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neo-Clerodane Diterpenoids from *Scutellaria barbata* with Activity against Epstein−Barr Virus Lytic Replication

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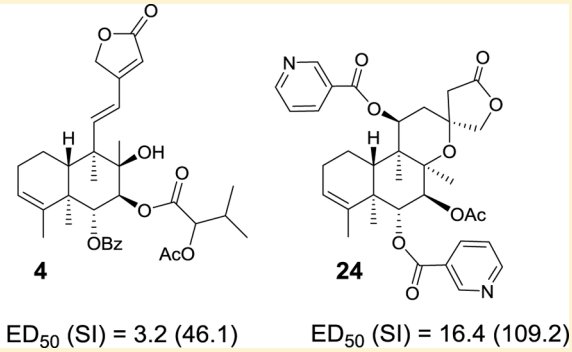
Supporting Information



ABSTRACT: Bioassay-guided fractionation was conducted on an EtOAc-soluble extract of the whole plants of *Scutellaria barbata*, monitored by inhibition of Epstein−Barr virus (EBV) lytic replication. Twenty-six *neo*-clerodane diterpenoids were isolated, of which 13 are new (**1**−**13**, scutolides A−L) and 13 previously known (**14**−**26**). The structures of **1**−**13** were elucidated by analysis of their NMR and MS spectroscopic data. Furthermore, the configurations of the new compounds **1** and **11** were confirmed by single-crystal X-ray diffraction. All of the isolated compounds were evaluated for inhibitory effects against EBV lytic replication. Eleven compounds (**3**, **4**, **6**, **11**, **12**, **15**, **16**, **17**, **20**, **22**, and **24**) exhibited moderate to potent inhibition, with EC_{50} values from 3.2 to 23.6 μM and selective index (SI) values from 2.1 to 109.2. More specifically, the new compound **4** showed the most potent activity, with EC_{50} and SI values of 3.2 μM and 46.1, respectively, while compound **24** (EC_{50} = 16.4 μM) exhibited the highest SI of 109.2. This study is the first to report that *neo*-clerodane diterpenoids demonstrate significant inhibition against EBV lytic replication.

Scutellaria barbata D. Don is a flowering plant belonging to a large cosmopolitan genus in the family Lamiaceae, which includes about 350 species.[1] This plant (Chinese name "Ban-Zhi-Lian") is used as a traditional Chinese medicine to treat digestive system cancers, hepatoma, lung cancer, breast cancer, and chorioepithelioma.[2] This species is also used as an herbal remedy for inflammation and as a diuretic.[3] Previous phytochemical investigations on *S. barbata* have resulted in the isolation of a series of cytotoxic[4−10] and antioxidative[11] *neo*-clerodane diterpenoids. Other reports have described *neo*-clerodane diterpenoids with antiviral,[12] especially anti-influenza virus FM1,[13] activity. However, to our knowledge, no chemical constituents with activity against Epstein−Barr virus (EBV) have been reported from this plant.

EBV is a human herpesvirus. Herpesvirus infections are common in humans and are not lethal. EBV is also associated with several forms of cancer, including nasopharyngeal carcinoma, gastric carcinoma, Burkitt lymphoma, and Hodgkin disease.[14] As part of our continuing studies to discover antiviral compounds from medicinal plants,[15−17] a 95% EtOH extract of

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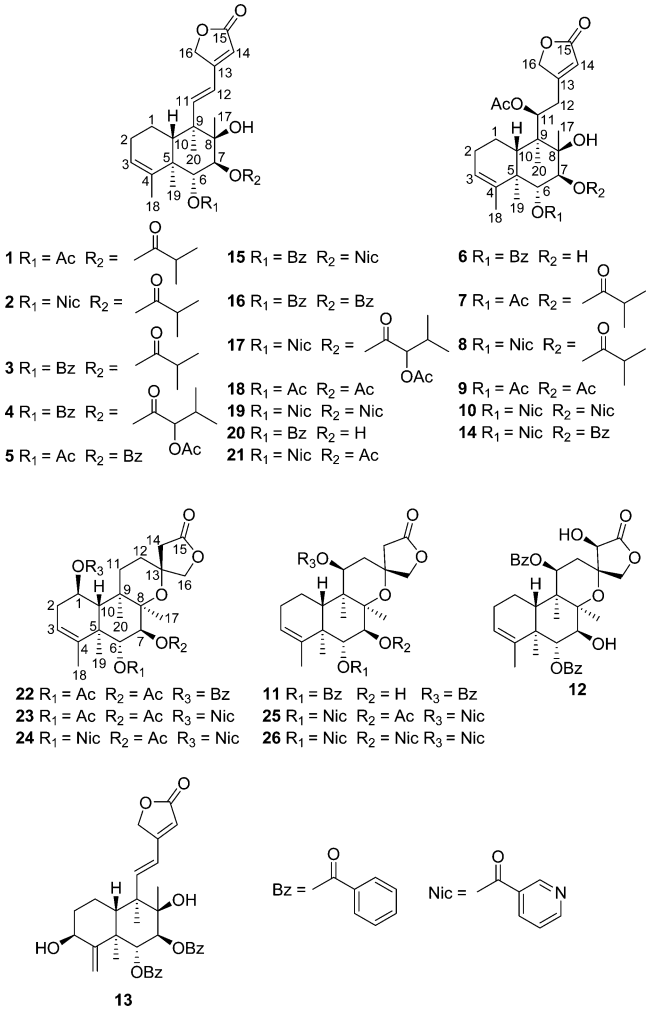
whole plants of *S. barbata* was found to potently inhibit EBV lytic replication with an EC_{50} value of 34.4 μg/mL using the P3HR-1 cell line. Further bioactivity-guided fractionation of this extract utilizing the EBV lytic replication assay led to the isolation of 13 new (**1**–**13**) and 13 known (**14**–**26**) *neo*-clerodane diterpenoids. Herein, we report the isolation and structural elucidation as well as bioactivity evaluation of these 26 *neo*-clerodane diterpenoids from *S. barbata*.



RESULTS AND DISCUSSION

A 95% EtOH extract of whole plants of *S. barbata* was suspended in water and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. Each portion was evaluated for anti-EBV activity. The EtOAc-soluble fraction showed significant inhibition of EBV lytic replication, with an EC_{50} of 27.6 μg/mL. Bioactivity-guided isolation of the EtOAc-soluble fraction was performed using the EBV DNA lytic replication assay.[18] As a result, 13 new (**1**–**13**) and 13 known (**14**–**26**) *neo*-clerodane diterpenoids were obtained.

The known compounds were identified as barbatellarine B (**14**),[19] scutebarbatine Y (**15**),[18] barbatin D (**16**),[20] scutebarbatine L (**17**),[21] 6,7-di-*O*-acetyl barbatin C (**18**),[4,22] scutebarbatine A (**19**),[11] scutebata J (**20**),[23] scutebarbatine K (**21**),[21] scutebata D (**22**),[8] scutebata F (**23**),[8] barbatine D (**24**),[11] barbatine A (**25**),[11] and barbatine B (**26**),[11] based on spectroscopic data analysis and comparison with literature values.

Compound **1** was obtained as white needles. Its molecular formula was found to be $C_{26}H_{36}O_7$, as established from an ion at m/z 461.25274 $[M + H]^+$ in the positive HRESIMS. Its IR spectrum showed absorptions for hydroxy (3460 cm^{-1}) and carbonyl ester (1739 cm^{-1}) groups. The ^1H NMR spectrum of **1** revealed the presence of an isopropyl group [δ_H 2.55 (1H, m, H-2″), 1.16 (3H, d, J = 7.1 Hz, H-3″), 1.19 (3H, d, J = 7.1 Hz, H-4″)]; an α,β-unsaturated-γ-lactone moiety [δ_H 5.90 (1H, br s, H-14), 5.00 (2H, s, H-16)]; and a double bond with *E* configuration [δ_H 6.32 (1H, d, J = 16.8 Hz, H-11), 6.39 (1H, d, J = 16.8 Hz, H-12)]. The ^{13}C NMR data of **1** revealed acetoxy (δ_C 170.5, s; 21.8, q) and isobutyryloxy (δ_C 176.5, s; 34.2, d; 18.8, q; 19.4, q) groups, as well as a double bond [δ_C 147.1 (d, C-11); 121.9 (d, C-12)]. In addition to the acetoxy and isobutyryloxy groups, the ^{13}C NMR spectrum of **1** showed 20 carbon signals. On the basis of the above data and previous studies of the *Scutellaria* genus, compound **1** was assigned as a *neo*-clerodane diterpenoid. Comparison of the NMR data of **1** and 6,7-di-*O*-acetyl barbatin C (**18**) suggested that the two compounds are similar structurally, except for the presence of a C-7 isobutyryloxy group in **1** rather than a C-7 acetoxy moiety in **18**. The HMBC correlations of **1** (Figure 1) confirmed that

