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Anti-inflammatory Sesquiterpene Derivatives from the Leaves of *Tripterygium wilfordii*

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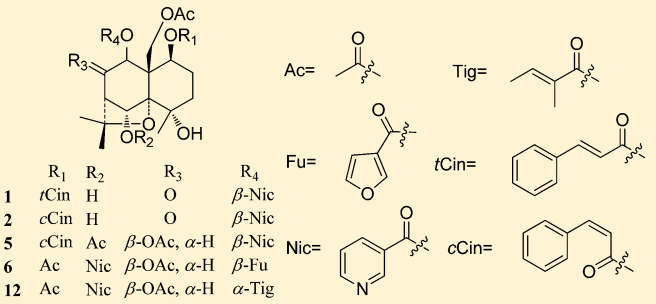
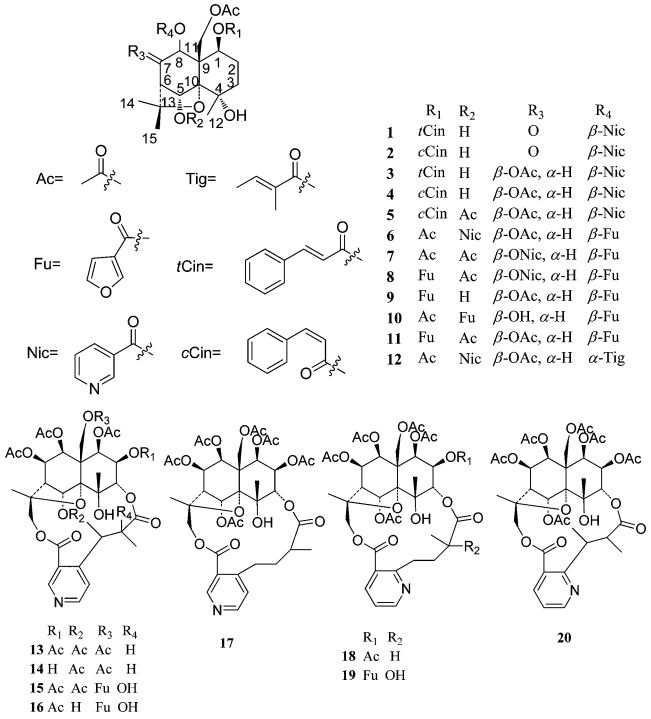
Supporting Information

ABSTRACT: Twelve new dihydroagarofuran sesquiterpene polyol esters, triptersinines A–L (**1**–**12**), and eight known sesquiterpene pyridine alkaloids were isolated from the leaves of *Tripterygium wilfordii*. Their structures were elucidated on the basis of spectroscopic analyses, including UV, IR, and NMR experiments (^{1}H–^{1}H COSY, NOESY, HSQC, and HMBC). Furthermore, in an in vitro bioassay, compounds **1**, **9**, **11**, **13**, **14**, and **18** showed moderate inhibitory effects on nitric oxide production in LPS-induced macrophages at 5 μM; all compounds were inactive when tested against five human cancer cell lines (IC_{50} values >1 μM).



	R_1	R_2	R_3	R_4
1	*t*Cin	H	O	*β*-Nic
2	*c*Cin	H	O	*β*-Nic
5	*c*Cin	Ac	*β*-OAc, *α*-H	*β*-Nic
6	Ac	Nic	*β*-OAc, *α*-H	*β*-Fu
12	Ac	Nic	*β*-OAc, *α*-H	*α*-Tig

Tripterygium wilfordii Hook. f. (Celastraceae) is a traditional Chinese medicine distributed in the southern part of China, the roots of which have been used to treat cancer and inflammation. Recently, a traditional Chinese medicine preparation derived from a water/chloroform extract of the roots of *T. wilfordii* (the so-called "total multi-glycoside") has been employed in the clinical treatment of rheumatoid arthritis, skin disorders, male-fertility control, and other inflammatory and autoimmune diseases.[1] On the basis of the significant pharmacological activities of *T. wilfordii*, many chemical investigations have been conducted to expound the bioactive substance. As a result, hundreds of compounds have been obtained from the roots of *T. wilfordii*, including sesquiterpenes, diterpenes, triterpenes, and lignans.[2] Compared with the investigations of the roots of *T. wilfordii*, few chemical studies have been conducted on the leaves, although the leaves exhibited marked anti-inflammatory activity.[3] It is known that the roots of *T. wilfordii* could be widely used in clinical practice after years of growth. So, the clinical use of the roots is restricted by the availability of *T. wilfordii*. On the contrary, the leaves of *T. wilfordii* could be collected every year without great damage to the plant. In order to promote the sustainable utilization of *T. wilfordii*, we performed extensive chemical and bioactive investigations on the ethanolic extract of the leaves. After the separation of the EtOAc extract of *T. wilfordii* by silica gel, MPLC, and preparative HPLC, 12 new dihydroagarofuran sesquiterpene polyol esters, triptersinines A–L (**1**–**12**), together with eight known sesquiterpene pyridine alkaloids (**13**–**20**) were obtained. Their structures were determined by spectroscopic analyses. The inhibitory effects on nitric oxide production in LPS-induced macrophages and cytotoxicity against five human cancer cell lines of **1**–**20** were also evaluated.



