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Bioactive Dibenzocyclooctadiene Lignans from the Stems of *Schisandra neglecta*

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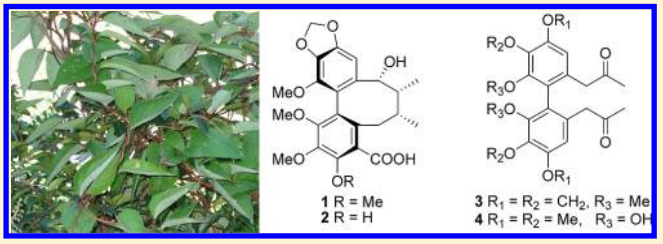
Supporting Information

ABSTRACT: Seven new unusual dibenzocyclooctadiene lignans, neglignans A–G (**1**–**7**), together with 16 known dibenzocyclooctadiene lignans, were isolated from the stems of *Schisandra neglecta*. Compounds **1** and **2** are the first dibenzocyclooctadiene lignans bearing a carboxyl group at C-4, and compounds **3** and **4** are the first 7,8-*seco*-dibenzocyclooctadiene lignans found from Nature. The new compounds (**1**–**7**) and several of the known compounds were evaluated for their anti-HIV activity and cytotoxicity. Compounds **2** and **6** showed anti-HIV-1 activities with therapeutic index values greater than 50, and compound **4** showed cytotoxicity against the NB4 and SHSY5Y cancer cell lines with IC_{50} values of 2.9 and 3.3 μM, respectively.



The stems and fruits of plants in the genus *Schisandra* are used commonly in Traditional Chinese Medicine for their diverse beneficial bioactivities.[1,2] Previous studies have shown that these species are rich in lignans and triperpenoids, especially dibenzocyclooctadiene lignans, which have been found to possess some potentially beneficial activities, including antihepatotoxic, anti-HIV, antioxidant, antitumor, and cytotoxic effects.[3−5]

Schisandra neglecta A. C. Smith (Schisandraceae) is a climbing plant distributed mainly in southwest mainland China. In previous studies, several new dibenzocyclooctadiene lignans were isolated from the fruits of *S. neglecta* from Dali Prefecture, Yunnan Province,[6] and from the stems of *S. neglecta* from Xizang Autonomous Region.[7,8] In our continuing efforts to identify bioactive natural products from the medicinal plants of the family Schisandraceae, a chemical investigation on the stems of *S. neglecta* was carried out, which were collected from the Xichang Prefecture, Sichuan Province, People's Republic of China. As a result, seven new dibenzocyclooctadiene lignans (**1**–**7**), together with 16 known dibenzocyclooctadiene lignans (**8**–**23**), were obtained from this plant. Compounds **1** and **2** are the first examples of dibenzocyclooctadiene lignans bearing a carboxyl group at C-4, and compounds **3** and **4** are the first naturally occurring 7,8-*seco*-dibenzocyclooctadiene lignans. In addition, the anti-HIV-1 activity of compounds **1**–**7**, **14**, **17**–**21**, and **23** and the cytotoxicity of compounds **1**–**7**, **11**–**14**, and **18**–**22** for a small cancer cell line panel were evaluated. Described in this paper are the structure elucidation of **1**–**7** and biological evaluation of the compounds isolated.

RESULTS AND DISCUSSION

The stems of *S. neglecta* were extracted with 70% acetone. The extract produced was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18, and RP-HPLC, to afford seven new dibenzocyclooctadiene lignans, neglignans A–G (**1**–**7**), together with 16 known dibenzocyclooctadiene lignans. The ^{1}H and ^{13}C NMR data of compounds **1**–**7** are listed in Tables 1–3. The known compounds (Figure S1, Supporting Information) were identified as rubrisandrin A (**8**),[9] gomisin J (**9**),[10] schisanhenol (**10**),[10] (±)-gomisin M_1 (**11**),[11] marlignan G (**12**),[12] isogomisin O (**13**),[10] rubschisantherin (**14**),[10] schisandrin A (**15**),[10] schizandrin (**16**),[10] gomisin T (**17**),[13] gomisin F (**18**),[10] angeloylgomisin Q (**19**),[10] schisphenone (**20**),[14] schisphenin F (**21**),[14] interiotherin C (**22**),[15] and gomisin D (**23**),[16] by comparing their spectroscopic data with values reported in the literature.