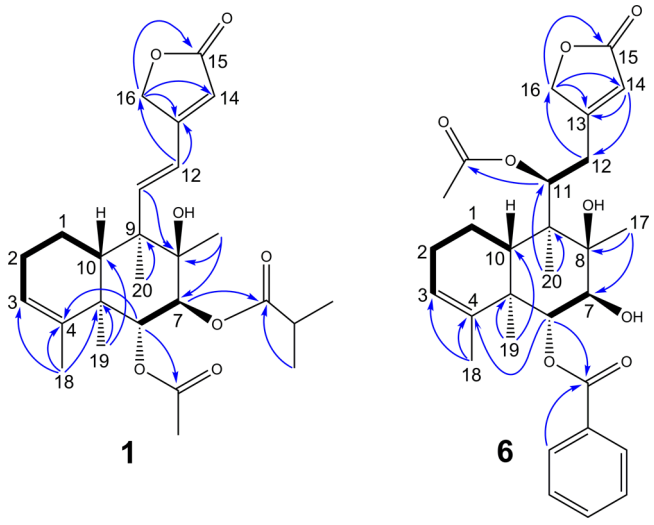


Figure 1. Key ^1H–^1H COSY (bold —) and HMBC (blue →) correlations of **1** and **6**.

the acetoxy (δ_H 1.96, s; δ_C 170.5, s; 21.8, q) and isobutyryloxy (δ_H 2.55, m; 1.16, d, J = 7.1 Hz; 1.19, d, J = 7.1 Hz; δ_C 176.5, s; 34.2, d; 18.8, q; 19.4, q) groups were connected to C-6 and C-7, respectively.

The relative configuration of **1** was identified from its ROESY data (Figure S8, Supporting Information). The α-orientation of H-7 was indicated by correlations of H-7 with H-17, H-19, and H-20, whereas a correlation of H-6 with H-10 suggested that these two protons are β-oriented on the opposite face of the molecule.

The absolute configurations of this compound type are optimally determined by CD spectroscopy.[11] However, single-crystal X-ray diffraction using Cu Kα radiation was performed to unambiguously identify the absolute configuration of **1** (Figure 2) as (5*R*,6*R*,7*S*,8*R*,9*R*,10*S*)-6-*O*-acetoxy-7-*O*-isobutyryl barbatin C. Compound **1** was assigned the trivial name scutolide A.

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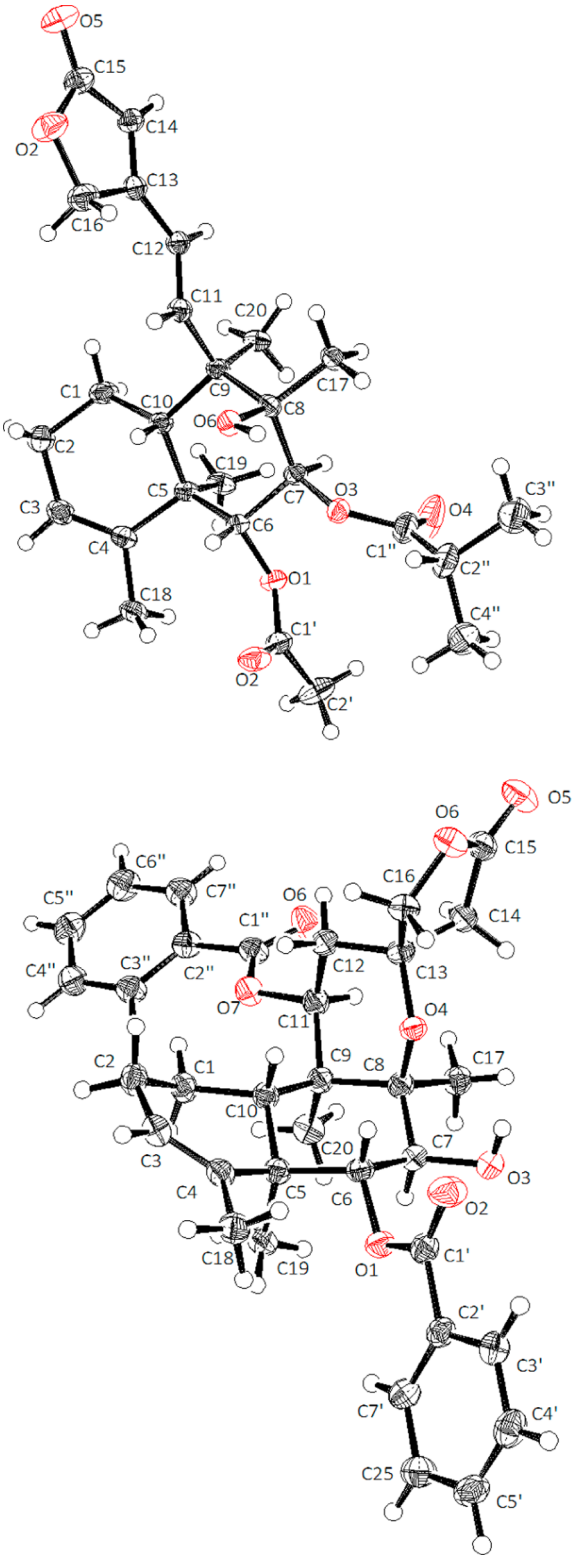

Figure 2. ORTEP drawings of **1** (above) and **11** (below).

Compound **2** was isolated as a white powder. Its molecular formula was determined to be $C_{30}H_{37}O_7N$ from an ion at m/z 524.26392 $[M + H]^+$ in the HRESIMS. The NMR data were very similar to those of **1** (Table 1), except for the presence of a nicotinoyloxy moiety and the absence of the acetoxy group. HMBC correlations from H-6 [δ_H 5.73 (d, J = 10.0 Hz)] to C-1′ (δ_C 164.8) and H-7 [δ_H 5.50 (d, J = 10.0 Hz)] to C-1″ (δ_C 176.3) indicated that the nicotinoyloxy group is connected to C-6 and the isobutyryloxy group [δ_H 2.34 (m); 0.77 (d, J = 6.9 Hz); 1.00 (d, J = 6.9 Hz); δ_C 34.1 (d); 18.5 (q); 19.2 (q)] was positioned at C-7. In the NOESY spectrum of **2** (Figure S16, Supporting Information), the correlations of H-7 with H-17, H-19, and H-20 suggested that these groups are cofacial and α-oriented, whereas the correlation of H-6 with H-10 indicated that these hydrogens are β-oriented. Therefore, the structure of **2** (scutolide B) was defined as (5*R*,6*R*,7*S*,8*R*,9*R*,10*S*)-6-*O*-nicotinoyl-7-*O*-isobutyryl barbatin C.

Compound **3** gave a molecular formula of $C_{31}H_{38}O_7$, as established by HRESIMS. Its ^{1}H and ^{13}C NMR spectra (Table 1) indicated the presence of a benzoyloxy moiety [δ_H 7.97 (m, 2H), 7.41 (t, J = 7.2 Hz, 2H), 7.53 (t, J = 7.2 Hz, 1H); δ_C 166.2 (s), 130.4 (s), 129.9 (d × 2), 128.7 (d × 2), 133.4 (d)] and the absence of an acetoxy group [δ_H 1.96 (3H, s) and δ_C 170.5 (s), 21.8 (q)] as found in **1**. Detailed analyses of the 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY, Figures S21−24, Supporting Information) data and comparison with the NMR data of **1** revealed that compound **3** (scutolide C) was (5*R*,6*R*,7*S*,8*R*,9*R*,10*S*)-6-*O*-benzoyl-7-*O*-isobutyryl barbatin C.

Compound **4** was isolated as a white powder. Its HRESIMS showed a molecular ion at m/z 593.27611 $[M - H]^-$, corresponding to the molecular formula $C_{34}H_{42}O_9$. Its NMR data (Table 1) were almost identical to those of **3**, except for signals representative of an additional methine (δ_H 4.89, d, J = 3.5 Hz; δ_C 76.3, d) and an acetoxy group (δ_H 2.15, s; δ_C 21.0, q, 172.4, s). In the COSY spectrum of **4**, the correlations of H-2″ (δ_H 4.89, d, J = 3.5 Hz) with H-3″ (δ_H 2.11, m) and of H-3″ (δ_H 2.11, m) with two doublet methyl signals (δ_H 0.39, d, J = 6.8 Hz; δ_H 0.74, d, J = 6.8 Hz) indicated the presence of a −OCH−CH$(CH_3)_2$ moiety. HMBC correlations (Figure S31, Supporting Information) from H-2″ and H-3″ to C-1″ (δ_C 168.9, s), H-2″ to an acetoxy carbon (δ_C 172.4, s), and H-7 (δ_H 5.39, d, J = 9.9 Hz) to C-1″ (δ_C 168.9, s) indicated that a 2-acetoxy-3-methylbutanoyloxy moiety is present at C-7. The relative configuration of **4**, as determined from its NOESY spectrum (Figure S32, Supporting Information), was the same as that of **1**. Thus, the structure of **4** (scutolide D) was found to be (5*R*,6*R*,7*S*,8*R*,9*R*,10*S*)-6-*O*-benzoyl-7-*O*-(2-acetyl-3-methyl)-butyryl barbatin C.

