Sesquiterpenoids from *Inula linearifolia* Inhibit Nitric Oxide Production

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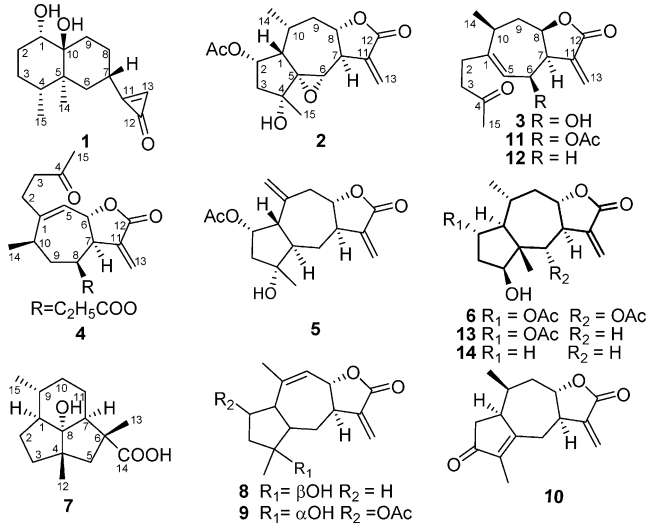
Fourteen sesquiterpenoids including six new analogues (**1**−**6**) were isolated from the aerial parts of *Inula linearifolia*.. The structures of the new sesquiterpenoids were determined by spectroscopic data analysis, and the structures of **1** and **6** were confirmed by single-crystal X-ray diffraction data and the modified Mosher method. All 14 compounds were examined for the inhibition of LPS-induced nitric oxide production in RAW264.7 macrophages. Compounds **6**, **13**, and **14** were found to inhibit nitric oxide production potently, with IC_{50} values of 0.11, 0.25, and 0.10 μM, respectively.

The genus *Inula* (Asteraceae family) is composed of about 100 plant species, a number of which are used as traditional herbal medicines throughout the world.[1] Recently, much attention has been paid to *Inula* species due to their diverse biological activities, which include anti-inflammatory, antitumor, and bactericidal effects.[1,2] Plants of *Inula* species are rich sources of sesquiterpenes, such as eudesmanes, macrophyllic acids, elemanes, germacranes, guaianes, bis-sesquiterpenes, and sesquiterpene-monoterpene dimers.[1] *Inula linearifolia* Turcz. is widely distributed in China and is used in the traditional Chinese medicine "JinFeiCao". However, only one sesquiterpenoid, britanin, has previously been reported from this plant.[3] As part of our ongoing search for biologically active sesquiterpenoids from *Inula* species, six new (**1**−**6**) and eight known sesquiterpenoids (**7**−**14**) were isolated from the aerial parts of *I. linearifolia*. Herein, we describe the isolation and structure elucidation of these six new sesquiterpenes and the inhibitory activities of all 14 compounds against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages.



Results and Discussion

The petroleum ether and $CHCl_3$-soluble fractions of the MeOH extract of the aerial parts of *I. linearifolia* were subjected to repeated column chromatography over silica gel, MCI, Sephadex LH-20, and preparative HPLC, using various solvent systems as eluants, to afford six new (**1**−**6**) and eight known (**7**−**14**) sesquiterpenoids.

