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Prenylated Benzoylphloroglucinols and Xanthones from the Leaves of *Garcinia oblongifolia* with Antienteroviral Activity

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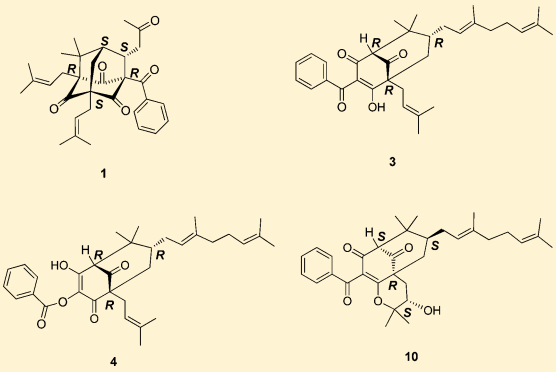
Supporting Information

ABSTRACT: An acetone extract of the leaves of *Garcinia oblongifolia* showed antiviral activity against enterovirus 71 (EV71) using a cytopathic effect inhibition assay. Bioassay-guided fractionation yielded 12 new prenylated benzoylphloroglucinols, oblongifolins J–U (**1**–**12**), and five known compounds. The structures of **1**–**12** were elucidated by spectroscopic analysis including 1D- and 2D-NMR and mass spectrometry methods. The absolute configurations were determined by a combination of a Mosher ester procedure carried out in NMR tubes and ECD calculations. Compared to ribavirin (IC_{50} 253.1 μM), compounds **1**, **4**, and **13** exhibited significant anti-EV71 activity in vitro, with IC_{50} values of 31.1, 16.1, and 12.2 μM, respectively. In addition, the selectivity indices of these compounds were 1.5, 2.4, and 3.0 in African green monkey kidney (Vero) cells, respectively.



Enterovirus 71 (EV71) is a small, positive-sense, single-stranded RNA virus in the genus *Enterovirus*, family Picornaviridae.[1] Infection with EV71 is of particular concern because this causes severe neurological complications and may result in death in infants and young children.[2] Currently, no vaccines or antiviral drugs against EV71 are clinically available.[1] Thus, it is imperative to develop new and effective antiviral drugs to combat human EV71 infections. In a program for discovering new compounds with anti-EV71 activities from Chinese herbs, an acetone extract of the leaves of *Garcinia oblongifolia* Champ. ex Benth (Clusiaceae) demonstrated anti-EV71 activity using an EV71 infection-induced cytopathic effect (CPE) assay (Figure S1, Supporting Information). Thus, it was speculated that the leaves of *G. oblongifolia* might contain secondary metabolites with anti-EV71 activity.

G. oblongifolia, a tree or medium-sized shrub, is widely distributed in southern mainland China and northern Vietnam.[3] Previous phytochemical investigations on this species resulted in the isolation of compounds including polycyclic prenylated acylphloroglucinols,[3,4] prenylated xanthones,[3,4] and biphenyls.[5] In this study, the chemical constituents of the acetone extract of the leaves of *G. oblongifolia* were investigated to find potential anti-EV71 lead compounds. As a result, a series of prenylated benzoylphloroglucinols, including 12 new compounds (**1**–**12**) and one known analogue, along with four known xanthones, were identified from the active petroleum ether-soluble fraction. All compounds were evaluated for their antiviral activities against EV71 using the CPE inhibition assay by an MTT method. Herein, the isolation, structure elucidation, and antiviral evaluation of the isolates obtained are described.

RESULTS AND DISCUSSION

The petroleum ether-soluble part of the acetone extract of *G. oblongifolia* was purified by column chromatography over MCI gel, silica gel, reversed-phase C_{18} silica gel, Sephadex LH-20, and preparative HPLC to afford 17 compounds, including 12 new (**1**–**12**) and five known compounds. The structures of the known compounds were identified as euxanthone (**13**),[6] oblongifolin C,[4a] garcihombronone C,[7] oblongixanthone B,[4b] and dulxanthone-B[8] by comparison of their spectroscopic data with published values.

Oblongifolin J (**1**) was isolated as a light brown gum and showed a pseudomolecular ion peak at m/z 503.2772 $[M + H]^+$ (calcd 503.2797) in the HRESIMS, corresponding to the molecular formula $C_{32}H_{38}O_5$. The ^1H NMR spectrum revealed the presence of a benzene ring [δ_H 7.51 (1H, m), 7.35 (2H, t, J = 7.6 Hz), and 7.18 (2H, d, J = 7.6 Hz)], a vinyl proton [δ_H 5.16 (1H, m)], two methine carbons [δ_H 3.95 (1H, m) and 1.88 (1H, m)], three methylene carbons [δ_H 2.48 and 2.43

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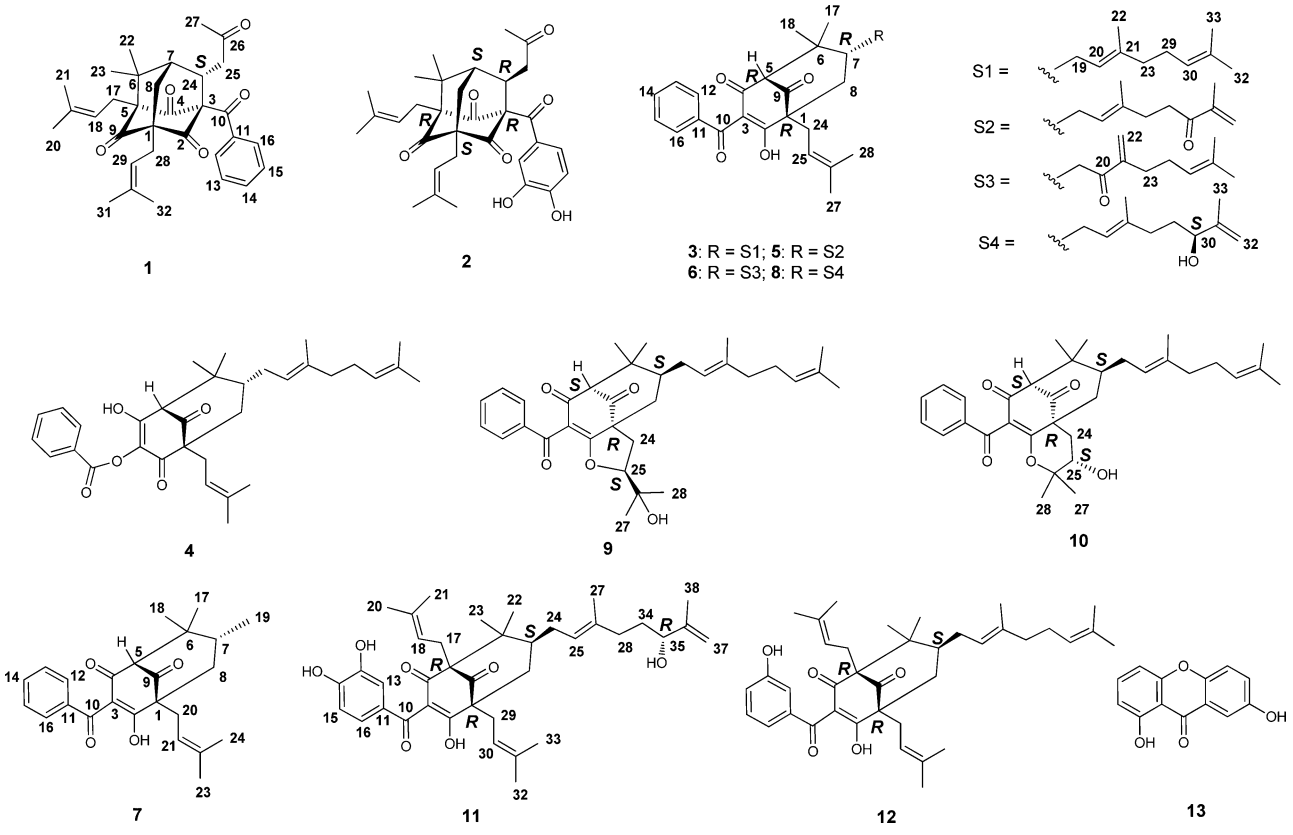
Chart 1