The molecular formula of **5** was assigned as $C_{29}H_{34}O_7$ according to its HRESIMS data at m/z 495.23724 $[M + H]^+$. The NMR spectra of **5** and **1** (Table 1) were similar, except for signals indicative of the presence of a benzoyloxy group in **5** rather than those for an isobutyryloxy moiety in **1**. HMBC correlations from H-6 [δ_H 5.62 (d, J = 10.0 Hz)] to C-1′ (δ_C 170.7) and H-7 [δ_H 5.55 (d, J = 10.0 Hz)] to C-1″ (δ_C 166.2) suggested that an acetoxy group is connected to C-6 and a benzoyloxy group substituted at C-7. The relative configuration of **5** was deduced to be the same as that of **1** based on the ROESY spectroscopic data and comparison with **1**. Thus, the structure of **5** (scutolide E) was identified as (5*R*,6*R*,7*S*,8*R*,9*R*,10*S*)-6-*O*-acetyl-7-*O*-benzoyl barbatin C.

Compound **6**, a white powder, showed the molecular formula $C_{29}H_{36}O_8$, as established by HRESIMS of the $[M + H]^+$ peak at m/z 513.24792. The IR spectrum showed absorption signals for hydroxy group (3480 cm^{-1}) and ester carbonyl (1735 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table 2) contained five singlet methyl resonances at δ_H 1.34, 1.53, 1.32, 0.82, and 2.02, a broad singlet at δ_H 5.24 (1H), an AB system [δ_H 5.26 (d, J = 9.4 Hz), 3.78 (dd, J = 9.4, 5.4 Hz)], and an ABX system [δ_H 5.60 (d, J = 9.9 Hz), 2.58 (dd, J = 9.9,

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Table 1. ^{1}H and ^{13}C NMR Chemical Shifts (δ) of Compounds 1−5[a]

	1		2		3		4		5	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1a	1.29 m	19.3	1.31 m	19.4	1.30 m	19.5	1.30 m	19.4	1.28 overlap	19.5
1b	1.58 m		1.61 m		1.60 m		1.59 m		1.28 overlap	
2a	1.99 m	26.2	2.00 m	26.3	2.11 overlap	26.4	1.99 overlap	26.5	2.00 overlap	26.4
2b			1.93 m		2.00 overlap		1.99 overlap		2.00 overlap	
3	5.21 br s	123.2	5.22 br s	123.6	5.19 br s	123.2	5.19 br s	123.3	5.21 br s	123.2
4		141.1		140.8		141.2		141.1		141.3
5		43.21		43.5		43.6		43.6		43.3
6	5.40 d (10.6)	74.9	5.73 d (10.0)	76.2	5.69 d (10.0)	75.5	5.73 d (9.9)	74.7	5.62 d (10.0)	74.9
7	5.29 d (10.6)	75.0	5.50 d (10.0)	75.0	5.48 d (10.0)	75.0	5.39 d (9.9)	77.3	5.55 d (10.0)	76.1
8		76.9		77.2		77.2		77.4		77.3
9		48.2		48.3		48.4		48.3		48.5
10	2.20 m	42.6	2.29 dd (8.4, 2.1)	42.8	2.29 m	42.9	2.24 d (11.2)	42.9	2.27 dd (12.8, 2.0)	42.9
11	6.32 d (16.8)	147.1	6.35 d (16.8)	146.9	6.35 d (16.8)	147.2	6.35 d (16.8)	147.2	6.37 d (17.2)	147.1
12	6.39 d (16.8)	121.9	6.40 d (16.8)	122.0	6.41 d (16.8)	122.0	6.39 d (16.8)	122.0	6.38 d (17.2)	122.0
13		162.3		162.2		162.3	5.90 s	162.2		162.5
14	5.90 br s	114.9	5.93 br s	115.1	5.91 s	115.1	5.90 s	115.0	5.89 s	115.0
15		174.2		174.1		174.2		174.2		174.2
16	5.00 s	70.8	4.98 s	70.9	4.98 br s	70.9	4.97 br s	70.8	4.96 d (1.6)	70.9
17	0.95 s	22.4	0.98 s	22.6	0.96 s	22.5	0.99 s	22.8	1.00 s	22.7
18	1.56 s	19.4	1.52 s	20.3	1.55 s	20.3	1.53 s	20.3	1.58 br s	20.2
19	1.23 s	17.4	1.39 s	17.6	1.38 s	17.4	1.36 s	17.6	1.28 s	17.4
20	1.16 s	15.4	1.21 s	15.5	1.19 s	15.5	1.18 s	15.6	1.20 s	15.6
1′		170.5		164.8		166.2		165.8		170.7
2′	1.96 s	21.8		126.4		130.4		130.6	1.74, s	21.6
3′			9.17 dd (1.9, 0.8)	151.2	7.97 m	129.9	7.99, m	129.9		
4′					7.41 t (7.2)	128.7	7.40 t (7.8)	128.6		
5′			8.77 dd (4.9, 1.8)	153.8	7.53 t (7.2)	133.4	7.52 t (7.4)	133.3		
6′			7.40 ddd (8.0, 4.9, 0.7)	123.6	7.41 t (7.2)	128.7	7.40 t (7.8)	128.6		
7′			8.24 dt (8.0, 1.9)	137.2	7.97 m	129.9	7.99 m	129.9		
1″		176.5		176.3		176.4		168.9		166.2
2″	2.55 m	34.2	2.34 m	34.1	2.31 m	34.1	4.89 d (3.5)	76.3		129.2
3″	1.16 d (7.1)	18.8	0.77 d (6.9)	18.5	0.70 d (7.6)	18.4	2.11 m	29.3	8.01 dd (8.4, 1.2)	130.1
4″	1.19 d (7.1)	19.4	1.00 d (6.9)	19.2	0.97 d (7.6)	19.3	0.39 d (6.8)	16.3	7.44 t (7.8)	128.8
5″							0.74 d (6.8)	18.9	7.57 t (7.8)	133.8
6″									7.44 t (7.4)	128.8
7″									8.01 dd (8.4, 1.2)	130.1
11-OAc								172.4		
							2.15 s	21.0		

[a] ^{1}H NMR measured at 400 MHz, ^{13}C NMR measured at 100 MHz, and spectra obtained in $CDCl_3$ with TMS as internal standard. Assignments were supported with HSQC and HMBC NMR spectra.

15.1 Hz), 3.53 (d, J = 15.1 Hz)]. The ^{13}C NMR spectrum contained 20 resonances representing four methyls, four methylenes, six methines (2 × sp^2, 4 × sp^3), and six quaternary carbons (3 × sp^2, 3 × sp^3), in addition to signals for a benzoyloxy [δ_H 8.03 (2H, m), 7.43 (2H, t, J = 7.7 Hz), 7.56 (1H, t, J = 7.4 Hz); 5.60 (d, J = 9.9 Hz); δ_C 168.5 (s), 130.4 (s), 129.9 (d × 2), 128.8 (d × 2), 133.6 (d)] and an acetoxy [(δ_H 2.02 (3H, s); δ_C 171.3 (s), 21.0 (q)] group. On the basis of the above data, compound **6** was deduced to be a *neo*-clerodane diterpenoid with an oxygenated substituent at C-11, which was confirmed by the 2D NMR spectra. HMBC correlations (Figure 3) of H-11 [δ_H 5.60 (d, J = 9.9 Hz)] with a carbonyl carbon (δ_C 171.3) showed that an acetoxy group is substituted at C-11. The HMBC correlation of H-6 [δ_H 5.26 (d, J = 9.4 Hz)] with C-1′ (δ_C 168.5) corroborated that the benzoyloxy group is positioned at C-6. Furthermore, the HMBC correlations (Figure 3) from H-16 to C-13 (δ_C 168.6), C-14 (δ_C 116.7), and C-15 (δ_C 174.2) indicated the presence of an α,β-unsaturated lactone ring. Thus, the structure of compound **6** was deduced as shown.

The relative configuration of compound **6** was deduced from its ROESY spectrum (Figure S48, Supporting Information). Correlations of H-7 with H-17, H-19, H-20 and of H-11 with H-17, H-19, H-20 indicated that these groups are cofacial and α-oriented, whereas the ROESY correlation of H-10 with H-6 indicated these hydrogens to be on the opposite face and β-oriented. The relative configuration of C-11 was also deduced as *S* from the ROESY correlations of H-11/H-17, H-20, and H-7 (Figure S48, Supporting Information). Therefore, the structure of **6** (scutolide F) was determined as (11*S*)-11-acetoxy-6α-benzoyloxy-7β,8β-dihydroxy-3(4),13(14)-neoclerodien-15,16-olide.

