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Limonoids from the Seeds of *Swietenia macrophylla* with Inhibitory Activity against Dengue Virus 2

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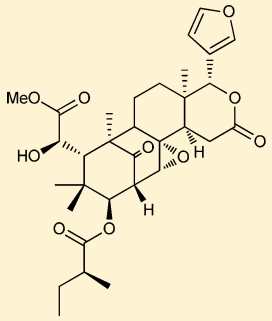
Supporting Information

ABSTRACT: Fractionation of an ethanol-soluble extract of the seeds of *Swietenia macrophylla* yielded six new limonoids, swielimonoids A–F (**1**–**6**), along with 20 known compounds. Compounds **1** and **2**, mexicanolide-type limonoids, were assigned with an α,β-unsaturated δ-lactone moiety (ring D) and a C═C bond between C-8 and C-30. Compounds **3**–**6** could be categorized as highly oxygenated phragmalin-type limonoids. The structures of these new compounds were elucidated through the interpretation of spectroscopic data. The antidengue virus 2 activities of the isolated components from *S. macrophylla* were investigated, and of 12 compounds subjected to bioassay, compounds **2** and **7**–**10** were found to show inhibitory activity in the range 3.5 to 12.5 μM. Among these, the new limonoid **2** exhibited significant antiviral activity (EC_{50} = 7.2 ± 1.33 μM) with a selectivity index (CC_{50}/EC_{50}) value of >27.7.



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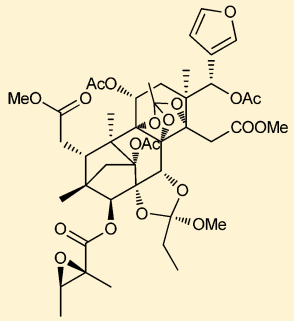

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Dengue virus is transmitted in tropical and subtropical areas, especially in Southeast Asia and Latin America. Around the world, 2.5 billion people live in areas susceptible to dengue fever, and more than 50 million people suffer from dengue fever every year. This insect-borne disease may result in dengue hemorrhagic fever and dengue shock syndrome, leading to a 20% death rate. Since there is currently no vaccine or drug for dengue virus, developing a new drug for curing this disease is of high priority. In a preliminary screening procedure, the ethanol extract of the seeds from *Swietenia macrophylla* showed inhibitory activity against dengue virus 2 (DENV-2), the most highly transmitted dengue virus in Asia. Thus, this plant was selected for further phytochemical and biological investigation.

Big-leaf mahogany (*Swietenia macrophylla* King) is a timber tree in the family Meliaceae and originates from Mexico and Central America. This plant was imported and widely planted in Southeast Asia in the early 20th century. Apart from its original use as a timber source, *S. macrophylla* has several folk medicine applications in Taiwan and East Asia. For example, the fruits of this plant are reported to treat hypertension and diabetes.[1] As a result of earlier phytochemical studies, limonoids,[2−4] lignans,[5] steroids,[6] coumarins,[6] and various phenols[7] were isolated as the major constituents of *S. macrophylla*. Among these secondary metabolites, limonoids here aroused the most attention due to their unusual structures and multiple biological effects. Limonoids, which are tetranortriterpenoids with a furan ring attached at C-17, usually exhibit high degrees of oxidation and skeletal rearrangements. Examples of such carbon frameworks are the andirobin-, gedunin-, mexicanolide-, phragmalin-, and D-ring-opened phragmalin-type limonoids. In earlier biological studies of purified limonoids from *S. macrophylla*, acaricidal,[8] antidiabetic,[9,10] antidiarrheal,[11] antifeedant,[3] anti-inflammatory,[6] antimalarial,[12] antimicrobial,[13,14] antimutagenic,[15] antinociceptive,[16] antioxidant,[17] antitumor,[1,15] and hypolipidemic[17] activities have been demonstrated. Herein, the isolation, structure elucidation, and anti-DENV-2 activities of isolated limonoids from the seeds of *S. macrophylla* are reported.

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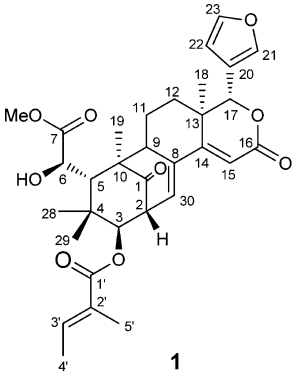
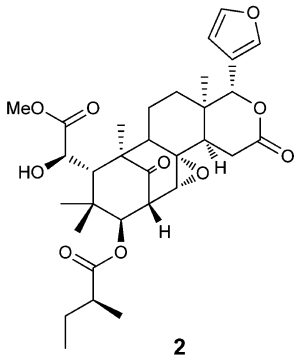
RESULTS AND DISCUSSION

The plant was collected in Tainan County, Taiwan, and the seeds were ground and then extracted with ethanol. The solvent was removed under vacuum, and the residue was partitioned between H_2O and EtOAc to yield an organic extract. Repeated HPLC chromatography of this extract afforded six new limonoids (**1**–**6**), together with 20 known compounds, swietenolide (**7**),[18] swietenine acetate (**8**),[18] 7-deacetoxy-7α-hydroxygedunin (**9**),[3] methyl angolensate (**10**),[18] 3-*O*-tigloylswietenolide (**11**),[18] proceranolide (**12**),[3] 6-*O*-acetylswietenolide (**13**),[3,18] 3,6-*O*,*O*-diacetylswietenolide (**14**),[18] 3-*O*-acetylswietenolide (**15**),[18] khayasin T (**16**),[18] febrifugin (**17**),[3,19] swietenine (**18**),[18] swietemahonin G (**19**),[20] swietemahonolide (**20**),[20] swietemahonin F (**21**),[20] 6-*O*-acetylswietemahonin G (**22**),[21] swietemahonin E (**23**),[19] 7-deacetoxy-7-oxogedunin (**24**),[17] andirobin (**25**),[22] and seco-mahoganin (**26**).[18] The structures of all compounds were established by interpretation of their 2D NMR and other spectroscopic data.



