sup> Xin Fang,<sup>†</sup> Ting-Quan Yang,<sup>†</sup> Xiao-Jiang Hao,<sup>\*,†</sup> and Hong-Ping He<sup>\*,†</sup>

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, People's Republic of China, and Graduate University of the Chinese Academy of Sciences, Beijing 100049, Beijing, People's Republic of China

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Seven new neoclerodane diterpenoids, scutebatas A–G (**1**–**7**), have been isolated from *Scutellaria barbata*. Compounds **1**–**3** possess a rare  $\alpha$ -hydroxy group in their  $\alpha,\beta$ -unsaturated lactone rings. Their structures were elucidated by spectroscopic analysis, and the relative configuration of scutebata A was deduced using ROESY data and the computational DFT method. Compounds **1**, **2**, **4**, **5**, and **6** were evaluated for *in vitro* cytotoxicity against six human cancer cell lines: HL-60, SMMC-7721, A-549, SK-BR-3, CACO-2, and PANC-1. Scutebata A (**1**) showed weak cytotoxicity against SK-BR-3 with an  $IC_{50}$  value of 15.2  $\mu$ M.

*Scutellaria* is a cosmopolitan genus of the Lamiaceae (or Labiatae) family.<sup>1</sup> The use of species belonging to this genus in Chinese traditional medicine has a long history. For example, dried whole plants of *Scutellaria barbata* D. Don, named “Ban zhi lian” in Chinese, are commonly used in folk medicines to treat tumors, hepatitis, cirrhosis, and other diseases.<sup>2–4</sup> In recent years, several cytotoxic neoclerodane diterpenoids have been isolated from *S. barbata*.<sup>5–9</sup> With the aim of discovering compounds with potential antitumor properties, we initiated a phytochemical study of the aerial parts of *S. barbata*, which led to the isolation of seven new neoclerodane diterpenoids, scutebatas A–G (**1**–**7**). Importantly, scutebatas A–C (**1**–**3**) were shown to possess a rare  $\alpha$ -hydroxy group in their  $\alpha,\beta$ -unsaturated lactone rings. In this paper, we describe the isolation and structural identification of scutebatas A–G and the *in vitro* cytotoxic activities of compounds **1**, **2**, **4**, **5**, and **6** against six human cancer cell lines: HL-60, SMMC-7721, A-549, SK-BR-3, CACO-2, and PANC-1.



Scutebata A (**1**), obtained as white powder, had the molecular formula  $C_{36}H_{40}O_{10}$  according to its HRESIMS at  $m/z$  655.2527 [ $M + Na$ ]<sup>+</sup> (calcd 655.2519). Its IR data exhibited absorption bands for a hydroxy ( $3431\text{ cm}^{-1}$ ), ester carbonyls ( $1727, 1762\text{ cm}^{-1}$ ), an olefinic bond ( $1638\text{ cm}^{-1}$ ), and phenyl ( $1604, 1026, 710\text{ cm}^{-1}$ ) groups. In addition to the signals of one acetoxy and two benzoyloxy

groups, the  $^{13}C$  NMR spectrum revealed 20 carbon resonances, which were classified into seven quaternary carbons ( $4 \times sp^2, 3 \times sp^3$ ), five methines ( $1 \times sp^2, 4 \times sp^3$ ), four  $sp^3$  methylenes, and four methyl groups. The  $^1H$  NMR spectrum of compound **1** showed signals of four methyl singlets [ $\delta_H$  0.89 (s); 1.13 (s); 1.38 (s); 1.50 (s)], a broad singlet at  $\delta_H$  5.27 (1H), an AB system [ $\delta_H$  5.63 (d,  $J = 10.2$  Hz), 5.53 (d,  $J = 10.2$  Hz)], and an ABX system [ $\delta_H$  5.37 (d,  $J = 10.8$  Hz), 2.74 (dd,  $J = 13.8, 10.8$  Hz), 3.22 (d,  $J = 13.8$  Hz)]. Comparison of  $^1H$  and  $^{13}C$  NMR spectra of compound **1** (Table 1) with scuteulein A<sup>10</sup> suggested that compound **1** is a neoclerodane diterpenoid with an oxygenated substituent at C-11, which was confirmed by analysis of the 2D NMR data. HMBC correlations of H-6/C-1', H-7/C-1'', and H-11/C-1''' also established that benzoyloxy groups were present at C-6 and C-7 and that the acetoxy group was present at C-11. Furthermore, the HMBC correlations (Figure 1) between H<sub>2</sub>-16 and C-13 (129.9 s), C-14 (138.3 s), and C-15 (169.7 s) indicated the presence of an  $\alpha,\beta$ -unsaturated lactone ring with a hydroxy group at C-14.