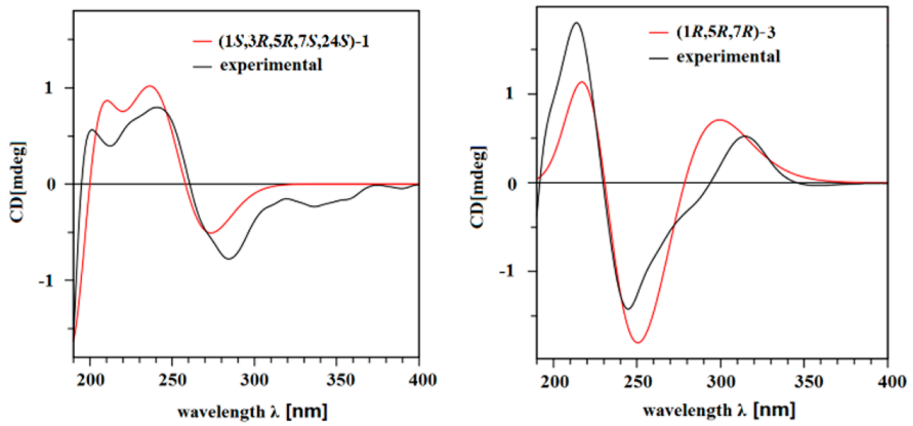


Figure 1. Calculated ECD spectra of **1** and **3** and their experimental curves.

(each 1H, m), 2.49 (2H, m), and 2.96 and 2.46 (each 1H, m)], and seven methyl groups [δ_H 2.22 (3H, s), 1.70 (6H, s), 1.68 (3H, s), 1.62 (3H, s), 1.23 (3H, s), and 1.19 (3H, s)]. The ^{13}C NMR and DEPT spectra indicated the presence of four carbonyl carbons (δ_C 208.4, 204.4, 203.7, and 203.0), a benzoyl group (δ_C 195.8, 135.9, 134.1, 130.5 × 2, and 129.3 × 2), two double bonds [δ_C 135.8, 135.5, 120.8, and 120.2], four quaternary carbons [δ_C 80.1, 78.4, 70.5, and 55.2], two methines, four methylenes, and seven methyls. On the basis of these data, compound **1** was proposed as a trioxygenated adamantyl ketone bearing two prenyl units.[9] When the NMR data of **1** were compared with those of (+)-hyperibone K (Figure S3, Supporting Information),[9b] it was found that they exhibited very similar structures. The only structural difference between the two compounds was the presence of an acetonyl group (CH_3−CO−CH_2−) in **1** [as shown by the presence of the methyl resonance (δ_C 30.5; δ_H 2.22) and a methylene (δ_C 43.1; δ_H 2.96, 2.46), which showed HMBC correlations to the low-field ketone carbonyl (δ 208.4)] instead of a 2-methylpropenyl group in (+)-hyperibone K. The location of the acetonyl group was assigned at C-24 by the HMBC correlations from H-25 (δ_H 2.96, 2.46) to C-3 (δ_C 80.1), C-7 (δ_C 47.2), and C-24 (δ_C 49.3). Other key HMBC correlations are shown in Figure S4 (Supporting Information).

The relative configurations at the chiral centers C-1, C-3, C-5, and C-7 were evident for the adamantyl core.[9c,10] The relative configuration of the remaining chiral carbon at C-24 was deduced from the NOE correlations of H-8a/CH_3-23 and H-25b/CH_3-22 (Figure S4, Supporting Information). Thus, there were two possible isomers: (1*S*,3*R*,5*R*,7*S*,24*S*)-**1** and

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(1*R*,3*S*,5*S*,7*R*,24*R*)-**1**. The absolute configuration of **1** was determined by comparison of their experimentally measured electronic circular dichroism (ECD) curves with the TDDFT-predicted curves. The results showed that the calculated ECD curve of (1*S*,3*R*,5*R*,7*S*,24*S*)-**1** was consistent with the experimental ECD spectrum of **1** (Figure 1). Thus, the structure of **1** was assigned as shown.

Oblongifolin K (**2**) was obtained as a light brown gum. The HRESIMS data of **2** exhibited an ion at *m*/*z* 533.2562 $[M - H]^-$ (calcd for $C_{32}H_{37}O_7$ 533.2539), suggesting a molecular formula of $C_{32}H_{38}O_7$, 32 mass units greater than that of **1**. The similarities of the ^1H and ^{13}C NMR spectroscopic data of **2** (Table 1) when compared to **1** indicated that they possess a similar structure, and the only difference observed was that the benzoyl group (δ_C 195.8, 135.9, 134.1, 130.5 × 2, and 129.3 × 2) in **1** was replaced by a 3,4-dihydroxybenzoyl group (δ_C 151.8, 146.3, 128.2, 124.5, 117.5, and 115.0) in **2**. The planar structure of **2** was confirmed by DEPT, HSQC, and HMBC experiments. Key HMBC correlations are shown in Figure 2. The relative configuration was assigned in a similar manner to **1**, and key NOESY correlations are shown in Figure 2. In order to determine the absolute configuration of **2**, ECD calculations were conducted. The calculated ECD spectrum of (1*S*,3*R*,5*R*,7*S*,24*R*)-**2** matched well with the experimental ECD spectrum of **2** (Figure S5, Supporting Information), thus establishing the structure and absolute configuration of **2** as depicted.

Table 1. ^1H and ^{13}C NMR Data (400 and 100 MHz, CD_3OD) for Compounds 1 and 2[a]

	1		2	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		70.5		70.3
2		203.7		203.9
3		80.1		79.8
4		203.0		203.0
5		78.4		78.3
6		55.2		54.9
7	1.88, m	47.2	1.79, m	47.3
8	2.62, m	43.8	2.58, m	43.7
	2.37, m		2.30, m	
9		204.4		204.7
10		195.8		194.4
11		135.9		128.2
12	7.18, d (7.6)	130.5	6.89, d (1.9)	117.5
13	7.35, t (7.6)	129.3		146.3
14	7.51, m	134.1		151.3
15	7.35, t (7.6)	129.3	6.57, d (8.4)	115.0
16	7.18, d (7.6)	130.5	6.25, dd (1.9, 8.)	124.5
17	2.48, m	24.3	2.46, m	24.3
	2.43, m		2.36, m	
18	4.92, m[b]	120.8	4.85, m	121.1
19		135.5		135.2
20	1.68, s	26.4	1.65, s	26.4
21	1.62, s	18.4	1.59, s	18.4
22	1.23, s	22.9	1.17, s	22.9
23	1.19, s	23.8	1.13, s	23.8
24	3.95, m	49.3	3.87, m	49.8
25a	2.96, m	43.1	2.93, dd (18.1, 3.2)	43.3
25b	2.46, m		2.34, m	
26		208.4		208.4
27	2.22, s	30.5	2.17, s	30.5
28	2.49, m	28.9	2.50, m	28.9
			2.46, m	
29	5.16, m	120.2	5.14, t (6.8)	120.4
30		135.8		135.8
31	1.70, s	26.3	1.67, s	26.3
32	1.70, s	18.3	1.67, s	18.3

[a]Assignments based on HSQC, HMBC, and NOESY experiments. Chemical shifts in ppm. [b]Overlapped with the solvent peak.