As shown in Table 2, the NMR data indicated that compounds **7**−**10** have similar structures to **6**, except for the substituents at C-6 and C-7. Therefore, the structures of

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Table 2. ^{1}H and ^{13}C NMR Chemical Shifts (δ) of Compounds 6−10[a]

	6		7		8		9		10	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1a	1.76 m	19.6	1.71 m	19.6	1.76 m	19.6	1.70 m	19.6	1.81 m	19.7
1b	1.93 m		1.89 m		1.94 m		1.88 m		1.97 m	
2a	2.07 br s	26.0	2.08 br s	26.0	2.11 br s	26.0	2.07 br s	26.0	2.13 br s	26.0
2b	2.07 br s									
3	5.24 br s	123.4	5.25 br s	123.4	5.26 br s	123.6	5.25 br s	123.3	5.28 br s	123.6
4		141.9		141.4		141.3		141.5		141.4
5		42.9		43.0		43.4		42.9		43.4
6	5.26 d (9.4)	78.9	5.26 d (10.0)	74.7	5.57 d (10.0)	75.8	5.24 m	74.7	5.80 d (10.1)	76.0
7	3.78 dd (9.4, 5.4)	74.9	5.27 d (10.0)	75.4	5.45 d (10.0)	75.2	5.21 m	75.8	5.68 d (10.1)	77.0
8		79.1		78.7		78.8		78.5		78.9
9		47.0		47.5		47.5		47.7		47.8
10	2.25 d (11.5)	40.5	2.25 d (11.0)	40.6	2.34 m	40.8	2.23 m	40.6	2.43 d (11.4)	40.7
11	5.60 d (9.9)	75.2	5.56 dd (10.4, 1.2)	74.6	5.59 d (9.6)	74.7	5.55 d (10.6)	74.8	5.61 d (10.0)	74.7
12a	2.58 dd (15.1, 9.9)	33.2	2.8 m	33.2	2.61 dd (15.1, 10.7)	33.1	2.58 m	33.1	2.64 dd (10.0, 15.2)	33.2
12b	3.53 d (15.1)		3.46 d (15.1)		3.51 d (15.2)		3.46 d (14.6)		3.57 d (15.3)	
13		168.6		168.2		168.1		168.2		168.2
14	5.83 s	116.7	5.83 s	116.8	5.84 s	117.0	5.82 s	117.0	5.84 s	116.9
15		174.2		174.0		174.0		174.0		174.3
16a	4.62 dd (17.5, 1.5)	73.4	4.62 dd (17.4, 1.6)	73.3	4.64 dd (17.4, 1.6)	73.3	4.62 d (17.4)	73.3	4.62 dd (1.6, 17.5)	73.3
16b	4.87 dd (1.5, 17.5)		4.83 dd (17.4, 1.6)		4.85 dd (17.4, 1.6)		4.83 d (17.4)		4.83 dd (1.6, 17.5)	
17	1.34 s	22.0	1.18 s	19.4	1.21 s	22.5	1.18 s	22.5	1.31 s	22.5
18	1.53 br s	21.0	1.57 d (1.2)	20.4	1.52 d (1.2)	20.5	1.56 s	20.4	1.55 d (1.0)	20.5
19	1.32 s	17.2	1.23 s	17.4	1.37 s	17.6	1.22 s	17.2	1.43 s	17.5
20	0.82 s	16.3	0.87 s	16.2	0.92 s	16.2	0.87 s	16.1	1.00 s	16.3
1′		168.5		170.4		164.8		170.7		165.0
2′		130.4	1.96 s	21.9		126.3	1.97 s	21.7		125.8
3′	8.03 m	129.9			9.16 d (1.6)	151.3			8.93 d (1.6)	150.8
4′	7.43 t (7.7)	128.8								
5′	7.56 t (7.4)	133.6			8.76 dd (4.8, 1.6)	154.0			8.60 dd (1.7, 4.8)	153.8
6′	7.43 t (7.7)	128.8			7.37 dd (7.9, 4.8)	123.6			7.97 dt (1.9, 8.0)	136.9
7′	8.03 m	129.9			8.21 dt (7.9, 1.9)	137.3			7.19 m	123.4
1″				176.2		176.3		170.1		164.7
2″			2.57 m	34.3	2.30 m	34.1	2.05 s	20.8		124.9
3″			1.14 d (6.8)	18.9	0.74 d (7.0)	18.5			8.98 d (1.6)	151.1
4″			1.18 d (6.8)	19.6	0.98 d (7.0)	19.4				
5″									8.62 dd (1.7, 4.8)	153.9
6″									8.02 dt (1.9, 8.0)	137.4
7″									7.19 m	123.6
11- OAc		171.3		171.0		171.0		171.0		171.1
	2.02 s	21.0	2.01 s	21.0	2.03 s	20.9	2.01 s	20.9	2.04 s	21.0

[a] ^{1}H NMR measured at 400 MHz, ^{13}C NMR measured at 100 MHz, and spectra obtained in $CDCl_3$ with TMS as internal standard. Assignments were supported with HSQC and HMBC NMR spectra.

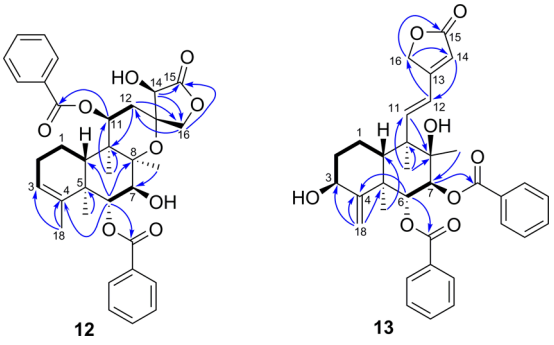

Figure 3. Key $^{1}H-^{1}H$ COSY (bold —) and HMBC (blue →) correlations of **12** and **13**.

compounds **7**−**10** were identified by comparison of their NMR data with those of **6**.

Compound **7** was assigned the molecular formula $C_{28}H_{40}O_9$, as established by HRESIMS (m/z 521.27390, $[M + H]^+$). The NMR data of **7** were similar to those of **6** (Table 2), but with signals indicative of acetoxy [(δ_H 2.01 (3H, s); δ_C 171.0 (s), 21.0 (q)] and isobutyryloxy [(δ_H 2.57 (1H, m), 1.14 (3H, d, J = 6.8 Hz), 1.18 (3H, d, J = 6.8 Hz); δ_C 176.2 (s), 34.3 (d), 18.9 (q), 19.6 (q)] groups, while those for a benzoyloxy moiety were absent. The acetoxy group was placed at C-6 from an HMBC correlation (Figure S55, Supporting Information) of H-6 [δ_H 5.26 (d, J = 10.0 Hz)] with a carbonyl carbon (δ_C 170.4). HMBC correlations from H-7 [δ_H 5.27 (d, J = 10.0 Hz)] and H-2″ (δ_H 2.57, m) to C-1″ (δ_C 176.2) and from H-2″ (δ_H 2.57, m) to C-7 (δ_C 75.4, d) indicated the isobutyryloxy group to be located at C-7. The relative configuration of compound **7** was

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Table 3. ^{1}H and ^{13}C NMR Chemical Shifts (δ) of Compounds 11−13[a]

