Article

**Lignan Glycosides from *Urena lobata***

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**Abstract:** Four new lignan glycosides; urenalignosides A–D (1–4), along with 12 known ones (5–16) were isolated from *Urena lobata*. Their structures were determined on the basis of extensive spectroscopic and spectrometric data (1D and 2D NMR; IR; CD; and HRESIMS). Compounds 2–4; 6; 7; 10; and 11 showed inhibition of nitric oxide production in lipopolysaccharide-induced RAW 264.7 macrophage cells with IC₅₀ values in the range of 25.5–98.4 µM (positive control; quercetin; IC₅₀ = 7.2 ± 0.2 µM).

**Keywords:** *Urena lobata* L.; lignan glycosides; urenalignosides A–D; nitric oxide production

1. Introduction

*Urena lobata*, belonging to the family Malvaceae, is an annually shrubby herbage widely distributed around the world, particularly in the tropical and subtropical areas of Asia, South America, and Africa [1]. This plant is also known as Caesar weed, Congo jute, and Bachita, the local name varies from region to region. In Africa, the leaves and flowers of *U. lobata* could be eaten as food during famine time and the bast fiber of *U. lobata* is used as cordage material [2]. More interestingly, *U. lobata* is also commonly used in folk medicines for the treatment of diabetes, abdominal colic, malaria, gonorrhea, dysentery, fever, rheumatism, and edema [3,4]. Pharmacological studies indicated that the extract of *U. lobata* showed significant antibacterial, antihyperglycemic, antinociceptive, antidiarrheal, anti-inflammatory, and wound healing activities [5–7]. In China, *U. lobata* is also named “Ditaohua,” which is dominantly distributed in the south of China, such as Guangxi, Yunnan, and Guizhou provinces and clinically used to treat pathological leucorrhea and gonorrhea [8]. Promoted by these significant activities, great efforts have been made to clarify the bioactive constituents of *U. lobata* leading to the separation and elucidation of flavonoids, phenylethyl glycosides, lignans, coumarins, and triglycerides [1,9–13]. In our previous report, 16 megastigmane glycosides were identified from *U. lobata* [14]. As an ongoing study, four new lignan glycosides, urenalignosides A–D (1–4) together with 12 known ones (5–16) were obtained from *U. lobata* (Figure 1). Herein, the isolation and structural elucidation of the new compounds, as well as their inhibitory effects on NO production on LPS-stimulated RAW264.7 macrophage cells, are described.