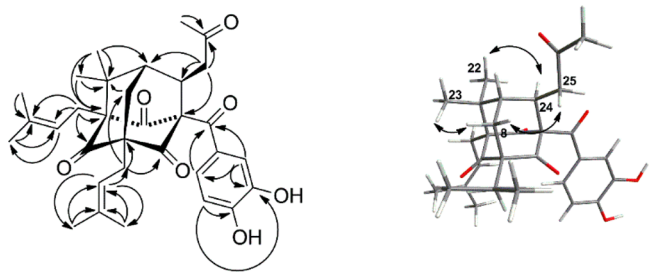

Figure 2. Key correlations observed in the HMBC and NOESY NMR spectra of **2**.

Compounds **1** and **2** both contain an acetonyl functionality in their molecules, consistent with these two compounds being isolated from an acetone-soluble fraction, so they could be considered as extraction artifacts. To determine if **1** and **2** are artifacts, another sample of the leaves of *G. oblongifolia* was extracted using petroleum ether, and subsequent isolation procedures and analytical processes were carried out in the absence of acetone (Experimental Section, Supporting Information). As shown in Figure S2 (Supporting Information), **1** and **2** could also be detected in this new plant extract by UPLC-ESIMS/MS, thus indicating that they are natural products rather than extraction artifacts.

Oblongifolin L (**3**) was obtained as a yellow gum. Its negative-ion HRESIMS revealed a pseudomolecular ion $[M - H]^-$ consistent with the molecular formula, $C_{33}H_{42}O_4$. The ^1H NMR data (Table 3) indicated that **3** possesses a monosubstituted benzene ring [δ_H 7.58 (2H, m), 7.55 (1H, m), and 7.41 (2H, m)]. There were also resonances for two angular methyl groups [δ_H 1.23 and 0.98 (each 3H, s)], three olefinic protons [δ_H 5.17 (1H, m), 5.04 (1H, m), and 5.02 (1H, m)], five vinylic methyl groups [δ_H 1.72, 1.65, 1.62 (3H each, s), and 1.57 (6H, s)], and four allylic methylene carbons, which suggested the presence of one prenyl group and one geranyl group. The ^{13}C NMR spectrum showed resonances for six aromatic carbons and a conjugated carbonyl group (δ_C 198.1), revealing the occurrence of a benzoyl group. Resonances for a bicyclo[3.3.1]nonane ring system including a nonconjugated ketone (δ_C 207.4), an enolized 1,3-diketone (δ_C 196.9, 185.7, and 118.9), two quaternary carbons (δ_C 65.3 and 45.5), two methines (δ_C 69.8 and 42.4), and a methylene (δ_C 43.2) were observed. In addition, a characteristic proton single peak was present at δ_H 3.06, demonstrating HSQC correlation with a carbon signal at δ_C 69.8. This suggested that a methine group occurs at C-5 in **3**,[11] which was confirmed by the HMBC correlations from the proton signal at δ_H 3.06 to C-1 (δ_C 65.3), C-3 (δ_C 118.9), C-4 (δ_C 185.7), C-6 (δ_C 45.5), C-9 (δ_C 207.4), C-17 (δ_C 27.6), and C-18 (δ_C 21.2). These data suggested that **3** is an isoprenyl benzophenone derivative bearing both a prenyl group and a geranyl group. Comparison of the NMR data with those of oblongifolin B (Figure S3, Supporting

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Table 2. ^{1}H NMR Data (400 MHz, CD_3OD/0.1% TFA) for Compounds 3−6 and 8−10[a]

position	3	4	5	6	8	9	10
5	3.06, s	2.93, s	3.06, s	3.12, s	3.08, s	2.88, s	2.89, s
7	1.73, m	2.27, m	1.72, m	2.58, m	1.76, m	2.06, m	1.57, m
8	2.02, m	1.95, m	1.96, m	1.91, m	2.02, m	2.38, m	3.02, dd (14.4, 4.4)
	1.99, m	1.43, m	1.48, m	1.56, m	1.51, m	1.85, m	1.55, m
12	7.58, m	8.15, m	7.59, m	7.72, m	7.60, m	7.82, m	7.84, m
13	7.41, m	7.53, m	7.42, m	7.48, m	7.44, m	7.49, m	7.52, m
14	7.55, m	7.68, m	7.55, m	7.59, m	7.58, m	7.64, m	7.65, m
15	7.41, m	7.53, m	7.42, m	7.48, m	7.44, m	7.49, m	7.52, m
16	7.58, m	8.15, m	7.59, m	7.72, m	7.60, m	7.82, m	7.84, m
17	1.23, s	1.21, s	1.22, s	1.19, s	1.25, s	0.97, s	0.94, s
18	0.98, s	0.94, s	0.97, s	1.02, s	1.01, s	1.20, s	1.15, s
19	2.16, m	2.20, m	2.16, m	2.81, m	2.20, m	2.34, m	2.12, m
	1.74, m	1.78, m	1.76, m	2.57, m	1.76, m	1.88, m	2.07, m
20	5.02, m	5.12, m	5.05, m		5.10, m	5.26, t (7.1)	5.24, m
22	1.57, s	1.63, s	1.61, s	6.12, s	1.62, s	1.67, s	1.68, s
				5.83, s			
23	1.97, m	2.01, m	2.25, t (7.4)	2.30, t (7.3)	2.05, m	2.08, m	2.06, m
					1.98, m		
24	2.46, m	2.44, m	2.46, m	2.41, m	2.45, m	2.75, dd (13.0, 10.9)	2.82, dd (15.1, 3.8)
	2.38, m	2.35, m	2.36, m		2.39, m	1.95, dd (13.0, 5.6)	1.72, m
25	5.17, m	5.07, m	5.16, m	5.15, m	5.18, m	4.74, dd (10.9, 5.6)	3.73, m
27	1.72, s	1.65, s	1.72, s	1.71, s	1.74, s	1.09, s	1.25, s
28	1.62, s	1.65, s	1.62, s	1.63, s	1.64, s	1.09, s	1.00, s
29	2.06, m	2.10, m	2.80, t (7.4)	2.30, m	1.60, m	2.14, m	2.26, m
	1.98, m			2.08, m		2.09, m	1.85, m
30	5.04, m	5.10, m		5.09, m	3.95, t (6.1)	5.11, m	5.15, m
32	1.65, s	1.69, s	6.03, s	1.67, s	4.89, s	1.61, s	1.65, s
			5.80, s		4.80, s		
33	1.57, s	1.61, s	1.81, s	1.58, s	1.70, s	1.58, s	1.60, s

[a]Assignments based on DEPT, HSQC, and HMBC experiments. Chemical shifts in ppm, J in Hz.

Information) revealed that the structure of **3** was closely comparable to that of oblongifolin B,[4a] except for the unsubstituted benzoyl group and the absence of a prenyl group at C-5. All these assignments were confirmed by DEPT, HSQC, and HMBC NMR experiments. Key HMBC correlations are shown in Figure 3. The relative configurations of H-5, H-7, and CH_3-18 were assigned using NOE correlations, as shown in Figure 3. Furthermore, the calculated ECD curve (Figure 1) for **3** led to the absolute structure being determined as shown.

Oblongifolin M (**4**) was obtained as a pale yellow gum. The ^{1}H and ^{13}C NMR data (Tables 2 and 3) were similar to those of **3**, except for the differences of C-3, C-4, and C-10 (**4**: δ_C 130.8, 169.9, and 165.8, respectively; **3**: δ_C 118.9, 185.7, and 198.1, respectively). In addition, the HRESIMS showed an ion peak at m/z 517.2950 $[M - H]^-$, consistent with a molecular formula of $C_{33}H_{42}O_5$. The molecular weight was found to differ from that of **3** by 16 Da, corresponding to the presence of an additional oxygen. These findings indicated that **4** is a derivative of **3** with an additional oxygen located between C-3 and C-10 or between C-10 and C-11.[12] The HMBC correlations showed long-range correlations from the proton signal at δ_H 8.15 (2H, m, H-12 and H-16) to C-10 (δ_C 165.8), as well as from δ_H 2.93 (H-5) to C-3 (δ_C 130.8) and C-4 (δ_C 169.9) (Figure S7, Supporting Information), so the additional oxygen atom was assigned between C-3 and C-10. In addition, an alkaline hydrolysis reaction was conducted for **4**, and its hydrolysate analyzed by UPLC-ESI-QTOF-MS (Experimental Section, Supporting Information). A deprotonated molecular ion peak at m/z 121.0268 for $[M - H]^-$ was detected, which was assigned unambiguously as benzoic acid by comparing the retention times and m/z value with a reference compound (Figure S6, Supporting Information).