	R_1	R_2	R_3	R_4
1	*t*Cin	H	O	*β*-Nic
2	*c*Cin	H	O	*β*-Nic
3	*t*Cin	H	*β*-OAc, *α*-H	*β*-Nic
4	*c*Cin	H	*β*-OAc, *α*-H	*β*-Nic
5	*c*Cin	Ac	*β*-OAc, *α*-H	*β*-Nic
6	Ac	Nic	*β*-OAc, *α*-H	*β*-Fu
7	Ac	Ac	*β*-ONic, *α*-H	*β*-Fu
8	Fu	Ac	*β*-ONic, *α*-H	*β*-Fu
9	Fu	H	*β*-OAc, *α*-H	*β*-Fu
10	Ac	Fu	*β*-OH, *α*-H	*β*-Fu
11	Fu	Ac	*β*-OAc, *α*-H	*β*-Fu
12	Ac	Nic	*β*-OAc, *α*-H	*α*-Tig

	R_1	R_2	R_3	R_4
13	Ac	Ac	Ac	H
14	H	Ac	Ac	H
15	Ac	Ac	Fu	OH
16	Ac	H	Fu	OH

RESULTS AND DISSCUSSION

Compound **1** was obtained as a white, amorphous solid. Its molecular formula was established as $C_{32}H_{35}NO_{10}$ by HRESIMS (594.2329 $[M + H]^+$, calcd for 594.2334), implying

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16 degrees of unsaturation. The UV spectrum displayed the presence of an aromatic moiety (239, 272, 285 nm). The ^{1}H NMR spectroscopic data of **1** revealed the presence of an acetyl methyl group [δ_H 1.97 (3H, s)], a nicotinoyl group [δ_H 8.95 (1H, d, J = 1.8 Hz), 8.34 (1H, dd, J = 4.8, 1.8 Hz), 8.18 (1H, dt, J = 7.8, 1.8 Hz), 7.21 (1H, dd, J = 7.8, 4.8 Hz)], a *trans*-cinnamoyl group [δ_H 7.34 (1H, m), 7.27 (1H, d, J = 16.2 Hz), 7.26 (2H, m), 7.13 (2H, d, J = 7.2 Hz), 6.04 (1H, d, J = 16.2 Hz)], an oxygenated methylene [δ_H 5.38 (1H, d, J = 12.6 Hz), 4.42 (1H, d, J = 12.6 Hz)], three methyls [δ_H 1.72, 1.68, 1.59 (each 3H, s)], and three methine proton signals (δ_H 6.02, 5.69, 5.38). The ^{13}C NMR data confirmed the presence of the above moieties and exhibited one ketone carbon (δ_C 201.0). On the basis of the NMR data, the structure of compound **1** was established as a dihydroagarofuran sesquiterpene, attached with an acetyl, a nicotinoyl, and a *trans*-cinnamoyl group.[4] The ^{1}H−^{1}H COSY spectrum of **1** revealed a separated spin−spin system (H-1/H-2/H-3). The remaining dihydroagarofuran proton signal at δ_H 3.03 (H-6) was correlated with the carbon signals at δ_C 201.0 (C-7) and 82.3 (C-8), the proton signal at δ_H 6.02 (H-8) was correlated with the signals at δ_C 53.5 (C-9) and 61.2 (C-11), and the proton signal at δ_H 1.72 (H-12) was correlated with the signals at δ_C 36.2 (C-3) and 73.6 (C-4) in the HMBC spectrum. On the basis of the above information, the C-1, C-4, C-5, C-8, C-11 highly oxygenated 7-oxo-dihydroagarofuran was established.

In the HMBC spectrum of **1**, the correlations of H-1/C-9$_{t\text{-Cin}}$ (δ_C 167.1), H-8/C-7$_{Nic}$ (δ_C 165.1), and H-11/C-2$_{Ac}$ (δ_C 171.5) determined the attachments of acetyl, nicotinoyl, and *trans*-cinnamoyl groups. The NOESY spectrum of **1** exhibited the correlations of H-8/H-1, H-14, H-5/H-6, H-11, and H-12. Thus, the relative configurations of the ester groups were elucidated as 1β, 8β oriented. Therefore, compound **1** was determined as 1β-*trans*-cinnamoyl-4α,5α-dihydroxy-8β-nicotinoyl-11-acetoxy-7-oxo- dihydroagarofuran, named triptersinine A.

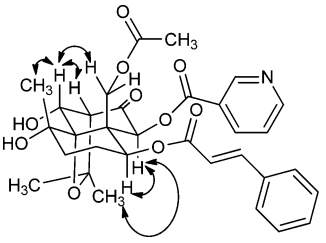

Figure 1. Key NOESY correlations of compound **1**.

The spectroscopic data revealed that **2** was also a dihydroagarofuran sesquiterpene attached with an acetyl, a nicotinoyl, and a *cis*-cinnamoyl group, similar to compound **1**. The *cis*-cinnamoyl group was deduced from the coupling constant between δ_H 6.54 (H-7$_{c\text{-Cin}}$) and δ_H 5.48 (H-8$_{c\text{-Cin}}$) (J = 12.6 Hz) in the ^{1}H NMR spectrum and confirmed by the correlation of δ_H 6.54/δ_H 5.48 in the NOESY spectrum. The *cis*-cinnamoyl group was assigned at position C-1, the nicotinoyl group was assigned at position C-8, and the acetyl group was assigned at position C-11 on the basis of correlations of H-1/C-9$_{c\text{-Cin}}$ (δ_C 166.0), H-8/C-7$_{Nic}$ (δ_C 165.1), and H-11/C-2$_{Ac}$ (δ_C 171.2) in the HMBC spectrum of **2**. The relative configuration of **2** was established by the NOESY correlations of H-8/H-1, H-14; H-5/H-6, H-11, H-12, the same as **1**. Thus, the structure of **2** was determined to be 1β- *cis*-cinnamoyl-4α,5α-dihydroxy-8β-nicotinoyl-11-acetoxy-7-oxo-dihydroagarofuran, named triptersinine B.

Compound **3** was obtained as a white, amorphous solid, and the HRESIMS displayed a quasi-molecular ion peak at m/z 638.2596 [M + H]$^+$ (calcd for 638.2594), corresponding to the molecular formula $C_{34}H_{39}NO_{11}$. The NMR data of **3** were similar to those of **1** and revealed a dihydroagarofuran sesquiterpene skeleton with two acetyl methyl groups, a nicotinoyl group, and a *trans*-cinnamoyl group. Compared with compound **1**, the carbon signal of a ketone was not observed in the ^{13}C NMR spectrum of **3**, and two separated spin−spin systems, (H-1/H-2/H-3) and (H-6/H-7/H-8), were observed in the ^{1}H−^{1}H COSY spectrum of **3**. From the above observations, a C-1, C-4, C-5, C-7, C-8, C-11 highly oxygenated dihydroagarofuran skeleton was established for compound **3**.