2. Results

The 95% EtOH extracts of *U. lobata* were suspended in H₂O and extracted successively with petroleum ether (PE), EtOAc, and n-BuOH. The n-BuOH soluble fraction was separated by D₁₀ macroporous adsorption resin, silica gel, and Sephadex LH-20 column chromatography and semi-preparative HPLC to afford four new lignan glycosides (1–4) together with 12 known ones (5–16) (Figure 1).
Compound 1 was obtained as a colorless powder. Its molecular formula was assigned as C_{30}H_{40}O_{15} due to the presence of a [M − H]^- ion at m/z 639.2282 (calcld for C_{30}H_{39}O_{15}, 639.2294) in the HRESIMS spectrum (Figure S1), which was also supported by the $^{13}$C-NMR data (Table 1). The IR spectrum of 1 showed the absorption bands contributing to hydroxy group (3385 cm$^{-1}$), benzene ring (1615 and 1518 cm$^{-1}$), and ester carbonyl (1735 cm$^{-1}$) group. The NMR spectra of 1 (Figures S2 and S3) showed the presence of two 1,3,4,5-tetrasubstituted benzene moieties [$\delta_{\text{H}}$ 6.78 (2H, s, H-2,6), 6.80 (2H, s, H-2',6'). $\delta_{\text{C}}$ 134.1 (C-1), 105.2 (C-2,6), 149.4 (C-3,5), 133.6 (C-3',5'), 136.3 (C-4'), 136.4 (C-4'), two oxygenated methylenes [$\delta_{\text{H}}$ 4.12 (1H, $J$ = 8.0 Hz, H-7), 4.98 (1H, $J$ = 9.0 Hz, H-7')]. $\delta_{\text{C}}$ 85.6 (C-7), 84.2 (C-7'), two $sp^3$ methines [$\delta_{\text{H}}$ 2.75 (1H, m, H-8), 2.45 (1H, m, H-8')). $\delta_{\text{C}}$ 52.7 (C-8), 51.1 (C-8')], two oxygenated methylenes [$\delta_{\text{H}}$ 4.36 (1H, overlapped, H-9'a) and 3.72 (1H, $J$ = 12.0, 5.0 Hz, H-9'b); 4.09 (1H, dd, $J$ = 10.5, 5.5 Hz, H-9'a) and 3.80 (1H, dd, $J$ = 10.0, 4.5 Hz, H-9'b). $\delta_{\text{C}}$ 69.3 (C-9), 64.8 (C-9')], and four methoxyl groups [$\delta_{\text{H}}$ 3.93 (12H, s), $\delta_{\text{C}}$ 56.9]. Comparison of the above NMR data with those of icariol A$_2$ [15], a lignan previously isolated from Epimedium sagittatum, revealing the presence of an icariol A$_2$ moiety in 1. In addition, signals due to an acetyl group [$\delta_{\text{H}}$ 1.95 (3H, s), $\delta_{\text{C}}$ 20.7, 172.8] and a glucopyranosyl moiety were also observed in the NMR spectra of 1. The anomeric proton was presented at $\delta_{\text{H}}$ 4.36 (1H, $J$ = 8.0 Hz), corresponding to the carbon at $\delta_{\text{C}}$ 104.6 assigned by HSQC experiment, and the relatively large coupling constant ($J$ = 8.0 Hz) of the anomeric proton suggested that the glucopyranosyl moiety was in $\beta$ configuration. Given that naturally occurring glucose is $D$-form, and limited by the small amount of 1, we tentatively determined the glucopyranosyl moiety in 1 was in $D$-form. In the HMBC spectra of 1, the correlations between the anomeric proton $\delta_{\text{H}}$ 4.36 (1H, $J$ = 8.0 Hz, H-1") and C-9 ($\delta_{\text{C}}$ 69.3) confirmed that the glucopyranosyl moiety was linked at C-9 (Figure 2). The acetyl group was linked at C-9' determined by the HMBC correlation between H-9' and the carbonyl carbon ($\delta_{\text{C}}$ 172.8). All the protons and carbons were unambiguously assigned (Table 1) by $^1$H-$^1$H COSY, HSQC, and HMBC experiments (Figures S4–S6).

The relative configuration of 1 was determined by NOESY spectrum (Figure S7), which showed the NOE correlations of H-7/H-8' and H-7'/H-8. The CD spectra (Figure S8) of 1 showed the positive Cotton effect at 246 nm suggested that both C-7 and C-7’ were in $R$ configuration [16,17], and thus the

![Figure 1. Structures of compounds 1–16 from U. lobate.](image-url)
configuration of C-8, and C-8’ were assigned as 8S, 8’S. Accordingly, the structure of 1 was determined as shown in Figure 1, named as urenalignoside A.