	11		12		13	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1a	1.79 m	18.4	1.76 m	18.4	1.47 br s	21.7
1b	2.62 dd (13.4, 5.8)		2.64 m		1.71 m	
2a	2.14 overlap	26.4	2.15 overlap	26.5	1.23 m	32.3
2b					2.21 m	
3	5.29 br s	123.3	5.29 br s	123.3	4.56 dd (4.8, 11.8)	82.7
4		141.8		141.8		151.9
5		43.0		43.0		46.8
6	5.31 d (9.8)	77.7	5.30 d (9.9)	78.0	6.23 d (10.1)	74.1
7	3.64 dd (11.8, 10.0)	75.2	3.68 dd (11.7, 9.9)	75.0	5.77 d (10.1)	75.5
8		78.5		85.0		77.5
9		43.5		43.5		48.6
10	2.45 d (11.9)	40.2	2.45 d (11.2)	40.6	2.17 m	46.2
11	5.67 dd (12.8, 4.1)	74.5	5.98 dd (12.8, 4.3)	75.1	6.34 d (16.9)	146.2
12a	2.09 dd (12.9, 4.0)	35.3	2.06 d (12.7)	30.9	6.40 d (16.9)	122.2
12b	2.17 overlap		2.33 dd (12.7, 4.3)			
13		78.1		81.0		161.9
14	2.89 d (17.3)	42.9	4.45 d (3.4)	76.4	5.93 s	115.4
	2.84 d (17.3)					
15		174.3		175.6		174.2
16a	4.36 d (9.4)	79.3	4.27 d (9.4)	76.0	4.97 br s	70.9
16b	4.23 d (9.4)		4.23 d (9.4)			
17	1.55 s	20.0	1.69 s	18.2	1.05 s	22.8
18	1.59 s	21.1	1.59 s	21.2	4.92 s	102.6
					5.08 s	
19	1.36 s	17.4	1.37 s	17.4	1.51 s	17.4
20	1.11 s	17.2	1.12 s	17.2	1.26 s	15.4
1′		167.5		167.5		166.3
2′		129.8		130.1		129.0
3′	8.05 d (7.2)	129.9	8.06 d (7.7)	129.9	7.75 d (7.4)	129.7
4′	7.47 t (7.8)	128.9	7.43 t (7.7)	128.8	7.31 t (7.7)	128.5
5′	7.54 t (7.7)	132.2	7.55 t (7.7)	133.2	7.39 t (7.6)	133.1
6′	7.47 t (7.8)	128.9	7.43 t (7.7)	128.8	7.31 t (7.7)	128.5
7′	8.05 t (7.2)	129.9	8.06 d (7.7)	129.9	7.75 d (7.4)	129.7
1″		166.6		166.6		166.2
2″		130.9		130.8		130.0
3″	7.98 d (7.2)	129.8	7.98 d (7.7)	129.8	7.87 d (7.4)	130.0
4″	7.43 t (7.7)	128.6	7.46 t (7.7)	128.6	7.23 t (7.6)	128.4
5″	7.60 t (7.7)	133.7	7.58 t (7.7)	133.5	7.46 d (7.4)	133.5
6″	7.43 t (7.7)	128.6	7.46 t (7.7)	128.6	7.23 t (7.6)	128.4
7″	7.98 d (7.2)	129.8	7.98 d (7.7)	129.8	7.87 d (7.4)	130.0

[a] ^{1}H NMR measured at 400 MHz, ^{13}C NMR measured at 100 MHz, and spectra obtained in $CDCl_3$ with TMS as internal standard. Assignments were supported with HSQC and HMBC NMR spectra.

established by its NOESY spectrum (Figure S56, Supporting Information) and comparison with compound **6**. Thus, the structure of **7** (scutolide G) was found to be (11*S*)-6*α*,11-diacetoxy-8*β*-dihydroxy-7*β*-isobutyryloxy-3(4),13(14)-neocleroden-15,16-olide.

Compound **8** was obtained as a white, amorphous solid. Its molecular formula was established as $C_{32}H_{41}O_9N$ based on the protonated molecular ion peak $[M + H]^+$ at m/z 584.28430 in the HRESIMS. The IR spectrum contained an absorption band for a carbonyl ester (1735 cm^{-1}). The NMR data of **8** (Table 2) were very similar to those of **7**, but with additional signals evident for a nicotinoyloxy group [δ_H 9.16 (1H, d, J = 1.6 Hz), 8.76 (1H, dd, J = 4.8, 1.6 Hz), 7.37 (1H, dd, J = 7.9, 4.8 Hz), 8.21 (1H, dt, J = 7.9, 1.9 Hz); δ_C 164.8 (s), 126.3 (s), 151.3 (d), 154.0 (d), 123.6 (d), 137.3 (d)] and those representative of an acetoxy group being absent. HMBC correlations (Figure S63, Supporting Information) of H-6 [δ_H 5.57 (d, J = 10.0 Hz)] with C-1′ (δ_C 164.8) indicated that the nicotinoyloxy group is substituted at C-6. The relative configuration of **8** was determined by comparison with that of **7** and confirmed by NOESY correlations of H-7/H-17, H-19, H-20, H-11, and H-17 of and H-6/H-10. Thus, the structure of **8** (scutolide H) was defined as (11*S*)-6*α*-nicotinoyloxy-7*β*-isobutyryloxy-8*β*-dihydroxy-11-acetoxy-3(4),13(14)-neocleroden-15,16-olide.

The NMR data of **9** were almost identical to those of **7** (Table 2), except for the absence of signals for a $-CH(CH_3)_2$ moiety and the presence of additional acetoxy group signals [δ_H 2.05 (3H, s); δ_C 170.1 (s), 20.8 (q)]. The NMR data indicated the presence of three acetoxy groups in **9**, which were located at C-6, C-7, and C-11, respectively, from the HMBC correlations (Figure S71, Supporting Information) of H-6 (δ_H 5.24, m) with C-1′ (δ_C 170.7), H-7 (δ_H 5.21, m) with C-1″ (δ_C 170.1), and

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H-11 (δ_H 5.55, d, J = 10.6 Hz) with $C_{11\text{-}CO}$ (δ_C 171.0). The structure of **9** (scutolide I) was defined as (11*S*)-6α-nicotinoyloxy-7β-isobutyryloxy-8β-dihydroxy-11-acetoxy-3-(4),13(14)-neocleroden-15,16-olide by comparison with compounds **6**−**8**.

Compound **10** was obtained as a white powder with a molecular formula of $C_{34}H_{38}O_9N_2$. The NMR data of **10** (Table 2) resembled those of compound **8**, except for the lack of signals for an isobutyryloxy group and the presence of signals for an additional nicotinoyloxy group. The acetoxy group (δ_H 2.04, s; δ_C 171.1, s; 21.0, q) is substituted at C-11 from the HMBC correlation of H-11 [δ_H 5.61 (d, J = 10.0 Hz)] with the carbonyl carbon $C_{11\text{-}CO}$ (δ_C 171.1). HMBC correlations of H-6 [δ_H 5.80 (d, J = 10.1 Hz)] with C-1′ (δ_C 165.0) and H-7 with C-1″ (δ_C 164.7) confirmed the location of two nicotinoyloxy groups at C-6 and C-7. The relative configuration of **10** was determined from its ROESY spectrum (Figure S80, Supporting Information). Thus, the structure of **10** (scutolide J) was defined as (11*S*)-6α-nicotinoyloxy-7β-nicotinoyloxy-8β-dihydroxy-11-acetoxy-3(4),13(14)-neocleroden-15,16-olide.

Compound **11** gave the molecular formula $C_{34}H_{38}O_8$. The IR spectrum showed absorption bands at 2966, 1784, 1713, 1267, 1095, 1037, and 711 cm^{-1}, which were assignable to carbonyl and aromatic groups. In addition to two benzoyloxy moieties, the NMR data (Table 3) exhibited signals indicative of a *neo*-clerodane diterpenoid with a 3-ene-γ-13-spiro-15,16-lactone moiety [δ_H 1.55 (s, H_3-17), 1.59 (s, H_3-18), 1.36 (s, H_3-19), 1.11 (s, H_3-20), 5.29 (br s, H-3), 2.89 (d, J = 17.3 Hz, H_a-14), 2.84 (d, J = 17.3 Hz, H_b-14), 4.36 (d, J = 9.4 Hz, H_b-16), 4.23 (d, J = 9.4 Hz, H_b-16), δ_C 20.0 (q, C-17), 21.1 (q, C-18), 17.4 (q, C-19), 17.2 (q, C-20), 123.3 (d, C-3), 141.8 (s, C-4), 42.9 (t, C-14), 79.3 (t, C-16)]. Detailed analysis of the 2D NMR data (^{1}H−^{1}H COSY, HMQC, and HMBC; Figures S84−86, Supporting Information) confirmed the above findings. The positions of the two benzoyloxy groups were determined from the HMBC correlations (Figure S87) from H-6 [δ_H 5.31 (d, J = 9.8 Hz)] to C-1′ (δ_C 167.5) and H-11 [δ_H 5.67 (dd, J = 4.1, 12.8 Hz)] to C-1″ (δ_C 166.6), respectively. Thus, the structure of **11** was defined as shown.

The relative configuration of **11** was deduced from its ROESY spectrum. ROESY correlations of H-7/H-17, H-19, H-20 and H-6/H-10, H-16 indicated that they are all α-oriented, while the correlations of H-6/H-10 and H-16 suggested that H-6 and H-16 are β-oriented. Since the absolute configuration of this type of neo-clerodane was not reported before, a single-crystal X-ray diffraction analysis was conducted using Cu Kα radiation to define the absolute configuration of **11** as (5*R*,6*R*,7*S*,8*R*,9*S*,10*S*,11*S*,13*R*) (Figure 2). Compound **11** was named scutolide K.

The molecular formula of **12** was determined as $C_{34}H_{38}O_9$ by HRESIMS (m/z 589.24469 [M − H]$^-$). Comparison of the NMR data (Table 3) with those of **11** suggested that the two compounds have similar structures, but an additional oxygenated methine (δ_H 4.45, d, J = 3.4 Hz) and hydroxy group are present in **12**, while one methylene found in **11** is absent. The HMBC correlations (Figure 3) from the oxygenated methine proton [δ_H 4.45, d, J = 3.4 Hz] to the carbons with chemical shifts of δ_C 175.6 (C-15), 81.0 (C-13), 76.4 (C-14), and 30.9 (C-12) indicated that the hydroxy group is located at C-14. The relative configuration of **12** was determined by comparison with **11** and confirmed by a ROESY experiment. The ROESY correlations (Figure S95, Supporting Information) of H-7/H-17, 19, 20, H-11/H-17, and H-14/H-17 suggested that they were cofacial and α-oriented, whereas the ROESY correlation of H-6/H-10 indicated that they were on opposite faces and β-oriented. Thus, the configuration of C-14 was established as *R*. The structure of **12** was defined as (14*R*)-14β-hydroxyscutolide K.