The ROESY correlations of H-7/H<sub>3</sub>-17, H<sub>3</sub>-19, and H<sub>3</sub>-20 indicated that they were cofacial and  $\alpha$ -orientated, whereas the ROESY correlation of H-10/H-6 indicated that they were on the opposite face and  $\beta$ -oriented. The relative configuration of C-11 was deduced from the ROESY experiment and molecular modeling (Gaussian 03 D.01)<sup>11</sup> using *ab initio* calculations. The ROESY correlations of H-11/H<sub>3</sub>-17, H<sub>3</sub>-20, and H<sub>b</sub>-16 suggested that in the preferred conformation of the pendant chain, H-11 was close to these groups. DFT calculations applied to compound **1** indicated that, in the minimum energy conformation, the calculated interatomic distances were H-11...Me-20 = 2.66 Å, H-11...Me-17 = 1.96 Å, and H-11...H<sub>b</sub>-16 = 2.93 Å, which accounts for the cross-peaks observed in the ROESY spectrum and indicated that compound **1** had an 11*S*\* relative configuration. Together, these results showed that compound **1** was 11(*S*\*)-11-acetoxy-6 $\alpha$ ,7 $\beta$ -dibenzoyloxy-8 $\beta$ ,14-dihydroxy-3,13(14)-neocleroden-15,16-olide.

Scutebata B (**2**), obtained as a white, amorphous powder, had a molecular formula of  $C_{35}H_{39}NO_{10}$  according to its HRESIMS at  $m/z$  656.2470 [ $M + Na$ ]<sup>+</sup> (calcd 656.2471). Comparison of the  $^1H$  and  $^{13}C$  NMR spectra of compounds **2** and **1** showed that they were closely related, with the exception that one benzoyloxy group in compound **1** was replaced by one nicotinoyloxy group in compound **2**. The  $^1H$ – $^1H$  COSY, HMQC, HMBC, and ROESY data further confirmed that the structure of compound **2** was 11(*S*\*)-11-acetoxy-6 $\alpha$ -nicotinoyloxy-7 $\beta$ -benzoyloxy-8 $\beta$ ,14-dihydroxy-3,13(14)-neocleroden-15,16-olide.

The molecular formula of scutebata C (**3**) was found to be  $C_{28}H_{35}NO_9$  according to its HRESIMS at  $m/z$  552.2212 [ $M + Na$ ]<sup>+</sup>

\* To whom correspondence should be addressed. Tel: +86-871-5223263. Fax: +86-871-5223070. E-mail: haoxj@mail.kib.ac.cn; hehongping@mail.kib.ac.cn.

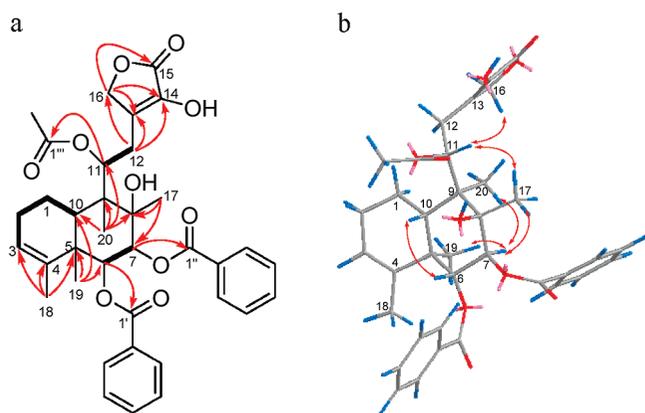
<sup>†</sup> Kunming Institute of Botany.

<sup>‡</sup> Graduate University of the Chinese Academy of Sciences.