1



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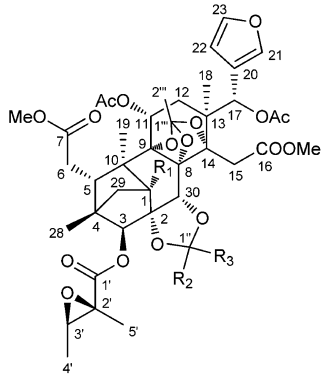

3 R_1 = OAc, R_2 = α-Et, R_3 = β-OMe
4 R_1 = OAc, R_2 = β-Et, R_3 = α-OMe
5 R_1 = OH, R_2 = β-Et, R_3 = α-OMe
6 R_1 = OAc, R_2 = β-Me, R_3 = α-OMe

Swielimonoid A (**1**), $[\alpha]_D^{23}$ +23 (*c* 1.0, CH_2Cl_2), gave a molecular formula of $C_{32}H_{38}O_9$, representing 14 degrees of unsaturation, as inferred from the HRESIMS (*m*/*z* 589.2413 $[M + Na]^+$) and ^{13}C NMR spectra. The IR spectrum indicated the presence of hydroxy group (3497 cm^{-1}), carbonyl (1649, 1715, and 1728 cm^{-1}), and furan ring (875 cm^{-1}) functionalities. The ^1H NMR data of **1** (Table 1) indicated clearly the presence of five methyl singlets (δ_H 0.87, 1.04, 1.11, 1.47, and 1.91), a methyl doublet (δ_H 1.90, *J* = 7.3 Hz), a methoxy singlet (δ_H 3.83), three typical methines from a furan ring (δ_H 6.49, 7.44, and 7.51 s), and a methine quartet for a tigloyloxy moiety (δ_H 6.97).[23] The ^{13}C NMR (Table 3) and

Table 1. ^1H NMR Spectroscopic Data of Compounds 1 and 2[a]

position	1	2
2	3.73, dd (9.1, 6.2)	3.56, dd (9.6, 2.6)
3	4.72, d (9.1)	4.93, d (9.7)
5	3.33, s	3.25, m
6	4.38, s	4.46, s
9	2.29, m	2.00, dd (14.2, 4.3)
11	1.33, m	1.76, m
	1.85, m	1.89, m
12	1.26, m	1.33, m
	1.98, m	2.07, dt (14.8, 3.4)
15	6.10, s	2.82, dd (17.0, 6.7)
		3.26, m
17	5.10, s	5.10, s
18	1.04, s	1.04, s
19	1.47, s	1.34, s
21	7.51, s	7.42, br s
22	6.49, s	6.37, br s
23	7.44, s	7.44, t (1.6)
28	0.87, s	1.11, s
29	1.11, s	0.89, s
30	6.21, dd (6.2, 2.9)	3.22, d (2.6)
MeO-7	3.83, s	3.93, s
2′		2.51, m
3′	6.97, q (7.3)	1.54, m
		1.77, m
4′	1.90, d (7.3)	0.97, t (7.4)
5′	1.91, s	1.26, d (7.3)

[a]Measured in $CDCl_3$ at 400 MHz for **1** and at 600 MHz for **2**; chemical shifts are in ppm; *J* values in Hz are in parentheses.

DEPT spectra of **1** showed 32 carbon signals, consisting of a carbonyl (δ_C 213.8), three ester carbonyls (δ_C 164.8, 166.6, and 175.8), six olefinic methines (δ_C 110.2, 112.4, 129.6, 139.1, 141.4, and 143.2), four olefinic quaternary carbons (δ_C 120.1, 128.0, 136.4, and 161.1), three oxymethines (δ_C 72.2, 79.4, and 79.6), a methoxy group (δ_C 53.1), two aliphatic methines (δ_C 44.3 and 55.4), three aliphatic quaternary carbons (δ_C 37.6, 39.3, and 52.6), three aliphatic methylenes (δ_C 22.6, 33.5, and 49.0), and six methyls (δ_C 12.0, 14.7, 16.2, 22.4, 22.5, and 23.3). Comparison of the ^1H and ^{13}C NMR data of **1** with related limonoids showed that **1** possesses the same mexicanolide-type carbon skeleton as 2-hydroxyseneganolide A.[24] However, the oxymethine chemical shift that resonated at δ_H 3.52 (H-3) in 2-hydroxyseneganolide A was replaced by a downfield shifted signal at δ_H 4.72, leading to the inference that the hydroxy group is modified to form an ester group in **1**. The COSY proton spin–spin coupling systems and the key HMBC correlations of **1** are shown in Figure 1. A methyl 2-hydroxyacetate moiety was assigned at C-5 by virtue of the HMBC correlations from H-6 (δ_H 4.38, s) to C-4/C-5/C-7 (δ_C 175.8)/C-10. Moreover, the HMBC correlations from H-17 (δ_H 5.10) to C-20 (δ_C 120.1)/C-21 (δ_C 141.4)/C-22 (δ_C 110.2) supported the attachment of a furan ring to C-17. Another proton spin system was found from correlations between H-3′ (δ_H 6.97)/H-4′ (δ_H 1.90) in the COSY spectrum. The HMBC correlations from H-3 to C-1′ and from H-5′ to C-1′/C-2′/C-3′ pointed to the presence of a tigloyloxy moiety at C-3. Full 2D NMR spectroscopic analysis was used to identify **1** as a mexicanolide-type limonoid with a tigloyloxy moiety at C-3. The relative configuration of **1** was

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Table 2. ^{1}H NMR Spectroscopic Data of Compounds 3−6[a]

position	3	4	5	6
3	5.36, s	5.22, s	5.24, s	5.27, s
5	2.68, m	2.67, m	2.72, d (11.5)	2.68, d (11.4)
6	2.23, dd (16.8, 11.3)	2.24, dd (17.1, 11.5)	2.23, dd (17.0, 11.5)	2.24, dd (17.1, 11.4)
	2.35, d (16.8)	2.40, d (17.1)	2.40, d (17.0)	2.41, d (17.1)
11	5.38, dd (11.6, 4.7)	5.42, dd (11.2, 4.6)	5.42, dd (11.2, 4.8)	5.45, dd (11.2, 4.7)
12	1.97, m	1.96, m	1.95, m	1.96, m
	1.73, d (4.6)	1.82, dd (13.4, 4.4)	1.88, m	1.87, dd (13.6, 4.5)
15	2.97, d (14.2)	3.04, d (14.3)	3.10, d (12.1)	3.09, d (14.1)
	3.31, d (14.2)	3.35, d (14.3)	3.38, d (14.3)	3.38, d (14.1)
17	6.44, s	6.52, s	6.51, s	6.55, s
18	1.13, s	1.10, s	1.11, s	1.09, s
19	1.07, s	1.06, s	1.11, s	1.06, s
21	7.90, s	7.92, s	7.94, s	7.91, s
22	6.62, m	6.66, m	6.65, d (1.7)	6.67, m
23	7.35, t (1.5)	7.34, t (1.4)	7.34, t (1.7)	7.34, t (1.7)
28	0.83, s	0.82, s	0.79, s	0.82, s
29	2.52, d (12.2)	2.53, d (12.1)	1.79, m	2.54, d (12.0)
	2.65, d (12.2)	2.68, d (12.1)	1.95, m	2.65, d (12.0)
30	5.02, s	4.82, s	4.81, s	4.82, s
MeO-7	3.66, s	3.66, s	3.66, s	3.66, s
MeO-16	3.65, s	3.65, s	3.64, s	3.65, s
MeO-1″	3.11, s	3.44, s	3.47, s	3.45, s
AcO-1	2.04, s	2.02, s		2.02, s
AcO-11	1.97, s	1.97, s	1.97, s	1.97, s
AcO-17	2.11, s	2.11, s	2.11, s	2.11, s
2′				
3′	3.39, q (5.4)	3.46, q (5.4)	3.48, q (5.3)	3.49, q (5.3)
4′	1.26, d (5.4)	1.23, d (5.4)	1.25, d (5.3)	1.32, d (5.3)
5′	1.68, s	1.63, s	1.63, s	1.62, s
2″	1.90, m	1.25, m	1.26, m	1.39, s
		1.53, m	1.61, m	
3″	0.95, t (7.4)	0.92, t (7.2)	0.94, t (7.2)	
2‴	1.77, s	1.76, s	1.80, s	1.75, s