Oblongifolin N (**5**) showed a deprotonated molecular ion peak at m/z 515.2789 $[M - H]^-$ in the HRESIMS, corresponding to the elemental formula, $C_{33}H_{40}O_5$ (calcd 515.2797). Comparison of its NMR data (Tables 2 and 3) with those of **3** revealed these compounds to be closely related, except for differences between C-29 (δ_C 37.2), C-30 (δ_C 203.7), C-31 (δ_C 145.9), and C-32 (δ_C 126.0) of **5** and C-29 (δ_C 27.6), C-30 (δ_C 125.3), C-31 (δ_C 132.5), and C-32 (δ_C 26.1) of **3**. This indicated the presence of a 2-carbonyl-3-methylbutenyl group at C-23 of **5**. This conclusion was supported by HMBC correlations between H-23/C-30, H-23/C-29, H-23/C-20, H-23/C-22, H-29/C-30, H-33/C-31, H-33/C-32, H-32/C-30, and H-32/C-31 (Figure S8, Supporting Information). The planar structure of oblongifolin N was further confirmed by DEPT, HSQC, and HMBC experiments.

Oblongifolin O (**6**) gave the same molecular formula ($C_{33}H_{40}O_5$) as **5** (m/z 515.2806 $[M - H]^-$ (calcd 515.2797)) by HRESIMS. The ^{1}H and ^{13}C NMR spectra were very similar to those of **5**, suggesting that **6** is also a prenylated benzoylphloroglucinol with a terminal double bond and one additional carbonyl group in the side chain. Key HMBC correlations (Figure 5) indicated that the carbonyl group and the terminal double bond were located at C-20 and C-21 in **6**, respectively. This differed from **5**, since these functionalities occurred at C-30 and C-31.

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Table 3. ^{13}C NMR Data (100 MHz, CD_3OD/0.1% TFA) for Compounds 3−6 and 8−10[a]

position	3	4	5	6	8	9	10
1	65.3	63.4	65.3	65.3	65.6	62.7	51.4
2	196.9	186.8	196.9	196.1	197.1	180.4	174.9
3	118.9	130.8	119.0	119.3	119.1	117.6	127.2
4	185.7	169.9	185.8	185.1	185.7	193.8	194.1
5	69.8	69.5	70.1	70.1	70.1	75.0	75.4
6	45.5	42.9	45.4	44.8	45.4	44.5	44.1
7	42.4	40.7	42.4	37.5	42.5	42.9	42.3
8	43.2	41.1	43.1	43.0	43.1	39.4	42.9
9	207.4	207.4	207.7	206.9	207.6	203.7	206.5
10	198.1	165.8	198.2	198.1	198.2	194.3	195.9
11	138.9	130.6	139.1	139.2	139.1	138.7	138.8
12	129.9	131.3	129.9	130.1	129.9	130.4	130.2
13	129.3	129.5	129.3	129.3	129.3	129.9	129.9
14	133.9	134.6	133.9	133.9	133.9	134.9	135.0
15	129.3	129.5	129.3	129.3	129.3	129.9	129.9
16	129.9	131.3	129.9	130.1	129.9	130.4	130.2
17	27.6	27.2	27.5	27.4	27.5	20.9	20.8
18	21.2	20.1	27.2	21.4	21.2	27.5	27.3
19	28.8	28.8	28.9	38.7	29.0	28.5	28.4
20	123.8	124.1	124.3	202.2	123.9	123.7	123.9
21	138.2	137.7	137.4	150.2	138.0	138.5	138.4
22	16.4	18.2	16.7	126.0	16.5	16.5	16.6
23	40.9	40.9	35.7	32.4	36.8	40.9	41.3
24	31.2	30.6	31.2	30.9	31.1	29.8	30.8
25	121.1	121.1	121.1	121.0	121.1	94.8	69.6
26	135.5	134.6	135.5	135.5	135.6	71.1	86.9
27	26.4	26.2	26.4	26.3	26.3	26.1	27.3
28	18.4	18.2	18.3	18.3	18.2	25.9	23.9
29	27.6	27.6	37.2	28.4	34.6	27.7	28.2
30	125.3	125.3	203.9	124.8	76.3	125.3	125.5
31	132.5	132.3	145.9	133.3	148.9	132.4	132.4
32	25.1	25.9	126.0	26.0	177.0	26.0	26.0
33	18.4	17.7	17.9	18.0	17.8	17.9	17.8

[a]Assignments based on DEPT, HSQC, and HMBC experiments. Chemical shifts in ppm.

Oblongifolin P (7) was obtained as a yellow gum, and its molecular formula, $C_{24}H_{28}O_5$, was determined on the basis of the HRESIMS peak at m/z 379.1913 $[M - H]^-$. The ^1H NMR spectrum indicated that 7 possesses one olefinic proton, attributed to a prenyl group, three methyl groups on sp^3 carbons, and two vinyl methyl groups. There were also resonances evident for a monosubstituted benzene ring. The ^{13}C NMR spectrum of 7 exhibited the presence of a nonconjugated carbonyl group at δ_C 207.4 (C-9), an enolized 1,3-diketone group (δ_C 197.3, C-2; δ_C 119.1, C-3; δ_C 185.8, C-4), two quaternary carbons at δ_C 65.6 (C-1) and 45.6 (C-6), a methylene carbon (δ_C 45.7), two methine carbons (δ_C 69.7 and 35.9), three methyl groups at δ_C 27.6 (C-17), 20.4 (C-18), and 14.9 (C-19), and five other signals that were attributed to an isoprenyl group. On comparing the NMR data of 7 with those of 3, it was found that the two compounds have the same benzoylphloroglucinol skeleton, but a difference was observed only in the structure of the side chain at C-7, since the geranyl group of 3 was replaced at this position by a methyl group in 7. The HMBC correlations from this methyl to C-7, C-6, and C-8 confirmed its location at C-7. Other key HMBC correlations are shown in Figure 5.

According to the observed NOESY correlations (Figures S7−S10, Supporting Information), the relative configurations of compounds 4−7 were deduced to be the same as those of 3, with H-5, H-7, and CH_3-18 all on the same side of the 2,2-dimethylbicyclo[3.3.1]nonane ring. In addition, the experimental ECD spectra of 4−7 were in near agreement with that of 3 (Figure 4), thus suggesting that the absolute configurations of 4−7 are the same as that of 3.

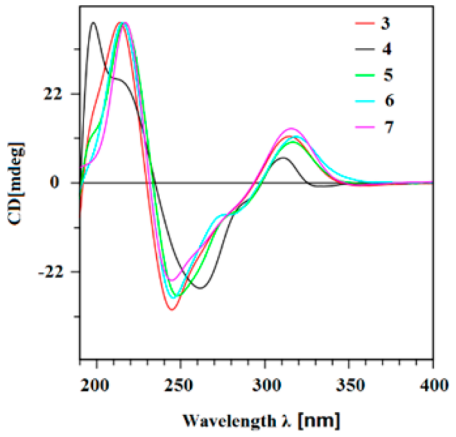

Figure 4. Experimental ECD spectra of 3−7.