The attachments of these ester groups were determined by an HMBC experiment, showing long-range correlations of H-1/C-9$_{t\text{-Cin}}$ (δ_C 167.1), H-7/C-2$_{Ac}$ (δ_C 171.6), H-8/C-7$_{Nic}$ (δ_C 164.8), and H-11/C-2$_{Ac}$ (δ_C 171.9). The relative configuration of **3** was established on the basis of the NOESY correlations between H-8 and H-1, H-7, H-14 and between H-5 and H-6, H-11, H-12. Therefore, compound **3** was established as 1β-*trans*- cinnamoyl-4α,5α-dihydroxy-7β,11-diacetoxy-8β-nicotinoyldihydroagarofuran, named triptersinine C.

Analyses of the 1D and 2D NMR data of compound **4** indicated that **4** possessed the same skeleton and substitution mode as **3**, except for the cinnamoyl group. A *cis*-cinnamoyl group was deduced from the ^{1}H NMR data of **4** and confirmed by a NOESY correlation between δ_H 6.48 (H-7$_{c\text{-Cin}}$) and δ_H 5.32 (H-8$_{c\text{-Cin}}$). The positions of these ester groups were established by the HMBC correlations of H-1/C-9$_{c\text{-Cin}}$ (δ_C 166.3), H-7/C-2$_{Ac}$ (δ_C 171.5), H-8/C-7$_{Nic}$ (δ_C 164.9), and H-11/C-2$_{Ac}$ (δ_C 171.6), and the relative configuration of **4** was determined by the NOESY correlations between H-8 and H-1, H-7, H-14 and between H-5 and H-6, H-11, H-12. Therefore, compound **4** was determined as 1β-*cis*-cinnamoyl-4α,5α-dihydroxy-7β,11-diacetoxy-8β-nicotinoyldihydroagarofurn, named triptersinine D.

Compound **5**, obtained as a white, amorphous solid, had the molecular formula $C_{36}H_{41}NO_{12}$, deduced from the [M + Na]$^+$ ion peak at m/z 702.2521 in its HRESIMS. The NMR data of compound **5** indicated that it was similar to **4**, except that it contained one extra acetyl group. In the same manner as described above, the three acetyl groups were assigned to positions C-5, C-7, and C-11, and the *cis*-cinnamoyl and nicotinoyl groups were located at C-1 and C-8 by the HMBC correlations of H-5, H-7, H-11/δ_C 171.72 (Ac × 2), 171.66 (Ac); H-1/δ_C 166.3 (*cis*-Cin); and H-8/δ_C 164.8 (Nic). The relative configuration was determined on the basis of the NOESY correlations between H-8 and H-1, H-7, H-14 and between H-5 and H-6, H-11, H-12. Thus, compound **5** was elucidated as 1β-*cis*-cinnamoyl-4α-hydroxy-5α,7β,11-triacetoxy-8β-nicotinoyldihydroagarofuran, named triptersinine E.

Compound **6**, a white, amorphous solid, gave the molecular formula $C_{32}H_{37}NO_{13}$, as established by HRESIMS (m/z 644.2332 calcd for [M + H]$^+$ 644.2338). Analyses of the spectroscopic data revealed that compound **6** and the known compound 1α-nicotinoyloxy-2α-acetoxy-6β-acetoxy-9β-furoyloxy-11-acetoxy-4β-dihydroxydihydro-β-agarofuran[5] had the same molecular formula, dihydroagarofuran skeleton, and ester groups. Indeed, the differences between the two compounds were the positions of these ester groups. The long-range correlations of H-1/δ_C 171.4, H-5/δ_C 163.6, H-7/δ_C

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171.7, H-8/δ_C 164.9, and H-11/δ_C 171.9 in the HMBC spectrum indicated that three acetyl groups were located at C-1, C-7, and C-11, the nicotinoyl group was located at C-5, and the furanoyl group was located at C-8. The observed NOESY correlations of H-8/H-1, H-7, H-14 and H-5/H-6, H-11, H-12 determined the relative configuration of **6**. Therefore, compound **6** was elucidated as 1β,7β,11-triacetoxy-4α-hydroxy-5α-nicotinoyl-8β-furanoyldihydroagarofuran, named triptersinine F.

Compound **7** was determined to have the same molecular formula as **6** by the HRESIMS. The NMR data showed that **7** was similar to **6**, except for the locations of these ester groups. The long-range correlations of H-1/δ_C 170.1 (Ac), H-5/δ_C 170.0 (Ac), H-11/δ_C 170.7 (Ac), H-7/δ_C 164.4 (Nic), and H-8/δ_C 161.3 (Fu) in its HMBC spectrum determined the locations of the ester groups. The relative configuration of **7** was established by the NOESY correlations of H-8/H-1, H-7, H-14 and H-5/H-6, H-11, H-12. All these data established the structure of **7** as 1β,5α,11-triacetoxy-4α-hydroxy-7β-nicotinoyl-8β-furanoyldihydroagarofuran, named triptersinine G.

Compound **8** was isolated as a white, amorphous solid, and the molecular formula, $C_{35}H_{37}NO_{14}$, was calculated from the $[M + H]^+$ ion at m/z 696.2287 in its HRESIMS. The NMR data revealed that **8** was a dihydroagarofuran derivative, containing a nicotinoyl, two furanoyl, and two acetyl groups. Compound **8** was similar to compound **7**, except for the number and positions of furanoyl and acetyl groups. In its HMBC spectrum, the correlations of H-1/δ_C 163.5 (Fu), H-5/δ_C 171.9 (Ac), H-7/δ_C 165.8 (Nic), H-8/δ_C 162.7 (Fu), and H-11/δ_C 172.0 (Ac) confirmed the positions of these ester groups. The NOESY correlations of H-8/H-1, H-7, H-14 and H-5/H-6, H-11, H-12 established the relative configuration of **8**. Therefore, compound **8** was elucidated as 1β,8β-difuranoyl-4α-hydroxy-5α,11-diacetoxy-7β-nicotinoyldihydroagarofuran, named triptersinine H.