Compound **1** had the molecular formula $C_{15}H_{22}O_3$ by positive HRESIMS at *m*/*z* 251.1651 $[M + H]^+$, indicating five degrees of unsaturation. Its IR spectrum exhibited the presence of two OH groups (3450 and 3268 cm^{-1}) and a cyclopropenone (1845, 1556 cm^{-1}) group. The ^1H, ^{13}C, and DEPT NMR spectra showed the presence of one singlet and one methyl doublet [δ_H 0.92 (3H, s, H_3-14), δ_C 17.0; 0.77 (3H, d, J = 6.9 Hz, H_3-15), δ_C 15.5], five methylenes, four methines, including an oxygen-bearing carbon [δ_H 3.42 (m); δ_C 76.5 (C-1)], one olefinic carbon [δ_H 8.74 (1H, d, J = 12.5 Hz); δ_C 147.9 (C-13)], four quaternary carbons, including an olefinic carbon [δ_C 161.9 (C-11)], one carbonyl [δ_C 176.1 (C-12)], and one oxygenated quaternary carbon [δ_C 74.3 (C-10)] (Tables 1 and 2). Analysis of the $^1H-^1H$ COSY plot of **1** suggested the following correlations: H-1/H_2-2/H_2-3/H-4/H_3-15 and H_2-6/H-7/H_2-8/H_2-9 (Supporting Information). The connections of these moieties through a quaternary carbon was established by analyzing HMBC correlations. In the HMBC spectrum, the cross-peaks from H-1 to C-3, C-5, and C-10; H_2-9 to C-1, C-5, and C-10; H_3-14 to C-4, C-6, and C-10; H_3-15 to C-3 and C-5; H-7 to C-12 and C-13; and H-13 to C-12 indicated that **1** had a planar structure. The relative configuration of **1** was confirmed by single-crystal X-ray diffraction (Figure 1), which was consistent with the NOESY correlations: H_3-15 to H-3α; H_3-14 to H-3α and H-6α; and H-1 to H-3β and H-4. The absolute configuration of **1** was determined by a combination of the modified Mosher method[4,5] and X-ray data (Figure 2). Thus, the absolute configuration of **1** was established as 1*S*, 4*R*, 5*S*, 7*S*, and 10*S*, and thus the structure of **1** was elucidated and it was named inearifolianone.

Compound **2** was isolated as a light yellow gum. The molecular formula was established as $C_{17}H_{22}O_6$ by HRESIMS at *m*/*z* 321.1339 $[M - H]^-$, indicating seven degrees of unsaturation. Its IR spectrum exhibited absorption bands corresponding to an OH group (3427 cm^{-1}), an ester carbonyl (1738 cm^{-1}), and an olefinic bond (1627, 1456 cm^{-1}). The similarities between the NMR spectra of **2** (Tables 1 and 2) and gaillardin (**9**) suggested that **2** was also a sesquiterpene lactone with a guaianolide skeleton, esterified with an acetyl group.[6] The main difference between the ^{13}C NMR spectra of **2** and **9** was that the signals of the two olefinic carbons [δ_C 136.2 (C-10), δ_C 128.2 (C-9)] in **9** shifted upfield to δ_C 34.1 and δ_C 43.9, whereas the methine group at δ_C 51.0 (C-5) and the methylene group at δ_C 34.4 (C-6) in **9** moved downfield to δ_C 74.3 and δ_C 56.3 in **2**, respectively. These observations suggested that the olefinic carbons in **9** were hydrogenated in **2** and that an epoxide group was present between C-5 and C-6 in **2** due to the remaining unsaturation. The assumption was confirmed by 2D NMR data, in which HMBC correlation of H-2 to the carbonyl carbon at δ_C 170.3 indicated that the acetoxyl group was located at C-2. In NOESY spectra, the

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Table 1. ^{1}H NMR Spectroscopic Data of **1**−**6** (*J* values in Hz) (500 MHz)

