Terpenoids from *Daphne aurantiaca* and Their Potential Anti-inflammatory Activity

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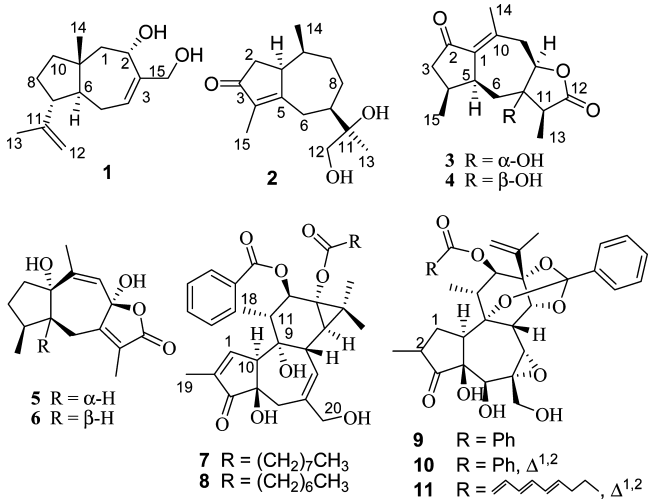
Phytochemical examination of the methanolic extract from the stem bark of *Daphne aurantiaca* led to the isolation of six new sesquiterpenoids, dauca-3,11-dien-2α,15-diol (**1**), 3-oxoguai-4-ene-11,12-diol (**2**), 4α,5α,8α,11αH-3-oxoguai-1(10)-en-12,8-olide-7α-diol (**3**), 4α,5α,8α,11*β*H-3-oxoguai-1(10)-en-12,8-olide-7*β*-diol (**4**), 4α,5*β*H-guai-9,7(11)-dien-12,8-olide-1α,8α-diol (**5**), 4α,5αH-guai-9,7(11)-dien-12,8-olide-1α,8α-diol (**6**), and a new diterpenoid, 12-*O*-benzoylphorbol 13-nonanoate (**7**), together with 10 known terpenoids. All compounds were tested for inhibitory activity against LPS-induced NO production in RAW 264.7 macrophages. Compounds **7**, **8**, **9**, **10**, and **11** showed potent inhibitory activities against NO production with IC_{50} values of 0.01, 0.01, 0.06, 0.07, and 0.03 μM, respectively.

Daphne aurantiaca Diels (Thymelacaceae) is a common evergreen shrub native to Yunnan and Sichuan Provinces of the People's Republic of China. Its stem bark is used in folk medicine for the treatment of bruises and injuries from falls.[1] So far, there are no reports on the phytochemical investigation of this plant. In the course of a study on chemical constituents of Thymelaeaceous plants,[2−5] six new sesquiterpenoids (**1**−**6**) and a new diterpenoid (**7**), together with 10 known terpenoids, were isolated from the stem bark of title plant. In this paper, we describe the structural elucidation of the new compounds and the inhibitory activities of all 17 compounds against LPS-induced NO production in macrophages.



Results and Discussion

The petroleum ether-soluble and EtOAc-soluble fractions of the methanolic extract of the stem bark of *D. aurantiaca* were subjected to repeated column chromatography over silica gel, RP-18, and Sephadex LH-20, eluting with various solvent systems, to afford six new sesquiterpenoids, **1**−**6**, and a new diterpenoid, **7**, together with 10 known terpenoids. By comparing physical and spectroscopic data with reported data, 10 known compounds were identified: oleadaphnal,[6] 4,11-guaiadien-3-one,[7] loliolide,[8] lupenone,[9] lupenyl acetate,[10] 20(29)-lupen-3-ol,[11] 12-*O*-benzoylphorbol 13-octanoate (**8**),[12] yuanhuatin (**9**),[13] genkwadaphnin (**10**),[14] and gniditrin (**11**).[15]