**Table 1.**  $^1\text{H}$  NMR Data of Scutebatas A–G (1–7) ( $\delta$  in ppm,  $J$  in Hz)

no.	1 <sup>b</sup>	2 <sup>b</sup>	3 <sup>b</sup>	4 <sup>c</sup>	5 <sup>c</sup>	6 <sup>c</sup>	7 <sup>c</sup>
1 $\alpha$	1.76, m	1.76, m	1.69, m	5.76, dt (9.6, 6.2)	5.40, dt (9.6, 6.2)	5.76, dt (9.6, 6.2)	5.84, dt (9.6, 6.1)
1 $\beta$	2.05, <sup>a</sup>	2.05, <sup>a</sup>	2.00, <sup>a</sup>				
2 $\alpha$	2.09, <sup>a</sup>	2.10, <sup>a</sup>	2.07, <sup>a</sup>	2.71, m	2.59, m	2.68, <sup>a</sup>	2.77, m
2 $\beta$	2.17, <sup>a</sup>	2.18, <sup>a</sup>	2.13, <sup>a</sup>	2.17, m	1.97, <sup>a</sup>	2.15, m	2.23, m
3	5.27, br s	5.29, br s	5.23, br s	5.31, br s	5.28, br s	5.29, br s	5.36, br s
6	5.63, d (10.2)	5.65, d (10.2)	5.20, d (10.2)	5.41, d (10.2)	5.36, d (10.2)	5.38, d (10.2)	5.88, d (10.2)
7	5.53, d (10.2)	5.54, d (10.2)	3.69, d (10.2)	5.25, d (10.2)	5.22, d (10.2)	5.22, d (10.2)	5.66, d (10.2)
10	2.35, d (12.0)	2.35, d (12.0)	2.21, d (12.0)	2.72, d (9.6)	2.53, d (9.6)	2.70, d (9.6)	2.87, d (9.6)
11 $\alpha$	5.37, br d (10.8)	5.37, br d (10.8)	5.36, br d (10.8)	1.56, m	1.59, <sup>a</sup>	1.58, m	1.64, <sup>a</sup>
11 $\beta$				2.07, <sup>a</sup>	2.02, <sup>a</sup>	2.01, <sup>a</sup>	2.16, <sup>a</sup>
12 $\alpha$	2.74, dd (13.8, 10.8)	2.73, dd (13.8, 10.8)	2.69, dd (13.8, 10.8)	1.68, <sup>a</sup>	1.70, <sup>a</sup>	1.68, <sup>a</sup>	1.74, m
12 $\beta$	3.22, br d (13.8)	3.22, br d (13.8)	3.15, br d (13.8)	2.09, <sup>a</sup>	1.98, <sup>a</sup>	2.03, <sup>a</sup>	2.17, <sup>a</sup>
14 $\alpha$				2.57, d (17.2)	2.53, d (17.4)	2.54, d (17.2)	2.68, d (17.2)
14 $\beta$				3.14, d (17.2)	3.10, d (17.4)	3.09, d (17.2)	3.15, d (17.2)
16 $\alpha$	4.59, d (16.4)	4.59, d (16.4)	4.57, d (16.4)	4.12, d (8.6)	4.12, d (8.6)	4.10, d (8.6)	4.12, d (8.9)
16 $\beta$	4.67, d (16.4)	4.67, d (16.4)	4.64, d (16.4)	4.19, d (8.6)	4.22, d (8.6)	4.16, d (8.6)	4.20, d (8.9)
17	1.13, 3H, s	1.13, 3H, s	1.20, 3H, s	1.12, 3H, s	1.12, 3H, s	1.11, 3H, s	1.20, 3H, s
18	1.50, 3H, s	1.51, 3H, s	1.52, 3H, s	1.67, 3H, s	1.63, 3H, s	1.65, 3H, s	1.68, 3H, s
19	1.38, 3H, s	1.38, 3H, s	1.28, 3H, s	1.36, 3H, s	1.29, 3H, s	1.34, 3H, s	1.57, 3H, s
20	0.89, 3H, s	0.89, 3H, s	0.78, 3H, s	1.09, 3H, s	1.05, 3H, s	1.07, 3H, s	1.20, 3H, s
2'					2.24, m		
3'	7.66, m	8.76, d (1.6)	9.15, br s	7.95, br d (7.2)	1.12, 3H, d (7.0)	9.12, d (1.7)	7.99, m
4'	7.34, m			7.46, br t (7.2)	1.14, 3H, d (7.0)		7.31, m
5'	7.50, m	8.66, dd (4.8, 1.6)	8.85, d (5.0)	7.59, br t (7.2)		8.78, br d (4.9)	7.47, m
6'	7.34, m	7.39, <sup>a</sup>	7.63, dd (7.9, 5.0)	7.46, br t (7.2)		7.40, dd (4.9, 7.9)	7.31, m
7'	7.66, m	7.97, dt (8.0, 1.6)	8.37, br d (7.9)	7.95, br d (7.2)		8.18, br d (7.9)	7.99, m
2''				2.01, 3H, s	1.99, 3H, s	1.99, 3H, s	
3''	7.82, m	7.81, m					8.92, d (1.9)
4''	7.34, m	7.36, <sup>a</sup>					
5''	7.50, m	7.54, m					8.61, d (4.8)
6''							7.19, m
7''							7.96, m
2'''	1.99, 3H, s	2.00, 3H, s	1.96, 3H, s	2.09, 3H, s	2.08, 3H, s	2.06, 3H, s	
3'''	7.82, m						7.82, m
4'''	7.49, m						7.49, m
5'''	7.61, br t						7.61, br t