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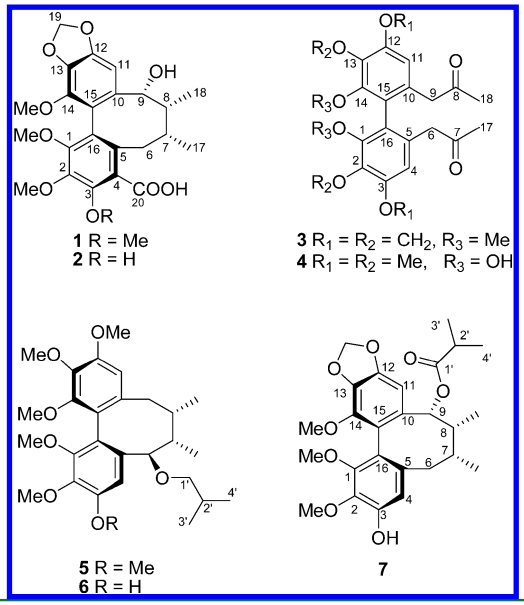

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Compounds 1 and 2 (δ in ppm)

	1[a]		2[b]	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	154.0 s		154.1 s	
2	141.1 s		142.0 s	
3	151.9 s		148.7 s	
4	106.4 s		106.9 s	
5	134.5 s		133.1 s	
		1.77 dd (J = 12.4, 6.7)		1.79 dd (J = 12.5, 6.5)
6	33.4 t		33.7 t	
		2.37 d (J = 12.4)		2.46 d (J = 12.5)
7	36.6 d	2.00 m	36.6 d	2.00 m
8	40.2 d	2.06 m	40.3 d	2.04 m
9	80.0 d	4.77 d (J = 8.5)	80.7 t	4.70 d (J = 8.5)
10	132.9 s		132.0 s	
11	105.2 d	6.40 s	105.2 d	6.62 s
12	149.9 s		149.1 s	
13	138.9 s		137.9 s	
14	142.6 s		142.5 s	
15	117.4 s		117.9 s	
16	118.3 s		119.0 s	
17	20.5 q	1.05 d (J = 7.2)	20.1 q	1.09 d (J = 7.1)
18	11.5 q	0.71 d (J = 7.1)	11.2 q	0.75 d (J = 6.9)
19	175.1 s	9.20 s	175.0 s	9.28 s
20	101.7 t	5.98, 6.00 s	101.3 t	5.92, 5.99 s
OMe-1	60.7 q	3.86 s	60.3 q	3.79 s
OMe-2	60.1 q	3.99 s	60.0 q	3.93 s
OMe-3	55.9 q	4.01 s		
OMe-14	60.9 q	3.89 s	60.8 q	3.82 s
Ar-OH-3				10.04 s

[a]In $CDCl_3$. [b]In C_5D_5N.

Compound **1** was obtained as a yellow gum. Its molecular formula was determined as $C_{24}H_{28}O_9$ by HRESIMS, m/z 483.1639 $[M + Na]^+$ (calcd 483.1631). Its ^{1}H and ^{13}C NMR spectra showed 24 hydrogen and 28 carbon signals, respectively, corresponding to two aromatic rings (δ_C 101.7–154.0) with an aromatic proton (δ_H 6.40), a methylene carbon (δ_C 33.4), two methine carbons (δ_C 36.6, 40.2), an oxygenated methine carbon (δ_C 80.0), two methyl groups (δ_C 11.5, 20.5; δ_H 0.71, δ_H 1.05), a methylenedioxy unit (δ_C 101.7; δ_H 5.98, 6.00), four methoxy groups (δ_C 55.9, 60.1, 60.7, 60.9; δ_H 3.86, 3.89, 3.99, 4.01), and a carboxyl group (δ_C 175.1, δ_H 9.20). The UV absorption bands at 210 and 248 nm, the ^{1}H–^{1}H COSY correlations of H-6/H-7/H-8/H-9, H-7/H-17, and H-8/H-18, and the HMBC correlations of H-11 (δ_H 6.40) with C-9 (δ_C 80.0), C-10 (δ_C 132.9), and C-15 (δ_C 117.4), of H-9 (δ_H 4.77) with C-10 (δ_C 132.9), C-11 (δ_C 105.2), and C-15 (δ_C 117.4), and of H-6 (δ_H 1.77, 2.37) with C-4 (δ_C 106.4), C-5 (δ_C 134.5), and C-16 (δ_C 118.3) indicated that **1** is a dibenzocyclooctadiene lignan.[13,16] The ^{1}H and ^{13}C NMR spectra of **1** were found to be similar to those of yunnankadsurin B.[17] The obvious differences were the disappearance of an aromatic proton signal and appearance of one additional carboxyl group signal in **1**. This indicated that a carboxyl group is substituted on the aromatic ring in **1**. The HMBC correlation (Figure 1) of the carboxyl proton signal (δ_H 9.20) with C-4 (δ_C 106.4) indicated the carboxyl group to be located at C-4. The HMBC correlations of H-19 (δ_H 5.98, 6.00) with C-12 (δ_C 149.9) and C-13 (δ_C 138.9) were used to place the methylenedioxy group between C-12 and C-13. Four methoxy groups could be located at C-1, C-2, C-3, and C-14, as supported by HMBC correlations of the four methoxy proton signals (δ_H 3.86, 3.89, 3.99, 4.01) with C-1 (δ_C 154.0), C-2 (δ_C 141.1), C-3 (151.9), and C-14 (δ_C 142.6), respectively. In addition, a hydroxy group was positioned at C-9 to substantiate the oxygenated methine carbon (C-9), which was also consistent with the molecular formula. Thus, the planar structure of **1** was established.