Compound **1** gave the molecular formula $C_{15}H_{24}O_2$, as deduced from HRESIMS ($[M + Na]^+$ at *m*/*z* 259.1674). The ^1H NMR spectrum exhibited three olefinic protons at δ_H 4.86 (brs), 4.76 (brs), and 5.78 (d, $J = 5.6$ Hz), together with two singlet methyls at δ_H 1.75 and 0.96. Accordingly, the ^{13}C and DEPT NMR of **1** (Table 1) gave characteristic carbon resonances due to an exocyclic double bond [δ_C 148.2 (s), 113.6 (t)], an endocyclic double bond [δ_C 143.8 (s), 127.4 (d)], an oxymethine (δ_C 70.0), a hydroxymethyl (δ_C 68.2), and two methyl groups (δ_C 23.8 and 18.8). All of the NMR data pointed to **1** being based on a typical daucane sesquiterpene skeleton. The NMR data of **1** were very similar to those of a known compound, dauca-3,11-dien-2-one,[16] except for the occurrence of signals due to a hydroxymethyl group [δ_C 68.2 (C-15), δ_H 4.08 (1H, d, $J = 12.4$ Hz), 4.19 (1H, d, $J = 12.4$ Hz)] and an oxygen-bearing methine [δ_C 70.0, δ_H 4.48 (1H, dd, $J = 2.4$, 10.4 Hz)], instead of a methyl group at C-15 and a carbonyl at C-2 in dauca-3,11-dien-2-one. The HMBC correlations of H_2-15 with C-2 (δ_C 70.0) and C-3 (δ_C 143.8) and of H-2 (δ_H 4.48) with C-1 and C-3 provided further evidence for the structure of **1** proposed. The relative configuration of OH-2 was determined as α through the NOESY correlation between H-2 and Me-14. Therefore, the structure of **1** was determined as dauca-3,11-diene-2α,15-diol.

Compound **2** showed a pseudomolecular ion peak at *m*/*z* 275.1611 ($[M + Na]^+$) in the HRESIMS, in agreement with the molecular formula, $C_{15}H_{24}O_3$. The NMR data of **2** (Table 1) indicated the presence of a carbonyl [δ_C 211.3 (s)], a double bond [δ_C 180.6 (s); 138.4 (s)], a hydroxymethyl [δ_C 69.0 (t), δ_H 3.47 (1H, d, $J = 11.6$ Hz), 3.57 (1H, d, $J = 11.6$ Hz)], and three methyl groups [δ_C 19.7 (q), δ_H 1.09 (3H, s); δ_C 12.5 (q), δ_H 0.63 (3H, d, $J = 7.2$ Hz); δ_C 7.9 (q), δ_H 1.66 (3H, s)], suggesting the compound is a guaiane-type sesquiterpene. The NMR data were found to be analogous to those of the known compound 3-oxoguai-4-en-12-ol,[7] except for an additional oxygenated quaternary carbon (δ_C 75.8) instead of the methine at C-11 [(δ_C 42.1, δ_H 1.65 (1H, m)] in 3-oxoguai-4-en-12-ol. The relative configuration of **2** was obtained on the basis of the NOE correlations of H-1/H-10, H-7/Me-13, and H-7/H-10. The structure of **2** was thus deduced as 3-oxoguai-4-ene-11,12-diol.

Compound **3** was isolated as a colorless oil. Its HRESIMS (negative) gave a pseudomolecular ion peak at *m*/*z* 263.1280 ($[M - H]^-$), corresponding to the molecular formula $C_{15}H_{20}O_4$. The ^{13}C NMR (Table 1) spectrum exhibited 15 carbon resonances, including a ketone carbonyl (δ_C 206.7), an ester carbonyl (δ_C 178.4),

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Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data of Compounds **1**−**3** (**1** and **2** in CD_3OD, **3** in DMSO-d_6)