<sup>a</sup> Overlapped, without denoting multiplicity. <sup>b</sup> In DMSO- $d_6$ . <sup>c</sup> In CDCl<sub>3</sub>.



**Figure 1.** (a)  $^1\text{H}$ – $^1\text{H}$  COSY (—) and selected HMBC (→) correlations of **1**. (b) ROESY (↔) correlations of **1**.

(calcd 552.2209 for C<sub>28</sub>H<sub>35</sub>NO<sub>9</sub>Na). Its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra showed a similar signal pattern to those of compound **2**, with the exception that a benzyloxy group was absent in compound **2**. The 2D NMR data established that compound **3** was 11(*S*\*)-11-acetoxy-6 $\alpha$ -nicotinoyloxy-7 $\beta$ -hydroxy-8 $\beta$ ,14-dihydroxy-3,13(14)-neocleroden-15,16-olide.

Scutebata D (**4**) was assigned the molecular formula C<sub>31</sub>H<sub>38</sub>O<sub>9</sub> from its HRESIMS, which displayed a quasi-molecular ion at  $m/z$  577.2431 [ $M + \text{Na}$ ]<sup>+</sup> (calcd 577.2413). Its IR data showed absorptions for a  $\gamma$ -spirolactone (1787 cm<sup>-1</sup>), ester carbonyls (1749, 1720, 1710 cm<sup>-1</sup>), an olefinic bond (1638, 1629 cm<sup>-1</sup>), and a phenyl group (1026, 716 cm<sup>-1</sup>). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Tables 1 and 2) exhibited one benzyloxy and two acetoxy groups. In addition, the 1D NMR data showed signals of a neoclerodane diterpenoid

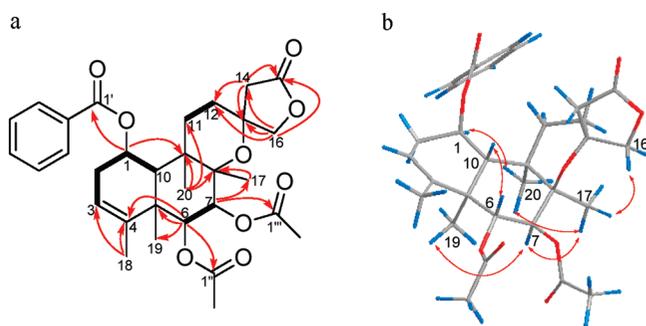
with a 3-en- $\gamma$ -13-spiro-15,16-lactone moiety [ $\delta_{\text{H}}$  1.09 (s, H<sub>3</sub>-20), 1.12 (s, H<sub>3</sub>-17), 1.36 (s, H<sub>3</sub>-19), 1.67 (s, H<sub>3</sub>-18), 5.31 (br s, H-3), 2.57 (d,  $J = 17.3$  Hz, H $_{\alpha}$ -14), 3.14 (d,  $J = 17.3$  Hz, H $_{\beta}$ -14), 4.12 (d,  $J = 8.6$  Hz, H $_{\alpha}$ -16), 4.19 (d,  $J = 8.6$  Hz, H $_{\beta}$ -16),  $\delta_{\text{C}}$  21.1 (q, C-20), 19.6 (q, C-17), 16.6 (q, C-19), 20.0 (q, C-18), 120.2 (d, C-3), 143.1 (s, C-4), 44.3 (t, C-14), 76.5 (t, C-16)].<sup>4,6</sup> Detailed analysis of the 2D NMR data, including the HMQC,  $^1\text{H}$ – $^1\text{H}$  COSY, and HMBC data (Figure 2), confirmed the above findings. The locations of benzyloxy and acetoxy groups were determined by the HMBC correlations from H-1 ( $\delta$  5.76) to C-1', H-6 ( $\delta$  5.41) to C-1'', and H-7 ( $\delta$  5.25) to C-1''', respectively.

