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Structures and biological evaluation of phenylpropanoid derivatives from *Murraya koenigii*

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ABSTRACT

Four new phenylpropanoid derivatives (**1**–**4**), together with eleven known analogues (**5**–**15**) were isolated and identified by comparison with their references and extensive spectroscopic methods from *Murraya koenigii* for the first time. Compounds (**1**–**15**) were assayed for their inhibitory activities by measuring IL-6-induced STAT3 promoter activities in HepG2 cells, and found compounds **1**, **2**, **6**, and **15** showed inhibitory effects with IC_{50} values of 11.5, 18.7, 8.9, and 22.7 μM, respectively. The inhibitory activities of compounds (**1**–**15**) were screened against NO production in lipopolysaccharide (LPS)-induced RAW264.7 macrophage cells, and found compounds **3**, **4**, **9**, **11**, and **14** exhibited inhibitions against LPS-induced NO production in RAW264.7 macrophages, with IC_{50} values of 32.7, 7.9, 42.1, 58.9, and 62.4 μM, respectively.

1. Introduction

Murraya koenigii is a favorite condiment from the Rutaceae family. It is mainly distributed in the tropical and subtropical regions [1]. The leaves, stems, and roots of this plant have been served as folk medicine for treating various diseases [2]. Phytochemical investigations of *M. koenigii* have revealed a wide array of natural products including alkaloids [3], sesquiterpenes [4], volatile oils [5], and alkenes [6]. Different parts of *M. koenigii* have varied biological activities such as anti-inflammatory [7], anti-oxidative [6], nephroprotective [8], hepatoprotective [4], anti-listerial [9], and PTP1B inhibitory [3] activities.

After consulting a large of references, there were no reports on inhibitory activities by measuring IL-6-induced STAT3 promoter activities in HepG2 cells and inhibition against lipopolysaccharide (LPS)-induced NO production in RAW264.7 macrophages of *M. koenigii*. With the aim of identifying new bioactive compounds from *M. koenigii*, we analyzed the different extraction sites by carrying out a bioassay-guided investigation of *M. koenigii* in order to evaluate its further bioactivities. As a result, four new phenylpropanoid derivatives (**1**–**4**), and eleven known analogues (**5**–**15**) were isolated and identified from the active fraction (EtOAc soluble fraction) of *M. koenigii* for the first time. Described herein are the isolation, structural elucidation, and bioactivity evaluation of compounds (**1**–**15**) from *M. koenigii* in this work.

2. Materials and methods

2.1. General experimental procedures

The optical rotations were recorded on a Perkin-Elmer 241 digital polarimeter at 20 °C. The melting points were measured by a XT5B microscopic melting point apparatus which was uncorrected. The CD spectra were recorded on a JASCO J-815CD spectrometer. The UV and IR spectra were measured by a Hitachi UV-240 spectrophotometer and a Nicolet 5700 FTIR microscope spectrometer with KBr pellets, respectively [4]. The 1D and 2D NMR measurements were performed on Varian Mercury-300, Inova-501, DD2-500, Bruker Avance III HD-600

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spectrometers with tetramethylsilane as an internal standard. The ESI-MS and HR-ESI-MS data were measured by a Q-Trap LC/MS/MS spectrometer and an Agilent 1100 series LC/MSD Trap SL mass spectrometer. The HPLC separation was performed on an Agilent 1200 series with a DIKMA (4.6 × 250 mm) analytical column packed with C18 (5 μm) and the preparative HPLC was conducted by a Shimadzu LC-6AD instrument with a SPD-20A detector and an YMC-Pack ODS-A column (250 × 20 mm, 5 μm) [6]. The column chromatography was performed with Sephadex LH-20, Toyopearl HW-40, and silica gel (160–200 mesh, 200–300 mesh). The TLC was carried out on precoated silica gel GF254 plates and the spots were visualized under UV light (254 or 365 nm) or by spraying with 10% sulfuric acid in EtOH followed by heating [10].

2.2. Plant material

Murraya koenigii was harvested from Xi Shuang Ban Na Tropical Botanical Garden, the Chinese Academy of Sciences, Kunming, China in August 2010. This plant was identified by Prof. Lin Ma (Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College). A voucher specimen (No. ID-S-2436) was deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China [4].

2.3. Extraction and isolation

The air-dried *Murraya koenigii* (22.50 kg) was extracted three times with 95% EtOH (60 L) of heating under reflux for 3 h each time. The solvent was concentrated to afford 2.10 kg of dark crude extract, which was successively partitioned with $CHCl_3$ (3 × 6000 mL), EtOAc (3 × 6000 mL), and *n*-butanol (3 × 6000 mL) to yield three fractions: $CHCl_3$ soluble fraction (33.50 g), EtOAc soluble fraction (24.90 g), and *n*-butanol soluble fraction (221.20 g), respectively [11]. According to the screening results of bioactivity-guided investigation, the EtOAc soluble fraction exhibited potential inhibitory activities by measuring IL-6-induced STAT3 promoter activities in HepG2 cells and inhibition against lipopolysaccharide (LPS)-induced NO production in RAW264.7 macrophages.

