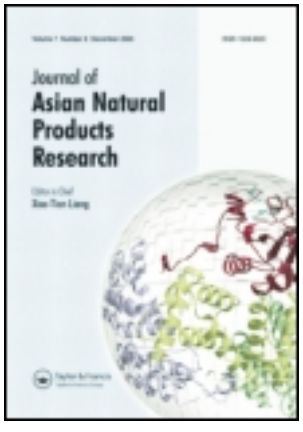

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Inhibitory activities on nitric oxide production of stilbenoids from *Pholidota yunnanensis*

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Three new stilbenoids, 1-(4′-hydroxybenzyl)-imbricatin (**1**), (*E*)-4′-hydroxy-2′,3,3′,5-tetramethoxystilbene (**2**), and (*E*)-3,4′-dihydroxy-2,6-bis(4-hydroxybenzyl)-2′,3′,5-trimethoxystilbene (**3**), together with 15 known stilbene derivatives, were isolated from *Pholidota yunnanensis*. Their structures were elucidated by spectroscopic methods and by comparison of their NMR data with those of related compounds. Furthermore, the inhibitory activities on nitric oxide (NO) production of the isolated compounds were examined in murine macrophages (RAW 264.7) activated by lipopolysaccharide. The cytotoxicity of 18 compounds was determined by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. Among the tested compounds, eight stilbenoids, including three dihydrophenanthrenes, three stilbenes, and one bibenzyl derivative showed inhibitory effects on NO production without cytotoxicity with IC_{50} values ranging from 4.07 to 7.77 μM, as compared to MG-132, which was used as a positive control (IC_{50} of 0.10 μM). One dihydrophenanthrene, phoyunnanin C (**5**), showed cytotoxic effects at the test concentrations.

Keywords: *Pholidota yunnanensis*; Orchidaceae; stilbenoids; RAW 264.7 macrophages; nitric oxide

1. Introduction

Pholidota yunnanensis Rolfe (Orchidaceae), belonging to the genus *Pholidota*, is a perennial herb distributed in Yunnan, Guangxi, Sichuan, and Guizhou provinces in China [1]. The whole plant or pseudobulb has been used as traditional medicine, and Yi-nationality herbal medicine for the treatment of various diseases, including cough, rheumatism, stomach ache, and trauma [2,3]. Previous investigations on this species have resulted in the isolation of stilbenoids, triterpenes, and steroids [4–6]. To further research on the chemical constituents of this genus, three new stilbenoids **1**–**3**, together with 15 known stilbene derivatives **4**–**18**, were isolated from *P. yunnanensis*. The structural elucidations of all the compounds were based on the spectroscopic evidences and compared with the literature data. To endow a scientific base for the traditional applications of this herbal medicine, the 18 isolates were tested for suppressing the production of nitric oxide (NO). The NO radical produced by the oxidation of L-arginine by NO synthase is an effective molecule for the anti-inflammatory and antimicrobial effects of macrophages. Macrophages play major roles in inflammation and host defense mechanisms against bacterial and viral infections [7]. However, excessive production of NO may lead to severe injury

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to host cells and tissues during acute and chronic inflammation, and thus, suppression of NO production in lipopolysaccharide (LPS)-stimulated macrophages is a useful method for evaluating anti-inflammatory activities [8]. Herein, we describe the isolation, the structural elucidation of the new stilbenoids, and inhibitory effects on NO production by activated murine macrophages of all the compounds **1**–**18**.

2. Results and discussion

The aqueous acetone extract of the whole fresh plant of *P. yunnanensis* was suspended into H_2O and then partitioned successively with EtOAc and *n*-BuOH. Further purification of the EtOAc fraction was carried out with silica gel (200–300 mesh), MCI-gel CHP-20P, Sephadex LH-20, RP-8, and RP-18 column chromatography (CC). This led us to obtain three new compounds, 1-(4′-hydroxybenzyl)-imbricatin (**1**), (*E*)-4′-hydroxy-2′,3,3′,5-tetramethoxystilbene (**2**), and (*E*)-3,4′-dihydroxy-2,6-bis(4-hydroxybenzyl)-2′,3′,5-trimethoxystilbene (**3**), together with 15 known stilbene derivatives, imbricatin (**4**) [9], phoyunnanin C (**5**) [5], eulophiol (**6**) [10], 2,5-dihydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene (**7**) [11], lusianthridin (**8**) [12], 2,5-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene (**9**) [13], 2,4,7-trihydroxy-9,10-dihydrophenanthrene (**10**) [14], coelonin (**11**) [15], bulbophyllanthrin (**12**) [16], moscatin (**13**) [17], 2,5-dihydroxy-3,4-dimethoxyphenanthrene (**14**) [9], phoyunbene B (**15**) [4], (*E*)-2′,3-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxystilbene (**16**) [18], bulbocol (**17**) [19], and 3,3′-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl (**18**) [20] (Figure 1).