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Compounds 3 and 4 (δ in ppm, in C_5D_5N)

	3		4	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1, 14	142.8 s		148.0 s	
2, 13	137.8 s		138.9 s	
3, 12	148.6 s		152.0 s	
4, 11	106.6 d	6.55 s	107.0 d	6.59 s
5, 10	128.9 s		128.5 s	
6, 9	49.3 t	4.04 s	49.8 t	4.02 s
7, 8	209.3 s		208.9 s	
15, 16	118.5 s		119.9 s	
17, 18	30.7 q	2.21 s	30.3 q	2.24 s
OMe-1, 14	60.9 q	3.80 s		
OMe-2, 13			61.0, s	3.88 s
OMe-3, 12			55.8, s	3.89 s
$-OCH_2O-$	101.7 t	5.92, 5.98 s		
Ar-OH				10.85 s

The configurations of the biphenyl groups in all isolated dibenzocyclooctadiene lignans in this investigation were determined on the basis of their characteristic circular dichroism (CD) spectra. The CD spectra of *S*-biphenyl-configured lignans show a positive Cotton effect at 215–225 nm and a negative Cotton effect at 240–260 nm. However, lignans with the *R*-biphenyl configuration show a negative Cotton effect at 215–230 nm and a positive Cotton effect at 240–260 nm.[18,19] The CD spectrum of **1** exhibited a negative Cotton effect at 252 nm and a positive Cotton effect at 222 nm, indicating that **1** has an *S*-biphenyl configuration.[18,19] The ROESY correlations of H-20 (carboxyl proton)/CH_3-17 and H-11/H-8 in **1** suggested a twist-boat-chair (TBC) con-

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Table 3. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Compounds 5−7 (δ in ppm)

	5[a]		6[a]		7[b]	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	151.6 s		151.4 s		152.0 s	
2	141.7 s		142.9 s		139.8 s	
3	152.7 s		149.3 s		150.5 s	
4	111.2 d	6.42 s	111.9 d	6.44 s	111.3 d	6.97 s
5	133.5 s		132.3 s		135.0 s	
6α	88.2 d	4.04 d (J = 8.2)	88.6 d	4.00 (J = 8.2)	38.1 t	2.60 d (J = 11.5)
6β						2.74 dd (J = 11.5, 6.5)
7	38.5 d	1.72 m	38.9 d	1.66 m	36.3 d	2.02 m
8	36.4 d	1.85 m	36.4 d	1.83 m	39.0 d	2.14 m
9α	38.0 t	1.98 m	38.2 t	2.03 m	83.9 t	5.41 d (J = 8.1)
9β		2.28 m		2.32 m		
10	136.4 s		136.5 s		137.3 s	
11	106.3 d	6.49 s	106.3 d	6.52 s	105.5 d	6.75 s
12	151.8 s		151.9 s		148.6 s	
13	139.7 s		139.5 s		136.2 s	
14	151.2 s		150.8 s		141.9 s	
15	122.4 s		122.1 s		121.8 s	
16	123.8 s		123.1 s		122.9 s	
17	17.5 q	0.87 overlap	17.1 q	0.87 overlap	15.2 q	1.02 d (J = 7.6)
18	17.5 q	0.87 overlap	17.2 q	0.87 overlap	20.1 q	1.14 d (J = 7.5)
1′	72.3 t	3.61 d (J = 6.8)	72.8 t	3.61 (J = 6.8)	175.2 s	
2′	28.6 d	2.06 m	28.2 d	2.13 m	34.9 d	2.47 m
3′, 4′	19.9 q	0.93 d (J = 6.8)	19.5 q	0.97 (J = 6.8)	18.5 q	0.93 d (J = 7.0)
OMe-1	60.2 q	3.71 s	60.0 q	3.78 s	60.1 q	3.86 s
OMe-2	60.6 q	3.86 s	60.4 q	3.86 s	60.4 q	3.91 s
OMe-3	56.0 q	3.84 s				
OMe-12	55.7 q	3.84 s	55.7 q	386 s		
OMe-13	60.3 q	3.86 s	60.1 q	3.83 s		
OMe-14	60.8 q	3.74 s	60.6 q	3.76 s	60.7 q	3.82 s
−OCH_2O−					101.2 t	6.00, 6.09 s
Ar-OH				9.24 brs		10.60 brs

[a] In $CDCl_3$. [b] In C_5D_5N.