Compound 2 was obtained as colorless powder. Its molecular formula was assigned as C27H38O13 by the [M + HCOO]− ion at m/z 615.2284 (calcd for C28H39O15, m/z 615.2294) in the HRESIMS spectrum (Figure S9), which was also supported by the 13C-NMR-NMR data (Table 1). The NMR spectra of 2 (Figures S10 and S11) showed the presence of a 1,3,4-trisubstituted [δH 7.28 (1H, d, J = 1.5 Hz, H-2), 6.83 (1H, d, J = 8.0 Hz, H-5), 6.96 (1H, dd, J = 8.0, 1.5 Hz, H-6). δC 130.8 (C-1), 113.3 (C-2), 148.7 (C-3), 147.1 (C-4), 115.5 (C-5), 122.1 (C-6)] and a 1,3,4,5-tetrasubstituted benzene moieties [δH 6.53 (2H, s, H-2’,6’). δC 135.1 (C-1’), 106.7 (C-2’,6’), 154.3 (C-3’,5’), 139.9 (C-4’)], two oxygen-bearing methines [δH 5.31 (1H, d, J = 3.0 Hz, H-7), 4.23 (1H, m, H-8). δC 77.7 (C-7), 86.8 (C-8)], two oxygen-bearing methylenes [δH 3.61 (2H, t, J = 6.4 Hz, H-9), 3.16 (2H, m, H-8). δC 33.4 (C-7’), 35.4 (C-8’)], and three methoxy groups [δH 3.89 (3H, s, 3′-OCH3). δC 56.4 (3, 3′,5′-OCH3)]. Comparison of the above-mentioned NMR data with those of 1-(4’-hydroxy-3′-methoxy-phenyl)-2-[4’′-(3-hydroxypropyl)-2’,6’′-dimethoxyphenoxymethyl]-1,3-diol, a lignan previously isolated from Bursera tonkinensis [18], suggested the occurrence of an 8-O-4’-neolignan moiety in 2. In addition, signals due to a glucopyranosyl moiety were also observed in the NMR spectra of 2. The relatively large coupling constant (J = 7.5 Hz) of the anomeric proton resonated at δH 4.23 (1H, d, J = 7.5 Hz, H-1′′) suggested the glucopyranosyl moiety was in β configuration. The linkage of the glucopyranosyl moiety was determined at C-7 by the HMBC correlation between the anomeric proton and C-7 (Figure 2). Unambiguous assignments of the protons and carbons (Table 1) were achieved by 1H-1H COSY, HSQC, HMBC, and NOESY experiments (Figures S12–S15).

It has been well reported that the relative configurations of C-7 and C-8 could be solved by the analysis of the coupling constant between H-7 and H-8. Regularly, a relatively small coupling constant (J = 3–4 Hz) between H-7 and H-8 defines the erythro configurations of C-7 and C-8, while a relatively large coupling constant (J = 6–8 Hz) give rise to the threo configurations of C-7 and C-8 [19–24]. Accordingly, the stereochemistry of C-7 and C-8 in 2 were assigned as erythro according to the small coupling constant (J = 3.0 Hz) between H-7 and H-8. The positive Cotton effect at 233 nm in the CD spectrum (Figure S16) of 2 suggested that the configuration of C-8 was 5 [22,24–26], and thus the configuration of C-7 was determined as R. Therefore, the structure of 2 namely urenalignoside B was elucidated as shown in Figure 1.

Compound 3 was obtained as a colorless powder, with a molecular formula of C25H34O13 determined by the presence of a [M − H]− ion at m/z 541.1920 (calcd for C25H33O13, m/z 541.1927) in the HRESIMS spectrum (Figure S17). The NMR data of 3 (Figures S18–S23) is comparable to those of 2, except the absence of one methoxy group in 3. In the HMBC spectrum of 3, the correlation between the anomeric proton [δH 4.93 (1H, J = 7.5 Hz, H-1′′)] of the glucopyranosyl moiety and the C-3′ of the aglycon demonstrated that the glucopyranosyl moiety was linked at C-3′ in 3 (Figure 2). The large coupling constant (J = 8.5 Hz) between H-7 and H-8 suggested that the C-7 and C-8 were in threo orientation. The negative Cotton effect at 233 nm in the CD spectrum (Figure S24) of 3 suggested
that the configuration of C-8 was R \[23,24\], and thus the configuration of C-7 was 7R. Therefore, the structure of 3 namely *urenaalignoside C* was determined as shown in Figure 1.