Compound **13** was obtained as a white, amorphous solid. It gave the molecular formula $C_{34}H_{36}O_8$. The IR spectrum showed absorption bands at 3485, 2932, 1729, 1273, 1108, 1028, 978, and 710 cm^{-1}. Its NMR data (Table 3) were somewhat different from those of compounds **1**−**5**. Comparison of the NMR data of **13** and compounds **1**−**5** revealed the presence of an exocyclic double bond (CH_2═) moiety (δ_C 102.6, t, C-18) and the absence of one methyl group and a CH═ moiety. Thus, the CH_3-18 group in compounds **1**−**5** was altered to an exocyclic double bond in **13**. Detailed analysis of the 1D and 2D NMR data suggested that the exocyclic double bond (CH_2═) moiety is located at C-4, which was confirmed by HMBC correlations of H-18 [δ_H 4.92 (s); 5.08 (s)] with C-10 (δ_C 46.2), C-3 (δ_C 82.7), and C-4 (δ_C 151.9). The location of the hydroxy group at C-3 was suggested from HMBC (Figure 3) correlations of H-3 [δ_H 4.56 (dd, J = 4.8, 11.8 Hz)] with C-4 (δ_C 151.9) and C-18 (δ_C 102.6) and the COSY correlation of H-3 with H-2 [δ_H 1.23 (m); 2.21 (m)]. HMBC correlations of H-6 [δ_H 6.23 (d, J = 10.1 Hz)] with C-1′ (δ_C 166.3) and H-7 [δ_H 5.77 (d, J = 10.1 Hz)] with C-1″ (δ_C 166.2, s) suggested that the two benzoyloxy groups are connected to C-6 and C-7, respectively. The relative configuration of **13** was determined from its ROESY spectrum (Figure S104, Supporting Information). Thus, the structure of **13** (scutolide L) was defined as shown.

All isolated compounds (**1**−**26**) were evaluated for activity against EBV DNA lytic replication in P3HR-1 cells. Initially, the 26 compounds were tested for inhibition of EBV DNA lytic replication at a concentration of 20 μM. (+)-Rutamarin was used as control.[24] Among them, 11 compounds (**3**, **4**, **6**, **11**, **12**, **15**−**17**, **20**, **22**, and **24**) exhibited more than 50% inhibition against EBV lytic replication. These 11 compounds were further analyzed to obtain their EC_{50}, CC_{50}, and SI values (Table 4). On the basis of these results, preliminary SAR (structure−activity relationship) correlations could be established. Structurally, the 26 compounds tested can be divided

Table 4. Anti-EBV Lytic Replication Activities of the Isolates from *S. barbata*

cmpd	EC_{50}[a]	R^2[d]	CC_{50}[b]	R^2[d]	SI[c]
3	9.2	0.9377	49.7	0.9172	5.4
4	3.2	0.9659	147.7	0.4217	46.1
6	8.7	0.9594	37.8	0.9691	4.3
11	18.3	0.9271	66.5	0.6776	3.6
12	5.2	0.8703	15.1	0.8101	2.9
15	23.6	0.9612	1442	0.5732	61.1
16	14.3	0.8389	30.0	0.8344	2.1
17	18.7	0.8699	307.4	0.9824	16.4
20	9.1	0.9689	68.3	0.7152	7.5
22	12.9	0.9424	737.7	0.9691	57.2
24	16.4	0.9567	1791	0.7324	109.2
(+)-rutamarin[e]	5.43	0.879	>150		>39.6

[a]The inhibitory effects of compounds against EBV lytic replication were tested and expressed as EC_{50} values (μM). [b]Cytotoxicities were measured after 2 days of compound treatment and expressed as CC_{50} values (μM). [c]Selective index (SI) = CC_{50}/EC_{50}. [d]Regression coefficients of the dose−response curves. [e]Positive control.

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into two groups. In group 1, all of the compounds (**1**−**10**, **13**−**21**) possess an α,β-unsaturated-γ-lactone moiety, while the compounds of group 2 (**11**, **12**, **22**−**26**) are characterized by a γ-spirolactone moiety. As shown in Table 4, compounds from both groups showed activity. Compound **4** from group 1 exhibited the most potent activity (EC_{50} = 3.2 μM), while compound **24** from group 2 demonstrated the highest SI (109.2). Of the 19 compounds in group 1, only those with benzoyl substitution on C-6 exhibited antiviral activity, and replacement of the benzoyl group with an acetoxy or nicotinoyloxy group generally resulted in complete loss of activity (e.g., **3** vs **2** and **16** vs **5**). The only exception was **17**, which has a nicotinoyloxy group at C-6, but still showed moderate activity (EC_{50} = 18.7 μM, SI = 16.4). However, its EC_{50} was nearly 6-fold higher than that of **4**, with a benzoyl group on C-6. These results implied that a benzoyl substituent at C-6 is required for activity in *neo*-clerodane diterpenoids with an α,β-unsaturated-γ-lactone. Structural variation at C-7 had much less effect on activity. Compound **4**, with a 2-acetoxy-3-methyl substituent on C-7, exhibited the most potent activity, while compound **15**, with a nicotinoyloxy group at C-7, demonstrated reduced activity, but improved selectivity. Thus, compound **15** had the highest SI (61.1) of all group 1 compounds. Other compounds with isobutyryl (**3**), benzoyl (**16**), and hydroxy (**20**) groups still showed moderate activity, although they were less potent than **4**. Compounds **6** and **20** have the same substituents at C-6 and C-7, while **6** has a β-OAc group at C-11, rather than the double bond between C-11 and C-12 as in **20**. Compound **6** had equivalent activity to that of **20**, while its cytotoxicity was almost 2-fold more than that of **20**.

Four of the seven compounds in group 2 (**11**, **12**, **22**, and **24**) showed moderate to potent activity (EC_{50} values of 5.2−18.3 μM). Unlike compounds in group 1, substitution at C-6 of compounds in group 2 could be varied without reduction of the activity. Compound **24**, with two nicotinoyloxy groups at C-6 and C-11, respectively, showed moderate activity (16.4 μM) as well as the lowest toxicity (1791 μM) and, thus, the highest SI (109.2).

In summary, bioactivity-guided investigation of the whole plants of *S. barbata* led to the isolation of 13 new *neo*-clerodane diterpenoids, as well as 13 related known compounds. Biological assays revealed that 11 of the compounds isolated exhibited moderate to potent anti-EBV activity. The new compound **4** exhibited the most potent activity (EC_{50} = 3.2 μM, SI = 46.1). Compound **24** showed moderate activity (EC_{50} = 16.4 μM), but low cytotoxicity (CC_{50} = 1791 μM) and a high selective index (SI = 109.2). On the basis of the results obtained, SAR correlations have also been determined. This report is the first to reveal that *neo*-clerodane diterpenoids possess inhibitory activity against EBV lytic replication.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 341 polarimeter. UV spectra were recorded using a Shimadzu UV2450 spectrophotometer. IR spectra were recorded on a Bruker Tensor 37 infrared spectrophotometer with KBr pellets. The NMR spectra were recorded on a Bruker AVANCE-400 NMR spectrometer with TMS as an internal reference operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively. The HRESIMS and HREIMS data were determined on a Shimadzu LCMS-IT-TOF mass spectrometer and were measured on an Agilent 1200 series LC-MS/MS system. TLC analysis was carried out on silica gel plates (Marine Chemical Ltd., Qingdao, People's Republic of China). Macroporous resin D101 (Sinopharm Chemical Reagent Co., Ltd., Shanghai, People's Republic of China), RP-C_{18} silica gel (Fuji, 40−75 μm), MCI gel (CHP20P, 75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (200−300 Mesh, Marine Chemical Ltd.), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography (CC). HPLC separations were carried out on an LC-20AT Shimadzu liquid chromatography system to an Agilent SB-C_{18} column (250 × 9.4 mm, 5 μm) connected with an SPD-M20A diode array detector.