position	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]
1	3.42 m	1.98 dd (12.5, 3.5)			2.51 dd (12.5, 5.0)	1.91 m
2	1.51 m; 2.20 m	5.02 m	2.59 m; 2.59 m	2.54 m; 2.54 m	5.30 ddd (11.5, 8.5, 6.0)	4.90 t (5.0)
3	1.59 m; 1.21 m	2.44 m; 1.81 m	2.29 m; 2.29 m	2.27 m; 2.27 m	2.20 m, 1.98 m	1.83 m, 2.07 m
4	1.90 m					4.13 dd (11.0, 9.0)
5			5.30 d (2.0)	5.08 d (2.5)	2.27 ddd (12.0, 9.5, 3.0)	
6	1.87 m; 2.08 m	3.23 d (5.5)	4.69 dd (10.0, 1.5)	5.69 dd (11.0, 5.0)	1.16 m; 2.38 m	5.05 d (12.0)
7	3.05 m	2.71 m	3.28 m	3.46 ddd (11.5, 8.5, 3.0)	2.71 m	3.04 m
8	2.27 m; 2.06 m	4.06 ddd (12.5, 9.5, 2.0)	4.61 ddd (12.5, 9.5, 3.5)	4.64 ddd (12.0, 8.5, 3.0)	3.91 dt (15.0, 3.0)	4.53 m
9	2.31 m; 1.24 m	2.44 m; 1.53 m	2.17 m; 1.80 m	2.10 m; 1.86 m	2.59 m; 3.06 dd (14.0, 3.0)	1.45 m; 2.45 m
10		1.81 m	2.36 m	2.39 m		1.90 m
13	8.74 d (12.5)	5.88 d (3.5)	6.36 d (2.0)	6.31 d (2.5)	6.20 d (3.0)	6.15 d (3.0)
		6.36 d (3.5)	6.09 d (2.0)	5.72 d (2.5)	5.53 d (3.0)	5.39 d (3.0)
14	0.92 s	1.07 d (6.5)	1.14 d (6.0)	1.13 d (7.0)	4.99 d (3.0); 5.15 d (3.0)	0.99 d (6.0)
15	0.77 d (6.9)	1.29 s	2.17 s	2.15 s	1.23 s	1.03 s
2-OAc		2.07 s			2.04 s	2.05 s
6-OAc						2.25 s
8-C_2H_5COO				2.39 m; 2.39 m 1.16 t (7.0)		

[a] Recorded in methanol-d_4. [b] Recorded in $CDCl_3$.

Table 2. ^{13}C NMR Spectroscopic Data of **1**−**6** (125 MHz)

position	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]
1	76.5, CH	53.5, CH	141.9, C	142.7, C	51.0, CH	51.1, CH
2	29.8, CH_2	75.3, CH	42.3, CH_2	42.3, CH_2	75.6, CH	75.3, CH
3	26.7, CH_2	44.7, CH_2	30.0, CH_2	30.0, CH_2	48.0, CH_2	36.3, CH_2
4	36.0, CH	70.9, C	207.7, C	207.7, C	78.5, C	72.9, CH
5	40.4, C	73.4, C	129.3, CH	125.9, CH	53.5, CH	51.1, C
6	36.9, CH_2	56.3, CH	67.7, CH	70.0, CH	27.1, CH_2	76.5, CH
7	34.3, CH	50.8, CH	48.9, CH	46.1, CH	49.3, CH	52.4, CH
8	23.2, CH_2	78.0, CH	77.2, CH	76.9, CH	83.5, CH	76.1, CH
9	29.5, CH_2	43.9, CH_2	37.0, CH_2	36.9, CH_2	42.1, CH_2	44.0, CH_2
10	74.3, C	34.1, CH	35.2, CH	35.1, CH	141.8, C	30.2, CH
11	161.9, C	137.7, C	136.5, C	135.8, C	139.6, C	138.6, C
12	176.1, C	168.8, C	169.9, C	169.3, C	169.9, C	168.8, C
13	147.9, CH	121.9, CH_2	125.4, CH_2	125.0, CH_2	119.1, CH_2	120.0, CH_2
14	17.0, CH_3	19.9, CH_3	20.7, CH_3	20.6, CH_3	116.5, CH_2	15.9, CH_3
15	15.5, CH_3	24.9, CH_3	29.9, CH_3	29.9, CH_3	24.6, CH_3	20.4, CH_3
2-OAc		170.3, C			170.4, C	170.2, C
		21.1, CH_3			21.2, CH_3	21.2, CH_3
6-OAc						172.6, C
						21.2, CH_3
8-C_2H_5COO				173.3, C		
				27.6, CH_2		
				9.0, CH_3		

[a] Recorded in methanol-d_4. [b] Recorded in $CDCl_3$.

following correlations was observed: H-2 to H_3-15 and H-10; H-6 to H-8 and H_3-15; H-8 to H-6 and H-10. On the basis of these findings and by comparing its NMR data with that of the known compound gaillardin, **2** was identified as 5α,6α-epoxy-2α-acetoxy-4α-hydroxy-1β,7α-guaia-11(13)-en-12,8α-olide.