### Table 1. Data of compounds 1–4 (500 MHz for \(^1\)H and 125 MHz for \(^{13}\)C, CD3OD, J in Hz).

| No. | 1 \(^a\) | 2 \(^a\) | 3 \(^a\) | 4 \(^a\) |
|-----|---------|---------|---------|---------|
| 1   | \(\delta_H\) 134.1 | \(\delta_C\) 130.8 | \(\delta_H\) 133.5 | \(\delta_C\) 133.3 |
| 2   | 6.78, s | 105.2 | 7.28, d, (1.5) | 113.3 | 7.01, d, (1.5) | 111.6 | 7.08, d, (1.5) | 112.2 |
| 3   | 149.4 | 148.7 | 149.0 | 148.5 |
| 4   | 136.5 | 147.1 | 147.5 | 147.0 |
| 5   | 149.4 | 6.83, d, (8.0) | 115.5 | 6.77, d, (8.5) | 116.1 | 6.78, d, (8.0) | 115.7 |
| 6   | 6.78, s | 105.2 | 6.96, dd, (8.0, 1.5) | 122.1 | 6.88, dd, (8.0, 1.5) | 121.1 | 6.94, dd, (8.0, 1.5) | 121.0 |
| 7   | 5.12, d, (8.0) | 85.6 | 5.31, d, (3.0) | 77.7 | 4.97, d, (8.5) | 75.0 | 5.12, d, (7.0) | 74.4 |
| 8   | 2.75, m | 52.7 | 4.23, m | 86.8 | 4.01, m | 89.7 | 4.16, m | 88.3 |
| 9   | 3.80, dd, (10.0, 4.5) | 69.3 | 3.16, m | 61.4 | 3.69, m | 61.3 | 3.62, m | 62.1 |
|     | 4.09, dd, (10.0, 5.5) |     |     |     |     |     |     |     |

\(^a\) Assignments were carried out based on HSQC and HMBC experiments.

Compound 4 was obtained as a colorless powder, with a molecular formula of C\(_{27}\)H\(_{38}\)O\(_{13}\) by the [M – H\(^+\)]\(^-\) ion m/z 569.2258 (calcd for C\(_{27}\)H\(_{38}\)O\(_{13}\) m/z 569.2240) in the HRESIMS spectrum (Figure S25). Comparison of the NMR data of 4 (Figures S26–S31) with those of 2 revealed that these two compounds share a highly similar skeleton, except the significantly deshielded chemical shift of C-9' (\(\delta_C\) 69.2; \(\delta_C\) + 6.3), suggesting that the O-glucopyranosyl moiety was linked at C-9' in 4, but not like that at C-7 in 2. The deduction was confirmed by HMBC correlation between the anomeric proton (\(\delta_H\) 7.1) and C-9' (Figure 2). The relatively large coupling constant (J = 7.0 Hz) between H-7 and H-8 suggested that C-7 and C-8 were in *threo* orientation. The absolute configuration of C-8 was assigned as S based on the positive Cotton effect at 233 nm presented in the CD spectrum (Figure S32) of 4 \[22,24–26\], and thus the configuration of C-7 was assigned as S. Accordingly, the structure of 4 namely *urenaalignoside D* was determined as shown in Figure 1.