Plant Material. The dried whole plants of *S. barbata* (3.5 kg) were collected in Nanning District, Guangxi Province, People's Republic of China, in September 2012 and were identified by Dr. Chunyan Han from Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (ZY-20120304) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

Extraction and Isolation. The air-dried and powdered whole plants of *Scutellaria barbata* (3.5 kg) were extracted with 95% EtOH (3 × 20 L) by maceration at room temperature for 3 days. The solvent was evaporated under reduced pressure to yield a crude extract (362 g), which was suspended in H_2O and extracted successively with petroleum ether (3 × 3 L), EtOAc (3 × 3 L), and *n*-BuOH (3 × 3 L). Then, the EtOAc-soluble extract (183 g) was chromatographed on D101 macroporous resin eluting with a step gradient of EtOH−H_2O to give six fractions (A−F). Fraction C (20 g) was subjected to RP-C_{18} CC using a step gradient of MeOH−H_2O from 50% to 100% to afford five subfractions (C1−C5). Fraction C1 was chromatographed on a silica gel column with CH_2Cl_2−MeOH (100:1) as eluent to yield compounds **23** (525 mg), **24** (53 mg), and **25** (42 mg). Fraction C3 was separated on Sephadex LH-20 eluting with MeOH and further purified by semipreparative HPLC using 65% CH_3CN−H_2O as the solvent to produce compounds **10** (7.4 mg) and **19** (24 mg). Compounds **9** (17 mg) and **18** (15 mg) were isolated from fraction C4 by semipreparative HPLC eluted with 60% MeOH−H_2O. Fraction D (16 g) was subjected to Sephadex LH-20 with MeOH as eluent to separate diterpenoid and flavonoid fractions. The diterpenoid portion was then chromatographed on a silica gel column eluting with CH_2Cl_2−MeOH (50:1) to afford three subfractions (D1−D3), which were further purified by RP-HPLC eluting with MeCN−H_2O (75:25, 65:35, 60:40) to afford compounds **7** (47 mg), **8** (48 mg), **14** (4 mg), **1** (32 mg), **2** (50 mg), and **26** (15 mg). Fraction E (45 g) was chromatographed on a silica gel column using CH_2Cl_2−MeOH (100:0, 100:1, 98:2, 95:5) to give 10 subfractions (E1−E10). Fraction E6 was further separated using MCI gel eluting with an increasing gradient of MeOH−H_2O (30−100%) to give compounds **11** (56 mg), **12** (42 mg), and **22** (419 mg). Fraction E7 was chromatographed successively over two silica gel columns eluting with CH_2Cl_2−MeOH (200:1) and CH_2Cl_2−MeOH (100:1), respectively, to obtain compounds **5** (20 mg), **6** (46 mg), **13** (8 mg), **16** (51 mg), and **20** (5 mg). Fraction E8 was chromatographed over MCI gel with an increasing gradient of MeOH−H_2O (70, 90, 100%) and then further purified by semipreparative RP-HPLC using MeOH−H_2O (70%) to obtain compounds **3** (29 mg), **4** (26 mg), and **21** (5 mg). Compounds **15** (14 mg) and **17** (16 mg) were isolated from fraction F by semipreparative RP-HPLC with MeCN−H_2O (70:30) as the solvent system.

*Scutolide A (**1**):* white powder; $[\alpha]_D^{30}$ −34.7 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 257 (4.24) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 211 (0.89), 263 (−0.37) nm; IR ν_{max} 3460, 2973, 1739, 1642, 1377, 1247, 1150, 1028, 884, 738 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HRESIMS *m*/*z* 461.25274 $[M + H]^+$ (calcd for $C_{26}H_{37}O_7$, 461.25338).

*Scutolide B (**2**):* white powder; $[\alpha]_D^{30}$ −57.2 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.19), 258 (4.48) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 205 (−0.87), 265 (−0.47) nm; IR ν_{max} 2925, 1735, 1647, 1458, 1274, 1141, 1026, 799, 630 cm$^{-1.}$ ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HRESIMS *m*/*z* 524.26392 $[M + H]^+$ (calcd for $C_{30}H_{38}O_7N_1$, 524.26428).

*Scutolide C (**3**):* white powder; $[\alpha]_D^{30}$ −41.3 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 237 (4.45), 258 (4.27) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (1.49), 222 (−0.59), 260 (−0.40) nm; IR ν_{max} 3475, 2971,

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1732, 1645, 1455, 1380, 1266, 1146, 1027, 716 cm^{-1}, ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HRESIMS m/z 523.26874 $[M + H]^+$ (calcd for $C_{31}H_{39}O_7$, 523.26903).

*Scutolide D (**4**):* white powder; $[\alpha]_D^{30}$ −26.4 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 258 (4.31) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−1.33), 223 (−0.62), 263 (0.29) nm; IR ν_{max} 3482, 2969, 1740, 1645, 1453, 1378, 1270, 1110, 1031, 711 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HRESIMS m/z 593.27611 $[M - H]^-$ (calcd for $C_{34}H_{41}O_9$, 593.27561).

*Scutolide E (**5**):* white powder; $[\alpha]_D^{30}$ −42.1 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 239 (4.46), 259 (4.53) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 200 (−1.61), 225 (−1.24), 253 (0.91) nm; IR ν_{max} 2925, 1734, 1642, 1447, 1267, 1104, 1022, 710 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HRESIMS m/z 495.23724 $[M + H]^+$ (calcd for $C_{29}H_{35}O_7$, 495.23773).

*Scutolide F (**6**):* white powder; $[\alpha]_D^{30}$ −14.5 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 223 (4.19) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (0.39), 231 (−0.55) nm; IR (KBr) ν_{max} 3480, 2971, 1735, 1272, 1025, 969, 714 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 2; HRESIMS m/z 513.24792 $[M + H]^+$ (calcd for $C_{29}H_{37}O_8$, 513.24829).

*Scutolide G (**7**):* white powder; $[\alpha]_D^{30}$ −18.1 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.10) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (−2.03), 224 (−1.84) nm; IR ν_{max} 3482, 2970, 1738, 1453, 1372, 1238, 1022, 602 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 2; HRESIMS m/z 521.27390 $[M + H]^+$ (calcd for $C_{28}H_{41}O_9$, 521.27451).

*Scutolide H (**8**):* white, amorphous solid; $[\alpha]_D^{30}$ −24.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.56), 261 (4.19) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 222 (−0.23), 258 (−0.06) nm; IR ν_{max} 3480, 2973, 1736, 1647, 1457, 1376, 1275, 1026, 972, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 2; HRESIMS m/z 584.28430 $[M + H]^+$ (calcd for $C_{32}H_{42}O_9N$, 584.28541).

*Scutolide I (**9**):* white, amorphous solid; $[\alpha]_D^{30}$ −26.5 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.06), 255 (3.25) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (−0.29), 223 (−0.17) nm; IR ν_{max} 3477, 2971, 1741, 1445, 1374, 1237, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 2; HRESIMS m/z 491.22919 $[M - H]^-$ (calcd for $C_{26}H_{35}O_9$, 491.22866).

*Scutolide J (**10**):* white powder; $[\alpha]_D^{30}$ −11.0 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.29), 262 (3.92) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (5.06), 233 (−6.44), 268 (−2.15) nm; IR ν_{max} 3475, 2974, 1734, 1429, 1282, 1120, 1025, 968, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 2; HRESIMS m/z 617.25160 $[M - H]^-$ (calcd for $C_{34}H_{37}O_9N_2$, 617.25045).

*Scutolide K (**11**):* white powder; $[\alpha]_D^{30}$ −14.5 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.34) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−1.10), 222 (−1.24), 243 (0.19) nm; IR ν_{max} 2966, 1784, 1713, 1267, 1095, 1037, 801, 711 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HRESIMS m/z 573.25096 $[M - H]^-$ (calcd for $C_{34}H_{37}O_8$, 573.25123).

*(14R)-14β-Hydroxyscutolide K (**12**):* white powder; $[\alpha]_D^{30}$ −52.8 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.24) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 213 (−2.87) nm; IR ν_{max} 3489, 2963, 1789, 1715, 1452, 1382, 1270, 1104, 1032, 711 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HRESIMS m/z 589.24469 $[M - H]^-$ (calcd for $C_{34}H_{37}O_9$, 589.24480).

*Scutolide L (**13**):* white, amorphous solid; $[\alpha]_D^{30}$ −63.5 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.32), 258 (4.27) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (−9.38), 238 (−6.27) nm; IR ν_{max} 3485, 2932, 1729, 1273, 1108, 1028, 978, 710 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS m/z 571.23480 $[M - H]^-$ (calcd for $C_{34}H_{35}O_8$, 571.23374).