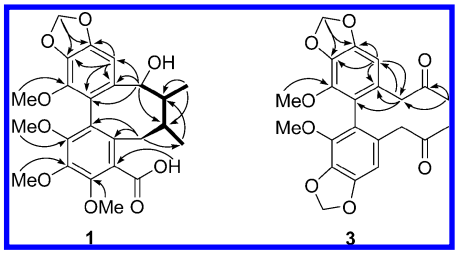

Figure 1. Selected HMBC (↷) and ^{1}H−^{1}H COSY (−) correlations of 1 and 3.

formation for the cyclooctadiene ring (Figure 2). The configuration of OH-9 was deduced as having an α-orientation

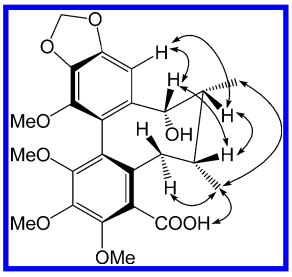

Figure 2. Key ROESY correlations of 1.

by the ROESY correlation between H-11/H-9.[18] The methine protons at C-7 and C-8 were also correlated with H-9, revealing the R orientation of both Me-17 and Me-18. This was also apparent from the observed ROESY correlations of Me-17 with Me-18 and of Me-17 with the H-20 signal.[18] Therefore, the structure of 1 was determined as shown, and it has been given the travial name neglignan A.

Compound 2 was obtained as a yellow gum and showed a quasi-molecular ion at m/z 469.1470 $[M + Na]^+$ in the HRESIMS (calcd for 469.1475), corresponding to the molecular formula $C_{23}H_{26}O_9$. The ^{1}H and ^{13}C NMR spectra of 2 were very similar to those of 1. Detailed comparison of the NMR spectra of 1 and 2 showed that the only differences between these compounds were due to the substitution at C-3. A methoxy group in 1 was substituted by a hydroxy group in 2, which was supported by the disappearance of a methoxy group and the appearance of a phenolic hydroxy group signal in 2. In dibenzocyclooctadiene lignans, the chemical shifts of methoxy groups at C-3 and C-12 occur at δ_C 55−56, whereas the methoxy groups at C-1, C-2, C-13, and C-14 resonated at δ_C 60−61.[3,20] This suggested that the substituted hydroxy group might be at C-3, and the three methoxy groups, in turn, occur at C-1, C-2, and C-14. The C-3 hydroxy group placement was supported by analysis of the HMBC correlations of the proton signal of OH-3 with C-2, C-3, and C-4. In addition, the CD spectrum of 2 gave a negative Cotton effect at 250 nm and a

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positive Cotton effect at 222 nm, indicating an *S*-biphenyl configuration, which is the same as that of **1**. Thus, the structure of **2** (neglignan B) was established as shown.

Compound **3** was obtained as a yellow gum. The molecular formula of this compound was determined as $C_{22}H_{22}O_8$ from its HRESIMS at *m/z* 437.1218 $[M + Na]^+$ (calcd 437.1212). The ^1H and ^{13}C NMR data indicated the presence of an aromatic ring (δ_C 142.8, 137.8, 148.6, 106.6, 128.9, and 118.5) with one aromatic proton (δ_H 6.55), a methylene (δ_C 49.3), a keto group (δ_C 209.3), a methyl group (δ_C 30.7), a methoxy group (δ_C 60.9), and a methylenedioxy functionality (δ_C 101.7). The molecular formula, $C_{22}H_{22}NaO_8$, was consistent with **3** being a 7,8-*seco*-dibenzocyclooctadiene lignan possessing a symmetrical structure with magnetic equivalence. The HMBC correlations of H-17, 18 (δ_H 2.21) with C-6, 9 (δ_C 49.3) and C-7, 8 (δ_C 209.3) and of H-6, 9 (δ_H 4.04) with C-7, 8 (δ_C 209.3) and C-17, 18 (δ_C 30.7) supported the presence of two 2-oxopropyl groups ($-CH_2C(O)CH_3$).[21] The HMBC correlations of H-6, 9 (δ_H 4.04) with C-4, 11 (δ_C 106.6), C-5, 10 (δ_C 128.9), and C-15, 16 (δ_C 118.5) and of H-4, 11 (δ_H 6.55) with C-6, 9 (δ_C 49.3) indicated that two 2-oxopropyl groups are located at C-5 and C-10, respectively. The HMBC correlations of the methylenedioxy protons (δ_H 5.92, 5.98) with C-2, 13 (δ_C 137.8) and C-3, 12 (δ_C 148.6) were used to locate the methylenedioxy group at C-2, 13 and C-3, 12. The methoxy group located at C-1, 14 was supported by the HMBC correlations of methoxy proton signals (δ_H 3.80) with C-1, 14 (δ_C 142.8), respectively. Thus, the structure of **3** (neglignan C) was established as shown.