By comparison of their spectroscopic and specific rotation data with those of the known compounds, the remaining 11 compounds were identified as (7R,8R)-*threo*-4,9,9'-trihydroxy-3,3',5'-trimethoxy-8-O-4'-neolignan-7-O-\(\beta\)-\(\beta\)-glucopyranoside \(\text{(5) \cite{21} , rourinoside (6) \cite{22}}\), (7R,8R)-*threo*-guaiaacylglycerol-8-O-4'-sinapyl ether-7-O-\(\beta\)-\(\beta\)-glucopyranoside \(\text{(7) \cite{23}}\), (7S,8R)-*erythro*-4,9,9'-trihydroxy-3,3'-dimethoxy-8-O-4'-neolignan-7-O-\(\beta\)-\(\beta\)-glucopyranoside \(\text{(8) \cite{24}}\), (7S,8S)-*threo*-4,9,9'-trihydroxy-3,3'-dimethoxy-8-O-4'-neolignan-7-O-\(\beta\)-\(\beta\)-glucopyranoside \(\text{(9) \cite{24}}\), (7S,8S)-*erythro*-4,9,9'-pentaerythro-3-methoxy-8-O-4'-neolignan-9'-O-\(\beta\)-\(\beta\)-glucopyranoside \(\text{(10) \cite{25}}\), (7S,8S)-*erythro*-4,9,9'-pentaerythro-3-methoxy-8-O-4'-neolignan-4-O-\(\beta\)-\(\beta\)-glucopyranoside
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(11) [26], (75,7′S,8R,8′R)-icariol A2-9-0-β-d-glucopyranoside (12) [16], (75,7′S,8S,8′S)-icariol A2-4-0-β-d-glucopyranoside (13) [27], lyoniresinol-9′-0-β-d-glucopyranoside (14) [28], (−)-isolariciresinol 4-0-β-d-glucopyranoside (15) [29], and cedrusin-4′-0-β-d-glucopyranoside (16) [30], respectively. Compounds 2–11 and 16 are neolignans which are classified as a subgroup of lignan family [31].

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were obtained on a Rudolph Autopol IV automatic polarimeter (Hackettstown, NJ, USA). IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrophotometer (Madison, WI, USA) with KBr pellets. UV spectra were obtained using a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). NMR spectra were recorded on a Varian INOVA-500 spectrometer (Palo Alto, CA, USA) operating at 500 MHz for 1H-NMR and 125 MHz for 13C-NMR. HRESIMS was recorded on an LCMS-IT-TOF system, fitted with a Prominence UFLC system and an ESI interface (Shimadzu, Kyoto, Japan). Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), LiChroprep RP-C18 gel (40–63 µm, Merck, Germany), D101 m acroporous adsorption resin (Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Qingdao Marine Chemical Inc., Qingdao, China) were used for open column chromatography (CC). HPLC was performed on a ShimadzuLC-20AT pump system (Shimadzu Corporation, Tokyo, Japan), equipped with an SPD-M20A photodiode array detector monitoring at 254 nm. A semi-preparative HPLC column (YMC-Pack C18, 250 × 10 mm, 5 µm) was employed for the isolation. TLC was performed using GF254 plates (Qingdao Marine Chemical Inc., Qingdao, China).

3.2. Plant Material

_Urena lobata_ L. was collected in Guangxi Province, People’s Republic of China, in September 2013. The plant material was authenticated by one of the authors (P. F. Tu) and a voucher specimen (DTH2013029) was deposited at the Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China.