The relative configuration of compound **4** was deduced from its ROESY spectrum. The ROESY correlations of H-7/H<sub>3</sub>-17, H<sub>3</sub>-19, H<sub>3</sub>-20 and H-1/H<sub>3</sub>-19, H<sub>3</sub>-20 indicated that they were cofacial and  $\alpha$ -orientated, whereas the ROESY correlation of H-10/H-6 indicated that they were on the opposite face and  $\beta$ -orientated. The configuration of C-13 was determined to be *S*\* by the ROESY correlation between H<sub>2</sub>-16 and H<sub>3</sub>-17.<sup>12</sup> Thus, the structure of compound **4** was found to be 13(*S*\*)-1 $\beta$ -benzyloxy-6 $\alpha$ ,7 $\beta$ -diacetoxy-8 $\beta$ ,13-epoxy-3-neocleroden-15,16-olide.

A comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR data showed that scutebata E (**5**, C<sub>28</sub>H<sub>38</sub>O<sub>9</sub>) and F (**6**, C<sub>30</sub>H<sub>37</sub>NO<sub>9</sub>) possessed the same 1 $\beta$ ,6 $\alpha$ ,7 $\beta$ -trihydroxy-8 $\beta$ ,13-epoxy-3-neocleroden-15,16-olide structural framework as scutebata D (**4**). The only difference between these compounds was the presence of an ester substituent at C-1 in compounds **5** and **6**, which was corroborated by their HMBC correlations. The 1 $\beta$ -benzyloxy group in compound **4** was replaced by an isobutyroyloxy group in compound **5** [ $\delta_{\text{H}}$  1.12 (3H, d,  $J = 7.0$  Hz), 1.14 (3H, d,  $J = 7.0$  Hz), 2.24 (1H, m);  $\delta_{\text{C}}$  176.3 (s), 34.3 (d), 19.2 (q), 18.5 (q)] and a nicotinoyloxy group in compound **6** [ $\delta_{\text{H}}$  9.12 (d,  $J = 1.7$  Hz), 8.78 (br d,  $J = 4.9$  Hz), 7.40 (dd,  $J = 4.9, 7.9$  Hz), 8.18 (br d,  $J = 7.9$  Hz);  $\delta_{\text{C}}$  164.3 (s), 125.8 (s), 150.7