	1		**2**		**3**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	52.1	2.10, dd (4.8, 12.8) 1.50, m	47.2	3.22, m	137.1	
2	70.0	4.48, dd (2.4, 10.4)	42.4	2.02, d (19.2) 2.58, dd (19.2, 7.2)	206.7	
3	143.8		211.3		47.7	1.99, dd (5.6, 17.2) 2.37, dd (7.2, 17.2)
4	127.4	5.78, d (5.6)	138.4		31.8	2.23, m
5	28.2	1.95, m 2.17, dd (8.0, 14.0)	180.6		39.2	3.14, m
6	51.2	1.97, m	34.3	2.33, dd (18.0, 10.8) 3.22, d (10.8)	31.8	1.54, dd (2.4, 14.0) 1.62, dd (10.8, 14.0)
7	51.0	2.97, dd (9.6, 19.6)	43.8	1.97, m	77.8	
8	29.2	1.78, m	28.2	1.30, m	83.4	4.28, dd (2.0, 9.6)
9	43.6	1.45, m 1.59, m	37.8	1.82, m	38.1	2.58, dd (2.4, 17.2) 2.67, ddd (1.2, 10.0, 17.2)
10	43.0		36.8	2.12, m	145.9	
11	148.2		75.8		48.6	2.56, q (8.4)
12	113.6	4.86, brs 4.76, brs	69.0	3.47, d (11.6) 3.57, d (11.6)	178.4	
13	23.8	1.75, s	19.7	1.09, s	11.2	1.11, d (8.0)
14	18.8	0.96, s	12.5	0.63, d (7.2)	21.6	2.13, d (2.0)
15	68.2	4.08, d (12.4) 4.19, d (12.4)	7.9	1.66, s	15.8	0.84, d (7.2)
OH						5.54, brs

two olefinic carbons [δ_C 137.1 (s); 145.9 (s)], four methines, three methylenes (δ_C 31.8, 38.1, 47.7), an oxygenated quarternary carbon (δ_C 77.8), and three methyl groups (δ_C 11.2, 15.8, 21.6). Detailed inspection of the NMR data of **3** revealed this compound to be a guaianolide-type sesquiterpene lactone.[17,18] In the HMBC spectrum of **3**, the oxygenated proton at δ_H 4.28 (1H, dd, J = 2.0, 9.6 Hz, H-8) showed a correlation with an ester carbonyl (δ_C 178.4), indicating **3** to be a 8,12-olide derivative. The key HMBC correlation of H_2-3 with the ketone carbonyl (δ_C 206.7) was used to place the ketone carbonyl at C-2. The double bond was located between C-1 and C-10 due to the HMBC correlations of H-5 (δ_H 2.13) with C-1 (δ_C 137.1) and C-10 (δ_C 145.9) and of Me-14 (δ_H 2.13) with C-1 (Figure 1). Additionally, the HMBC cross-peak between the hydroxy proton at δ_H 5.54 and C-7 revealed that the hydroxy group was attached at C-7. The relative configuration of **3** was determined through interpretation of the NOESY spectrum. The NOESY correlations of H-5 with H-8 and H-4 supported the α-orientation of H-8. Both OH-7 and H-11 were deduced to be α-oriented on the basis of the NOESY correlations from H-8 to OH-7 and H-11. Therefore, the structure of compound **3** was deduced as 4α,5α,8α,11αH-3-oxoguai-1(10)-en-12,8-olide-7α-diol.

Compound **4** was assigned the same molecular formula, $C_{15}H_{20}O_4$, as **3**, by HRESIMS ([M − H]$^-$ at m/z 263.1280). The NMR data of **4** were quite similar to those of **3**, and the same planar structure was deduced by interpretation of the 2D NMR spectra, suggesting these compounds to be diastereoisomers. Interpretation of the NOESY spectrum of **4** revealed that the only difference is the relative configuration of OH-7. The key correlation of OH-7 with Me-13 indicated a β-orientation of OH-7 for **4**. Consequently, the structure of compound **4** was established as 4α,5α,8α,11βH-3-oxoguai-1(10)-en-12,8-olide-7β-diol.