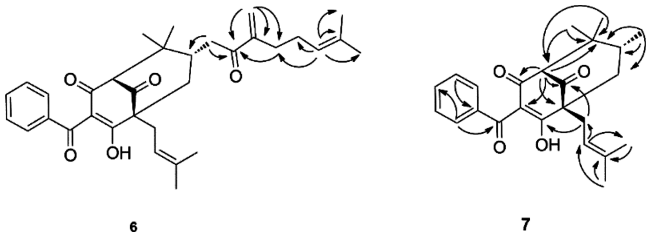

Figure 5. Key correlations observed in the HMBC NMR spectra of 6 and 7.

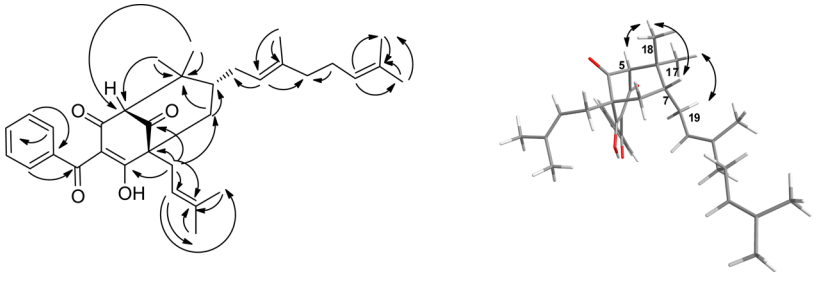

Figure 3. Key correlations observed in the HMBC and NOESY NMR spectra of 3.

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Oblongifolin Q (**8**) was isolated as a light yellow gum. The HRESIMS of **8** exhibited a deprotonated molecular ion $[M - H]^-$ at *m/z* 517.2993 (calcd 517.2954), consistent with a molecular formula of $C_{33}H_{42}O_5$. The ^{1}H NMR data of this compound were similar to values obtained for **3**, except for differences between H-30 [δ_H 3.95 (1H, t, *J* = 6.1 Hz)] and H-32 [δ_H 4.89 (1H, s); δ_H 4.80 (1H, s)] in **8** and H-30 [δ_H 5.04 (1H, m)] and H-32 [δ_H 1.65 (3H, s)] in **3**. This information indicated the occurrence of a 2-hydroxy-3-methylbutenyl group at C-23 in **8**.[13] This finding was also supported by the ^{13}C NMR data of C-30 (δ_C 76.3, CH), C-31 (δ_C 148.9, C), and C-32 (δ_C 117.0, CH_2) and HMBC correlations between H-30/C-33, H-32/C-30, H-32/C-31, H-32/C-32, H-33/C-30, H-33/C-31, and H-33/C-32. The relative configuration of **8** was determined by NOESY experiments. The NOE correlations of H-5/CH_3-18, CH_3-18/H-7, and CH_3-17/H-18a indicated that H-5, CH_3-18, and H-7 are all oriented on the same side of the 2,2-dimethylbicyclo[3.3.1]nonane ring, while CH_3-17 and the geranyl group are oriented on the opposite side. However, the configuration of C-30 could not be assigned from the NOESY spectrum. Thus, a convenient Mosher ester procedure carried out in NMR tubes[14] combined with calculated ECD data was used to establish the absolute configuration of **8**. Compound **8** were treated with (*R*)-(−)-*α*- and (*S*)-(+)-*α*-methoxy-*α*-(trifluoromethyl)phenylacetyl chloride in deuterated pyridine directly in separate NMR tubes at room temperature (Experimental Section, Supporting Information), which afforded the (*S*)- and (*R*)-MTPA ester derivatives (**8*S*** and **8*R***, respectively) of **8**. The ^{1}H NMR signals of the two MTPA esters were assigned unambiguously based on their DEPT and HSQC spectra. A consistent distribution of positive and negative $\Delta\delta_H$ values around C-30 allowed the assignment of the *S*-configuration for C-30 (Figure 6). Two possible isomers,

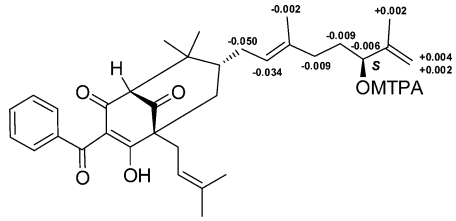

Figure 6. $\Delta\delta$ ($\delta_S - \delta_R$) values (in ppm) for the MTPA ester of **8**.

(1*R*,5*R*,7*R*,30*S*)-**8** and (1*S*,5*S*,7*S*,30*S*)-**8**, were considered, and the ECD spectra were calculated. Visual inspection suggested that the (1*R*,5*R*,7*R*,30*S*)-**8** curve was similar to the experimental curve (Figure 7). Thus the structure of **8** was designated as shown.

Oblongifolin R (**9**) was isolated as a yellow gum. The molecular formula, $C_{33}H_{42}O_5$, was deduced by HRESIMS at *m/z* 517.2949 $[M - H]^-$, which was the same as that of the known compound guttiferone R.[11] The ^{1}H and ^{13}C NMR data (Tables 2 and 3) were almost identical with those of guttiferone R (Figure S3, Supporting Information),[11] suggesting that **9** is also a prenylated benzoylphloroglucinol derivative with a (2-hydroxyisopropyl)dihydrofuran ring moiety. In the ^{13}C NMR spectrum, the signals of the carbons C-5 (δ_C 75.0) and C-21 (δ_C 138.5) in **9** differed significantly from C-5 (δ_C 69.7) and C-21 (δ_C 133.8) in guttiferone R, suggesting these compounds to differ structurally only in the structure of the side chain. HMBC correlations as shown in Figure S11 (Supporting Information) indicated *gem*-dimethyl substitution at C-5 and a geranyl substituent at C-7 (C-23 was substituted by a prenyl to form the geranyl) in **9**, whereas one of these methyl groups was substituted with a prenyl group and C-7 was substituted with a prenyl group in guttiferone R. The relative configuration of **9** was determined by a NOESY experiment. Key NOE correlations are shown in Figure S11 (Supporting Information).

Oblongifolin S (**10**) was obtained as a colorless gum. The positive ion at *m/z* 519.3128 $[M + H]^+$ indicated a molecular formula of $C_{33}H_{42}O_5$, which is similar to **9**. The ^{1}H and ^{13}C NMR data (Tables 2 and 3) showed close similarities to those of **9**, with the exception of differences at C-1 (**10**: δ_C 51.4, CH; **9**: δ_C 62.7, CH), C-3 (**10**: δ_C 127.2, CH; **9**: δ_C 117.6, CH), C-25 (**10**: δ_C 69.6, CH; **9**: δ_C 94.8, CH), and C-26 (**10**: δ_C 86.9, C; **9**: δ_C 71.1, C). This indicated that a 2,2-dimethyl-3-hydroxypyran moiety at C-1 and C-2 of **10** replaced the (2-hydroxyisopropyl)dihydrofuran ring moiety in **9**.[11,15] HMBC correlations between H-24/C-1, H-24/C-2, H-24/C-25, H-24/C-26, H-25/C-1, H-27/C-25, H-27/C-26, H-28/C-25, and H-28/C-26 confirmed the presence of a 2,2-dimethyl-3-hydroxypyran moiety fused with the phloroglucinol moiety at C-1 and C-2. Key HMBC and NOE correlations are shown in Figure S12 (Supporting Information). By comparing experimental and calculated ECD spectra (Figure S13, Supporting Information), the absolute configurations of **9** and **10** were both determined to be 1*R*,5*S*,7*S*,25*S*.