[a]Measured in $CDCl_3$ at 400 MHz; chemical shifts are in ppm; J values in Hz are in parentheses.

determined on the basis of ROESY correlations (Figure 1). From previous reports of limonoids,[25] H-17 is most often β-oriented. The ROESY spectrum of **1** showed the sequential correlations of H-17/H-12β/H-11β/H-5, indicating that these functionalities are on the same face of the molecule. On the other hand, the furan ring was assigned as α-oriented. In the ROESY spectrum, a cross-peak between Me-18 (δ_H 1.04) and H-22 (δ_H 6.49) suggested that these protons are on the α-face of the molecule of **1**. In turn, the ROESY cross-peaks of Me-18/H-9/H-19/H-6 and H-9/H-12α indicated the α-orientation of these protons. Moreover, the β-orientation of the tigloyloxy moiety at C-3 was assigned by the ROESY correlations of H-3/H-28 and H-3/H-29. On the basis of the above observations, the relative configuration of **1** was established as shown.

Swielimonoid B (**2**) exhibited the molecular formula $C_{32}H_{42}O_{10}$, with 12 degrees of unsaturation, as deduced from the HRESIMS (m/z 609.2672 $[M + Na]^+$). The close similarity of the UV, IR, and NMR spectroscopic data of **2** and cipadesin A[25] suggested these compounds to be close analogues, with a major difference between them being the replacement of the methylene signal at H-6 in cipadesin A with an oxymethine proton at δ_H 4.46 in **2**. This suggested there is a hydroxy group attached at C-6, analogous to the substitution at this same position in **1**. The presence of a methyl 2-hydroxyacetate moiety attached to C-5 was corroborated by the HMBC correlations (Figure 2) from H-6 to C-7 (δ_C 175.6)/C-5 (δ_C 46.2). The ROESY spectrum of **2** (Figure 2) exhibited correlations between H-17/H-11β/H-5, H-11β/H-30, and H-5/Me-29, indicating the β-orientation of these protons. Thus, the epoxide ring and the methyl 2-hydroxyacetate moiety were assigned as α-orientated. In addition, the ROESY correlations of H-17/H-15β, H-15α/H-14, and H-22/Me-18 revealed H-14 and Me-18 to be on the α-face of the molecule. From all of these data, structure **2** was assigned as swielimonoid B.

Compound **3** was obtained as an amorphous powder and found to possess the molecular formula $C_{45}H_{58}O_{20}$, as inferred from its sodiated molecular ion peak at m/z 941.3412 in the HRESIMS. The IR absorptions at 1020 and 1744 cm^{-1} suggested the presence of C−O and ester functionalities, respectively. The ^{1}H NMR data of **3** (Table 2) in $CDCl_3$ exhibited five methyl singlets (δ_H 0.83, 1.07, 1.13, 1.68, and 1.77), a methyl triplet (δ_H 0.95), three acetoxy group methyls (δ_H 1.97, 2.04, and 2.11), three methoxy groups (δ_H 3.11, 3.65, and 3.66), four oxymethine groups (δ_H 5.02, 5.36, 5.38, and 6.44), a β-furyl ring [δ_H 6.62 (1H, m, H-22), 7.35 (1H, t, J = 1.5 Hz, H-23), and 7.90 (1H, s, H-21)], and a characteristic 2,3-epoxy-2-methylbutyryl group [δ_H 1.26 (3H, d, J = 5.4 Hz, Me-4′), 1.68 (3H, s, Me-5′), and 3.39 (1H, q, J = 5.4 Hz, H-3′)].[1,24] The ^{13}C NMR (Table 3) and HSQC spectra of **3** showed 45 carbon signals, attributed to 13 methyls (including those from the three acetoxy and three methoxy groups), five aliphatic methylenes, nine methines (including five oxygenated and three olefinic), and 18 quaternary carbons (of which six are oxygenated, two are orthoester carbons, one is olefinic, and six are ester carbonyls). The above findings accounted for eight degrees of unsaturation (including two C═C double bonds and six carbonyls), indicating that **3** is composed of a nonacyclic ring system, containing a furan ring and a fused octacyclic ring system. Compound **3** was proposed as being a phragmalin-type limonoid with two unusual orthoacetate units.

In the HMBC spectrum of **3** (Figure 3), H_2-29 (δ_H 2.52 and 2.65) showed correlations with C-2 (δ_C 86.0)/C-3 (δ_C 85.0)/C-4(δ_C 44.4)/C-5 (δ_C 38.2) and C-10 (δ_C 53.5). Moreover, the HMBC correlations from Me-19 (δ_H 1.07) to C-5/C-9 (δ_C 87.5)/C-10, from Me-28 (δ_H 0.83) to C-3 (δ_C 85.0)/C-4/C-5, from H-3 (δ_H 5.36) to C-1 (δ_C 85.6)/C-30 (δ_C 70.5), and from H-30 (δ_H 5.02) to C-1/C-8 (δ_C 88.7) revealed the presence of a tricyclo[3.3.1^{2,10}.1^{1,4}]decane ring system with two tertiary methyls at C-4 and C-10.[26] In the COSY spectrum, correlations between H-11 (δ_H 5.38) and H-12 (δ_H 1.73 and 1.97) were observed. The proton sequence and the HMBC correlations from Me-18 (δ_H 1.13) to C-12 (δ_C 32.4)/C-13 (δ_C 43.8)/C-14 (δ_C 88.2)/C-17 (δ_C 69.4) and from H_2-15 (δ_H 2.97 and 3.31) to C-14/C-16 (δ_C 171.2) were used to establish the connectivity of C-11 to C-18. In addition, the HMBC correlations from H-17 (δ_H 6.44) to AcO-17 (δ_C 168.6)/C-20 (δ_C 121.4) and from MeO-16 (δ_H 3.65) to C-16 revealed a furyl group and an acetyl group to be attached to C-17, and a methoxy group was shown to be attached at C-16. A methyl propionate moiety at C-5 was supported by the HMBC correlations of H-6/C-7 (δ_C 172.6) and MeO-7 (δ_H 3.66)/C-7 and by the COSY correlation between H-5 (δ_H 2.68) and H_2-6 (δ_H 2.23 and 2.35). A 2,3-epoxy-2-methylbutyryl moiety was assigned at C-3 from the HMBC correlations between H-3 (δ_H 5.36) and C-1′ (δ_C 171.5). An acetyl group attached at C-11