Compound **1** was isolated as yellow crystal and its molecular formula $C_{23}H_{20}O_5$ was analyzed by HR-EI-MS at *m/z* 514.1987 $[M]^-$. The IR spectrum showed absorption bands at 3355, 1609, 1511, 1476, and 1447 cm^{-1}, ascribable to hydroxyl and aromatic functional groups. The UV spectrum exhibited the absorption maxima at 220, 284, and 311 nm, similar to those of dihydrophenanthrene derivatives [11]. The ^1H NMR spectrum displayed the signals assignable to one set of A_2B_2 aromatic protons at δ 6.95 (2H, d, $J = 8.4$ Hz, H-2′, 6′) and 6.63 (2H, d, $J = 8.4$ Hz, H-3′, 5′), and two additional aromatic protons at δ 6.58 (1H, s, H-8) and 6.31 (1H, s, H-3); three methylenes at δ_H 2.65 (2H, m, H_α-9, 10), 2.57 (2H, m, H_β-9, 10), and 3.88 (2H, s, H-a′); an oxygenated methylene at δ_H 5.06 (2H, s, H-11); and a phenyl methoxyl at δ 3.73 (3H, s, H-6). The ^{13}C NMR (Table 1) spectrum, combined with DEPT135 and HMQC spectra, showed the signals due to 18 aromatic carbons (five oxygenated

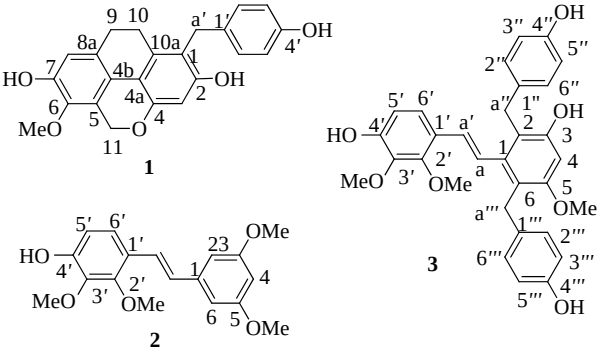

Figure 1. Structures of **1**–**3**.

Table 1. ^{1}H and ^{13}C NMR assignments and 2D NMR correlations of compound **1** in CD_3OD.

Position	δ_C (mult)	δ_H (mult, J in Hz)	HMBC (H → C)
1	113.2 (s)		
2	156.5 (s)		
3	102.0 (d)	6.31 (1H, s)	C-1, 4a
4	152.4 (s)		
4a	120.4 (s)		
4b	120.8 (s)		
5	122.9 (s)		
6	142.8 (s)		
7	149.5 (s)		
8	115.7 (d)	6.58 (1H, s)	C-4b, 6, 9
8a	129.8 (s)		
9	28.2 (t)	2.65 (H_α, m) 2.57 (H_β, m)	C-4b, 8, 10a
10	25.7 (t)	2.65 (H_α, m) 2.57 (H_β, m)	C-1, 4a, 8a
10a	135.4 (s)		
11	64.4 (t)	5.06 (2H, s)	C-4, 4b, 5, 6
6-OMe	61.4 (q)	3.73 (3H, s)	C-3
a′	30.6 (t)	3.88 (2H, s)	C-2, 2′, 6′, 10a
1′	133.8 (s)		
2′	130.1 (d)	6.95 (1H, d, $J = 8.4$ Hz)	C-a′, 4′, 6′
3′	115.9 (d)	6.63 (1H, d, $J = 8.4$ Hz)	C-1′, 5′
4′	155.9 (s)		C-4′
5′	115.9 (d)	6.63 (1H, d, $J = 8.4$ Hz)	C-1′, 3′
6′	130.1 (d)	6.95 (1H, d, $J = 8.4$ Hz)	C-a′, 2′, 4′