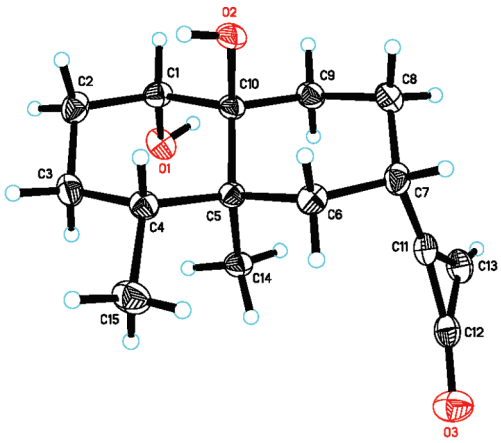
Compound **3** was obtained as a colorless gum and exhibited a molecular ion $[M + H]^+$ at *m*/*z* 265.1445 in the HRESIMS, which corresponded to the molecular formula $C_{15}H_{20}O_4$. Except for a signal of an additional oxygenated C atom resonating at δ_C 67.7 (C-6), its ^{1}H and ^{13}C NMR spectra resembled those of tomentosin (**12**).[7] These observations together with a 16 Da mass surplus indicated that **3** was a hydroxylated derivative of tomentosin. The relative configuration of **3** was determined using key NOESY correlations, namely, H-6 to H-9α, H-9α to H-10, H-6 to H-5, H-5 to H-3α, and H-3α to H-10. On the basis of the above-mentioned observations and literature values,[7] **3** was identified as 6β-hydroxytomentosin.

HRESIMS data showed **4** had the molecular formula $C_{18}H_{24}O_5$. The ^{1}H and ^{13}C NMR data closely resembled those of inusoniolide,[8] but three additional carbon resonances were observed at δ_C 9.0, 27.6, and 173.3 in **4**, which were assigned to a propionyl moiety using HMQC and HMBC data. These observations, coupled with HMQC and HMBC analyses, revealed that **4** was a C-8-propionylated derivative of inusoniolide. Thus, the structure of **4** was determined as 8β-propionylinusoniolide.

Compound **5** was obtained as light yellow oil. HRESIMS gave a quasimolecular ion peak $[M - H]^-$ at *m*/*z* 305.1402 consistent with the molecular formula $C_{17}H_{22}O_5$. The ^{1}H and ^{13}C NMR data of **5** resembled those of the reported otoimbricatin B,[9] suggesting that they have the same planar structure, which was also consistent with the 2D NMR data. Furthermore, the relative configuration of **5** was identical to that of **2** on the basis of NOESY correlations of H-1/H-2/H-8, H-2/H_3-15, and H-5/H-6α/H-7 (Figure 3). Thus, **5** was identified as 2α-acetoxy-4α-hydroxy-1β-guai-11(13),10(14)-dien-12, 8α-olide.

Compound **6** was obtained as colorless, tetragonal crystals after crystallization from MeOH. HRESIMS established a molecular formula of $C_{19}H_{26}O_7$ ($[M + H]^+$ at *m*/*z* 367.1767). A literature search revealed that the spectroscopic data of **6** resembled those of the known pseudoguaiaonlide-type sesquiterpene britanin.[3] X-ray diffraction analysis of **6** (Figure 1) established an α-orientaion of the acetoxy group at C-2 rather than the β-orientation in britanin. Consequently, the structure of **6** was determined to be 2α,6α-diacetoxy-4β-hydroxy-11(13)-pseudoguaien-12,8α-olide.

By comparing physical and spectroscopic data with literature values, the eight known sesquiterpenes (**7**−**14**) were identified as britanlin C (**7**),[10] 4-epi-isoinuviscolide (**8**),[11] gaillardin (**9**),[6] xerantholide (**10**),[12] inuchinenolide A (**11**),[13] tomentosin (**12**),[6] 2-*O*-acetyl-4-epipulchellin (**13**),[14] and 2-desoxy-4-epi-pucgellin (**14**).[15]