3.3. Extraction and Isolation

The air-dried _U. lobata_ (13.6 kg) were refluxed with 95% EtOH for three times (3 × 180 L, each for 1 h). After removing the solvent under reduced pressure, the residue (1.35 kg) was suspended in water (6 L), and partitioned with petroleum ether (3 × 6 L), EtOAc (5 × 6 L), and n-BuOH (3 × 6 L), successively. The n-BuOH-soluble fraction (158 g) was subjected to D101 m acroporous adsorption resin column and eluted with H2O–EtOH (100:0, 90:10, 50:50, 20:80, 0:100) to yield five fractions (Fr. 1-5). Fr. 2 (20 g) and Fr. 3 (40 g) were combined and subjected to silica gel chromatography and eluted with a stepwise gradient of EtOAc-MeOH-H2O from 30:2:1 to 5:2:1 to give five subfractions (Subfr. A–E). Subfr. B (8 g) was chromatographed on a Sephadex LH-20 column and eluted with MeOH to give six subfractions (Subfr. B1–B6). Subfr. B3 (1 g) was chromatographed on a silica gel column and eluted with gradient of CH2Cl2–MeOH (12:1, 10:1, 8:1, 5:1, 1:1, v/v) to give seven subfractions (Subfr. B3a–B3g). Subfr. B3d (0.2 g) was purified by semipreparative HPLC using 27% aqueous MeCN as the mobile phase to afford compound 7 (2.1 mg, _t_R_ 34.5 min). Subfr. B3g (0.1 g) was applied to semi-preparative HPLC using 25% aqueous MeCN to obtain two compounds 8 (3.1 mg, _t_R_ 23.0 min) and 9 (4.2 mg, _t_R_ 48.5 min). Subfr. B4 (4 g) was chromatographed on a Sephadex LH-20 column and eluted with MeOH to give six subfractions (Subfr. B4a–B4e). Subfr. B4a (1.2 g) was subjected to semi-preparative HPLC using 25% aqueous MeCN to give five compounds (Subfr. B4a1–B4a5). Subfr. B4c (1.1 g) was further separated by ODS column chromatography and eluted with MeOH–H2O (1:4, 1:3, 1:2, 2:3, 1:0, v/v), to afford five fractions (Subfr. B4c1–B4c5). Subfr. B4e (1.2 g) was applied to semi-preparative HPLC using 27% aqueous MeCN to give compound 1 (1.2 mg, _t_R_ 28.5 min). Subfr. B4c4 was repeatedly separated and purified by semi-preparative HPLC (27% aqueous MeCN) to give two fractions Subfr.
B4c4-1 (25.3 mg, tR 40.0 min), Subfr. B4c4-2 (7.4 mg, tR 49.0 min), and five compounds 3 (3.0 mg, tR 44.5 min), 4 (2.1 mg, tR 30.0 min), 5 (2.5 mg, tR 36.0 min), 12 (7.5 mg, tR 23.5 min), and 13 (2.5 mg, tR 27.5 min). Subfr. B4c4-2 was purified by semi-preparative HPLC (30% aqueous MeOH) to give compounds 10 (1.8 mg, tR 55.5 min) and 11 (2.0 mg, tR 57.0 min). Subfr. B4c5 was applied to semi-preparative HPLC using 10% aqueous MeOH to give compounds 2 (2.5 mg, tR 32.0 min), 6 (3.2 mg, tR 37.0 min), 14 (2.1 mg, tR 43.5 min), 15 (1.8 mg, tR 54.0 min), and 16 (1.2 mg, tR 55.5 min).

Urenalignoside A (1): Colorless powder, \([\alpha]_D^{25} = -45.7 (c 0.1, \text{MeOH})\); UV \(\lambda (\log \varepsilon)\): 208 (4.49), 317 (4.31), 383 (3.91) nm; IR (KBr) \(\nu_{\text{max}}\): 3385, 2921, 1735, 1615, 1518, 1462, 1428, 1367, 1331, 1217, 1114, 1076, 1036 cm\(^{-1}\); \(^1\)H and \(^{13}\)C-NMR data (see Table 1); negative-ion HRESIMS: \(m/z\) 639.2282 [M – H]\(^{-}\) (calcd for C\(_{30}\)H\(_{39}\)O\(_{15}\), 639.2294).

Urenalignoside B (2): Colorless powder, \([\alpha]_D^{25} = -64.0 (c 0.1, \text{MeOH})\); UV \(\lambda (\log \varepsilon)\): 202 (4.14), 226 (4.25), 277 (3.37), 298 (2.63), 317 (2.48), 329 (2.40), 341 (2.43), 348 (2.38) nm; IR (KBr) \(\nu_{\text{max}}\): 3423, 2926, 1630, 1384, 1253, 1119, 1076, 1037 cm\(^{-1}\); \(^1\)H and \(^{13}\)C-NMR data (see Table 1); negative-ion HRESIMS: \(m/z\) 615.2284 [M + HCOO]\(^{-}\) (calcd for C\(_{28}\)H\(_{39}\)O\(_{15}\), 615.2294).