carbons, six protonated carbons, and seven quaternary carbons); three methylenes carbons [C-9 (δ 28.2), C-10 (δ 25.7), and C-a′ (δ 30.6)]; an oxygenated methylene carbon C-11 (δ 64.4); and a phenyl-methoxyl C-OMe-6 (δ 61.4). All the above evidence indicated the presence of a dihydrophenanthrene skeleton, which was the same as imbricatin [13] and a *p*-hydroxybenzyl moiety. The COSY correlations from H-2′, 6′ (δ 6.95) to H-3′, 5′ (δ 6.63), and H-2′, 6′ (δ 6.95) to H-a′ (δ 3.88) confirmed the *p*-hydroxybenzyl moiety. The substituent patterns of **1** were further confirmed by the HMBC spectrum (Figure 2). In the HMBC spectrum, the long-range correlations between H-a′ and C-2, 2′, 6′, 10a determined that *p*-hydroxybenzyl moiety was linked to C-1; and between H-OMe-6 and C-6 indicated that the methoxy group was attached to C-6. Therefore, the structure of **1** was determined as 1-(4′-hydroxybenzyl)-imbricatin.

Compound **2** was obtained as pale yellow oil, and the molecular formula $C_{18}H_{20}O_5$ was determined by HR-ESI-MS at *m/z* 316.1308 $[M + Cl]^-$. The UV spectrum showed absorption maxima at 200, 221, and 317 nm, similar to those of stilbene derivatives [4]. The IR spectrum exhibited the presence of an OH group (3364 cm^{-1}) and aromatic rings (1587, 1494 and 1465 cm^{-1}). The ^{1}H NMR spectrum (Table 1) displayed signals due to two *trans*-olefinic protons at δ 7.25 (1H, d, $J = 16.4$ Hz, H-a) and 6.93 (1H, d, $J = 16.4$ Hz, H-a′), two *ortho*-coupled protons at δ 7.23 (1H, d, $J = 8.6$ Hz, H-6′) and 6.64 (1H, d, $J = 8.6$ Hz, H-5′), three *meta*-coupled protons at δ 6.62 (2H, t, $J = 2.2$ Hz, H-2, 6) and 6.37 (1H, t, $J = 2.2$ Hz, H-4), and four methoxyls at δ 3.85 (3H, s, H-3), 3.82 (3H, s, H-2′), and 3.79 (6H, s, H-3′, 5). The ^{13}C NMR

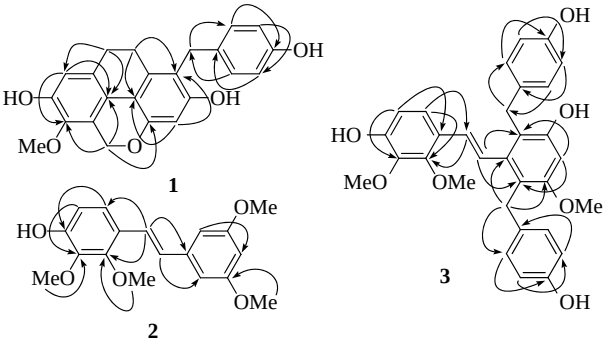

Figure 2. Key HMBC correlations of **1**–**3**.

(Table 2) spectrum, combined with DEPT135 and HMQC spectra, showed the signals ascribable to two olefinic carbons C-a′ (δ 128.2) and C-a (δ 124.4), four phenyl methoxyls, and 12 aromatic carbons (two quaternary carbons, five oxygenated carbons, and five protonated carbons). The stilbene skeleton was constructed on the basis of the $^{1}H-^{1}H$ COSY and HMBC experiments. In the $^{1}H-^{1}H$ COSY spectrum, the correlations between H-a (δ 7.25) and H-a′ (δ 6.93), H-4 (δ 6.37) and H-2, 6 (δ 6.62), and H-5′ (δ 6.64) and H-6′ (δ 7.23) suggested the presence of a *trans*-double bond, one 1,2,3,4-tetrasubstituted aromatic ring, and a 1,3,5-trisubstituted phenyl moiety. In the HMBC spectrum, the correlations between H-a and C-1′, 2, 6; H-6′ and C-a′, 2′, 4′; H-a′ and C-1, 2′, 6′; H-5′ and C-1′, 3′; H-2, 6 and C-a, 2, 6, 4; H-OMe-3′ and C-3′; H-OMe-2′ and C-2′ [4]; and H-OMe-3, 5 and C-3, 5 further confirmed the substituent patterns of **2**. In addition, the number and the position of hydroxyl could be established by the molecular formula and structural information. Consequently, the structure of **2** was elucidated as (*E*)-4′-hydroxy-2′,3,3′,5-tetramethoxystilbene (**2**).