Compound **4** was obtained as a yellow gum. The molecular formula, $C_{22}H_{26}O_8$, was inferred by HRESIMS at *m/z* 441.1529 $[M + Na]^+$ (calcd for $C_{22}H_{26}NaO_8$, 441.1525). The ^1H and ^{13}C NMR spectra of **4** (Table 2) were similar to those of **3**, and the major differences could be rationalized as being due to the different substitution patterns on the aromatic ring. The methylenedioxy group in **3** was replaced by a methoxy group and a hydroxy group in **4**, which was supported by the absence of the methylenedioxy group and the appearance of a phenolic proton (δ_H 10.85) and a methoxy group (δ_C 55.8; δ_H 3.89) in **4**. The HMBC correlations of the hydroxy group resonance (δ_H 10.85) with C-1, 14 (δ_C 148.0), C-2, 13 (δ_C 138.9), and C-15, 16 (δ_C 119.9) and the correlations of two methoxy signals (δ_H 3.88, 3.89) with C-2, 13 (δ_C 138.9) and C-3, 12 (δ_C 152.0) led to the assignment of two phenolic hydroxy groups at C-1 and C-14 and of four methoxy groups at C-2, C-3, C-12, and C-13, respectively. Thus, the structure of **4** (neglignan D) was established as shown.

Compounds **5** and **6** were both obtained as yellow gums. Compound **5** was assigned the molecular formula $C_{28}H_{40}NaO_7$ from its HRESIMS *m/z* at 511.2679 $[M + Na]^+$ (calcd *m/z* 511.2672). Its ^1H, ^{13}C, and DEPT NMR spectra showed signals for 28 carbons and 40 hydrogens. The NMR data (Table 3) and CD spectrum indicated that **5** is an *S*-biphenyl-configured dibenzocyclooctadiene lignan possessing six methoxy groups on the aromatic rings. The NMR data of **5** were similar to those of marlignan I.[12] The main structural differences between these two compounds were that an ethoxy group in marlignan I is substituted by a *O*-isobutyl group in **5**. This was supported by the absence of ethoxy group signals and the appearance of an *O*-isobutyl unit [δ_C 72.3, t, 28.6, d, 19.9, q (2C); δ_H 3.61, d, J = 6.8 Hz (2H), 2.06, m (1H), 0.93, d, J = 6.8 Hz (6H)] in **5**. The HMBC correlations of H-1′ (δ_H 3.61) with C-6 (δ_C 88.2) and of H-6 (δ_H 4.04) with C-1′ (δ_C 72.3) indicated that the *O*-isobutyl group should be located at C-6. On the basis of this evidence, the structure of **5** (neglignan E) was established as shown.

A HRESIMS parent ion *m/z* at 497.2508 $[M + Na]^+$ (calcd for 497.2515) was consistent with the molecular formula $C_{27}H_{38}O_7$ for compound **6**. The ^1H and ^{13}C NMR spectra of **6** (Table 3) were similar to those of **5**, with the major difference in **6** being the replacement of a methoxy group (δ_C 56.0, δ_H 3.84) by a phenolic hydroxy group (δ_H 9.24). The NMR data (δ_C 56.0) suggested that this substituted methoxy group occurs at either C-3 or C-12 on the aromatic ring. The HMBC correlations of the phenolic hydroxy group proton (δ_H 9.24) with C-2 (δ_C 142.9), C-3 (δ_C 149.3), and C-4 (δ_C 111.9) indicated that the phenolic hydroxy group is located at C-3. The upfield shift of C-3 from δ_C 152.7 to δ_C 149.3 also supported this change in substituent. The *S*-biphenyl configuration of the biphenyl groups in **6** were also determined by its CD spectrum. Therefore, the structure of **6** (neglignan F) was determined as shown.