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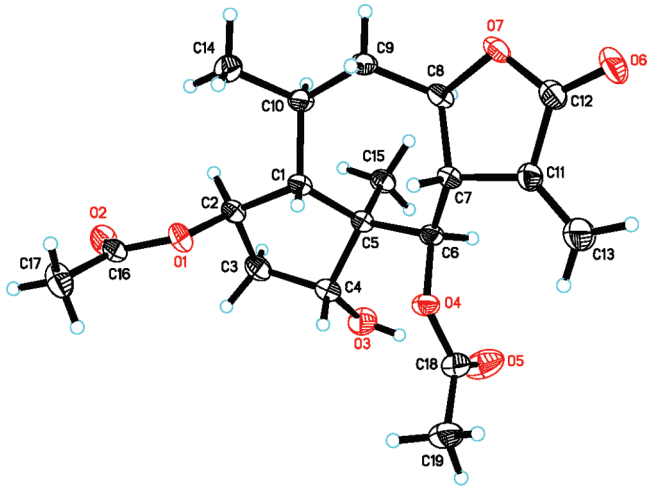

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Figure 1. X-ray crystal structures of **1** and **6**.

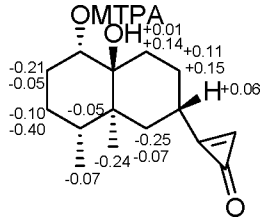

Figure 2. $\Delta\delta$ ($\delta_S - \delta_R$) values in ppm for MTPA derivatives of **1**.

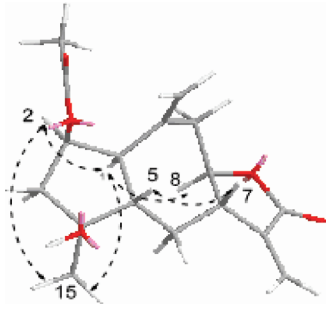

Figure 3. Selected NOESY correlations of **5**.

All 14 compounds were examined with respect to their inhibitory activities against LPS-induced NO production in RAW264.7 macrophages.[16,17] The pseudoguaianolide-type sesquiterpenoids **6**, **13**, and **14** exhibited potent inhibitory activities against NO production, with IC_{50} values of 0.11, 0.25, and 0.10 μM, respectively, compared to that of aminoguanidine, an iNOS inhibitor with an IC_{50} value of 2.70 μM (Table 3). Furthermore, these compounds were not significantly cytotoxic at the concentrations required for inhibiting NO production (as determined by MTT assay). Accordingly, these findings suggest that pseudoguaiaonlides should be viewed as promising lead compounds for the development of novel anti-inflammatory agents.

Table 3. Inhibitory Effects of Sesquiterpenoids against LPS-Induced NO Production in RAW 264.7 Macrophages

compound	IC_{50} (μM)
1	12.89
2	26.18
3	13.10
4	13.32
5	5.29
6	0.11
7	22.79
8	28.70
9	2.95
10	35.60
11	14.36
12	9.15
13	0.25
14	0.10
AG	2.70

[a] Inhibitory effects against LPS-induced NO production in RAW 264.7 macrophages. AG: aminoguanidine.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer-341 digital polarimeter (Perkin-Elmer, Norwalk, CT). IR spectra were recorded on a Bruker-Vector-22 spectrometer using KBr pellets. ESIMS was performed using an Agilent-1100-LC/MSD-Trap (ESIMS) and an Agilent Micro-Q-Tof, whereas HRESIMS was performed using a Waters Q-TOF micro mass spectrometer. Column chromatography (CC) was performed using silica gel (SiO_2, 200−300 mesh, Qingdao Haiyang Chemical & Special Silica Gel Co, Ltd., Qingdao, P. R. China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). TLC was performed using precoated SiO_2 GF_{254} plates (Qingdao Haiyang Chemical & Special Silica Gel Co, Ltd., Qingdao, P. R. China), and compounds were visualized by exposure to UV light at 254 nm and spraying with a solution of vanillin in 5% H_2SO_4. Preparative HPLC using a Shimadzu PRC-ODS EV0233 and a C_{18}, 5 μm, 300 × 10 mm column and ^1H, ^{13}C, and 2D NMR spectra were obtained using a Bruker-DRX-500 spectrometer.

Plant Material. The aerial parts of *I. linearifolia* were collected from Changfeng County, Anhui Province, People's Republic of China, in late July 2007, and authenticated by Prof. Shou-Jin Liu of Anhui University of Traditional Chinese Medicine. A herbarium specimen (No. XX20070701) was deposited at the School of Pharmacy, Shanghai Jiao Tong University.