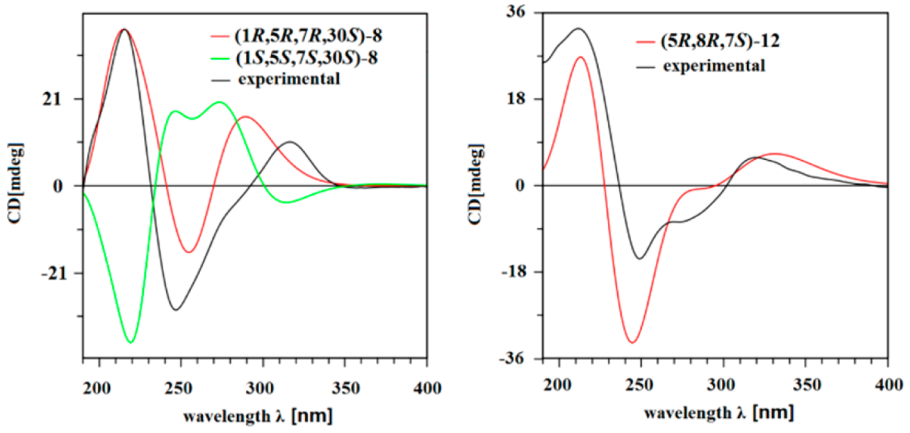

Figure 7. Calculated ECD spectra of **8** and **12** and their experimental curves.

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Oblongifolin T (**11**) was obtained as a yellow gum, and its molecular formula of $C_{38}H_{50}O_7$ was established by the negative HRESIMS ion at m/z 617.3505 $[M - H]^-$ (calcd for $C_{38}H_{49}O_7$, 617.3478). The ^1H and ^{13}C NMR data (Table 4) were identical with values reported for oblongifolin A (Figure S3, Supporting Information),[4a] except for differences between C-34 (δ_C 34.3), C-35 (δ_C 76.0), C-36 (δ_C 149.1), and C-37 (δ_C 111.7) of **11** and C-34 (δ_C 27.5), C-35 (δ_C 125.1), C-36 (δ_C 132.1), and C-37 (δ_C 26.0) of oblongifolin A, suggesting the existence of a 2-hydroxy-3-methylbutenyl group at C-23 in **11**.

Table 4. ^1H and ^{13}C NMR Data (400 and 100 MHz, CD_3OD) for Compounds 11 and 12[a]

	11		12	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		62.2		63.8
2		195.4		194.7
3		117.9		119.4
4		194.9		193.0
5		68.2		69.8
6		49.4		48.7
7	1.55, m	47.7	1.64, m	44.3
8	2.18, m	40.8	2.07, m	40.9
	2.09, m		2.04, m	
9		210.1		208.9
10		195.9		198.8
11		129.5		140.7
12	7.17, d (1.9)	117.5	7.02, brs	116.3
13		146.4		158.7
14		152.8	6.99, m	120.7
15	6.69, d (8.3)	115.2	7.18, m	129.9
16	6.96, dd (8.3, 1.9)	125.4	6.92, m	121.4
17	2.74, m	27.2	2.06, m	27.7
	2.58, m		2.01, m	
18	5.17, m	120.8	5.17, m	121.0
19		135.7		135.5
20	1.72, s	26.5	1.73, s	26.1
21	1.68, s	18.4	1.70, s	18.4
22	1.25, s	23.4	0.82, s	16.4
23	1.03, s	27.5	1.19, s	23.2
24	2.14, m	30.1	2.18, m	29.3
	2.08, m		2.15, m	
25	4.92, m	125.9	5.02, m	124.1
26		137.2		138.1
27	1.48, s	16.6	1.58, s	16.5
28	1.99, m	37.1	1.52, m	43.4
29	2.52, m	32.4	2.50, m	31.4
	2.42, m			
30	4.95, m	120.8	4.92, m	121.1
31		135.9		135.6
32	1.66, s	26.5	1.67, s	26.3
33	1.67, s	18.4	1.67, s	18.3
34	1.55, m	34.3	2.71, m	26.2
			2.65, m	
35	3.97, dd (7.4, 5.7)	76.0	5.07, m	125.4
36		149.1		132.4
37	4.91, s	111.7	1.67, s	26.1
	4.79, s			
38	1.70, s	17.8	1.60, s	17.9

[a]Assignments based on HSQC, HMBC, and NOESY experiments. Chemical shifts in ppm.

This conclusion was supported by HMBC correlations between H-35/C-28, H-35/C-34, H-35/C-37, and H-35/C-38. In the NOESY spectrum, the correlations of CH_2-17/CH_3-23, CH_3-23/CH_2-24, and CH_3-22/H-7 indicated that CH_2-17, CH_3-23, and CH_2-24 are all oriented on the same side of the 2,2-dimethylbicyclo[3.3.1]nonane ring, while CH_3-22 and H-7 are oriented on the opposite side. The absolute configuration of **11** was assigned in a similar manner to **8**. The configuration of C-35 was determined to be *R* by a convenient Mosher ester method (Figure S14, Supporting Information). Furthermore, the ECD experiment and ECD calculation of **11** were conducted. The experimental ECD spectrum of **11** was in accordance with the calculated ECD spectrum for (1*R*,5*R*,7*S*,35*R*)-**11** (Figure S15, Supporting Information), thus establishing the assignment of the absolute configuration of **11** as depicted.

Oblongifolin U (**12**) gave the molecular formula $C_{38}H_{50}O_5$, as determined by HRESIMS (m/z 585.3585 $[M - H]^-$, calcd 585.3580). A comparison of the spectroscopic data of **12** with those reported for oblongifolin E (Figure S3, Supporting Information)[4b] revealed these compounds to have very similar structures and to represent a pair of epimers with a prenylated benzoylphloroglucinol backbone. The only structural difference between **12** and oblongifolin E found was the opposite configuration of C-7. This was deduced by the different ^{13}C NMR chemical shifts of C-7 and CH_3-22 [**12**: C-7 (δ_C 44.3), CH_3-22 (δ_C 16.4); oblongifolin E: C-7 (δ_C 47.7), CH_3-22 (δ_C 27.3)].[16] The structure of **12** was confirmed by DEPT, HSQC, and HMBC experiments. To determine the relative configuration of **12**, a NOESY experiment was performed. NOE correlations between CH_2-17/CH_3-23 and CH_2-24/CH_3-23 suggested that CH_2-17, CH_3-23, and CH_2-24 are on the same side of the molecule. The absolute configuration of **12** was also assigned by experimental and theoretically calculated ECD methods. As shown in Figure 7, the absolute configuration of **12** was determined as shown.

All isolates were evaluated for their anti-EV71 activity using a cytopathic effect inhibition assay determined using an MTT method. When compared to ribavirin (IC_{50} 253.1 μM), compounds **1**, **4**, and **13** exhibited significant protection against EV71-induced CPE in Vero cells, with IC_{50} values of 31.1, 16.1, and 12.2 μM, respectively. Their selectivity indices (SI) were 1.5, 2.4, and 3.0 in Vero cells, respectively (Table 5).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using an Autopol VI polarimeter. Ultraviolet absorption spectra were recorded on a UV-2401 PC spectrophotometer. ECD spectra were recorded on a JASCO J-810 spectrometer. IR spectra

Table 5. Anti-EV71 Activity, Cytotoxicity, and Selectivity Index of Compounds 1, 4, and 13[a]

compound	CC_{50}[b] (μM)	IC_{50}[b] (μM)	SI[c]
1	47.0 ± 7.7[d]	31.1 ± 2.5[d]	1.5
4	37.8 ± 0.9	16.1 ± 0.5	2.4
13	36.5 ± 5.3	12.2 ± 3.3	3.0
ribavirin	>1000	253.1 ± 13.0	>4.0

[a]Compounds **2**, **3**, **5**−**12**, oblongifolin C, garcihombronone C, oblongixanthone B, and dulxanthone-B are inactive. [b]Cytotoxicity (CC_{50}) and antiviral activity (IC_{50}) were determined using an MTT assay on Vero cells. [c]Selectivity index (SI) is the ratio of CC_{50} to IC_{50}. [d]Values represent the mean ± SD of three independent experiments.