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Table 3. ^{13}C NMR Spectroscopic Data of Compounds 1−6[a]

position	1	2	3	4	5	6
1	213.8 C	213.8 C	85.6 C	88.3 C	83.3 C	88.2 C
2	49.0 CH_2	48.7CH_2	86.0 C	83.1 C	83.7 C	83.1 C
3	79.4 CH	78.3 CH	85.0 CH	85.5 CH	86.1 CH	85.5 CH
4	39.3 C	39.9 C	44.4 C	44.4 C	44.0 C	44.5 C
5	44.3 CH	46.2 CH	38.2 CH	38.6 CH	40.1 CH	38.7 CH
6	72.2 CH	72.4 CH	33.7 CH_2	33.9 CH_2	34.2 CH_2	34.0 CH_2
7	175.8 C	175.6 C	172.6 C	172.7 C	172.9 C	172.7 C
8	136.4 C	60.3 C	88.7 C	89.1 C	90.2 C	90.0 C
9	55.4 CH	55.4 CH	87.5 C	86.4 C	87.1 C	86.5 C
10	52.6 C	48.5 C	53.5 C	53.9 C	52.5 C	54.0 C
11	22.6 CH_2	20.3 CH_2	71.5 CH	71.5 CH	70.9 CH	71.5 CH
12	33.5 CH_2	32.8 CH_2	32.4 CH_2	32.2 CH_2	32.1 CH_2	32.0 CH_2
13	37.6 C	35.8 C	43.8 C	43.7 C	43.9 C	43.7 C
14	161.1 C	44.1 C	88.2 C	88.1 C	88.1 C	88.1 C
15	112.4 C	32.8 C	39.6 CH_2	39.7 CH_2	39.6 CH_2	39.8 CH_2
16	164.8 C	171.2 C	171.2 C	171.4 C	171.1 C	171.4 C
17	79.6 CH	80.4 CH	69.4 CH	69.4 CH	69.3 CH	69.9 CH
18	22.5 CH_3	26.9 CH_3	19.2 CH_3	19.3 CH_3	19.2 CH_3	19.5 CH_3
19	16.2 CH_3	17.0 CH_3	17.2 CH_3	18.0 CH_3	17.6 CH_3	18.1 CH_3
20	120.1 C	120.5 C	121.4 C	121.6 C	121.5 C	121.6 C
21	141.4 CH	140.6 CH	142.6 CH	142.6 CH	142.7 CH	142.5 CH
22	110.2 CH	109.8 CH	110.4 CH	110.7 CH	110.7 CH	110.7 CH
23	143.2 CH	143.4 CH	142.0 CH	141.8 CH	141.8 CH	141.8 CH
28	22.4 CH_3	22.6 CH_3	15.2 CH_3	15.5 CH_3	15.3 CH_3	15.5 CH_3
29	23.3 CH_3	23.2 CH_3	39.2 CH_2	39.1 CH_2	40.5 CH_2	39.0 CH_2
30	129.6 CH	63.2 CH	70.5 CH	68.1 CH	68.5 CH	68.1 CH
MeO-7	53.1 CH_3	53.5 CH_3	51.9 CH_3	51.9 CH_3	51.9 CH_3	51.9 CH_3
MeO-16			51.3 CH_3	51.3 CH_3	51.4 CH_3	51.4 CH_3
MeO-33			49.6 CH_3	49.3 CH_3	49.5 CH_3	49.2 CH_3
AcO-1			168.8 C	169.5 C		169.5 C
			22.3 CH_3	22.4 CH_3		22.4 CH_3
AcO-11			168.8 C	168.8 C	168.8 C	168.8 C
			21.3 CH_3	21.3 CH_3	21.2 CH_3	21.3 CH_3
AcO-17			168.6 C	168.6 C	168.5 C	168.5 C
			21.3 CH_3	21.4 CH_3	21.4 CH_3	21.3 CH_3
1′	166.6 C	175.6 C	171.5 C	171.8 C	171.6 C	171.9 C
2′	128.0 C	41.6 CH	57.8 C	57.8 C	57.7 C	57.7 C
3′	139.1 CH	26.5 CH_2	58.8 CH	58.9 CH	58.8 CH	58.8 CH
4′	14.7 CH_3	12.0 CH_3	13.4 CH_3	13.4 CH_3	13.4 CH_3	13.4 CH_3
5′	12.0 CH_3	17.5 CH_3	13.6 CH_3	13.6 CH_3	13.5 CH_3	13.6 CH_3
1″			124.5 C	123.6 C	123.6 C	121.8 C
2″			27.8 CH_2	30.1 CH_3	29.4 CH_3	24.1 CH_3
3″			8.1 CH_3	8.2 CH_3	8.1 CH_3	
1‴			119.1 C	119.1 C	119.5 C	119.2 C
2‴			16.3 CH_3	16.3 CH_3	16.2CH_3	16.2 CH_3

[a]Measured in $CDCl_3$ at 100 MHz for **1** and **3** and at 150 MHz for **2** and **4**−**6**; assignments were made using HSQC and HMBC techniques.

was verified from the HMBC correlation of H-11 (δ_H 5.38) with AcO-11 (δ_C 168.8). Moreover, the COSY correlations between H-2″ (δ_H 1.90) and H-3″ (δ_H 0.95) together with the HMBC correlations from both MeO-1″ and H-3″ to C-1″ (δ_C 124.5) revealed an ethyl group and a methoxy group attached at the orthoacetate quaternary carbon. This moiety was assigned as being connected to C-30 and C-2 by means of the HMBC correlations from H-30 to C-1″ and the downfield chemical shift of C-2, respectively. Also, a tertiary methyl group at δ_H 1.77 (Me-2‴) could be linked to another orthoacetate quaternary carbon, because it showed ^3J-coupling with C-1‴ (δ_C 119.1). This moiety was determined to be an 8,9,14-orthoacetate by considering the carbon chemical shifts of C-8, C-9, and C-14, as well as by comparison of the ^1H and ^{13}C NMR data with those of the closely related compound swietenitin J.[23] Thus, the planar structure of **3** could be elucidated.

The relative configuration of **3** was determined on the basis of ROESY correlations (Figure 3) and compared with spectroscopic data for previously known limonoids. The ROESY spectrum of **3** showed correlations of H-17/H-11β/H-30/H-5/H-28 and H-30/MeO-1″, indicating them all to be on the β-face of the molecule. Therefore, two orthoacetate groups, the ethyl group at C-1″, and the acetyl group at C-11 were assigned as being α-oriented. On the other hand, ROESY cross-peaks of H-22/Me-18/Me-2‴/AcO-11 and Me-2‴/Me-

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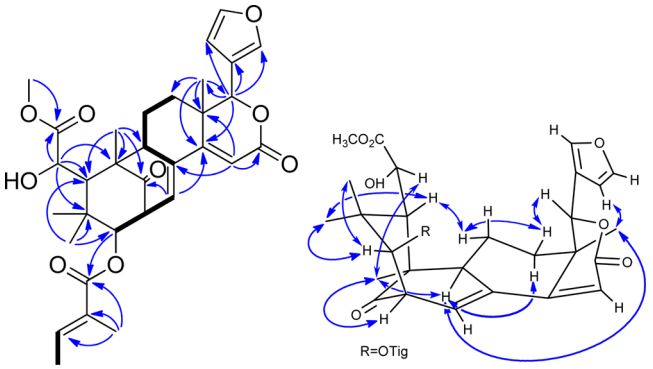

Figure 1. COSY (bold bond), selected HMBC (arrow), and key ROESY (left right arrow) correlations of **1**.