The molecular formula of compound **5** was determined as $C_{15}H_{20}O_4$ by HRESIMS ([M − H]$^-$ at m/z 263.1281). The NMR data (Table 2) showed typical features of a guaianolide sesquiterpene with an 8,12-lactone ring. The ^1H NMR spectrum exhibited two hydroxy protons at δ_H 4.33 (brs) and 5.04 (brs) and one olefinic proton at δ_H 5.46 (s). The ^{13}C NMR spectrum displayed 15 carbon resonances due to two double bonds (δ_C 119.2, 124.9, 147.6, 151.6), two oxygenated quarternary carbons (δ_C 74.0, 83.9), an ester carbonyl (δ_C 170.0), three methyls (δ_C 8.9, 16.2, 26.3), three methylenes (δ_C 23.5, 32.7, 35.7), and two sp^3 methines (δ_C 37.6, 41.1). One double bond was assigned between C-7 and C-11 on the basis of the HMBC correlations of Me-13 (δ_H 1.80) with C-7, C-11, and C-12 (Figure 1). Another double bond was positioned between C-9 and C-10 from the HMBC correlations from Me-14 (δ_H 1.30) to C-9 (δ_C 119.2) and C-10 (δ_C 147.6). Two hydroxy groups were substituted at C-1 (δ_C 74.0) and C-8 (δ_C 83.9), respectively, due to the key HMBC correlations of OH-1 (δ_H 4.33) with C-1, and OH-8 (δ_H 5.04) with C-8. The relative configuration of H-5 was deduced as β through the NOESY correlations of H-5 with Me-15. Both OH-1 and OH-8 were determined to be α-oriented on the basis of the NOE correlations of H-4/OH-8 and OH-1/OH-8. Thus, the structure of compound **5** was deduced as 4α,5βH-guai-9,7(11)-dien-12,8-olide-1α,8α-diol.

Compound **6** was assigned the same molecular formula, $C_{15}H_{20}O_4$, as **5** by HRESIMS ([M − H]$^-$ at m/z 263.1281). Inspection of the NMR data of **6**, including the 2D NMR spectra, revealed that **6** has the same planar structure, indicating the compounds as being diastereoisomers. The relative configuration of **6** was identical with those of **5** through analysis of the NOESY spectrum, except that the relative configuration of H-5 was determined to be α-oriented on the basis of the NOESY correlation of H-5 with H-4 (δ_H 2.27). The structure of compound **6** was consequently established as 4α,5αH,1α,8α-dihydroxy-9,7(11)-guaiadien-12,8-olide.

Compound **7** was formulated as $C_{36}H_{48}O_8$ by HRESIMS ([M + K]$^+$ at m/z 649.2986). In the ^1H NMR spectrum, five aromatic protons at δ_H 7.49 (2H, dt, J = 1.2, 7.2 Hz), 7.62 (1H, t, J = 7.2 Hz), and 7.99 (2H, dt, J = 1.2, 7.2 Hz) indicated the presence of a monosubstituted aromatic ring, while two protons at δ_H 7.57 (1H, brs) and 6.56 (1H, d, J = 4.4 Hz) exhibited the presence of two CH═C groups. The protons at δ_H 0.94 (3H, d, J = 6.4 Hz, H-18), 0.89 (3H, d, J = 7.2 Hz, H-9″), 1.21 (3H, s, H-16), 1.39 (3H, s, H-17), and 1.73 (3H, d, J = 1.6 Hz, H-19) revealed five methyls.

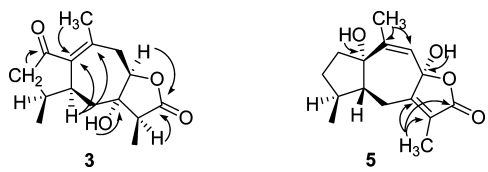

Figure 1. Key HMBC correlations of compounds **3** and **5**.