Compound **7** was obtained as a yellow gum and was assigned the molecular formula $C_{26}H_{32}O_8$, by HRESIMS at *m/z* 495.1990 $[M + Na]^+$ (calcd *m/z* 495.1995). The ^1H and ^{13}C NMR spectroscopic data implied that **7** is a dibenzocyclooctadiene lignan possessing three methoxy groups, a methylenedioxy group, and a phenolic hydroxy group on the aromatic ring.[22] The NMR spectra of **7** were very similar to those of schilancifolignan C.[22] Differences resulted from the signal due to an *O*-isobutyryl group [175.2, 34.9, 18.5 (2C); δ_H 2.47 (1H), 0.93 (6H)] and the lack of an acetoxy group in **7**. Further analysis of the HMBC spectrum showed that the *O*-isobutyryl group could be located at C-9, the three methoxy groups at C-1, C-2, and C-14, the methylenedioxy group at C-12 and C-13, and the phenolic hydroxy group at C-3. The *S*-biphenyl configuration of the biphenyl groups in **7** was also determined by its CD spectrum. Thus, the structure of **7** (neglignan G) was determined as shown.

Since some dibenzocyclooctadiene lignans from *Schisandra* species are reported to possess anti-HIV-1 activity and cytotoxicity for cancer cell lines,[12,22,25] these activities were tested for the new compounds (**1**−**7**) and the known compounds (**1**−**7**, **14**, **17**−**21**, and **23** for anti-HIV-1 activity and **1**−**7**, **11**−**14**, and **18**−**22** for cytotoxicity).

In the anti-HIV-1 assay, a cytotoxicity assessment against C8166 cells (CC_{50}) and the anti-HIV-1 activity were evaluated by an inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using azidothymidine (AZT) as a positive control (EC_{50} = 0.034 μM and CC_{50} > 200 μM).[24] The results revealed that compounds **2** and **6** showed anti-HIV-1 activity with therapeutic index (TI) values of greater than 50 (Table 4).

Cytotoxicity tests for compounds were performed against the NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by an MTT assay, with paclitaxel as the positive control.[25] The results are shown in Table 5. Compound **4** showed cytotoxicity against the NB4 and SHSY5Y cell lines with IC_{50} values of 2.9 and 3.3 μM, respectively. Compounds **1**−**3**, **12**, **19**, and **21** also showed cytotoxicity with IC_{50} values of less than 10 μM for some of the cell lines used.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Horiba SEPA-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer, and CD spectra were measured on a JASCO J-810 spectropolarimeter. A Tenor

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Table 4. Anti-HIV-1 Activities of Compounds 1−7, 14, 17−21, and 23

compound	CC_{50} (μM)	EC_{50} (μM)	TI[a]
1	86.7 ± 3.8	2.2 ± 0.23	40.3
2	75.2 ± 2.9	1.4 ± 0.14	55.3
3	54.5 ± 4.0	3.9 ± 0.22	14.1
4	38.8 ± 3.5	4.2 ± 0.31	9.2
5	>200 ± 8.2	5.9 ± 0.34	>33.7
6	>200 ± 6.4	3.5 ± 0.26	>58.0
7	148.6 ± 3.8	8.2 ± 0.53	18.1
14	85.2 ± 3.6	4.7 ± 0.47	18.2
17	124.5 ± 4.5	8.2 ± 0.54	15.1
18	61.9 ± 4.0	8.3 ± 0.38	7.5
19	84.7 ± 4.2	11.5 ± 0.56	7.4
20	156.4 ± 5.3	12.7 ± 0.58	12.3
21	146.8 ± 4.8	8.3 ± 0.49	17.8
23	185.7 ± 5.2	9.8 ± 0.36	18.9
AZT	>200	0.034 ± 0.01	5882 ± 12

[a]TI = CC_{50}/EC_{50}.

Table 5. Cytotoxicity Data for Cancer Cell Lines of Compounds 1−7, 11−14, and 18−22

	IC_{50} values (μM)				
compound	NB4	A549	SHSY5Y	PC3	MCF7
1	>10	6.8	>10	>10	7.4
2	>10	5.9	>10	9.2	>10
3	7.3	>6.5	8.2	>10	>10
4	2.9	8.6	3.3	5.1	7.8
5	>10	>10	>10	>10	>10
6	>10	>10	>10	>10	>10
7	>10	>10	>10	>10	>10
11	>10	>10	>10	>10	>10
12	>10	8.6	>10	9.5	>10
13	>10	>10	>10	>10	>10
14	>10	>10	>10	>10	>10
18	>10	>10	>10	>10	>10
19	>10	>10	7.8	>10	>10
20	>10	>10	>10	8.9	>10
21	7.4	>10	>10	>10	9.7
22	>10	>10	>10	>10	>10
paclitaxel	0.03	0.02	0.2	0.2	0.1

27 spectrophotometer was used for scanning IR spectra (KBr pellets). 1D- and 2D-NMR spectra were recorded on a DRX-500 spectrometer with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to TMS. HRESIMS was performed on an API QSTAR spectrometer or a VG Autospec-3000 spectrometer. Preparative HPLC was performed on a Shimadzu LC-8A liquid chromatograph equipped with a Zorbax PrepHT GF (21.2 mm × 25 cm, 7 μm) column or Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed using silica gel (200−300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich Co., St. Louis, MO, USA), or MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC, and the spots were visualized by heating the plates after spraying with 5% H_2SO_4 in EtOH.