**Table 2.**  $^{13}\text{C}$  NMR Data of Scutebata A–G (1–7)

no.	1 <sup>a</sup>	2 <sup>a</sup>	3 <sup>a</sup>	4 <sup>b</sup>	5 <sup>b</sup>	6 <sup>b</sup>	7 <sup>b</sup>
1	18.9 CH <sub>2</sub>	18.9 CH <sub>2</sub>	18.8 CH <sub>2</sub>	70.8 CH	70.3 CH	71.6 CH	70.9 CH
2	25.4 CH <sub>2</sub>	25.4 CH <sub>2</sub>	25.4 CH <sub>2</sub>	33.0 CH <sub>2</sub>	32.8 CH <sub>2</sub>	33.0 CH <sub>2</sub>	33.1 CH <sub>2</sub>
3	123.2 CH	123.4 CH	122.7 CH	120.2 CH	120.1 CH	119.9 CH	120.5 CH
4	140.9 C	140.8 C	141.4 C	143.1 C	143.4 C	143.2 C	143.1 C
5	42.6 C	42.5 C	42.1 C	44.2 C	44.1 C	44.2 C	44.7 C
6	74.9 CH	75.7 CH	78.3 CH	73.2 CH	73.1 CH	73.0 CH	74.6 CH
7	75.3 CH	75.3 CH	72.2 CH	74.1 CH	74.0 CH	73.9 CH	74.5 CH
8	76.9 C	76.8 C	77.8 C	80.8 C	80.7 C	80.7 C	81.2 C
9	47.0 C	47.0 C	46.4 C	38.7 C	38.6 C	38.6 C	38.9 C
10	40.1 CH	40.1 CH	39.9 CH	43.1 CH	43.1 CH	43.0 CH	43.5 CH
11	75.1 CH	75.0 CH	75.3 CH	28.5 CH <sub>2</sub>	28.3 CH <sub>2</sub>	28.5 CH <sub>2</sub>	28.6 CH <sub>2</sub>
12	28.2 CH <sub>2</sub>	28.2 CH <sub>2</sub>	28.0 CH <sub>2</sub>	29.3 CH <sub>2</sub>	29.3 CH <sub>2</sub>	29.2 CH <sub>2</sub>	29.3 CH <sub>2</sub>
13	129.9 C	129.9 C	130.2 C	76.5 C	76.3 C	76.4 C	77.0 C
14	138.3 C	138.3 C	138.1 C	44.3 CH <sub>2</sub>	44.2 CH <sub>2</sub>	44.2 CH <sub>2</sub>	44.5 CH <sub>2</sub>
15	169.7 C	169.8 C	169.6 C	173.7 C	173.7 C	173.7 C	173.7 C
16	68.7 CH <sub>2</sub>	68.7 CH <sub>2</sub>	68.6 CH <sub>2</sub>	76.5 CH <sub>2</sub>	76.4 CH <sub>2</sub>	76.3 CH <sub>2</sub>	76.6 CH <sub>2</sub>
17	20.5 CH <sub>3</sub>	20.5 CH <sub>3</sub>	21.0 CH <sub>3</sub>	19.6 CH <sub>3</sub>	19.6 CH <sub>3</sub>	19.5 CH <sub>3</sub>	19.8 CH <sub>3</sub>
18	19.8 CH <sub>3</sub>	19.8 CH <sub>3</sub>	19.9 CH <sub>3</sub>	20.0 CH <sub>3</sub>	20.0 CH <sub>3</sub>	20.0 CH <sub>3</sub>	20.2 CH <sub>3</sub>
19	17.0 CH <sub>3</sub>	16.9 CH <sub>3</sub>	16.8 CH <sub>3</sub>	16.6 CH <sub>3</sub>	16.4 CH <sub>3</sub>	16.6 CH <sub>3</sub>	16.8 CH <sub>3</sub>
20	16.1 CH <sub>3</sub>	16.1 CH <sub>3</sub>	15.9 CH <sub>3</sub>	21.1 CH <sub>3</sub>	21.1 CH <sub>3</sub>	21.0 CH <sub>3</sub>	21.2 CH <sub>3</sub>
1'	165.2 C	164.2 C	164.4 C	165.6 C	176.3 C	164.3 C	165.7 C
2'	128.9 C	125.4 C	126.8 C	130.0 C	34.3 CH	125.8 C	128.9 C
3'	128.8 CH	149.6 CH	149.6 CH	129.4 CH	18.5 CH <sub>3</sub>	150.7 CH	129.5 CH
4'	128.1 CH			128.7 CH	19.2 CH <sub>3</sub>		128.3 CH
5'	133.1 CH	153.6 CH	152.9 CH	133.4 CH		153.8 CH	133.3 CH
6'	128.1 CH	123.7 CH	124.0 CH	128.7 CH		123.5 CH	128.3 CH
7'	128.8 CH	136.6 CH	137.2 CH	129.4 CH		136.7 CH	129.5 CH
1''	165.7 C	165.7 C		169.9 C	169.8 C	169.7 C	163.5 C
2''	129.8 C	128.7 C		21.5 CH <sub>3</sub>	21.4 CH <sub>3</sub>	21.4 CH <sub>3</sub>	125.9 C
3''	129.5 CH	129.4 CH					150.7 CH
4''	128.4 CH	128.2 CH					
5''	133.2 CH	133.4 CH					153.3 CH
6''	128.4 CH	128.2 CH					123.1 CH
7''	129.5 CH	129.4 CH					136.7 CH
1'''	170.6 C	170.6 C	170.4 C	170.9 C	170.9 C	170.8 C	166.3 C
2'''	20.6 CH <sub>3</sub>	20.7 CH <sub>3</sub>	20.6 CH <sub>3</sub>	20.8 CH <sub>3</sub>	20.8 CH <sub>3</sub>	20.7 CH <sub>3</sub>	130.0 C
3''', 7'''							129.8 CH
4''', 6'''							128.7 CH
5'''							133.5 CH

<sup>a</sup> In DMSO-*d*<sub>6</sub>. <sup>b</sup> In CDCl<sub>3</sub>.**Figure 2.** (a)  $^1\text{H}$ – $^1\text{H}$  COSY (—) and selected HMBC (→) correlations of **4**. (b) ROESY (↔) correlations of **4**.