Compound **9** was obtained as a white, amorphous solid, and its molecular formula $C_{29}H_{34}O_{13}$ was established by the HRESIMS (608.2343, calcd for $[M + NH_4]^+$ 608.2338). The spectroscopic data revealed that **9** was a dihydroagarofuran sesquiterpene polyol ester. The ^{1}H NMR exhibited the presence of two acetyl groups and two furanoyl groups. The HMBC correlations of H-1/δ_C 163.6 (Fu), H-7/δ_C 171.5 (Ac), H-8/δ_C 162.7 (Fu), and H-11/δ_C 171.9 (Ac) determined the locations of these ester groups. The relative configuration of **9** was established by the NOESY correlations of H-8/H-1, H-7, H-14 and H-5/H-6, H-11, H-12. Thus, the structure of compound **9** was elucidated as 1β,8β-difuranoyl-4α,5α-dihydroxy-7β,11-diacetoxydihydroagarofuran, named triptersinine I.

Compound **10**, with the molecular formula $C_{29}H_{34}O_{13}$ (HRESIMS), was shown to be a dihydroagarofuran sesquiterpene with two acetyl and two furanoyl groups, which was similar to **9** in a study of its IR, UV, and NMR experiments, except for the locations of these ester groups. The HMBC correlations between H-1 and δ_C 169.9 (Ac), H-5 and δ_C 162.0 (Fu), H-8 and δ_C 161.4 (Fu), and H-11 and δ_C 170.5 (Ac) established the regiosubstitution partners, and the NOESY correlations of H-8/H-1, H-7, H-14 and H-5/H-6, H-11, H-12 determined the relative configuration of **10**. Therefore, compound **10** was elucidated as 1β,11β-diacetoxy-4α,7β-dihydroxy-5α,8β-difuranoyldihydroagarofuran, named triptersinine J.

Compound **11** showed the molecular formula $C_{31}H_{36}O_{14}$ by HRESIMS. The NMR data revealed that **11** possessed the same skeleton and substitution mode as compound **9**, except that **11** contained one extra acetyl group. The furanoyl groups were assigned at C-1 and C-8, and three acetyl groups were assigned at C-5, C-7, and C-11 by the corresponding HMBC correlations of H-1/δ_C 163.5, H-8/δ_C 162.7, H-5/δ_C 171.8, H-7/δ_C 171.6, and H-11/δ_C 172.1. The relative configuration was established by the NOESY correlations between H-8 and H-1, H-7, H-14 and between H-5 and H-6, H-11, H-12. Thus, the structure of **11** was determined as 1β,8β-difuranoyl-4α-hydroxy-5α,7β,11-triacetoxydihydroagarofuran, named triptersinine K.

Compound **12**, a white, amorphous solid, had the molecular formula $C_{32}H_{41}NO_{12}$, calculated from the $[M + H]^+$ ion at m/z 632.2702 in its HRESIMS. A dihydroagarofuran sesquiterpene skeleton was deduced from the ^{1}H, ^{13}C, and 2D NMR spectra of **12**. The ^{1}H NMR data revealed the presence of a nicotinoyl group, three acetyl groups, and a tigloyl group [δ_H 6.92 (1H, q, J = 6.4 Hz), 1.83 (3H, s), 1.82 (3H, d, J = 6.4 Hz)]. The characteristic ^{13}C NMR signals of the nicotinoyl and tigloyl groups were observed and further assigned by a combination of HSQC and HMBC experiments. On the basis of HMBC correlations of H-1/δ_C 169.9 (Ac), H-5/δ_C 165.5 (Nic), H-7/δ_C 169.7 (Ac), H-8/δ_C 165.8 (Tig), and H-11/δ_C 170.6 (Ac), the positions of acyl groups were assigned. The NOESY correlations of H-5/H-6, H-11, H-12; H-6/H-15; H-7/H-14, and H-8/H-11 determined the relative configuration of **12**. Consequently, the structure of **12** was elucidated as 1β,7β,11-triacetoxy-4α-hydroxy-5α-nicotinoyl-8α-tiglyloxydihydroagarofuran, named triptersinine L.

The eight known compounds were identified as peritassine A (**13**),[6] wilfordinine A (**14**),[7] hypoglaunine A (**15**),[8] hypoglaunine E (**16**),[9] wilfordinine E (**17**),[6] euonine (**18**),[6] wilfortrine (**19**),[10] and euonymine (**20**)[11] from their spectroscopic data upon comparisons with values reported in the literature.

The inhibitory effects on nitric oxide production in LPS-induced macrophages of compounds **1**−**20** were evaluated. As shown in Table 3, compounds **1**, **9**, **11**, **13**, **14**, and **18** showed moderate inhibitory abilities on NO production and no influence on cell viability by the MTT method; the other compounds exhibited weak effects. All compounds were inactive when tested against five human cancer cell lines (IC_{50} values >1 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P2000 automatic digital polarimeter. UV spectra were recorded on a JASCO V-650 spectrophotometer, and IR spectra were recorded on a Nicolet 5700 spectrometer using a FT-IR microscope transmission method. NMR spectra were acquired with VNS-600 and Mercury-400 spectrometers. HRESIMS spectra were collected on an Agilent 1100 series LC/MSD ion trap mass spectrometer. Preparative HPLC was conducted using a Shimadzu LC-6AD instrument with an SPD-20A detector and a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Column chromatography was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and ODS (50 μm, YMC, Japan). TLC was carried out on glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 10% sulfuric acid in EtOH followed by heating.