Plant Material. The stems of *S. neglecta* were collected in Luoji Mountain Village, Xichang County, Sicuan Province, People's Republic of China, in September 2011. The identification of the plant material was verified by Prof. Xi-Wen Li of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KIB 11-9-47) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered stems of *S. neglecta* (8.0 kg) were extracted four times with 70% $(CH_3)_2CO$ (4 × 60 L) at room temperature and filtered, with the filtrate evaporated under reduced pressure and partitioned with EtOAc (3 × 2 L). The EtOAc partition (426 g) was applied to silica gel (200−300 mesh) column chromatography, eluting with a $CHCl_3$−Me_2CO gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give five fractions, A−E. The further separation of fraction B (42.7 g) by silica gel column chromatography, eluted with petroleum ether−acetone (20:1−1:2), yielded mixtures B1−B6. Fraction B2 (5.28 g) was subjected to silica gel column chromatography using petroleum ether−acetone and semipreparative HPLC (75% MeOH−H_2O, flow rate 12 mL/min) to give **3** (11.8 mg), **5** (18.4 mg), and interiotherin C (31.5 mg). Fraction B3 (6.5 g) was subjected to silica gel column chromatography using petroleum ether−acetone and semipreparative HPLC (65% MeOH−H_2O, flow rate 12 mL/min) to give **6** (14.8 mg), **7** (12.5 mg), schisanhenol (22.4 mg), isogomisin O (16.8 mg), schisandrin A (18.2 mg), schizandrin (26.9 mg), gomisin F (33.2 mg), angeloylgomisin Q (25.7 mg), schisphenone (18.8 mg), schisphenin F (15.4 mg), and gomisin D (28.2 mg). Fraction B4 (3.8 g) was subjected to silica gel column chromatography using petroleum ether−acetone and semi-preparative HPLC (60% MeOH−H_2O, flow rate 12 mL/min) to afford **4** (13.6 mg), rubrisandrin A (10.2 mg), gomisin J (15.8 mg), rubschisantherin (19.0 mg), and gomisin T (20.8 mg). Fraction C (60.0 g) was subjected to silica gel column chromatography using petroleum ether−acetone (20:1−6:4) for elution followed by passage over a reversed-phase column (RP-18), eluting with MeOH−H_2O (30−90%), and then by Sephadex LH-20, using MeOH as eluant. Further purifications were performed by semipreparative HPLC and preparative HPLC separation (50% MeOH−H_2O) to give compounds **1** (12.2 mg), **2** (8.8 mg), (±)-gomisin M_1 (14.3 mg), and marlignan G (11.8 mg).

*Neglignan A (**1**):* yellow gum; $[\alpha]_D^{25}$ +15.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.62), 248 (3.72), 320 (0.64) nm; CD (*c* 0.05, MeOH) λ_{max} nm ($\Delta\varepsilon$) 252 (−45.6), 228 (−22.3), 222 (+6.62), 215 (−3.16); IR (KBr) ν_{max} 3396, 3087, 2919, 2857, 1685, 1626, 1562, 1468, 1397, 1326, 1232, 1116, 1069, 972, 864 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; ESIMS *m/z* (positive-ion mode) 483 $[M + Na]^+$; HRESIMS (positive-ion mode) *m/z* 483.1639 $[M + Na]^+$ (calcd $C_{24}H_{28}NaO_9$ for 483.1631).

*Neglignan B (**2**):* yellow gum; $[\alpha]_D^{24}$ +23.8 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.58), 250 (3.64), 320 (0.68) nm; CD (*c* 0.05, MeOH) λ_{max} nm ($\Delta\varepsilon$) 250 (−38.6), 238 (−15.3), 222 (+8.14), 215 (−3.05); IR (KBr) ν_{max} 3401, 3092, 2922, 2855, 1687, 1628, 1559, 1466, 1323, 1235, 1168, 1070, 973, 862 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), see Table 1; ESIMS (positive-ion mode) *m/z* 469 $[M + Na]^+$; HRESIMS (positive-ion mode) *m/z* 469.1470 $[M + Na]^+$ (calcd $C_{23}H_{26}NaO_9$ for 469.1475).