(d), 153.8 (d), 123.5 (d), 136.7 (d)]. The ROESY data also showed that the relative configurations of compounds **4**, **5**, and **6** were identical.

Scutebata G (**7**) was assigned to the molecular formula  $\text{C}_{40}\text{H}_{41}\text{NO}_9$  by positive HRESIMS. Its NMR data were similar to those of compound **4**, with the exception that a nicotinoyloxy group and a benzyloxy group in compound **7** replaced two acetoxy groups in compound **4**. HMBC correlations of H-1 ( $\delta_{\text{H}}$  5.84)/C-1', H-6 ( $\delta_{\text{H}}$  5.88)/C-1'', C-19, and H-7 ( $\delta_{\text{H}}$  5.66)/C-1''' indicated that a nicotinoyloxy group is present at C-6 and that benzyloxy groups are present at C-1 and C-7. The structure of scutebata G was thus determined to be 13(*S*\*)-1 $\beta$ ,7 $\beta$ -dibenzyloxy-6 $\alpha$ -nicotinoyloxy-8 $\beta$ ,13-epoxy-3-neocleroden-15,16-olide.

Compounds **1**, **2**, **4**, **5**, and **6** were tested for *in vitro* cytotoxicity against six human cancer cell lines (HL-60, SMMC-7721, A-549,

SK-BR-3, CACO-2, and PANC-1) using the MTT method. Scutebata A (**1**) showed weak cytotoxic activity against SK-BR-3 with an  $\text{IC}_{50}$  value of 15.2  $\mu\text{M}$ .

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectropolarimeter. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. ESIMS were recorded using a Finnigan MAT 90 instrument, and FABMS spectra were recorded using a VG Auto Spec-3000 spectrometer. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Sephadex LH-20 (40–70  $\mu\text{m}$ , Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (40–63  $\mu\text{m}$ , Merck Darmstadt, Germany). HPLC separations were performed using an Agilent 1100 series pump equipped with a UV detector and a Zorbax SB-C18 (10  $\mu\text{m}$ , Agilent Co. Ltd. Wilmington, DE) column (i.d. 9.4  $\times$  250 mm).

**Plant Material.** The dried aerial parts of *S. barbata* (20 kg) were collected in Guangxi Province of China and were sourced from Kunming Juhua medicinal material market in September 2007. A voucher specimen (HH2007092801) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and Isolation.** Dried and powered aerial parts of *S. barbata* (20 kg) were extracted with MeOH (3  $\times$  80 L) at 50  $^{\circ}\text{C}$ . After evaporation of the solvent, the residue was dissolved in H<sub>2</sub>O and extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract (220 g) was chromatographed over silica gel (200–300 mesh) and eluted with petroleum ether/EtOAc (9:1, 7:3, 1:1, 0:1) to

produce four fractions (A–D). Fraction B (54 g) was purified by column chromatography over MCI gel using 90% MeOH/H<sub>2</sub>O and MeOH as eluents. The fraction eluted by 90% MeOH was further purified by Sephadex LH-20 (eluted with MeOH) and silica gel (eluted with petroleum ether/Me<sub>2</sub>CO, 9:1) to produce compounds **1** (63 mg), **4** (28 mg), and **5** (31 mg). Fraction C (48 g) was subjected to an RP-18 column (eluted with MeOH/H<sub>2</sub>O at 30, 45, 60, 75, 90%) to produce five fractions (C1–C5). Fraction C3 was purified by Sephadex LH-20 in MeOH to produce fractions C3-1, C3-2, and C3-3. Fraction C3-1 was subjected to semipreparative HPLC using MeOH/H<sub>2</sub>O (70%) as the mobile phase (2 mL/min, 30 °C) to yield compound **3** (2.9 mg), whereas fraction C3-2 was subjected to CC on silica gel eluted with petroleum/Me<sub>2</sub>CO (8:2–7:3) to produce compounds **2** (31 mg) and **6** (74 mg). Fraction C3-3 was subjected to CC on silica gel eluted with petroleum/EtOAc (7:3) to yield compound **7** (4.2 mg).

**Scutebata A (1):** white, amorphous powder;  $[\alpha]_D^{21} -62.1$  (*c* 0.065, MeOH); IR (KBr)  $\nu_{\max}$  3432, 1762, 1727, 1638, 1604, 1278, 1119, 710 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR data see Tables 1 and 2; positive FABMS *m/z* 633 [M]<sup>+</sup>; HRESIMS *m/z* 655.2527 [M + Na]<sup>+</sup> (calcd 655.2519 for C<sub>36</sub>H<sub>40</sub>O<sub>10</sub>Na).