Table 2. ^{13}C and ^{1}H NMR Spectroscopic Data of Compounds **4**−**6** (in DMSO-d_6)

	4		**5**		**6**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	134.7		74.0		72.9	
2	207.3		35.7	2.16, m	35.2	1.40, m
				1.60, m		2.13, m
3	48.2	1.92, dd (10.4, 17.6)	32.7	1.62, m	32.1	1.85, m
		2.50, dd (7.2, 17.6)		1.38, m		1.26, m
4	32.2	2.24, m	37.6	2.16, m	37.4	2.27, m
5	40.1	3.16, m	41.1	1.58, m	54.1	1.97, ddd (3.6, 7.2, 16.4)
6	36.2	1.82, dd (3.6, 18.0)	23.5	2.66, dt (1.2, 14.4)	24.9	3.02, dt (1.2, 14.4)
				2.51, dd (4.0, 14.4)		2.48, dd (4.0, 14.4)
7	77.8		151.6		152.1	
8	85.2	4.35, dd (2.4, 12.8)	83.9		88.0	
9	38.9	2.24, m	119.2	5.46, s	120.0	5.73, s
		3.00, ddd (4.0, 13.6, 17.2)				
10	146.4		147.6		151.1	
11	42.6	2.92, q (6.8)	124.9		122.8	
12	178.4		170.0		170.9	
13	7.6	1.03, d (6.8)	8.9	1.80, s	8.5	1.80, s
14	21.6	2.16, d (1.6)	26.3	1.30, s	28.2	1.34, s
15	15.5	0.72, d (6.8)	16.2	0.96, d (7.2)	15.3	0.88, d (7.2)
OH-1				4.33, s		4.28, s
OH-7		5.34, brs				
OH-8				5.04, s		5.16, s

Table 3. Inhibition against LPS-Induced NO Production in RAW264.7 Macrophages ($n = 4$, mean ± SD)[a]

compound	IC_{50} (μM)
AG	0.05
2	0.34
3	0.20
4	0.13
5	0.31
7	0.01
8	0.01
9	0.06
10	0.07
11	0.03

[a] LPS: negative control; AG: aminoguanidine, positive control. The other compounds **1**, **6**, oleadaphnal, 4,11-guaiadien-3-one, loliolide, lupenone, lupenyl acetate, and 20(29)-lupen-3-ol were all inactive (IC_{50} > 0.4 μM)

A series of protons centered at δ_H 1.29 (m) implied the presence of a straight-chain alkane moiety. The ^{13}C NMR data of **7** displayed carbon resonances due to a ketone carbonyl (δ_C 210.3) and two ester carbonyls (δ_C 168.0, 178.0). The NMR spectra were very similar to those of 12-*O*-benzoylphorbol 13-octadecanoate,[12] except for an additional methylene in the alkane chain. Thus, the structure of **7** was deduced as 12-*O*-benzoylphorbol 13-nonanoate.

All 17 isolates were tested for their inhibitory activity against LPS-induced NO production in RAW 264.7 macrophages. Compounds **7**−**11** showed potent inhibitory activities against the production of NO (Table 3).

Since nitric oxide (NO) plays an important role in the inflammatory process,[19] inhibitors of NO release may be considered as potential therapeutic agents in inflammatory diseases.[20] Although a number of natural products have been reported to inhibit NO release,[21−23] only a limited number of terpenoids were studied so far.[24,25] The present investigation has shown that diterpenoids, such as compounds **7** and **8**, are potent nitric oxide synthase inhibitors.

Experimental Section

General Experimental Procedures. Optical rotations were acquired with a Perkin-Elmer 341 polarimeter, whereas UV spectra were obtained by using a Shimadzu UV-2550 UV−vis spectrophotometer. IR spectra were recorded on a Bruker Vector 22 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer with TMS as interal standard. HRESIMS were measured using a Q-TOF micro mass spectrometer (Waters, Millford, MA). Materials for column chromatography were silica gel (100−200 mesh; Huiyou Silica Gel Development Co. Ltd., Yantai, People's Republic of China), silica gel H (10−40 μm; Yantai), Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-gel ODS-A (50 μm; YMC, Allentown, PA). Preparative TLC (0.4−0.5 mm) was conducted with glass precoated silica gel GF_{254} plates (Yantai).