Table 2. ^{1}H and ^{13}C NMR assignments and 2D NMR correlations of compound **2** in CD_3OD.

Position	δ_C (mult)	δ_H (mult, *J* in Hz)	HMBC (H → C)
1	142.2 (s)		
2	105.3 (d)	6.62 (1H, t, *J* = 2.2)	C-4, 6
3	162.5 (s)		
4	100.3 (d)	6.37 (1H, t, *J* = 2.2)	
5	162.5 (s)		
6	105.3 (d)	6.62 (1H, t, *J* = 2.2)	C-2, 4
1′	124.1 (s)		
2′	152.1 (s)		
3′	141.5 (s)		
4′	153.0 (s)		
5′	113.2 (d)	6.64 (1H, d, *J* = 8.6)	C-1′, 3′
6′	122.3 (d)	7.23 (1H, d, *J* = 8.6)	C-a′, 2′, 4′
a	124.4 (d)	7.25 (1H, d, *J* = 16.4)	C-1′, 2, 6
a′	128.2 (d)	6.93 (1H, d, *J* = 16.4)	C-1, 2′, 6′
2′-OMe	61.6 (q)	3.82 (3H, s)	C-2′
3-OMe	55.7 (q)	3.85 (3H, s)	C-3
3′-OMe	61.1 (q)	3.79 (3H, s)	C-3′
5-OMe	55.7 (q)	3.79 (3H, s)	C-5

The key HMBC correlations are shown in Figure 2.

Compound **3** was yielded as colorless oil and the HR-ESI-MS at *m/z* 514.1987 $[M]^-$ displayed the molecular formula to be $C_{31}H_{30}O_7$. The UV spectrum exhibited the absorption maxima at 204, 222, and 282 nm ascribable to benzyl moieties. The IR spectrum showed the presence of hydroxyl (3397 cm^{-1}) and aromatic functional groups (1597, 1511, 1459, 1443 cm^{-1}). The ^1H NMR spectrum displayed resonances typical of a substituted stilbene, including a *trans*-double bond at δ 6.76 (1H, d, $J = 16.8$ Hz, H-a) and 6.39 (1H, d, $J = 16.8$ Hz, H-a′), a 1,2,3,4-tetrasubstituted phenyl moiety [a pair of *ortho*-coupled protons at δ 6.91 (1H, d, $J = 8.4$ Hz, H-6′) and 6.55 (1H, d, $J = 8.4$ Hz, H-5′)], and a pentasubstituted phenyl moiety [a proton singlet at δ 6.51 (1H, s, H-4)]. Furthermore, the molecular formula suggested the presence of two hydroxybenzyl moieties. Indeed, the ^1H NMR spectrum did show two methylene proton resonances at δ 3.93 (2H, s, H-a″) and 3.89 (2H, s, H-a‴), as well as two A_2B_2 spin systems at, respectively, δ 6.93 (2H, d, $J = 8.4$ Hz, H-2″, 6″) and 6.63 (2H, d, $J = 8.4$ Hz, H-3″, 5″), and δ 6.84 (2H, d, $J = 8.4$ Hz, H-2‴, 6‴) and 6.61 (2H, d, $J = 8.4$ Hz, H-3‴, 5‴). The ^{13}C NMR (Table 3) spectrum, together with DEPT135 and HSQC spectra, exhibited the signals attributable to an olefinic bond (δ 128.9, C-a′ and δ 126.5, C-a), two

Table 3. ^1H and ^{13}C NMR assignments and 2D NMR correlations of compound **3** in CD_3OD.