Plant Material. The leaves of *T. wilfordii* were collected in Taining, Fujian, China, in September 2009 and identified by Professor Lin Ma

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1–12[a]

position	1	2	3	4	5	6	7	8	9	10	11	12
	δ_H[b] (J in Hz)	δ_H[b] (J in Hz)	δ_H[b] (J in Hz)	δ_H[b] (J in Hz)	δ_H[b] (J in Hz)	δ_H[b] (J in Hz)	δ_H[c] (J in Hz)	δ_H[b] (J in Hz)	δ_H[b] (J in Hz)	δ_H[c] (J in Hz)	δ_H[b] (J in Hz)	δ_H[c] (J in Hz)
1	5.69 dd (12.0, 4.8)	5.58 dd (12.6, 4.8)	5.53 dd (12.0, 4.8)	5.43 dd (11.4, 4.2)	5.40 m	5.37 dd (11.4, 4.8)	5.35 dd (12.0, 4.2)	5.62 dd (12.0, 4.8)	5.58 dd (12.0, 4.8)	5.31 dd (12.0, 4.8)	5.57 dd (12.0, 4.8)	5.33 dd (12.4, 4.8)
2	1.81 m	1.75 m	1.96 m	1.80 m	1.78 m	1.79 m	1.85 m	1.95 m	1.94 m	1.82 m	1.90 m	2.03 m
	1.75	1.61 m	1.81 m	1.78 m	1.73 m	1.75 m	1.69 m	1.81 m	1.82 m	1.55 m	1.72 m	1.60 m
3	2.15 m	2.10 m	2.17 m	2.11 m	2.07 m	2.06 m	1.98 m	2.14 m	2.16 m	1.92 m	2.10 m	1.96 m
	1.77 m	1.73 m	1.67 m	1.62 m	1.69 m	1.72 m	1.72 m	1.73 m	1.65 m	1.74 m	1.78 m	1.76 m
5	5.38 brs	5.33 s	5.46 brs	5.44 s	6.79 s	6.97 s	6.81 s	6.90 s	5.45 s	6.90 s	6.82 s	6.66 s
6	3.03 brs	3.00 s	2.36 d (4.2)	2.34 d (4.2)	2.40 d (4.2)	2.52 d (4.2)	2.56 d (3.0)	2.64 d (3.6)	2.32 d (4.2)	2.49 d (4.2)	2.38 d (4.2)	2.48 d (3.2)
7			5.61 dd (5.4, 4.2)	5.59 dd (5.4, 4.2)	5.57 dd (5.4, 4.2)	5.64 dd (6.0, 4.2)	5.75 m	5.77 dd (5.4, 3.6)	5.56 dd (6.0, 4.2)	4.40 dd (5.4, 4.2)	5.54 dd (6.0, 4.2)	5.22 d (3.2)
8	6.02 s	5.99 s	5.77 d (5.4)	5.74 d (5.4)	5.73 d (5.4)	5.75 d (6.0)	5.72 d (5.4)	5.78 d (5.4)	5.64 d (6.0)	5.51 d (5.4)	5.63 d (6.0)	5.43 s
11	5.38 d (12.6)	5.03 d (13.2)	5.13 d (13.8)	4.83 d (13.2)	4.88 d (12.6)	5.21 d (13.8)	4.79 d (13.2)	4.98 d (13.2)	5.07 d (13.2)	5.11 d (13.2)	5.24 d (13.2)	4.67 d (12.8)
	4.42 d (12.6)	4.26 d (13.2)	4.86 d (13.8)	4.50 d (13.2)	4.33 d (12.6)	4.57 d (13.8)	4.76 d (13.2)	4.85 d (13.2)	4.75 d (13.2)	4.40 d (13.2)	4.59 d (13.2)	4.58 d (12.8)
12	1.72 s	1.662 s	1.70 s	1.64 s	1.39 s	1.40 s	1.36 s	1.44 s	1.68 s	1.37 s	1.44 s	1.30 s
14	1.59 s	1.59 s	1.64 s	1.63 s	1.63 s	1.65 s	1.68 s	1.68 s	1.60 s	1.573 s[d]	1.61 s	1.62 s
15	1.68 s	1.659 s	1.60 s	1.58 s	1.57 s	1.60 s	1.58 s	1.61 s	1.58 s	1.570 s[d]	1.57 s	1.54 s

[a]For signals of other ester groups see Experimental Section. [b]In methanol-d_4 (600 MHz). [c]In chloroform-d_1 (600 MHz for **7**, **10**; 400 MHz for **12**). [d]Signals may be reversed in the same column.

from the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (No. 20090034) is deposited at the herbarium of the Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

Extraction and Isolation. Air-dried leaves of *T. wilfordii* (50 kg) were extracted with 80% ethanol (400 L × 2h × 3). After evaporation of EtOH in vacuo, the aqueous residue was diluted with water and then partitioned with EtOAc (30 L × 3). The EtOAc extract (4000 g) was subjected to passage over polyamide by elution with water and 30%, 60%, and 95% EtOH–water in sequence to give fractions A_1 (478 g), A_2 (743 g), A_3 (828 g), and A_4 (1000 g). Fraction A_1 (478 g) was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (1:0–10:1) to afford 10 fractions (B_1–B_{10}). Fraction B_7 (52 g) was separated by a silica gel column (200–300 mesh) eluted with $CHCl_3$–MeOH (80:1–10:1) to afford 43 fractions (F_1–F_{43}). Subfraction F_{11} (3.5 g) was passed over an RP-18 column with MeOH–water (20–80%) and finally purified by preparative HPLC (detected at 210 nm, 8 mL/min) to give **1** (4 mg), **2** (6.2 mg), **3** (5.7 mg), **4** (6 mg), **5** (8 mg), **6** (14 mg), 7 (7 mg), **8** (9 mg), **9** (9 mg), **10** (7 mg), **11** (4 mg), **12** (6 mg), **13** (6.5 mg), **14** (9 mg), **15** (10 mg), **16** (8 mg), **17** (6 mg), **18** (5 mg), **19** (9 mg), and **20** (11 mg).