Extraction and Isolation. The air-dried and powdered aerial parts of *I. linearifolia* (60.0 kg) were extracted with 95% EtOH three times each for 24 h at room temperature. The extract was concentrated, and the residue was suspended in H_2O and then partitioned successively with petroleum ether (PE), $CHCl_3$, EtOAc, and *n*-BuOH. A portion of the PE extract (116.8 g from 1000.0 g) was subjected to silica gel column chromatography (100 mesh, 1000 g) and eluted with a PE−EtOAc (100:1 → 1:2) gradient to give 14 fractions (Fr.1−Fr.14) based on TLC analysis. Fr.5 and Fr.6 were chromatographed on silica gel using PE−EtOAc (20:1) as eluent to yield subfractions 5a−5e and 6a−6f, respectively. Subfractions 5e and 6c were further purified by preparative HPLC (MeOH−H_2O). Subfraction 5e gave **11** (t_R 32.3 min, 29.0 mg) (MeOH−H_2O, 40:60, flow rate 10 mL/min), whereas 6c yielded **3** (t_R 26.1 min, 2.0 mg), **4** (t_R 20.2 min, 4.8 mg), **5** (t_R 30.8 min, 4.8 mg), and **9** (t_R 35.2 min, 7.1 mg) (MeOH−H_2O, 35:65, flow rate 10 mL/min). Subfraction 6d was subjected to a silica gel column using PE−EtOAc (15:1) as eluent to yield **7** (6.6 mg). Compound **1** (31.0 mg) was crystallized (MeOH) from Fr.10, and **6** (330.2 mg) was crystallized (MeOH) from Fr.1. A portion of the $CHCl_3$ fraction (240.0 g from 600.0 g) was chromatographed over silica gel (100 mesh,

2000 g) and eluted with increasing amounts of MeOH (0−100%) in CH_2Cl_2 to afford 11 fractions (F1−F11). F1 and F2 were subjected to a Sephadex LH-20 column using MeOH as eluent to give subfractions 1A−1G and 2A−2J, respectively. Subfraction 1D was further separated into six fractions (1D1−1D6) using the same system. The subfractions 2C, 2D, 2E, 4D4, 1D4, and 1D6 were further separated by preparative HPLC (MeOH−H_2O). Subfractions 2C and 2E yielded **2** (t_R 25.6 min, 12.8 mg) and **13** (t_R 20.3 min, 10.2 mg) using MeOH−H2O as eluent at 35:65 and 45:55 (flow rate 10 mL/min), respectively. Subfraction 2D yielded **12** (t_R 34.0 min, 38.6 mg) and **8** (t_R 36.2 min, 2.8 mg) (MeOH−H_2O, 40:60), and subfraction 1D6 yielded **10** (t_R 28.1 min,7.3 mg) (MeOH−H_2O, 60:40). Subfraction 4D4 was further purified to give **14** (t_R 18.4 min, 4.0 mg) (MeOH−H_2O, 55:45).

Compound 1: monoclinic crystals (MeOH); $[\alpha]_D^{20}$ −16.2 (*c* 1.2, MeOH); IR (KBr) ν_{max} 3450, 3268, 1845, 1797, 1627, 1556, 1382, 1081, 944 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 523.3 $[2\ M + Na]^+$, *m/z* 499.3 $[2\ M - H]^-$; HRESIMS (positive ion) *m/z* 251.1651 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1647).

Compound 2: light yellow gum; $[\alpha]_D^{20}$ +46.7 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3427, 1738, 1456, 1100 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 345.1 $[M + Na]^+$, *m/z* 687.2 $[2\ M - H]^-$; HRESIMS (negative ion) *m/z* 321.1339 $[M - H]^-$ (calcd for $C_{17}H_{21}O_6$, 321.1339).

Compound 3: colorless gum; $[\alpha]_D^{20}$ −10.9 (*c* 0.3, MeOH); ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 287.2 $[M + Na]^+$, *m/z* 263.1 $[M - H]^-$; HRESIMS (positive ion) *m/z* 265.1445 $[M + H]^+$ (calcd for $C_{15}H_{21}O_4$, 265.1440).