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were obtained from a Perkin-Elmer 577 spectrometer. NMR spectra were measured on a Bruker AV-400 spectrometer and calibrated based on the solvent peak used. Mass spectrometry was performed on a Waters Q-TOF Premier spectrometer (Micromass MS Technologies, Manchester, UK), with an electrospray ion source (Waters, Milford, MA, USA) connected to a lock-mass apparatus, which performed real-time calibration correction. Column chromatography was performed with CHP20P MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (100−200 or 200−300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden), and reversed-phase C_{18} silica gel (50 μm, YMC, Kyoto, Japan). Precoated TLC sheets of silica gel 60 GF254 (Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China) were used. A Waters 2535 series machine equipped with an Xbridge C_{18} column (4.6 × 250 mm, 5 μm) was used for HPLC analysis, and a preparative Xbridge Prep C_{18} OBD column (19 × 250 mm, 5 μm) was used for the sample preparation. Ribavirin (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive compound with anti-EV71 activity.

Plant Material. The leaves of *G. oblongifolia* were collected at Bobai, Guangxi Province, People's Republic of China, in December 2005. The sample was identified by Dr. Chun-Feng Qiao. A voucher specimen (herbarium no. 20120843) has been deposited at the Innovative Research Laboratory of TCM, Shanghai University of Traditional Chinese Medicine.

Extraction and Isolation. Air-dried and powdered leaves of the plant (4.97 kg) were extracted with acetone (3 × 20 L, each two days) at room temperature. The combined extracts were evaporated to dryness under vacuum to afford the acetone-soluble portion (347.8 g). The residue was suspended in H_2O (3 L) and extracted in turn with petroleum ether (5 × 3 L) and EtOAc (5 × 3 L) to obtain the dried petroleum ether- (80 g), EtOAc- (200 g), and H_2O-soluble (63 g) extracts. The petroleum ether-soluble extract demonstrated anti-EV71 activity using cytopathic effect inhibition assays (Figure S16, Supporting Information). This bioactive extract was subjected to passage over a chromatography column (CC) on MCI and successively eluted with H_2O, 95% EtOH, and EtOAc. The 95% EtOH-eluting fraction was shown to have anti-EV71 activity in the CPE inhibition assay (Figure S2, Supporting Information). The bioactive fraction was chromatographed by silica gel CC using a gradient of petroleum ether−acetone (100:0 to 0:100, v/v) and yielded 12 fractions, A−L, according to the analysis of their TLC profiles.

Fraction E (3.7 g) was subjected to reversed-phase C_{18} silica gel CC and eluted in a step-gradient manner with MeOH−H_2O (60:40 to 100:0), to obtain 15 subfractions, Ea−Er, and compound **13** (25 mg). Subfraction Eb was further separated by preparative HPLC (MeOH−MeCN−H_2O, 6.5:58.5:35, with 0.1% formic acid in H_2O, 20 mL/min) to give compound **7** (4 mg). Subfractions Eh and Ej were purified by preparative HPLC (MeOH−MeCN−H_2O, 7:63:30, with 0.1% formic acid in H_2O, 20 mL/min) to yield compounds **1** (5 mg) and **4** (68 mg), respectively. Subfraction Em was chromatographed on a Sephadex LH-20 column (MeOH) to obtain compound **3** (108 mg). Compound **12** (7 mg) was obtained by preparative HPLC (MeCN−H_2O, 75:25, with 0.1% formic acid in H_2O, 20 mL/min) from subfraction En. Fraction F (7.9 g) was separated using a reversed-phase C_{18} silica gel column eluted with MeOH−H_2O (60:40 to 100:0) as a gradient system to obtain 20 subfractions (Fa−Ft) and oblongifolin C (45 mg). Garcihombronone C (5 mg), oblongixanthone B (7 mg), and dulxanthone-B (8 mg) were obtained from subfractions Fe, Fm, and Fn by recrystallization in acetone, respectively. Subfraction Fh was purified by preparative HPLC (MeOH−MeCN−H_2O, 6:54:40, with 0.1% formic acid in H_2O, 20 mL/min) to yield compounds **10** (2.8 mg), **9** (25 mg), **5** (17 mg), and **6** (8 mg). Subfraction H (3.8 g) was subjected to reversed-phase C_{18} silica gel CC and eluted in a step-gradient manner with MeOH−H_2O (55:45 to 100:0), to obtain subfractions Ha−Ho. Subfractions Hf and Hl were further purified by preparative HPLC (MeOH−H_2O, 75:25, and MeOH−H_2O, 85:15, respectively, both with 0.1% formic acid in H_2O, 20 mL/min) to obtain compounds **2** (3.2 mg) and **11** (6 mg), respectively. Subfraction G (3.9 g) was subjected to CC on reversed-phase C_{18} silica gel, eluted with MeOH−H_2O in a gradient (50:50 to 100:0), to obtain 20 subfractions (Ga−Gt). Compound **8** (8 mg) was obtained from subfraction Gg by preparative HPLC (MeCN−H_2O, 55:45, with 0.1% formic acid in H_2O, 20 mL/min).

*Oblongifolin J (**1**):* light brown gum; $[\alpha]^{25}_D$ +8.6 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 246 (4.28) nm; ECD (*c* 5.18 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 199 (+0.74), 240 (+0.95), 284 (−0.94); IR (KBr) ν_{max} 2956, 2925, 2852, 1743, 1700, 1596, 1448, 1376, 1253, 1172 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) data, see Table 1; ^{13}C NMR (CD_3OD, 100 MHz) data, see Table 1; HRESIMS *m/z* 503.2772 $[M + H]^+$ (calcd for $C_{32}H_{39}O_5$, 503.2797).

*Oblongifolin K (**2**):* light brown gum; $[\alpha]^{25}_D$ +55.4 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 236 (4.06), 284 (3.91), 315 (3.86) nm; ECD (*c* 8.80 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 201 (+5.12), 247 (−0.38), 278 (+2.97), 334 (−5.12); IR (KBr) ν_{max} 3403, 2964, 2919, 1745, 1700, 1600, 1521, 1438, 1376, 1288, 1189, 773 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) data, see Table 1; ^{13}C NMR (CD_3OD, 100 MHz) data, see Table 1; HRESIMS *m/z* 533.2562 $[M - H]^-$ (calcd for $C_{32}H_{37}O_7$, 533.2539).

*Oblongifolin L (**3**):* yellow gum; $[\alpha]^{25}_D$ −15.1 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 247 (4.04) nm; ECD (*c* 12.5 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 214 (+20.44), 245 (−16.03), 314 (+5.79); IR (KBr) ν_{max} 3399, 2964, 2929, 2875, 1721, 1673, 1635, 1594, 1573, 1521, 1448, 1376, 1286, 1120, 1074 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) data, see Table 2; ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) data, see Table 3; HRESIMS *m/z* 501.2997 $[M - H]^-$ (calcd for $C_{33}H_{41}O_4$, 501.3005).

*Oblongifolin M (**4**):* pale yellow gum; $[\alpha]^{25}_D$ −4.2 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.12), 269 (3.94) nm; ECD (*c* 9.36 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 196 (+8.69), 212 (+4.84), 261 (−4.92), 312 (+1.24); IR (KBr) ν_{max} 3423, 2967, 2917, 1745, 1671, 1602, 1452, 1376, 1263, 1209, 1122, 1022, 703 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) data, see Table 2; ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) data, see Table 3; HRESIMS *m/z* 517.2950 $[M - H]^-$ (calcd for $C_{33}H_{41}O_5$, 517.2954).