Triptersinine A (**1**)*:* white, amorphous solid; $[\alpha]_D^{25}$ −8.2 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (3.55), 272 (3.77), 285 (3.80) nm; IR (microscope) ν_{max} 3398, 2955, 2924, 2852, 1621, 1422, 1124, 1049 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1 and δ_H 8.95 (1H, d, J = 1.8 Hz), 8.34 (1H, dd, J = 4.8, 1.8 Hz), 8.18 (1H, dt, J = 7.8, 1.8 Hz), 7.21 (1H, dd, J = 7.8, 4.8 Hz) (8-Nic), 7.34 (1H, m), 7.27 (1H, d, J = 16.2 Hz), 7.26 (2H, m), 7.13 (2H, d, J = 7.2 Hz), 6.04 (1H, d, J = 16.2 Hz) (1-*trans*-Cin), 1.97 (3H, s) (11-Ac); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2 and δ_C 167.1, 147.1, 134.9, 131.7, 129.8, 129.2, 118.0 (1-*trans*-Cin), 165.1, 153.9, 151.2, 139.1, 126.9, 125.2 (8-Nic), 171.5, 20.7(11-Ac); HRESIMS m/z 594.2334 (calcd for $C_{32}H_{36}NO_{10}$, 594.2329).

Triptersinine B (**2**)*:* white, amorphous solid; $[\alpha]_D^{25}$ −62.2 (*c* 0.11 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (3.59), 272 (3.74), 285 (3.70) nm; IR (microscope) ν_{max} 3392, 2954, 2920, 2851, 1730, 1621, 1423, 1124 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1 and δ_H 8.92 (1H, d, J = 1.8 Hz), 8.72 (1H, dd, J = 4.8, 1.2 Hz), 8.18 (1H, dt, J = 7.8, 1.8 Hz), 7.47 (1H, dd, J = 7.8, 4.8 Hz) (8-Nic), 7.38 (2H, m), 7.30–7.32 (3H, m), 6.54 (1H, d, J = 12.6 Hz), 5.48 (1H, d, J = 12.6 Hz) (1-*cis*-Cin), 1.92 (3H, s) (11-Ac); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2 and δ_C 166.0, 146.1, 135.7, 131.1, 130.5, 129.0, 119.3 (1-*cis*-Cin), 165.1, 154.4, 151.5, 139.2, 126.9, 125.1 (8-Nic), 171.2, 20.6 (11-Ac); HRESIMS m/z 594.2334 (calcd for $C_{32}H_{36}NO_{10}$, 594.2327).

Triptersinine C (**3**)*:* white, amorphous solid; $[\alpha]_D^{25}$ −27.6 (*c* 0.08 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 238 (3.71), 272 (3.95), 285 (4.97) nm; IR (microscope) ν_{max} 3309, 2955, 2917, 2851, 1733, 1463, 1376, 1286, 1233 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1 and δ_H 9.04 (1H, dd, J = 1.8, 1.2 Hz), 8.36 (1H, dd, J = 4.8, 1.8 Hz), 8.24 (1H, dt, J = 7.8, 1.8 Hz), 7.21 (1H, dd, J = 7.8, 4.8 Hz) (8-Nic), 7.33 (1H, m), 7.25 (2H, m), 7.19 (1H, d, J = 16.2 Hz), 7.07 (2H, brd, J = 7.8 Hz), 5.91 (1H, d, J = 16.2 Hz) (1-*trans*-Cin), 2.02 (3H, s) (7-Ac), 2.27 (3H, s) (11-Ac); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2 and δ_C 167.1, 146.5, 134.9, 131.6, 127.2, 125.2, 118.1 (1-*trans*-Cin), 164.8, 153.9, 151.2, 139.0, 127.2, 125.2 (8-Nic), 171.6, 20.9 (7-Ac), 171.9, 21.8 (11-Ac); HRESIMS m/z 638.2596 (calcd for $C_{34}H_{40}NO_{11}$, 638.2594).

Triptersinine D (**4**)*:* white, amorphous solid; $[\alpha]_D^{25}$ −82.0 (*c* 0.1 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 238 (3.57), 271 (3.75) nm; IR (microscope) ν_{max} 3314, 2956, 2921, 2851, 1733, 1461, 1377, 1285 1233, 1161, 1135 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1 and δ_H 9.03 (1H, d, J = 1.8 Hz), 8.73 (1H, dd, J = 4.8, 1.8 Hz), 8.29 (1H, dt, J = 7.8, 1.8 Hz), 7.51 (1H, dd, J = 7.8, 4.8 Hz) (8-Nic), 7.30–7.35 (5H, m), 6.48 (1H, d, J = 12.6 Hz), 5.32 (1H, d, J = 12.6 Hz) (1-*cis*-Cin), 2.00 (3H, s) (7-Ac), 2.20 (3H, s) (11-Ac); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2 and δ_C 166.3, 145.3, 135.8, 131.0, 130.4, 129.1, 119.7 (1-*cis*-Cin), 164.9, 154.3, 151.5, 139.3, 127.3, 125.2

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Table 2. ^{13}C NMR Spectroscopic Data (δ) of Compounds 1−12 (125 MHz)[a]

	1	2	3	4	5	6	7	8	9	10	11	12
position	δ_C[b]	δ_C[b]	δ_C[b]	δ_C[b]	δ_C[b]	δ_C[b]	δ_C[c]	δ_C[b]	δ_C[b]	δ_C[c]	δ_C[b]	δ_C[c]
1	77.2	77.2	79.7	79.6	79.5	79.9	78.3	79.3	79.6	77.7	79.3	72.6
2	26.2	25.9	26.2	25.9	25.8	25.9	25.1	26.3	26.2	24.8	26.2	23.9
3	36.2	36.1	36.1	36.0	38.2	38.8	37.9	38.2	36.0	38.3	38.2	38.5
4	73.6	73.5	73.9	73.8	71.8	71.8	70.7	72.0	73.9	70.3	71.9	70.7
5	77.3	77.3	75.8	75.8	76.5	76.4	75.3	76.7	75.9	74.2	76.7	76.6
6	68.4	68.4	55.5	55.6	54.4	54.5	53.5	54.6	55.5	55.3	54.4	53.4
7	201.0	201.0	71.5	71.4	71.3	71.3	72.1	73.1	71.4	68.8	71.2	76.7
8	82.3	82.2	75.5	75.3	74.8	74.8	72.2	73.8	74.0	74.5	73.6	73.1
9	53.5	53.2	53.1	52.9	53.7	53.7	52.3	54.0	53.0	52.7	54.0	53.7
10	94.1	94.0	92.4	92.3	93.0	93.1	91.9	92.9	92.4	91.9	93.1	91.8
11	61.2	61.2	61.4	61.2	60.8	60.9	60.9	61.8	61.4	60.1	61.0	65.3
12	26.0	26.1	24.5	24.5	23.3	23.1	22.7	23.1	24.4	22.7	23.2	22.9
13	85.6	85.5	83.8	83.7	83.8	83.8	82.6	83.9	83.7	82.5	83.8	83.2
14	25.3	25.3	24.8	24.9	29.5	24.3	24.5	24.3	24.8	24.2	24.3	25.6
15	29.8	29.8	29.9	29.9	24.3	29.5	29.5	29.4	29.9	29.5	29.5	29.7

[a]For signals of other ester groups see Experimental Section. [b]In methanol-d_4. [c]In chloroform-d_1.