Position	δ_C (mult)	δ_H (mult, *J* in Hz)	HMBC (H → C)
1	142.0 (s)		
2	119.1 (s)		
3	142.8 (s)		
4	98.8 (d)	6.51 (1H, s)	C-2, 5, 6
5	158.2 (s)		
6	119.9 (s)		
1′	124.4 (s)		
2′	152.7 (s)		
3′	142.1 (s)		
4′	151.6 (s)		
5′	113.0 (d)	6.55 (1H, d, $J = 8.4$ Hz)	C-1′, 3′
6′	122.1 (d)	6.91 (1H, d, $J = 8.4$ Hz)	C-a′, 2′, 4′
1″	134.5 (s)		
2″	130.3 (d)	6.93 (1H, d, $J = 8.4$ Hz)	C-a″, 4″, 6″
3″	115.8 (d)	6.63 (1H, d, $J = 8.4$ Hz)	C-1″, 5″
4″	155.8 (s)		
5″	115.8 (d)	6.63 (1H, d, $J = 8.4$ Hz)	C-1″, 3″
6″	130.3 (d)	6.93 (1H, d, $J = 8.4$ Hz)	C-a″, 2″, 4″
1‴	134.7 (s)		
2‴	130.1 (d)	6.84 (1H, d, $J = 8.4$ Hz)	C-a‴, 4‴, 6‴
3‴	115.7 (d)	6.61 (1H, d, $J = 8.4$ Hz)	C-1‴, 4‴, 5‴
4‴	155.7 (s)		
5‴	115.7 (d)	6.61 (1H, d, $J = 8.4$ Hz)	C-1‴, 3‴, 4‴
6‴	130.1 (d)	6.84 (1H, d, $J = 8.4$ Hz)	C-a‴, 2‴, 4‴
a	126.5 (d)	6.76 (1H, d, $J = 16.8$ Hz)	C-1′, 2, 6
a′	128.9 (d)	6.39 (1H, d, $J = 16.8$ Hz)	C-1, 2′, 6′
a″	32.5 (t)	3.93 (2H, s)	C-1″, 2, 3, 6″
a‴	32.5 (t)	3.89 (2H, s)	C-1‴, 5, 6, 6‴
OMe-2′	61.1 (q)	3.34 (3H, s)	C-2′
OMe-3′	61.3 (q)	3.77 (3H, s)	C-3′
OMe-5	55.9 (q)	3.72 (3H, s)	C-5

Table 4. Inhibitory activities of compounds **1**–**19** against LPS-induced NO production in RAW 264.7 macrophages.

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
MG-132	0.103 ± 0.005	**10**	12.354 ± 1.412
1	20.788 ± 1.685	**11**	6.563 ± 0.602
2	12.510 ± 2.581	**12**	> 25
3	4.077 ± 0.184	**13**	11.941 ± 1.758
4	4.214 ± 0.801	**14**	> 25
5	Cytotoxicity	**15**	4.783 ± 1.300
6	> 25	**16**	6.822 ± 0.627
7	> 25	**17**	7.815 ± 1.117
8	6.338 ± 0.453	**18**	14.618 ± 3.202
9	11.593 ± 1.315		

methylenes (δ 32.5, C-a″, a‴), three phenyl methoxyls (δ 61.3, C-3′; δ 61.1, C-2′; and δ 55.9, C-5), and four aromatic rings (six quaternary carbons, seven oxygenated carbons, and 11 protonated carbons). The correlations from H-2″, 6″ (δ 6.93) to H-3″, 5″ (δ 6.63); H-2‴, 6‴ (δ 6.84) to H-3‴, 5‴ (δ 6.61); and H-5′ (δ 6.55) to H-6′ (δ 6.91) in the $^{1}H-^{1}H$ COSY spectrum, together with the HMBC cross-peaks between H-a and C-1′, 2, 6; H-4 and C-2, 5, 6; H-a′ and C-1, 2′, 6′; H-5′ and C-1′, 3′; H-6′ and C-a′, 2′, 4′; H-a″ and C-1″, 2, 2″, 3, 6″; H-2″, 6″ and C-a″, 2″, 4″; H-3″, 5″ and C-1″, 3″, 5″; H-a‴ and C-1‴, 5, 6, 6‴; H-2‴, 6‴ and C-a‴, 2‴, 4‴, 6‴; H-3‴, 5‴ and C-1‴, 3‴, 5‴; H-OMe-3′ and C-3′; H-OMe-5 and C-5; and between H-OMe-2′ and C-2′ gave evidence of the position of the hydroxybenzyl moiety, the methoxyls, and the hydroxy groups [4]. Thus, compound **3** was determined as (*E*)-3,4′-dihydroxy-2,6-bis(4-hydroxybenzyl)-2′,3′,5-trimethoxystilbene.