Plant Material. The plant material was collected in July 2006 in Lijiang City, Yunnan Province, People's Republic of China, and identified as *Daphne aurantiaca* by Prof. Li-Shan Xie of Kunming Institute of Botany. A voucher specimen has been deposited in the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai (No. 200607-11).

Extraction and Isolation. The air-dried and powdered stem bark of *D. aurantiaca* (7.0 kg) was extracted with MeOH for 3 × 50 L × 2 h. The solvent was evaporated under vacuum. Then, the extract was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-butanol, successively. The petroleum ether extract (300 g) was subjected to column chromatography on silica gel (200−300 mesh, 1000 g), eluted with a gradient mixture of petroleum ether−EtOAc. The 5% EtOAc eluate was chromatographed on silica gel with $CHCl_3$−MeOH to give oleadaphnal (50 mg), 4,11-guaiadien-3-one (150 mg), lupenone (15 mg), lupenyl acetate (20 mg), 20(29)-lupen-3-ol (35 mg), and **11** (17 mg). The EtOAc extract (400 g) was subjected to column chromatography on silica gel (200−300 mesh, 1000 g), eluted with gradient $CHCl_3$−MeOH. The 2% MeOH eluate was chromatographed on silica gel with $CHCl_3$−MeOH followed by Sephadex LH-20 with MeOH to give **1** (12 mg), **7** (5 mg), **8** (15 mg), **9** (30 mg), and loliolide (23 mg). Similarly, the 4% MeOH eluate afforded **2** (9 mg), **3** (4 mg), **4** (5 mg), **5** (15 mg), and **6** (80 mg).

Compound 1: colorless oil; $[\alpha]^{20}_D$ +19 (*c* 0.64, CH_3OH); UV (MeOH) λ_{max} (log ε) 207 (3.16) nm; IR (KBr) ν_{max} 3396, 2922, 1717, 1636, 1455, 1383, 998, 845 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; positive HRESIMS *m/z* 259.1674 (calcd for $C_{24}H_{32}O_6Na$, 259.1696).

Compound 2: colorless oil; $[\alpha]^{20}_D$ +126 (*c* 0.20, CH_3OH); UV (MeOH) λ_{max} (log ε) 242 (4.23) nm; IR (KBr) ν_{max} 3408, 2930, 2847, 1680, 1624, 1382, 1049 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; positive HRESIMS *m/z* 275.1611 (calcd for $C_{15}H_{24}O_3Na$, 275.1623).

Compound 3: colorless oil; $[\alpha]^{20}_D$ −94 (*c* 0.20, CH_3OH); UV (MeOH) λ_{max} (log ε) 252 (3.09), 212 (2.08) nm; IR (KBr) ν_{max} 3445, 2957, 1773, 1704, 1630, 1382, 1121, 1013 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; negative HRESIMS *m/z* 263.1280 (calcd for $C_{15}H_{19}O_4$, 263.1283).

Compound 4: colorless oil; $[\alpha]^{20}_D$ −122 (*c* 0.08, CH_3OH); UV (MeOH) λ_{max} (log ε) 252 (3.61), 211 (3.28) nm; IR (KBr) ν_{max} 3437,

2958, 1776, 1707, 1626, 1002 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 2; negative HRESIMS *m/z* 263.1280 (calcd for $C_{15}H_{19}O_4$, 263.1283).

Compound 5: colorless oil; $[\alpha]^{20}_D$ −88 (*c* 0.08, CH_3OH); UV (MeOH) λ_{max} (log ε) 274 (3.22), 212 (2.60) nm; IR (KBr) ν_{max} 3446, 2955, 2934, 2876, 1740, 1384, 1094 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 2; negative HRESIMS *m/z* 263.1281 (calcd for $C_{15}H_{19}O_4$, 263.1283).