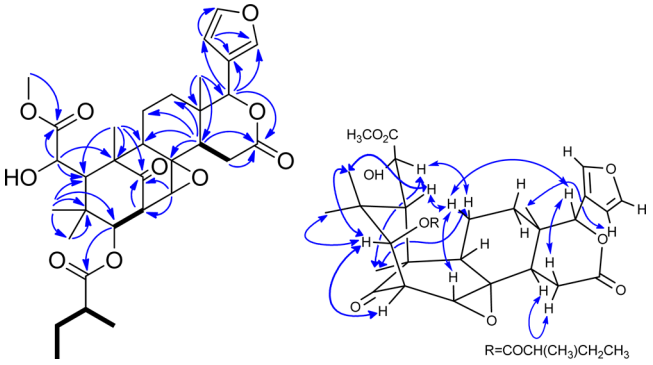

Figure 2. COSY (bold bond), selected HMBC (arrow), and key ROESY (left right arrow) correlations of **2**.

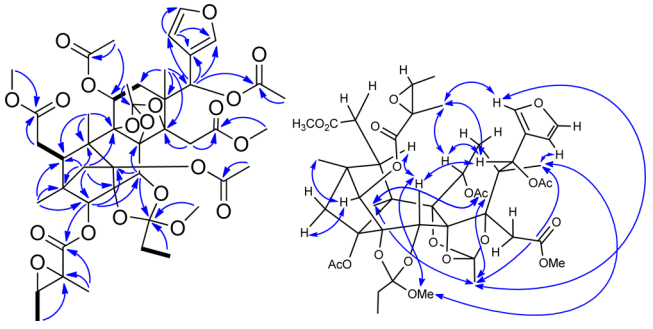

Figure 3. COSY (bold bond), selected HMBC (arrow), and key ROESY (left right arrow) correlations of **3**.

19/AcO-1 suggested these protons all to be located on the α-face of **3**. Moreover, the presence of ROESY cross-peaks of H-29/H-3 implied H-3 also to be α-oriented. Hence, the ester group attached at C-3 was determined as being β-oriented. The epoxy group in the 2′,3′-epoxy-2-methylbutyryl moiety was determined to be 2′*S*, 3′*R* from the ROESY correlations of H-3′/H-28 and Me-5′/H-11/H-17/H-21.[1,23] Therefore, the relative configuration of **3** was established as shown.

The HRESIMS (m/z 941.3412) indicated a molecular formula for swielimonoid D (**4**) identical to that of **3**. The IR and ^{1}H and ^{13}C NMR data were quite similar for these compounds, suggesting them to be isomers. In the COSY and HMBC spectra (Figure 4) of **4**, the correlations were similar to those observed from **3**, indicating the same planar structure. However, detailed comparison of the ^{1}H NMR spectra of these isolates showed some slight differences, including the chemical

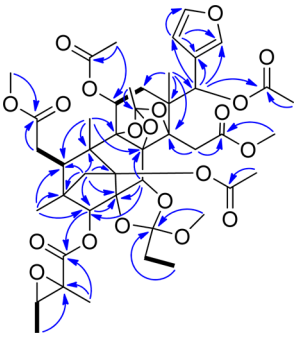

Figure 4. COSY (bold bonds) and selected HMBC (arrows) correlations of **4**.

shifts of MeO-1″ (δ_H 3.44 in **4** and δ_H 3.11 in **3**) and H-30 (δ_H 4.82 in **4** and δ_H 5.02 in **3**). These suggested that the orthoacetate units in these compounds have different configurations. To finalize the structural difference between **3** and **4**, a ROESY experiment was carried out. In this, **4** exhibited cross-peaks between MeO-1″ and Me-2‴ (δ_H 1.76), AcO-1 (δ_H 2.02), and AcO-11 (δ_H 1.97), while in the case of **3**, cross-peaks were found between MeO-1″ and H-30. Accordingly, the ethyl group at C-1″ was assigned as being β-oriented and opposite that of **3**. Thus, the structure of compound **4** was determined as shown.

Compound **5** gave a molecular formula of $C_{43}H_{56}O_{19}$ and was thus 42 amu less than that of **4** in molecular weight. Comparison of the NMR and IR data of **5** with those of **4** revealed these compounds to be closely related analogues. Compound **5** showed signals for two acetyl groups in the ^{1}H and ^{13}C NMR spectra and a hydroxy group functionality (3500 cm^{-1}) in the IR spectrum, implying that one acetyl group in **4** is replaced by a hydroxy group. The acetyl group attached at C-11 was assigned by HMBC correlations (Figure 5) from AcO-11

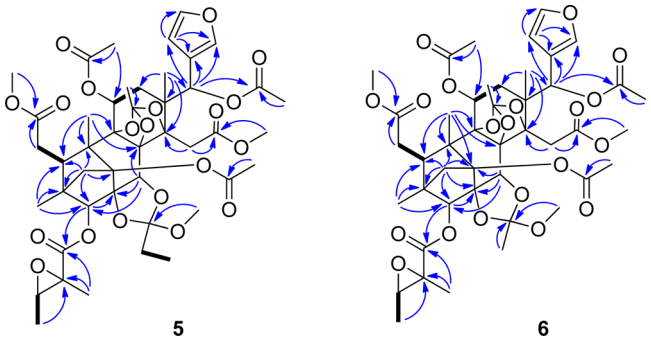

Figure 5. COSY (bold bond) and selected HMBC (arrow) correlations of **5** and **6**.

(δ_H 1.97) to C-11 (δ_C 70.9). The remaining OH group was attached to C-1, as confirmed by its chemical shift (δ_C 83.3). Other HMBC and ROESY correlations of **5** were identical to those of **4**. Compound **5**, swielimonoid E, was assigned as shown.

Swielimonoid F (**6**) gave the molecular formula $C_{44}H_{56}O_{20}$, as established from the HRESIMS (m/z 927.3256) and DEPT spectra. The IR and the ^{1}H and ^{13}C NMR spectra were also very similar to those of **4**, except that the ethyl group in **4** was replaced by a methyl group in **6**. The position of the methyl group attached at C-1″ (δ_C 121.8) was confirmed by HMBC correlations (Figure 5) of Me-2″ (δ_H 1.39) with C-1″. The

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ROESY spectrum of **6** showed correlations similar to those of **4**, suggesting the configurations of these two compounds to be the same. Thus, the structure of swielimonoid F (**6**) was established as shown.