Compound 4: light yellow oil; $[\alpha]_D^{20}$ −2.0 (*c* 0.5, MeOH); ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive ion) *m/z* 343.3 $[M + Na]^+$; HRESIMS (positive ion) *m/z* 343.1542 $[M + Na]^+$ (calcd for $C_{18}H_{24}O_5Na$, 343.1521).

Compound 5: light yellow oil; $[\alpha]_D^{20}$ +27.9 (*c* 0.2, MeOH); ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive ion) *m/z* 329.2 $[M + Na]^+$; HRESIMS (negative ion) *m/z* 305.1402 $[M - H]^-$ (calcd for $C_{15}H_{21}O_5$, 305.1389).

Compound 6: colorless, tetragonal crystals (MeOH); $[\alpha]_D^{20}$ −23.2 (*c* 1.0, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 389.2 $[M + Na]^+$, *m/z* 365.0 $[M - H]^-$; HRESIMS (positive ion) *m/z* 367.1767 $[M + H]^+$ (calcd for $C_{19}H_{27}O_7$, 367.1757).

Crystallographic data of compound **1**: $C_{15}H_{22}O_3$, M = 250.33, monoclinic, space group $P2(1)$, a = 7.6657(9) Å, α = 90°; b = 10.1466(12) Å, β = 111.211(2)°; c = 9.1327(11) Å, γ = 90°; V = 662.23(14) $Å^3$, Z = 2, D_{calc} = 1.255 mg/m^3, crystal size 0.425 × 0.330 × 0.278 mm^3. Mo Kα (λ = 0.71073 Å), $F(000)$ = 272, T = 293(2) K. The final R values were R_1 = 0.0361 and wR_2 = 0.0884 for 3988 observed reflections [$I > 2\sigma(I)$].

Crystallographic data of compound **6**: $C_{19}H_{26}O_7$, M = 366.43, tetragonal, space group $P4(3)2(1)2$, a = 9.3534(7) Å, α = 90°; b = 9.3534(7) Å, β = 90°; c = 42.209(5) Å, γ = 90°; V = 3692.7(6) $Å^3$, Z = 8, D_{calc} = 1.318 mg/m^3, crystal size 0.320 × 0.285 × 0.197 mm^3. Mo Kα (λ = 0.71073 Å), $F(000)$ = 1568, T = 293(2) K. The final R values were R_1 = 0.0458 and wR_2 = 0.1013 for 22 084 observed reflections [$I > 2\sigma(I)$].

Crystallographic data for **1** and **6** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 762277, 762278). Copies of the data may be obtained free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Preparation of (*R*)- and (*S*)-MTPA Esters of 1. The (*R*)- and (*S*)-MTPA esters of **1** were prepared using a modified Mosher method.[4,5] Briefly, **1** (3 mg) was treated with (*R*)- or (*S*)-MTPACl (each 5 μL) in pyridine-d_5 containing a small amount of DMAP in a clean NMR tube under an N_2 stream, and the NMR tube was immediately shaken to mix the sample and MTPACl. The tube was placed in a water bath at 50 °C for 4 h to afford the (*S*)-MTPA ester derivative (**1a**)/(*R*)-MTPA ester derivative (**1b**). The ^1H NMR data of the (*S*)-MTPA ester derivative (**1a**) and (*R*)-MTPA ester derivative (**1b**) were obtained directly from the reaction mixture (pyridine-d_5, 500 MHz).

Assay for Inhibitory Activities against LPS-Induced NO Production. This assay was conducted as previously described.[16,17] Briefly, RAW264.7 cells grown on a 100 mm culture dish were harvested and seeded in 96-well plates at 2 × 10^5 cells/well for NO production. The plates were pretreated with various concentrations of samples for 30 min and then incubated for 24 h with or without 1 μg/mL of LPS. The nitrite concentration in the culture supernatant was measured by the Griess reaction. Cell viability was measured by a MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Sigma-Aldrich).

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Supporting Information Available: 1D and 2D NMR spectra of compounds **1** to **6** and the X-ray crystallographic data (CIF files) of **1** and **6** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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