Compound 6: colorless oil; $[\alpha]^{20}_D$ −109 (*c* 0.20, CH_3OH); UV (MeOH) λ_{max} (log ε) 274 (3.52), 211 (2.66) nm; IR (KBr) ν_{max} 3456, 2959, 2952, 2873, 1746, 1665, 1368, 1217, 1029 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 2; negative HRESIMS *m/z* 263.1281 (calcd for $C_{15}H_{19}O_4$, 263.1283).

Compound 7: colorless oil; $[\alpha]^{20}_D$ +7 (*c* 0.09, CH_3OH); UV (MeOH) λ_{max} (log ε) 230 (3.34) nm; IR (KBr) ν_{max} 3394, 2927, 2857, 1718, 1452, 1384, 1271, 1175, 1105, 970, 711 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.99 (2H, dt, J = 1.2, 7.2 Hz, H-3′, H-7′), 7.62 (1H, t, J = 7.2, H-5′), 7.57 (1H, brs, H-1), 7.49 (2H, dt, J = 1.2, 7.2, H-4′, H-6′), 5.69 (1H, d, J = 10.4, H-12), 5.65 (1H, d, J = 4.4, H-7), 3.97, 3.93 (each 1H, d, J = 12.8, H-20), 3.38 (1H, dd, J = 4.4, 5.6, H-8), 3.20 (1H, d, J = 2.4, H-10), 2.53 (1H, d, J = 9.6, H-5), 2.37 (3H, m, H-11, H-2″), 1.73 (3H, d, J = 1.6, H-19), 1.62 (2H, m, H-3″), 1.39 (3H, s, H-17), 1.29 (10H, m, H-4″, H-5″, H-6″, H-7″, H-8″), 1.21 (3H, s, H-16), 1.18 (1H, d, J = 5.6, H-14), 0.94 (3H, d, J = 6.4, H-18), 0.89 (3H, t, J = 7.2, H-9″); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.5 (CH, C-1), 134.7 (C, C-2), 210.3 (C, C-3), 74.8 (C, C-4), 38.5 (CH_2, C-5), 143.0 (C, C-6), 129.3 (CH, C-7), 40.1 (CH, C-8), 67.0 (C, C-9), 57.4 (CH, C-10), 44.7 (CH, C-11), 79.3 (CH, C-12), 79.9 (C, C-13), 37.6 (CH, C-14), 27.5 (C, C-15), 24.2 (CH_3, C-16), 17.7 (CH_3, C-17), 15.0 (CH_3, C-18), 10.3 (CH_3, C-19), 68.0 (CH_2, C-20), 168.0 (C, C-1′), 131.3 (C, C-2′), 130.7 (CH, C-3′), 129.8 (CH, C-4′), 134.5 (CH, C-5′), 129.8 (CH, C-6′), 130.7 (CH, C-7′), 178.0 (C, C-1″), 35.3 (CH_2, C-2″), 25.8 (CH_2, C-3″), 32.9 (CH_2, C-4″), 30.1 (CH_2, C-5″, C-6″, C-7″), 23.7 (CH_2, C-8″), 14.5 (CH_3, C-9″); positive HRESIMS *m/z* 649.2963 (calcd for $C_{36}H_{48}O_8K^+$, 649.2986).

Assay for Inhibitory Activity against LPS-Induced NO Production. RAW 264.7 macrophages were seeded in 24-well plates (10^5 cells/well). The cells were co-incubated with compounds and LPS (1 μg/mL) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW 264.7 macrophage supernatants with Griess reagent. Aliquots of supernatants (100 μL) were incubated, in sequence, with 50 μL of 1% sulfanilamide and 50 μL of 0.1% naphthylethylenediamine in 2.5% phosphoric acid solution. The absorbances at 570 nm were read using a microtiter plate reader.[22]

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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