*Oblongifolin N (**5**):* pale yellow gum; $[\alpha]^{25}_D$ −17.5 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (logε) 259 (3.57) nm; ECD (*c* 10.85 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 216 (+9.46), 247 (−6.53), 316 (+2.36); IR (KBr) ν_{max} 3430, 2971, 2937, 1675, 1598, 1448, 1378, 1207, 1139, 844, 802 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) data, see Table 2; ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) data, see Table 3; HRESIMS *m/z* 515.2789 $[M - H]^-$ (calcd for $C_{33}H_{40}O_5$, 515.2797).

*Oblongifolin O (**6**):* pale yellow gum; $[\alpha]^{25}_D$ −20.8 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 257 (3.93) nm; ECD (*c* 15.5 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 215 (+13.55), 245 (−9.70), 319 (+3.79); IR (KBr) ν_{max} 3409, 2965, 2917, 1731, 1673, 1598, 1569, 1448, 1376, 1261, 1203, 1139, 804 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) data, see Table 2; ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) data, see Table 3; HRESIMS *m/z* 515.2806 $[M - H]^-$ (calcd for $C_{33}H_{39}O_5$, 515.2797).

*Oblongifolin P (**7**):* orange gum; $[\alpha]^{25}_D$ −56.3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 254 (4.03) nm; ECD (*c* 13.16 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 217 (+12.20), 244 (−7.16), 315 (+3.92); IR (KBr) ν_{max} 3442, 2967, 2921, 1733, 1677, 1635, 1554, 1450, 1382, 1209, 1143, 842, 804 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) δ_H 7.61 (2H, m, H-12 and H-16), 7.57 (1H, m, H-14), 7.44 (2H, m, H-13 and H-15), 5.19 (1H, t, *J* = 6.7 Hz, H-21), 3.10 (1H, s, H-5), 2.49 (1H, m, H-20a), 2.41 (1H, m, H-20b), 1.91 (1H, m, H-7), 1.87 (1H, m, H-8a), 1.74 (3H, s, H-23), 1.65 (3H, s, H-24), 1.60 (1H, m, H-8b), 1.19 (3H, s, H-17), 0.95 (3H, s, H-18), 0.90 (3H, s, H-19); ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) δ_C 207.4 (C-9), 198.2 (C-10), 197.3 (C-2), 185.8 (C-4), 138.9 (C-11), 135.5 (C-22), 133.9 (C-14), 129.9 (C-12 and C-16), 129.3 (C-13 and C-15), 121.0 (C-21), 119.1 (C-3), 69.7 (C-5), 65.6 (C-1), 45.7 (C-8), 45.1 (C-6), 35.9 (C-7), 31.1 (C-20), 27.6 (C-17), 26.4 (C-23), 20.4 (C-18), 18.2 (C-24), 14.9 (C-19); HRESIMS *m/z* 379.1913 $[M - H]^-$ (calcd for $C_{24}H_{27}O_4$, 379.1909).

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*Oblongifolin Q (**8**):* pale yellow gum; $[\alpha]^{25}_{D}$ −222.2 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.90) nm; ECD (*c* 8.69 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 215 (+15.36), 246 (−11.99), 317 (+4.22); IR (KBr) ν_{max} 3428, 2969, 2937, 1733, 1673, 1598, 1556, 1448, 1396, 1205, 1141, 804, 721 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) data, see Table 2; ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) data, see Table 3; HRESIMS *m*/*z* 517.2993 $[M - H]^-$ (calcd for $C_{33}H_{41}O_4$, 517.2954).

*Oblongifolin R (**9**):* light brown gum; $[\alpha]^{25}_{D}$ −54.5 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 255 (4.26) nm; ECD (*c* 8.40 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 211 (−14.19), 241 (+3.51), 269 (+8.15), 298 (−4.67); IR (KBr) ν_{max} 3430, 2967, 2921, 1737, 1679, 1623, 1450, 1370, 1286, 1213, 1170, 997, 842, 690 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) data, see Table 2; ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) data, see Table 3; HRESIMS *m*/*z* 517.2949 $[M - H]^-$ (calcd for $C_{33}H_{41}O_5$, 517.2954).

*Oblongifolin S (**10**):* light brown gum; $[\alpha]^{25}_{D}$ −37.8 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 253 (3.80) nm; ECD (*c* 5.02 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 210 (−6.16), 249 (+4.50), 270 (+4.16), 315 (+1.53); IR (KBr) ν_{max} 3411, 2967, 2931, 1729, 1677, 1594, 1448, 1363, 1249, 1122 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) data, see Table 2; ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) data, see Table 3; HRESIMS *m*/*z* 519.3128 $[M + H]^+$ (calcd for $C_{33}H_{43}O_5$, 519.3110).

*Oblongifolin T (**11**):* yellow gum; $[\alpha]^{25}_{D}$ +10.8 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 244 (3.80), 327 (3.56) nm; ECD (*c* 8.98 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 193 (+5.73), 222 (+2.27), 257 (−2.50), 351 (+0.30); IR (KBr) ν_{max} 3423, 2960, 2921, 1635, 1540, 1454, 1378, 1292, 1168 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) data, see Table 4; ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) data, see Table 4; HRESIMS *m*/*z* 617.3505 $[M - H]^-$ (calcd for $C_{38}H_{49}O_7$, 617.3478).

*Oblongifolin U (**12**):* orange gum; $[\alpha]^{20}_{D}$ +48.2 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 261 (3.90) nm; ECD (*c* 6.57 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 213 (+4.54), 248 (−2.25), 318 (+0.82); IR (KBr) ν_{max} 3423, 2967, 2925, 1681, 1554, 1448, 1384, 1209, 1143, 844, 725 cm^{-1}; ^1H NMR (CD_3OD/0.1% TFA, 400 MHz) data, see Table 4; ^{13}C NMR (CD_3OD/0.1% TFA, 100 MHz) data, see Table 4; HRESIMS *m*/*z* 585.3585 $[M - H]^-$ (calcd for $C_{38}H_{49}O_5$, 585.3580).

Antiviral Testing. Vero cells (ATCC CCL-81) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. After being dissolved in DMSO, the test extracts (100 mg/mL) and compounds (100 mM) were stored at −20 °C. Ribavirin, used as a positive control, was dissolved in H_2O at a concentration of 400 mM and stored at −20 °C. After being treated or untreated with compounds for four days, the viabilities of Vero cells were assessed by the MTT method (Experimental Section, Supporting Information).[17] The concentration of 50% cellular cytotoxicity (CC_{50}) of each test compound was calculated using the Forecast function of Microsoft Excel.[18]

Enterovirus 71 strain BrCr was propagated in Vero cells maintained with DMEM supplemented with 2% fetal bovine serum. The viral titer was determined by the end-point dilution assay of median tissue culture infective dose ($TCID_{50}$).[19] Vero cells were inoculated with the mixture of 100 × TCID50 EV71 and serially diluted extracts or compounds. After four days, the cell morphology was monitored under a light microscope, and the cell viability was tested with the MTT assay to determine the CPE inhibition rate (Experimental Section, Supporting Information).[20] The 50% inhibitory concentration (IC_{50}) of the test compounds was also calculated using the Forecast function of Microsoft Excel.[18] Data for all experiments are shown as mean values and calculated standard deviations from three independent assays in which each concentration of compounds was examined in quadruplicate. Selectivity index is the ratio of CC_{50} to IC_{50} for each compound.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed bioassay protocols used; analysis of compounds **1** and **2** in a new plant extract by UPLC-ESIMS/MS; computational details for compounds **1**−**3** and **8**−**12**; alkaline hydrolysis of compound **4** and UPLC-ESI-QTOF-MS analysis; preparation of the (*S*)- and (*R*)-MTPA ester derivatives of **8** and **11**; HRESIMS, ECD, NMR, and IR spectra of compounds **1**−**12**. 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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