Urenalignoside C (3): Colorless powder, \([\alpha]_D^{25} = -52.4 (c 0.1, \text{MeOH})\); UV \(\lambda (\log \varepsilon)\): 212 (4.58), 285 (4.00) nm; IR (KBr) \(\nu_{\text{max}}\): 3389, 2968, 2923, 2852, 1739, 1610, 1456, 1431, 1366, 1259, 1228, 1216, 1174, 1111, 1028 cm\(^{-1}\); \(^1\)H and \(^{13}\)C-NMR data (see Table 1); negative-ion HRESIMS: \(m/z\) 541.1920 [M – H]\(^{-}\) (calcd for C\(_{25}\)H\(_{33}\)O\(_{13}\), 541.1927).

Urenalignoside D (4): Colorless powder, \([\alpha]_D^{25} = -54.0 (c 0.1, \text{MeOH})\); UV \(\lambda (\log \varepsilon)\): 207 (4.62), 263 (4.70), 316 (4.23) nm; IR (KBr) \(\nu_{\text{max}}\): 3739, 3716, 3660, 3430, 2956, 2924, 2853, 1717, 1592, 1514, 1488, 1455, 1428, 1383, 1367, 1230, 1157, 1125, 1023 cm\(^{-1}\); \(^1\)H and \(^{13}\)C-NMR data (see Table 1); negative-ion HRESIMS: \(m/z\) 569.2258 [M – H]\(^{-}\) (calcd for C\(_{27}\)H\(_{39}\)O\(_{15}\), 569.2240).

3.4. Biological Assays

The murine macrophage RAW264.7 cell line was purchased from Peking Union Medical College (PUMC) Cell bank (Beijing, China), and was cultured in DMEM supplemented with 10% Fetal Bovine Serum, 100U/mL penicillin G and 100 µg/mL streptomycin, in a humidified 5% CO\(_2\) at 37 °C. Cell viability was evaluated using MTT assay. The NO concentration was detected by the Griess method. Briefly, RAW264.7 macrophage cells were seeded into 96-well plates at a density of 5 × 10\(^4\) cells/well and stimulated with 0.5 µg/mL LPS (Sigma, St. Louis, MO, USA) in the presence or absence of test compounds. After incubation for 24 h at 37 °C, treated RAW264.7 macrophage cells were incubated with 100 µL MTT solution (0.5 mg/mL in medium) for another 4 h at 37 °C, subsequently, the supernatants were removed and residues were dissolved using 150 µL DMSO for each well; 50 µL of cell-free supernatant was mixed with 100 µL of Griess reagent containing equal volumes of 2% (w/v) sulfanilamide in 5% (w/v) phosphoric acid and 0.2% (w/v) N-(1-naphthyl) ethylenediamine solution to measure nitrite production. The absorbance was detected at 540 nm using a microplate reader (Thermo, Waltham, MA, USA). Compared with a calibration curve prepared using NaNO\(_2\) standards. The experiments were performed in triplicate. quercetin was conducted as a positive control. All the compounds were prepared as stock solutions in DMSO (final solvent concentration less than 0.3% in all assays).

3.5. Bioactivity Evaluation

Compounds 1–16 were evaluated for their inhibitory effects on the NO production in LPS-stimulated RAW 264.7 macrophage cells. Quercetin was used as a positive control (IC\(_{50}\) = 7.2 ± 0.2 µM). Compounds 2–4, 6, 7, 10, and 11 exhibited weak inhibitory activity against NO production with IC\(_{50}\) values of 90.4 ± 3.2 µM, 74.3 ± 1.8 µM, 88.1 ± 2.2 µM, 98.4 ± 3.6 µM, 97.5 ± 2.6 µM, 97.7 ± 3.5 µM, 25.5 ± 1.2 µM, respectively.

Supplementary Materials: The following materials are available online: HRESIMS and NMR spectra data of compounds 1–4 as supporting information.
Author Contributions: S.S. designed and organized the study, Y.L. contributed the analysis and interpretation of data and wrote the draft, C.S. performed the isolation and structural elucidation of the chemicals and the bioassay experiments. N.D., B.Q., F.J., X.X., X.L., J.W. and X.W. contributed the analysis and interpretation of data, P.T. and S.S. reviewed and edited the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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**Sample Availability:** Samples of the compounds 1–16 are available from the authors.

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