Table 3. Inhibitory Effects of Compounds against LPS-Induced NO Production in RAW264.7 Macrophage Cells[a]

compound	concentration (μM)	inhibition (%)
dexamethasone[b]	5	70.4**
1	5	47.3*
9	5	45.5**
11	5	49.9*
13	5	48.2*
14	5	59.0**
18	5	54.7**

[a]The results are averages of three independent experiments; *$p < 0.05$, **$p < 0.01$, significantly different from control by Student's t-test. [b]Positive control.

(8-Nic), 171.5, 20.9 (7-Ac), 171.6, 21.7 (11-Ac); HRESIMS m/z 638.2596 (calcd for $C_{34}H_{40}NO_{11}$, 638.2592).

*Triptersinine E (**5**):* white, amorphous solid; $[\alpha]_D^{25}$ −95.8 (c 0.1$CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 238 (3.83), 272 (3.97), 284 (3.93) nm; IR (microscope) ν_{max} 3425, 2956, 2924, 2851, 1733, 1461, 1377, 1234, 1159 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1 and δ_H 9.04 (1H, brs), 8.76 (1H, d, J = 4.8 Hz), 8.31 (1H, dt, J = 7.8, 1.8 Hz), 7.54 (1H, dd, J = 7.8, 4.8 Hz) (8-Nic), 7.30−7.34 (5H, m), 6.49 (1H, d, J = 12.6 Hz), 5.34 (1H, d, J = 12.6 Hz) (1-*cis*-Cin), 2.27, 2.13, 2.02 (each 3H, s) (5, 7, 11-Ac); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2 and δ_H 166.3, 145.4, 135.9, 130.8, 130.3, 129.1, 119.7 (1-*cis*-Cin), 164.8, 154.4, 151.5, 139.3, 127.2, 125.3 (8-Nic), 171.72, 171.72, 171.66, 21.7, 21.4, 21.0 (5, 7, 11-Ac); HRESIMS m/z 702.2521 (calcd for $C_{36}H_{41}NO_{12}Na$, 702.2523).

*Triptersinine F (**6**):* white, amorphous solid; $[\alpha]_D^{25}$ −26.8 (c 0.1 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.67) nm; IR (microscope) ν_{max} 3552, 3378, 2954, 2923, 2852, 1740, 1370, 1311, 1284, 1228, 1159, 1140, 1092, 1034 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1 and δ_H 9.23 (1H, brs), 8.80 (1H, d, J = 3.6 Hz), 8.49 (1H, dt, J = 7.8, 1.8 Hz), 7.62 (1H, dd, J = 7.8, 3.6 Hz) (5-Nic), 8.25 (1H, brs), 7.61 (1H, m), 6.85 (1H, d, J = 1.8 Hz) (8-Fu), 1.50 (3H, s) (1-Ac), 2.08 (3H, s) (7-Ac), 2.33(3H, s) (11-Ac); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2 and δ_C 163.6, 154.8, 151.4, 139.1, 127.2, 125.5 (5-Nic), 164.9, 150.5, 145.8, 120.6, 110.7 (8-Fu), 171.4, 20.9 (1-Ac), 171.7, 21.0 (7-Ac), 171.9, 21.7 (11-Ac); HRESIMS m/z 644.2332 (calcd for $C_{32}H_{38}NO_{13}$, 644.2338).

*Triptersinine G (**7**):* white, amorphous solid; $[\alpha]_D^{25}$ −34.9 (c 0.11 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.67) nm; IR (microscope) ν_{max} 3452, 2955, 2920, 2851, 1739, 1371, 1278, 1230, 1139 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1 and δ_H 9.27 (1H, s), 8.83 (1H, s), 8.38 (1H, d, J = 7.2 Hz), 7.48 (1H, brs) (7-Nic), 8.00 (1H, s), 7.40 (1H, s), 6.67 (1H, s) (8-Fu), 2.14 (3H, s) (1-Ac), 1.64 (3H, s) (5-Ac), 2.03(3H, s) (11-Ac); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2 and δ_C 164.4, 153.0, 150.3, 138.2, 126.5, 124.0 (7-Nic), 161.3, 148.4, 144.4, 118.7, 109.6 (8-Fu), 170.1, 21.7 (1-Ac), 170.0, 20.9 (5-Ac), 170.7, 21.5 (11-Ac); HRESIMS m/z 644.2338 (calcd for $C_{32}H_{38}NO_{13}$, 644.2348).

*Triptersinine H (**8**):* white, amorphous solid; $[\alpha]_D^{25}$ −66.3 (c 0.12 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 242 (3.77) nm; IR (microscope) ν_{max} 3569, 3156, 2951, 2920, 2851, 1740, 1574, 1371, 1302, 1236, 1164, 1139 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1 and δ_H 9.18 (1H, s), 8.78 (1H, d, J = 4.8 Hz), 8.37 (1H, dt, J = 7.8, 1.8 Hz), 7.58 (1H, dd, J = 7.8, 4.8 Hz) (7-Nic), 7.83 (1H, brs), 7.42 (1H, t, J = 1.8 Hz), 6.41 (1H, d, J = 1.8 Hz) (1-Fu), 7.73 (1H, brs), 7.30 (1H, t, J = 1.8 Hz), 6.43 (1H, d, J = 1.8 Hz) (8-Fu), 2.17 (3H, s) (5-Ac); 1.99 (3H, s) (11-Ac); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2 and δ_C 163.5, 149.6, 145.5, 120.0, 110.2 (1-Fu), 165.8, 154.4, 151.4, 139.2, 125.3 (7-Nic), 162.7, 149.5, 145.1, 119.9, 110.1 (8-Fu), 171.9, 21.4, (5-Ac), 172.0, 21.6 (11-Ac); HRESIMS m/z 696.2287 (calcd for $C_{35}H_{38}NO_{14}$, 696.2287).