X-ray Crystallographic Analysis of Compound 1. The crystal structure and absolute configuration of **1** were determined by single-crystal X-ray diffraction analysis. A suitable crystal was selected and tested on an Xcalibur, Onyx, Nova diffractometer with the temperature kept at 100(2) K during data collection. Structures were solved using direct methods with SHELXS and refined with SHELXL. The H atoms were placed in calculated positions and refined using a riding model. There was one independent molecule per asymmetric unit. Molecular graphics were computed with ORTEP-3. Crystal data: compound **1**, $C_{26}H_{36}O_7$ (M = 460.55); orthorhombic, space group $P2_12_12_1$ (no. 19), a = 9.60332(9) Å, b = 9.87431(10) Å, c = 26.7500(2) Å, V = 2536.60(4) $Å^3$, Z = 4, T = 100(2) K, μ(Cu Kα) = 0.708 mm^{-1}, D_{calc} = 1.206 g/cm^3, 20 901 reflections measured, 4868 unique (R_{int} = 0.0329, R_{sigma} = 0.0222), which were used in all calculations. The final R_1 was 0.0315 and wR_2 was 0.0836 (all data). Crystallographic data for the structure of **1** have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 1024978.

X-ray Crystallographic Analysis of Compound 11. Crystal data for compound **11**: $C_{34}H_{38}O_8$ (M = 574.64); orthorhombic, space group $P2_12_12_1$ (no. 19), a = 9.67492(11) Å, b = 14.41358(14) Å, c = 20.62930(17) Å, V = 2876.76(5) $Å^3$, Z = 4, T = 100(2) K, μ(Cu Kα) = 0.767 mm^{-1}, D_{calc} = 1.327 g/cm^3, 27 926 reflections measured, 5604 unique (R_{int} = 0.0548, R_{sigma} = 0.0317), which were used in all calculations. The final R_1 was 0.0322 and wR_2 was 0.0831 (all data). Crystallographic data for the structure of **11** have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 1024913.

Assay for Inhibition on EBV Lytic Replication of Extracts and Isolated Compounds. The effects of the extracts and compounds on EBV lytic replication assay were evaluated using a previously reported method.[12] The crude extracts and all isolated compounds were dissolved with dimethyl sulfoxide (DMSO) to 100 mM and then diluted to the test concentration. The final DMSO concentration in culture medium was maintained at 0.5%. P3HR-1 cells, a Burkitt lymphoma cell line latently infected with EBV, were cultured in RPMI 1640 medium (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (Gibco-BRL), streptomycin (100 μg/mL), and penicillin (100 units/mL). For viral lytic replication, P3HR-1 cells were induced with TPA (20 ng/mL) and sodium butyrate (0.3 mM). After 3 h, the cells were treated with the extracts or compounds over a wide range of concentrations. TPA/NaB-induced and noninduced HR-1 cells were harvested at 48 h postinduction, and total DNA in the cells was purified using the Hipure Tissue DNA kits according to the manufacturer's protocol (Magen). The EBV genomic copy number was quantified by real-time PCR on a Roche 480 lightCycler instrument using the lightCyclerFastStart DNA Master^{Plus} SYBR green kit with primers for the detection of EBNA1 (sense: 5′-CATTGAGTCGTCTCCCCTTTGGAAT-3′; antisense: 5′-TCATAACAAGGTCCTTAATCGCATC-3′). The interacellular viral genomic DNA in each sample was normalized to GAPDH using the primers directed to GAPDH (sense: 5′-ACATCATCC-CTGCCTCTAC-3′; antisense: 5′-TCAAAGGTGGAGGAGTGG-3′). The half-maximal antiviral effective concentration (EC_{50}) values of the tested compounds were determined from dose−response curves of EBV DNA content values from TPA/butyrate-induced and compound-treated cells. The viral DNA contents were reduced by the contents of noninduced cells, divided by that from the control cells without drug treatment, and then represented on the y axes of the dose−response curves. Y axis value = $(TPA_X - \text{no } TPA_X)/(TPA_0 - \text{no } TPA_0)$, where X is any concentration of the drug and 0 represents no-drug treatment. The EC_{50} values for each compound were calculated with the aid of GraphPad Prism software. (+)-Rutamarin, which was previously reported to exhibit anti-EBV activity,[24] was used as the control in this study.

Cytotoxicity Assays. The viabilities of P3HR-1 cells after treatment with compounds were assessed by counting Trypan blue-stained cells 48 h post-treatment using a light microscope. Cell viabilities were defined relative to the control cells (non-drug-treated). The half-maximal cytotoxic concentration (CC_{50}) was calculated from the dose−response curves with GraphPad Prism software.

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■ ASSOCIATED CONTENT

Supporting Information

IR, MS, and 1D and 2D NMR spectra for compounds **1**−**13**. This material is 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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■ DEDICATION

Dedicated to Dr. William Fenical of Scripps Institution of Oceanography, University of California−San Diego, for his pioneering work on bioactive natural products.

■ REFERENCES

(1) Sato, Y.; Suzaki, S.; Nishikawa, T.; Kihara, M.; Shibata, H.; Higuti, T. *J. Ethnopharmacol.* **2000**, *72*, 483−488.

(2) Yu, J.; Lei, J.; Yu, H.; Cai, X.; Zou, G. *Phytochemistry* **2004**, *65*, 881−884.

(3) Lee, T. K.; Kim, D. I.; Han, J. Y.; Kim, C. H. *Immunopharmacol. Immunotoxicol.* **2004**, *26*, 315−327.

(4) Dai, S. J.; Tao, J. Y.; Liu, K.; Jiang, Y. T.; Shen, L. *Phytochemistry* **2006**, *67*, 1326−1330.

(5) Dai, S. J.; Wang, G. F.; Chen, M.; Liu, K.; Shen, L. *Chem. Pharm. Bull.* **2007**, *55*, 1218−1221.

(6) Dai, S. J.; Chen, M.; Liu, K.; Jiang, Y. T.; Shen, L. *Chem. Pharm. Bull.* **2006**, *54*, 869−872.

(7) Dai, S. J.; Peng, W. B.; Zhang, D. W.; Shen, L.; Wang, W. Y.; Ren, Y. *J. Nat. Prod.* **2009**, *72*, 1793−1797.

(8) Zhu, F.; Di, Y. T.; Liu, L. L.; Zhang, Q.; Fang, X.; Yang, T. Q.; Hao, X. J.; He, H. P. *J. Nat. Prod.* **2010**, *73*, 233−236.

(9) Li, Y. Y.; Tang, X. L.; Jiang, T.; Li, P. F.; Li, P. L.; Li, G. Q. *J. Asian Nat. Prod. Res.* **2013**, *15*, 941−949.

(10) Dai, S. J.; Qu, G. W.; Yu, Q. Y.; Zhang, D. W.; Li, G. S. *Fitoterapia* **2010**, *81*, 737−741.

(11) Nguyen, V. H.; Pham, V. C.; Nguyen, T. T. H.; Tran, V. H.; Doan, T. M. H. *Eur. J. Org. Chem.* **2009**, *33*, 5810−5815.

(12) Silva, L.; Gomes, A. C.; Rodilla, J. M. *Nat. Prod. Commun.* **2011**, *6*, 497−504.

(13) Gang, X.; Fang, Z.; Yang, X. W.; Zhou, J.; Yang, L. X.; Shen, X. L.; Hu, Y. J.; Zhao, Q. S. *Nat. Prod. Bioprospect.* **2011**, *1*, 81−86.

(14) Klein, E.; Kis, L. L.; Klein, G. *Oncogene* **2007**, *26*, 1297−1305.

(15) Gu, Q.; Chen, Y. Y.; Cui, H.; Huang, D. N.; Zhou, J. W.; Wu, T. Z.; Chen, Y. P.; Shi, L. N.; Xu, J. *RSC Adv.* **2013**, *3*, 10168−10172.

(16) Wu, T. Z.; Cui, H.; Cheng, B.; Fang, S.; Xu, J.; Gu, Q. *Biochem. Syst. Ecol.* **2014**, *54*, 65−67.

(17) Cui, H.; Xu, B.; Wu, T. Z.; Xu, J.; Yuan, Y.; Gu, Q. *J. Nat. Prod.* **2014**, *77*, 100−110.

(18) Wang, F.; Ren, F. C.; Li, Y. J.; Liu, J. K. *Chem. Pharm. Bull.* **2010**, *58*, 1267−1270.

(19) Lee, H.; Kim, Y.; Choi, I.; Min, B. S.; Shim, S. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 288−290.

(20) Dai, S. J.; Shen, L.; Ren, Y. *J. Integr. Plant Biol.* **2008**, *50*, 699−702.

(21) Dai, S. J.; Liang, D. D.; Ren, Y.; Liu, K.; Shen, L. *Chem. Pharm. Bull.* **2008**, *56*, 207−209.

(22) Dai, S. J.; Sun, J. Y.; Ren, Y.; Liu, K.; Shen, L. *Planta Med.* **2007**, *73*, 1217−1220.

(23) Zhu, F.; Di, Y. T.; Li, X. Y.; Liu, L. L.; Zhang, Q.; Li, Y.; Hao, X. J.; He, H. P. *Planta Med.* **2011**, *77*, 1536−1541.

(24) Wu, T.; Wang, Y.; Yuan, Y. *Antiviral Res.* **2014**, *107*, 95−101.

■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on February 3, 2015 with errors in several compound names. The corrected version was reposted on March 9, 2015.

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