**Scutebata B (2):** white, amorphous powder;  $[\alpha]_D^{23} -82.6$  (*c* 0.065, CHCl<sub>3</sub>/MeOH, 1:1); IR (KBr)  $\nu_{\max}$  3476, 1763, 1726, 1598, 1286, 1112, 711 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR data see Tables 1 and 2; positive ESIMS *m/z* 634 [M + 1]<sup>+</sup>; HRESIMS *m/z* 656.2470 [M + Na]<sup>+</sup> (calcd 656.2471 for C<sub>35</sub>H<sub>39</sub>NO<sub>10</sub>Na).

**Scutebata C (3):** white, amorphous powder;  $[\alpha]_D^{23} -34.8$  (*c* 0.13, MeOH); IR (KBr)  $\nu_{\max}$  3433, 1753, 1736, 1721, 1710, 1638, 1629, 1285, 1027, 592 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR data see Tables 1 and 2; positive ESIMS *m/z* 530 [M + 1]<sup>+</sup>; HRESIMS *m/z* 552.2212 [M + Na]<sup>+</sup> (calcd 552.2209 for C<sub>28</sub>H<sub>35</sub>NO<sub>9</sub>Na).

**Scutebata D (4):** white, amorphous powder;  $[\alpha]_D^{26} -40.4$  (*c* 0.095, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3431, 1786, 1749, 1720, 1710, 1638, 1629, 1247, 1026, 716 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR data see Tables 1 and 2; positive ESIMS *m/z* 577 [M + Na]<sup>+</sup>; HRESIMS *m/z* 577.2431 [M + Na]<sup>+</sup> (calcd 577.2413 for C<sub>31</sub>H<sub>38</sub>O<sub>9</sub>Na).

**Scutebata E (5):** white, amorphous powder;  $[\alpha]_D^{23} -49.0$  (*c* 0.26, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3436, 2976, 1791, 1749, 1374, 1248, 1027 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR data see Tables 1 and 2; positive ESIMS *m/z* 543 [M + Na]<sup>+</sup>; HRESIMS *m/z* 543.2589 [M + Na]<sup>+</sup> (calcd 543.2570 for C<sub>28</sub>H<sub>40</sub>O<sub>9</sub>Na).

**Scutebata F (6):** white, amorphous powder;  $[\alpha]_D^{23} -47.2$  (*c* 0.26, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3440, 2979, 1788, 1748, 1719, 1591, 1248, 1025 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR data see Tables 1 and 2; positive ESIMS *m/z* 578 [M + Na]<sup>+</sup>; HRESIMS *m/z* 578.2389 [M + Na]<sup>+</sup> (calcd 578.2366 for C<sub>30</sub>H<sub>37</sub>NO<sub>9</sub>Na).

**Scutebata G (7):** white, amorphous powder;  $[\alpha]_D^{26} -117.2$  (*c* 0.15, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3424, 1792, 1721, 1639, 1591, 1277, 1104, 1025, 713 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR data see Tables 1 and 2; positive ESIMS *m/z* 680 [M + 1]<sup>+</sup>; HRESIMS *m/z* 702.2668 [M + Na]<sup>+</sup> (calcd 702.2679 for C<sub>40</sub>H<sub>41</sub>NO<sub>9</sub>Na).

**Cytotoxicity Assays.** HL-60, SMMC-7721, A-549, SK-BR-3, CACO-2, and PANC-1 cell lines were maintained in RPMI 1640 medium and seeded in 96-well tissue culture plates. After 12 h incubation at 37 °C, the test compound (40 μM) was added and the plate was further incubated for 48 h. Cell growth was then evaluated using an MTT assay procedure.<sup>13</sup> Compounds that inhibited 50% of the growth of the cancer cells were evaluated again at five concentrations; each concentration of the compound was tested in three parallel wells. The IC<sub>50</sub> value was calculated using the Reed–Muench method. Cisplatin was included as a positive control.

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**Supporting Information Available:** 1D and 2D NMR spectra of scutebata A (1) and D (4) and 1D NMR spectra of compounds **2**, **3**, **5**, **6**, and **7** are available free of charge via the Internet at <http://pubs.acs.org>.

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