Compounds **1**–**18** were evaluated for their inhibitory activity against LPS-induced NO production in RAW 264.7 macrophages using the Griess assay. As shown in Table 4, compounds **3**–**4**, **8**, **11**, **13**, and **15**–**17** inhibited LPS-induced NO production significantly without cytotoxicity at concentrations ranging from 4.07 to 7.77 μM. For three new compounds, **1** and **2** exhibited moderate activity with an IC_{50} of 21.90 and 12.36 μM, respectively, while **3** showed obvious activity with an IC_{50} of 4.09 μM. In the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, only phoyunnanin C (**5**) showed cytotoxic effects at the test concentrations. Interestingly, previous studies showed that diverse stilbenoids possessed inhibitory activity against LPS-induced NO production [4], which was further confirmed by this study. The above-described data indicated that the stilbenoids of the plant played crucial roles in anti-inflammatory effects, which could contribute to a better understanding of the traditional applications of this plant in the treatment of cough, rheumatism, stomachache, bellyache, and trauma in Chinese folk medicine.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanaco micromelting point apparatus and uncorrected (Beijing TECH Instrument Co. Ltd, Beijing, China). Optical rotations were determined on a JASCO model 1020 polarimeter (Horiba, Tokyo, Japan). UV spectra were measured on a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrometer

(Bio-Rad, Hercules, CA, USA) using KBr pellets. MS and HR-MS were run on a VG Auto Spec-3000 mass spectrometer (VG, Manchester, UK). 1D and 2D NMR spectra were recorded on a Bruker AM-400 or DRX-500 spectrometer (Bruker, Bremerhaven, Germany) with tetramethylsilane as the internal standard. Silica gel (200–300 mesh) for CC and thin layer chromatography was obtained from Qindao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 was purchased from Amersham Biosciences (Buckinghamshire, UK).

3.2 Plant material

The whole fresh plant of *P. yunnanensis* was purchased from Puer (Yunnan province) in April 2011 and identified by Prof. Xiaohua Jin. A voucher specimen (No. 0798287) has been deposited in State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, China.

3.3 Extraction and isolation

The whole fresh plants (16 kg) were powdered and extracted four times with 80% acetone (35 liters) at room temperature for 2 h. The combined extracts were concentrated under reduced pressure to afford a dark-brown residue and then suspended in water (3 liters) and partitioned with EtOAc (3 liters × 5). The EtOAc layer (160 g) was subjected to silica gel column chromatography (200–300 mesh, 1.6 kg), eluted with a gradient of petroleum ether:acetone (100:0–0:100, v/v) to afford six fractions. Fraction 3 (3.9 g) was subjected to silica gel CC (200–300 mesh, 40 g) eluted by petroleum ether:acetone (20:1–1:1) to give three subfractions. Fraction 3-1 (0.73 g) were further separated by octadecylsilyl (ODS) column [MeOH:H_2O (65:35–90:10, v/v)] and then by Sephadex LH-20 column [$CHCl_3$:MeOH (1:1, v/v)] to yield compound **2** (63.4 mg). Fraction 3-3 (0.18 g) was separated and purified by Sephadex LH-20 column eluted with MeOH to afford **8** (5.3 mg). Fraction 4 (10.83 g) was subjected to repeated silica gel CC [200–300 mesh, 110 g, petroleum ether:acetone (18:1–1:1, v/v)] and then purified by CC (Sephadex LH-20, MeOH) to yield compounds **10** (7.9 mg), **13** (34 mg), and **15** (4.6 mg). Fraction 5 (23.24 g) was submitted to repeated silica gel CC [petroleum ether:acetone (10:1–0:1)] to give four subfractions (Fraction 5-1–5-4). Fraction 5-2 (5.30 g) was successively chromatographed over silica gel [(petroleum ether:acetone (9:1–5:1, v/v)], MCI gel [MeOH:H_2O (7:13–10:0, v/v)], RP-18 [MeOH:H_2O (2:3–9:1, v/v)], and then purified by Sephadex LH-20 column [$CHCl_3$:MeOH (1:1, v/v)] to yield **3** (25.6 mg), **4** (10.8 mg), **14** (23.6 mg), and **16** (9.7 mg). Similarly, fraction 5-3 (5.6 g) was further separated by CC [silica gel, petroleum ether:acetone (5:1–1:1)], and then passed over ODS column [MeOH:H_2O (2:3–1:0, v/v)] and purified by Sephadex LH-20 column eluted with MeOH to afford compounds **9** (14.32 mg), **18** (9.51 mg), and **19** (26.70 mg). Fraction 5-4 (5.6 g) was treated as fraction 5-3 to produce **5** (15.8 mg), **6** (11.6 mg), and **11** (23.5 mg). Fraction 6 (27.6 g) was subjected to repeated silica gel CC, eluted with a gradient of petroleum ether:acetone (8:1–4:1, v/v) to give three subfractions. Fraction 6-1 (6.85 g) was separated further by CC [silica gel, $CHCl_3$:MeOH:H_2O (10:2.5:1, v/v)], and then passed over ODS column [MeOH:H_2O (1:2–1:0, v/v)] and purified by Sephadex LH-20 column (MeOH) to afford **12** (24.5 mg) and **17** (6.4 mg). Fraction 6-2 (7.14 g) was treated as fraction 6-1 to give **1** (43.85 mg) and **7** (5.43 mg).