In the previous literature on pharragmalin-type limonoids, C-1″ is substituted by a β-oriented methoxy group. Interestingly, compounds **4**−**6** were found to possess an α-oriented methoxy group at C-1″. This is the first time that C-1″ isomers of D-ring-opened phragmalin-type limonoids have been isolated and characterized structurally. The ^{13}C NMR signals of C-30 were shifted from ca. 70 ppm in compound **3** to ca. 68 ppm in compounds **4**−**6**.

Swielimonoids A−C (**1**−**3**) and some of the major components isolated were tested for their in vitro half-maximal effective concentration against dengue virus 2 and for their half-maximal cytotoxicity concentration against the Huh-7 human liver cancer cell line (Table 4). Swielimonoid B (**2**) and compounds **7**, **8**, and **10** were seen to be the most active substances against DENV-2 (7.2 ± 1.33, 3.5 ± 0.34, 6.3 ± 1.12, and 4.3 ± 2.31 μM, respectively). In a half-maximal cytotoxicity test, compound **2** showed the weakest activity (CC_{50} >200 μM), whereas **7** and **8** were more toxic to Huh cells.

Table 4. Anti-DENV-2 Activity of Selected Compounds

compound	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]
2	7.2 ± 1.33	>200	>27.7
7	3.5 ± 0.34	68 ± 1.21	19.4
8	6.3 ± 1.12	83 ± 3.45	13.2
9	12.5 ± 2.35	105 ± 3.89	8.4
10	4.3 ± 2.31	116 ± 4.64	27
ribavirin[d]	12.6 ± 1.1	56 ± 2.3	4.47

[a]Half-maximal effective concentration. [b]Half-maximal cytotoxicity concentration. [c]Selectivity index, CC_{50}/EC_{50}. [d]Positive control.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with JASCO P-1020 digital polarimeter. UV spectra were obtained using JASCO UV-530 ultraviolet spectrophotometers. IR spectra were obtained on a PerkinElmer system 2000 FT-IR spectrophotometer. NMR spectra were obtained by JEOL JNM ECS 400 MHz, Bruker Avance 600 MHz, and Varian 600 MHz NMR. ESIMS data were collected on a VG Biotech Quattro 5022 mass spectrometer. High-resolution ESIMS data were obtained on a Bruker APEX II spectrometer (FT-ICR/MS, FTMS). Silica gel 60 (Merck) was used for column chromatography. The instrumentation for HPLC was composed of a Shimadzu LC-10AT pump and a Shimadzu SPD-20A UV−vis detector.

Plant Material. Specimens of *Swietenia macrophylla* seeds were collected in Tainan, Taiwan, in September 2012. The plant material was identified by two of the authors (S.-Y.W. and Y.-H.W.). A voucher specimen (no. KMU-S1) was deposited in the Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, and in the herbarium of Hsin-Hua Forest Station, National Chung-Hsing University, Taichung, Taiwan.

Extraction and Isolation. The seeds of *S. macrophylla* (2.7 kg) were extracted three times with ethanol at room temperature and concentrated under reduced pressure to afford a crude extract. This extract was partitioned between EtOAc and H_2O (1:1) to obtain an EtOAc-soluble layer (260.3 g). This layer was further partitioned between hexane and 75% EtOH to afford two layers. The 75% EtOH layer (116.0 g) was subjected to passage over a silica gel flash column (hexanes−EtOAc−MeOH, 2:1:0 to 0:0:1) to furnish fractions F1−F5 and a major compound, **7** (1.6 g). Fraction F1 (1.3 g) was separated on a Sephadex LH-20 column eluted with EtOAc−CH_2Cl_2−MeOH (1:1:1) to give limonoid-enriched fraction F1-S2 (91.3 mg). This fraction was subjected to NP-HPLC (Phenomenex Silica, 10 mm × 250 mm, flow rate, 2.0 mL/min, *n*-hexane−CH_2Cl_2−MeOH, 50:20:1) to give subfraction F1-S2-2 (30.5 mg). Subfraction F1-S2-2 was further purified by NP-HPLC (Phenomenex CN, 10 mm × 250 mm, flow rate, 2.0 mL/min, *n*-hexane−CH_2Cl_2−MeOH, 32:20:1) to give two further fractions, F1-S2-2-1 (5.6 mg) and F1-S2-2-2 (18.9 mg). F1-S2-2-1 was purified by RP-HPLC (Phenomenex phenyl-hexyl, 10 mm × 250 mm, flow rate, 2.0 mL/min, MeOH−H_2O, 80:20) to give **5** (2.3 mg). F1-S2-2-2 was separated by RP-HPLC (Phenomenex phenyl-hexyl, 10 mm × 250 mm, flow rate, 2.0 mL/min, MeOH−H_2O, 83:17) to yield **3** (10.6 mg), **4** (0.7 mg), and **6** (0.8 mg). Fraction F2 (7.7 g) was separated on a Sephadex LH-20 column eluted with EtOAc−CH_2Cl_2−MeOH (1:1:1) to afford a further limonoid-enriched fraction, F2-S4. This fraction was subjected to NP-HPLC (Phenomenex Si, 10 mm × 250 mm, flow rate, 2.3 mL/min, *n*-hexane−CH_2Cl_2−MeOH, 32:20:1) to give **11** (8.6 mg), **12** (14.0 mg), **13** (7.0 mg), and the additional subfractions F2-S4-1 (68.2 mg), F2-S4-2 (85.0 mg), and F2-S4-4 (103.3 mg). Subfraction F2-S4-1 was further purified by NP-HPLC (Phenomenex CN, 10 mm × 250 mm, flow rate, 2.0 mL/min, *n*-hexane−CH_2Cl_2−MeOH, 80:20:1) to give **16** (5.5 mg) and **21** (8.1 mg), together with the two subfractions, F2-S4-1-5 (4.1 mg) and F2-S4-1-6 (6.6 mg). F2-S4-1-5 was purified by RP-HPLC (Phenomenex C_{18}, 10 mm × 250 mm, flow rate, 2.0 mL/min, MeOH−H_2O, 75:25) to yield **20** (2.1 mg) and **25** (0.5 mg). F2-S4-1-6 was separated by RP-HPLC (Phenomenex C_{18}, 10 mm × 250 mm, flow rate, 2.0 mL/min, MeOH−H_2O, 72:28) to yield **26** (5.3 mg). Subfraction F2-S4-2 was also purified by NP-HPLC (Phenomenex CN, 10 mm × 250 mm, flow rate, 2.0 mL/min, *n*-hexane−CH_2Cl_2−MeOH, 70:20:1) to give five known compounds **17** (3.5 mg), **8** (15.5 mg), **14** (8.8 mg), **24** (1.6 mg), and **22** (0.9 mg). Subfraction F2-S4-4 was separated by NP-HPLC (Phenomenex CN, 10 mm × 250 mm, flow rate, 2.3 mL/min, *n*-hexane−CH_2Cl_2−MeOH, 68:20:1) to give **18** (31.6 mg) and fraction F2-S4-4-4 (25.0 mg). This fraction was further isolated by RP-HPLC (Phenomenex C_{18}, 10 mm × 250 mm, flow rate, 2.0 mL/min, MeOH−H_2O, 72:28) to yield **15** (13.6 mg) and **9** (4.0 mg). Fraction F5 (142.9 mg) was separated on a Sephadex LH-20 column eluted with EtOAc−CH_2Cl_2−MeOH (1:1:6) to give a limonoid-enriched fraction, F5-S1 (136.5 mg). Fraction F5-S1 was purified by NP-HPLC (Phenomenex Silica, 10 mm × 250 mm, flow rate, 2.3 mL/min, *n*-hexane−CH_2Cl_2−MeOH, 20:20:1) to give **19** (12.0 mg) and subfraction F5-S1-7. Fraction F4 (38.8 g) was subjected to passage over a Si gel column to afford subfraction F4-2 (181.0 mg). This subfraction was purified by NP-HPLC (Phenomenex Si, 10 mm × 250 mm, flow rate, 2.3 mL/min, *n*-hexane−CH_2Cl_2−MeOH, 74:20:1) to give **23** (41.8 mg) and subfractions F4-2-1 to F4-2-3. Subfraction F4-2-1 (26.2 mg) was purified by NP-HPLC (Phenomenex CN, 10 mm × 250 mm, flow rate, 2.3 mL/min, *n*-hexane−CH_2Cl_2−MeOH, 74:20:1) to furnish **14** (16.0 mg) and fraction F4-2-1-4 (8.2 mg). This fraction was purified further using RP-HPLC (Phenomenex C_{18}, 10 mm × 250 mm, flow rate, 2.0 mL/min, MeOH−H_2O, 80:20) to yield **10** (5.2 mg). Fraction F4-2-2 (6.7 mg) was purified by RP-HPLC (Phenomenex C_{18}, 10 mm × 250 mm, flow rate, 2.0 mL/min, MeOH−H_2O, 85:15) to yield **1** (5.0 mg). Fraction F4-2-3 (6.4 mg) was purified by RP-HPLC (Phenomenex C_{18}, 10 mm × 250 mm, flow rate, 2.0 mL/min, MeOH−H_2O, 80:20) to afford **2** (3.1 mg).