*Neglignan C (**3**):* yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.68), 276 (3.56), 320 (1.55) nm; IR (KBr) ν_{max} 3087, 2932, 2848, 1732, 1629, 1548, 1473, 1382, 1138, 1062, 978, 892 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), see Table 2; ESIMS (positive-ion mode) *m/z* 437 $[M + Na]^+$; HRESIMS (positive-ion mode) *m/z* 437.1218 $[M + Na]^+$ (calcd $C_{22}H_{22}NaO_8$ for 437.1212).

*Neglignan D (**4**):* yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.65), 276 (3.68), 320 (1.42) nm; IR (KBr) ν_{max} 3408, 3082, 2935, 2853, 1730, 1625, 1551, 1476, 1428, 1380, 1164, 1135, 1058, 976, 887 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), see Table 2; ESIMS (positive-ion mode) *m/z* 441 $[M + Na]^+$; HRESIMS (positive-ion mode) *m/z* 441.1529 $[M + Na]^+$ (calcd $C_{22}H_{26}NaO_8$ for 441.1525).

*Neglignan E (**5**):* yellow gum; $[\alpha]_D^{25}$ +38.2 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.87), 241 (3.65), 320 (0.68), nm; CD (*c* 0.05, MeOH), nm ($\Delta\varepsilon$) 250 (−48.9), 238 (−38.8), 220 (+25.4), 210 (+6.2); IR (KBr) ν_{max} 3086, 2948, 2874, 1620, 1572, 1493, 1458, 1415, 1324, 1276, 1021, 962, 871 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 3; ESIMS (positive-ion mode) *m/z* 511 [M

+ Na]$^+$; positive ESIMS m/z 511 [M + Na]$^+$; HRESIMS m/z 511.2679 [M + Na]$^+$ (calcd for $C_{28}H_{40}NaO_7$, 511.2672).

Neglignan F (6): yellow gum; $[\alpha]_D^{25}$ +33.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.80), 240 (3.62), 320 (0.89), nm; CD (*c* 0.05, MeOH), nm ($\Delta\varepsilon$) 250 (−42.6), 238 (−30.5), 220 (+28.8), 210 (+3.1); IR (KBr) ν_{max} 3412, 3090, 2947, 2876, 1623, 1576, 1495, 1457, 1412, 1327, 1273, 1025, 967, 873 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 3; ESIMS (positive-ion mode) m/z 497 [M + Na]$^+$; positive ESIMS m/z 483 [M + Na]$^+$; HRESIMS m/z 497.2508 [M + Na]$^+$ (calcd for $C_{27}H_{38}NaO_7$, 497.2515).

Neglignan G (7): white powder; $[\alpha]_D^{24}$ +11.8 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.76), 246 (3.85), 320 (1.22), nm; CD (*c* 0.05, MeOH) λ_{max} nm ($\Delta\varepsilon$) 250 (−44.8), 236 (−31.6), 222 (+18.8), 215 (+36.5); IR (KBr) ν_{max} 3496, 2968, 2933, 2870, 1732, 1608, 1495, 1459, 1412, 1382, 1318, 1245, 1197, 1126, 1101, 1012, 986, 932, 847 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; ESIMS (positive-ion mode) m/z 495 [M + Na]$^+$; HRESIMS (positive-ion mode) m/z 495.1990 [M + Na]$^+$ (calcd 495.1995 for $C_{26}H_{32}NaO_8$).

Anti-HIV-1 Assay. The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) using AZT as a positive control.[24] All experiments were performed in triplicate.

Cytotoxicity Assay. The cytotoxicity tests for these compounds were performed using NB4 (human acute promyelocytic leukemia), A549 (human lung adenocarcinoma epithelial), SHSY5Y (human neuroblastoma), PC3 (human prostate cancer), and MCF7 (human breast adenocarcinoma) cells using an MTT assay, with paclitaxel as the positive control.[25] All experiments were carried out in triplicate. The IC_{50} value was defined as the concentration of the test compound resulting in a 50% reduction of growth compared with untreated cells.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The structures of known compounds (**8**−**23**); the proposed biogenetic route of **1**−**3**; ^{1}H, ^{13}C, HSQC, HMBC, and HRMS spectra of **1**; ^{1}H, ^{13}C NMR, HSQC, and HMBC spectra of **3** and **5**; ^{1}H, ^{13}C, and HMBC spectra of **2** and **4**; ^{1}H and ^{13}C spectra of **6** and **7**; and the detailed experimental conditions for anti-HIV-1 and cytotoxicity assays. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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