3.3.1 Compound *1*

Yellow crystal; m.p. 159–162°C; $[\alpha]_D^{23}$ – 2.26 ($c = 0.57$, MeOH). UV (MeOH) λ_{max}

($\log \varepsilon_{max}$): 220 (4.59), 284 (4.17) and 311 (4.12) nm; IR (KBr) ν_{max}: 3355, 1610, 1511, 1475, 1447, 1412, 1345, 1299, 1255, 1169, 1158, 1112, 1016, 979 cm^{-1}. For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data, see Table 1. ESI-MS (positive): *m/z* 399 $[M + Na]^+$. HR-EI-MS (positive): *m/z* 376.1312 $[M]^+$ (calcd for $C_{23}H_{20}O_5$, 376.1311).

3.3.2 Compound **2**

Colorless oil; $[\alpha]_D^{23}$ −3.43 ($c = 0.27$, MeOH). UV (MeOH) λ_{max} ($\log \varepsilon_{max}$): 200 (4.43), 221 (4.42) and 317 (4.45) nm; IR (KBr) ν_{max}: 3364, 1587, 1494, 1465, 1428, 1323, 1268, 1199, 1156, 1065, 1019, 986, 963, 856 cm^{-1}. For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data, see Table 2. ESI-MS (positive): *m/z* 339 $[M + Na]^+$; HR-EI-MS (positive): *m/z* 316.1308 $[M]^+$ (calcd for $C_{18}H_{20}O_5$, 316.1311).

3.3.3 Compound **3**

Light yellow solid; $[\alpha]_D^{23}$ – 10.06 ($c = 0.11$, MeOH). UV (MeOH) λ_{max} ($\log \varepsilon$): 204 (4.76), 222 (4.68) and 282 (4.34) nm; IR (KBr) ν_{max}: 3396, 3272, 1597, 1511, 1459, 1443, 1379, 1309, 1231, 1172, 1106, 1047, 1018, 822 cm^{-1}. For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data, see Table 3. ESI-MS (positive): *m/z* 537 $[M + Na]^+$; HR-ESI-MS (positive): *m/z* 514.1987 $[M]^+$ (calcd for $C_{31}H_{30}O_7$, 514.1992).

3.4 Inhibition of NO production in LPS-stimulated RAW 264.7 macrophages cell line

The Murine monocytic RAW 264.7 macrophages were dispensed into 96-well plates (2×10^5 cells/well) containing RPMI 1640 medium (Hyclone, UT, USA) with 10% fetal bovine serum under a humidified atmosphere of 5% CO_2 at 37°C. After 24 h pre-incubation, cells were treated with serial dilutions of the compounds with the maximum concentration of 25 μM in the presence of 1 μg/ml LPS for 18 h. Each compound was dissolved in DMSO and further diluted in medium to produce different concentrations. NO production in each well was assessed by adding 100 μl of Griess reagent (Sigma, St. Louis, MO, USA) to 100 μl of each supernant from LPS-treated (Sigma) or LPS- and compound-treated cells in triplicate. After 5 min of incubation, the absorbance was measured at 570 nm with 2104 Envision Multilabel Plate Reader (Perkin-Elmer Life Sciences, Inc., Boston, MA, USA). Experiments on the cytotoxicity was conducted by the MTS assay [21]. MG-132 was used as a positive control.

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