*Swielimonoid A (**1**):* white, amorphous powder; $[\alpha]_D^{23}$ +23 (*c* 1.0, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 212 (4.02), 281 (3.68) nm; IR (CH_2Cl_2) ν_{max} 3497, 2951, 2928, 1728, 1715, 1649, 1258, 1131, 1030, 875, 736, 603 cm^{-1}; for ^{1}H NMR and ^{13}C NMR data, see Table 1 and Table 3, respectively; HRESIMS *m*/*z* 589.2413 [M + Na]$^+$ (calcd for $C_{32}H_{38}O_9Na$, 589.2410).

*Swielimonoid B (**2**):* white, amorphous powder; $[\alpha]_D^{26}$ −52 (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 209 (3.88) nm; IR (CH_2Cl_2) ν_{max} 3501, 2930, 2882, 1763, 1729, 1713, 1459, 1265, 1024, 867, 736, 601 cm^{-1}; for ^{1}H NMR and ^{13}C NMR data, see Table 1 and Table 3, respectively; HRESIMS *m*/*z* 609.2672 [M + Na]$^+$ (calcd for $C_{32}H_{42}O_{10}Na$, 609.2676).

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*Swielimonoid C (**3**):* white, amorphous powder; $[\alpha]_D^{23}$ −270 (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 2953, 1744, 1467, 1440, 1422, 1370, 1228, 1174, 1116, 1020, 960, 890, 736, 602 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 2 and Table 3, respectively; HRESIMS *m*/*z* 941.3412 $[M + Na]^+$ (calcd for $C_{45}H_{58}O_{20}Na$, 941.3419).

*Swielimonoid D (**4**):* white, amorphous powder; $[\alpha]_D^{26}$ +5 (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 2953, 2925, 2852, 1746, 1466, 1370, 1292, 1231, 1116, 1065, 893, 736, 604 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 2 and Table 3, respectively; HRESIMS *m*/*z* 941.3412 $[M + Na]^+$ (calcd for $C_{45}H_{58}O_{20}Na$, 941.3419).

*Swielimonoid E (**5**):* white, amorphous powder; $[\alpha]_D^{26}$ −61 (*c* 0.5, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3500, 2958, 2829, 2855, 1746, 1468, 1290, 1161, 1065, 907, 874, 733, 603 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 2 and Table 3, respectively; HRESIMS *m*/*z* 899.3307 $[M + Na]^+$ (calcd for $C_{43}H_{56}O_{19}Na$, 899.3313).

*Swielimonoid F (**6**):* white, amorphous powder; $[\alpha]_D^{26}$ −121 (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 2955, 2923, 1743, 1436, 1367, 1229, 893, 723, 601 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 2 and Table 3, respectively; HRESIMS *m*/*z* 927.3256 $[M + Na]^+$ (calcd for $C_{44}H_{56}O_{20}Na$, 927.3262).

*Andirobin (**26**):* colorless, amorphous powder; $[\alpha]_D^{25}$ −9 (*c* 1.0, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 208 (3.93), 230 (3.88) nm; IR (neat) ν_{max} 2925, 2854, 1744, 1678,1464, 1279, 1262, 1025, 931, 851 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.14 (1H, d, *J* = 10.4 Hz, H-1), 6.06 (1H, d, *J* = 10.4 Hz, H-2), 2.69 (1H, dd, *J* = 6.9, 3.0 Hz, H-5), 3.34 (1H, m, H-6), 2.50 (1H, dd, *J* = 17.0, 7.1 Hz, H-6), 3.71 (3H, s, MeO-7), 2.46 (1H, d, *J* = 7.0 Hz, H-9), 1.80 (1H, m, H-11), 1.97 (1H, m, H-11), 1.22 (1H, m, H-12), 1.67 (1H, dd, *J* = 13.8, 4.7 Hz, H-12), 4.04 (1H, s, H-15), 5.47 (1H, s, H-17), 0.94 (3H, s, H-18), 0.97 (3H, s, H-19), 7.39 (1H, brs, H-21), 6.34 (1H, m, H-22), 7.41 (1H, m, H-23), 1.11 (3H, s, H-28), 1.08 (3H, s, H-29), 5.27 (1H, s, H-30), 5.37 (1H, s, H-30); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 153.5 (d, C-1), 125.7 (d, C-2), 203.9 (s, C-3), 46.1 (s, C-4), 42.7 (d, C-5), 31.4 (t, C-6), 174.4 (s, C-7), 52.2 (q, MeO), 138.7 (s, C-8), 48.7 (d, C-9), 43.0 (s, C-10), 21.2 (t, C-11), 29.5 (t, C-12), 38.5 (s, C-13), 67.8 (s, C-14), 55.4 (d, C-15), 166.8 (s, C-16), 77.2 (d, C-17), 14.6 (q, C-18), 20.2 (q, C-19), 119.7 (d, C-20), 140.9 (d, C-21), 109.7 (d, C-22), 143.2 (d, C-23), 22.6 (q, C-28), 22.5 (q, C-29), 122.4 (t, C-30); ESIMS *m*/*z* 469 ($[M + H]^+$).