*Triptersinine I (**9**):* white, amorphous solid; $[\alpha]_D^{25}$ −46.6 (c 0.1 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 242 (3.70) nm; IR (microscope) ν_{max} 3392, 2954, 2924, 2852, 1733, 1371, 1310, 1240, 1165, 1135 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1 and δ_H 7.96 (1H, d, J = 1.2 Hz), 7.44 (1H, t, J = 1.8 Hz), 6.50 (1H, dd, J = 1.8, 1.2 Hz) (1-Fu), 7.73 (1H, d, J = 1.2 Hz), 7.30 (1H, t, J = 1.8 Hz), 6.43 (1H, dd, J = 1.8, 1.2 Hz) (8-Fu), 2.02 (3H, s) (7-Ac), 2.24 (3H, s) (11-Ac); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2 and δ_C 163.6, 149.7, 145.4, 120.0, 110.2 (1-Fu), 162.7, 149.4, 145.1, 119.9, 110.2 (8-Fu), 171.5, 20.9 (7-Ac), 171.9, 21.8 (11-Ac); HRESIMS m/z 613.1892 (calcd for $C_{29}H_{34}NaO_{13}$, 613.1889).

*Triptersinine J (**10**):* white, amorphous solid; $[\alpha]_D^{25}$ −27.9 (c 0.12 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 244 (3.40) nm; IR (microscope) ν_{max} 3429, 2955, 2919, 2851, 1731, 1370, 1312, 1228, 1161, 1085, 1062 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1 and δ_H 8.22 (1H, d, J = 0.6 Hz), 7.47 (1H, t, J = 1.8 Hz), 6.81 (1H, brs) (8-Fu), 8.13 (1H, d, J = 0.6 Hz), 7.44 (1H, t, J = 1.8 Hz), 6.81 (1H, brs) (5-Fu), 2.33 (3H, s) (11-Ac), 1.65 (3H, s) (1-Ac); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2 and δ_C 162.0, 148.7, 143.9, 119.4, 109.6 (5-Fu), 161.4, 148.8, 144.3, 118.7, 109.8 (8-Fu), 170.5, 21.3 (11-Ac), 169.9, 20.8 (1-Ac); HRESIMS m/z 613.1897 (calcd for $C_{29}H_{34}NaO_{13}$, 613.1897).

*Triptersinine K (**11**):* white, amorphous solid; $[\alpha]_D^{25}$ −31.2 (c 0.14 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.46) nm; IR (microscope) ν_{max} 3399, 2957, 2923, 2851, 1736, 1579, 1460, 1376, 1311, 1240, 1164 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1 and δ_H 7.99 (1H, brs), 7.45 (1H, t, J = 1.8 Hz), 6.51 (1H, d, J = 1.8 Hz) (1-Fu),

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7.75 (1H, brs), 7.31 (1H, t, J = 1.8 Hz), 6.44 (1H, d, J = 1.8 Hz) (8-Fu), 2.14 (3H, s) (5-Ac), 2.03 (3H, s) (7-Ac), 2.30 (3H, s) (11-Ac); ^{13}C NMR (CD_3OD, 125 MHz), see Table 2 and δ_C 163.5, 149.5, 145.1, 119.8, 110.1 (1-Fu), 162.7, 149.8, 145.5, 120.0, 110.1 (8-Fu), 171.8, 21.4 (5-Ac), 171.6, 21.0 (7-Ac), 172.1, 21.8 (11-Ac); HRESIMS m/z 655.1994 (calcd for $C_{31}H_{36}NaO_{14}$, 655.1997).

Triptersinine L (12): white, amorphous solid; $[\alpha]_D^{25}$ −30.6 (c 0.13 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243 (2.75), 258 (2.81), 264 (2.81) nm; IR (microscope) ν_{max} 3298, 2955, 2920, 2851, 1738, 1464, 1376, 1233, 1126, 1029 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1 and δ_H 9.38 (1H, brs), 8.88 (1H, brs), 8.53 (1H, d, J = 7.2 Hz), 7.51(1H, d, J = 7.2 Hz) (5-Nic), 6.92 (1H, q, J = 6.4 Hz), 1.83 (3H, s), 1.82 (3H, d, J = 6.4 Hz) (8-Tig), 1.79 (3H, s) (1-Ac), 2.24 (3H, s) (7-Ac), 2.29 (3H, s) (11-Ac); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2 and δ_C 165.5, 153.2, 151.2, 139.0, 126.1, 124.1 (5-Nic), 165.8, 140.8, 127.3, 14.8, 12.0 (8-Tig), 169.9, 21.1 (1-Ac), 169.7, 21.4 (7-Ac), 170.6, 21.5 (11-Ac); HRESIMS m/z 632.2702 (calcd for $C_{32}H_{42}NO_{12}$, 632.2705).

Inhibitory Effects on Nitric Oxide Production in LPS-Activated Macrophages. Compounds **1**−**20** were tested for their ability to inhibit LPS-activated nitric oxide production in RAW 264.7 macrophages. This assay was carried out as previously described.[12]

Cytotoxicty Assay. Compounds **1**−**20** were tested for cytotoxicity against A549 (human lung carcinoma), Bel-7402 (human liver carcinoma), BGC-823 (human stomach carcinoma), HCT-8 (human colon carcinoma), and A2780 (human ovarian carcinoma) cell lines by means of the MTT method as described in the literature.[13]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of spectra of compounds **1**−**12**. 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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