Anti-DENV-2 Assay. Naive Huh-7 cells were obtained from American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1% nonessential amino acids, and 1% antibiotic−antimycotic in a 5% CO_2 in air atmosphere at 37 °C. Huh-7 cells were seeded in 24-well plates at a density of 5×10^4 cells/mL/well for 12−16 h and then infected with DENV-2, strain 16681, at a multiplicity of infection (MOI) of 0.2 for 2 h at 37 °C. Cells were washed with PBS and then refed with DMEM−10% FBS medium containing test compounds. Cells were harvested at 72 h postinfection in a Western blot assay with anti-DENV NS2B (1:2000; Abcam, Cambridge, MA, USA) or anti-GAPDH antibody (1:10 000; GeneTex, Irvine, CA, USA), a loading control. The signal was detected using an ECL detection kit (PerkinElmer, Shelton, CT, USA). Ribavirin served as positive control.[27]

Cytotoxicity Assay. Huh-7 cells (the same origin as anti-DENV-2 assay) were seeded in 96-well plates at a density 5×10^4 cells/mL/well for 12−16 h and then infected with DENV-2 strain 16681 at an MOI of 0.2 for 2 h at 37 °C. Cells were washed with PBS and then refed with DMEM−10% FBS medium containing test compounds. Cells were harvested at 72 h postinfection for the cytotoxicity assay by using a standard MTS assay (CellTiter 96 Aqueous One Solution Cell Proliferation assay system, Promega, Madison, WI, USA) according to the manufacturer's instructions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of compounds **1**−**6**. 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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■ REFERENCES

(1) Goh, B. H.; Kadir, A. *J. Med. Plants Res.* **2011**, *5*, 1395−1404.

(2) Lin, B. D.; Zhang, C. R.; Yang, S. P.; Wu, Y.; Yue, J. M. *Chem. Pharm. Bull.* **2011**, *59*, 458−465.

(3) Mootoo, B. S.; Ali, A.; Motilal, R.; Pingal, R.; Ramlal, A.; Khan, A.; Reynolds, W. F.; McLean, S. *J. Nat. Prod.* **1999**, *62*, 1514−1517.

(4) Liu, J. Q.; Wang, C. F.; Chen, J. C.; Qiu, M. H. *Nat. Prod. Res.* **2012**, *26*, 1887−1891.

(5) Wu, S. F.; Lin, C. K.; Chuang, Y. S.; Chang, F. R.; Tseng, C. K.; Wu, Y. C.; Lee, J. C. *J. Viral Hepat.* **2012**, *19*, 364−370.

(6) Chen, J. J.; Huang, S. S.; Liao, C. H.; Wei, D. C.; Sung, P. J.; Wang, T. C.; Cheng, M. J. *Food Chem.* **2010**, *120*, 379−384.

(7) Falah, S.; Suzuki, T.; Katayama, T. *Pak. J. Biol. Sci.* **2008**, *11*, 2007−2012.

(8) El-Zalabani, S. M.; El-Askary, H. I.; Mousa, O. M.; Issa, M. Y.; Zaitoun, A. A.; Abdel-Sattar, E. *Exp. Parasitol.* **2012**, *130*, 166−170.

(9) Dutta, M.; Biswas, U. K.; Chakraborty, R.; Banerjee, P.; Maji, D.; Mondal, M. C.; Raychaudhuri, U. *Int. J. Diabetes Dev. Countries* **2013**, *33*, 60−65.

(10) Maiti, A.; Dewanjee, S.; Kundu, M.; Mandal, S. C. *Pharm. Biol.* **2009**, *47*, 132−136.

(11) Maiti, A.; Dewanjee, S.; Mandal, S. C. *Trop. J. Pharm. Res.* **2007**, *6*, 711−716.

(12) Murnigsih, T.; Matsuura, H.; Takahashi, K.; Yamasaki, M.; Yamato, O.; Maede, Y.; Katakura, K.; Susuki, M.; Kobayashi, S.; Yoshihara, T. *J. Vet. Med. Sci.* **2005**, *67*, 829−831.

(13) Richards, M. J.; Edwards, J. R.; Culver, D. H.; Gaynes, R. P. *Crit. Care Med.* **1999**, *27*, 887−892.

(14) Maiti, A.; Dewanjee, S.; Mandal, S. C.; Annadurai, S. *Iran. J. Pharm. Therap.* **2007**, *6*, 99−102.

(15) Guevara, A.; Apilado, A.; Sakurai, H.; Kozuka, M.; Takuda, H. *Philipp. J. Sci.* **1996**, *125*, 271−277.

(16) Das, A.; Sunilson, J. A. J.; Gopinath, R.; Radhamani, S.; Nilugal, K. *J. Pharm. Res.* **2009**, *2*, 1367−1369.

(17) Kalpana, K.; Pugalendi, K. V. *J. Basic Clin. Physiol. Pharmacol.* **2011**, *22*, 11−21.

(18) Kadota, S.; Marpaung, L.; Kikuchi, T.; Ekimoto, H. *Chem. Pharm. Bull.* **1990**, *38*, 639−651.

(19) Jimenez, A.; Villarreal, C.; Toscano, A.; Cook, M.; Arnason, J. T.; Bye, R.; Mata, R. *Phytochemistry* **1998**, *49*, 1981−1988.

(20) Kadota, S.; Marpaung, L.; Kikuchi, T.; Ekimoto, H. *Chem. Pharm. Bull.* **1990**, *38*, 894−901.

(21) Saad, M. M. G.; Iwagawa, T.; Okamura, H.; Doe, M.; Nakatani, M. *Heterocycles* **2004**, *63*, 389−400.

(22) Ollis, W. D.; Ward, A. D.; Oliveira, H. M. D.; Zelnik, R. A. *Tetrahedron* **1970**, *26*, 1637−1645.

(23) Lin, B. D.; Zhang, C. R.; Yang, S. P.; Zhang, S.; Wu, Y.; Yue, J. M. *J. Nat. Prod.* **2009**, *72*, 1305−1313.

(24) Leite, A. C.; Fernandes, J. B.; Silva, M. F.; Vieira, P. C. *Z. Naturforsch.* **2005**, *60b*, 351−355.

(25) Abdelgaleil, S. A.; Iwagawa, T.; Doe, M.; Nakatani, M. *Fitoterapia* **2004**, *171*, 566−572.
(26) Abdelgaleil, S. A. M.; Okamura, H.; Iwagawa, T.; Sato, A.; Miyahara, I.; Doe, M.; Nakatani, M. *Tetrahedron* **2001**, *57*, 119−126.
(27) Hsu, Y. C.; Chen, N. C.; Chen, P. C.; Wang, C. C.; Cheng, W. C.; Wu, H. N. *Arch. Virol.* **2012**, *157*, 681−688.