The EtOAc soluble fraction was chromatographed over silica gel (200–300 mesh, 100 g, 3.0 × 60 cm) eluting with a gradient elution (petroleum ether/acetone = 10:1 → 1:1 → 1:5, v/v) to yield three fractions: SMJY-B (4.20 g), XMJY-Y (10.50 g), and SMJY-Y (8.80 g). The SMJY-Y fraction was chromatographed over Sephadex LH-20 eluting with 90% MeOH to give two sub-fractions: SMJY-Y-a (2.35 g) and SMJY-Y-b (3.10 g). The separation of SMJY-Y-a was separated by MPLC (25–40% MeOH) and preparative HPLC (detection at 203 nm, 6 mL/min), successively, yielded **1** (7.34 mg, purity > 96% by HPLC), **2** (8.14 mg, purity > 97% by HPLC), and **5** (5.08 mg, purity > 96% by HPLC). The separation of SMJY-Y-b was separated by MPLC (20–40% MeOH) and preparative HPLC (detection at 205 nm, 6 mL/min), successively, yielded **6** (9.22 mg, purity > 95% by HPLC) and **14** (51.46 mg, purity > 96% by HPLC). In a similar way, the XMJY-Y fraction was also chromatographed over Sephadex LH-20 eluting with 95% MeOH to give two sub-fractions: XMJY-Y-a (3.70 g) and XMJY-Y-b (4.05 g). The separation of XMJY-Y-a was separated by MPLC (30–40% MeOH) and preparative HPLC (detection at 204 nm, 6 mL/min), successively, yielded **3** (6.22 mg, purity > 96% by HPLC), **7** (4.37 mg, purity > 97% by HPLC), **8** (6.21 mg, purity > 95% by HPLC), **9** (4.24 mg, purity > 97% by HPLC), **10** (4.25 mg, purity > 95% by HPLC), **11** (5.21 mg, purity > 96% by HPLC), and **15** (14.30 mg, purity > 96% by HPLC). The separation of XMJY-Y-b was separated by MPLC (35–45% MeOH) and preparative HPLC (detection at 204 nm, 6 mL/min), successively, yielded **4** (6.26 mg, purity > 96% by HPLC), **12** (5.33 mg, purity > 97% by HPLC), and **13** (2.47 mg, purity > 96% by HPLC). The structures of compounds (**1–15**) were shown in Fig. 1.

2.4. IL-6-induced STAT3 luciferase assay

Interleukin-6 (IL-6) is involved in a broad spectrum of inflammatory diseases, it has been postulated to be an effective therapy in the pathogenesis of several inflammatory diseases [12]. These diseases are commonly characterized by excessive IL-6 levels that lead to the induction of IL-6 signaling cascades, resulting in activation of the Janus kinase (Jak)/signal transducer and activator of transcription (STAT) pathways in the intracellular environment [13]. STAT3, one of the STAT family proteins, functions as a signal transducer in the cytoplasm and as a transcription factor in the nucleus [14]. STAT3-regulated genes are closely related to the production of pro-inflammatory cytokines or enzymes that cause the above-mentioned inflammatory diseases [15].

The HepG2 cells were put in Dulbecco's modified Eagle's medium (10% fetal bovine serum) and were cultured in the 96-well culture plate (5×10^4 cells/mL) with 5% CO_2 at 37 °C for 24 h. The HepG2 cells were exposed to a variety of concentrations of compounds (**1–15**), and these cells were incubated for an hour with IL-6 (10 ng/mL). After 6 h, the HepG2 cells were washed with PBS and treated with a lysis buffer (50 μL) for 15 min, followed by intermittent shaking at room temperature. The cell lysate (25 μL) was transferred to a white microtiter plate, and 50 μL of a luciferase assay reagent (Promega, Madison, WI) was mixed into each well. The luciferase activity was measured by a luminometer. The HepG2 cells were measured using a MTT assay which incubated in a 96-well plate for 24 h and treated with compounds (**1–15**) at the indicated concentrations for 48 h [16].

2.5. Inhibitory assay of NO production

The RAW264.7 macrophages were cultured in 24-well plates (105 cells/well), and they were maintained in a water-saturated atmosphere of 5% CO_2 at 37 °C. These cells were co-incubated with drugs and LPS (1 μg/mL) for 24 h. The amount of NO was assessed by determined the nitrite concentration in the cultured RAW264.7 macrophage supernatants with Griess reagent. Aliqueots of supernatants (100 μL) were incubated with 1% sulphanilamide (50 μL), 1% naphthylethylenediamine (50 μL) in 2.5% phosphoric acid solution, sequencely. The absorbances of compounds (**1–15**) were read using a microtiter plate reader at 570 nm [17].

3. Results and discussion

3.1. Spectroscopic data

(7′*E*,8*S*)-9′-hydroxy-7′-propen-3′,5′-dimethoxyphenyl-3-methoxyphenyl-7,9-propanediol-4-O-*β*-D-glu-copyranoside **(1)**: colourless powder; $[\alpha]_D^{20}$ −11.20 (*c* 0.30, MeOH); mp 214.3–215.5 °C; UV (MeOH) λ_{max}: 203 and 274 nm; IRν_{max} 3372.3, 1660.9, 1598.9, and 1501.5 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 1; HR-ESI-MS: *m/z* 575.2112 $[M+Na]^+$ (calcd. for $C_{27}H_{36}NaO_{12}$, 575.2099).

(7*R*)-2,6-dimethoxyphenyl-7,9-propanediol-1-O-*β*-D-glucopyranoside **(2)**: colourless powder; $[\alpha]_D^{20}$ –6.10 (*c* 0.12, MeOH); mp 184.2–185.6 °C; UV (MeOH) λ_{max}: 203 nm; IRν_{max} 3369.0, 1642.3, 1597.8, and 1509.3 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 1; HR-ESI-MS: *m/z* 413.1425 $[M+Na]^+$ (calcd. for $C_{17}H_{26}NaO_{10}$, 413.1418).

(2′*R*,4′*R*,7*S*)-2′,4-dihydroxy-3-methoxyphenyl-4′-hydroxymethyl-tetrahydro-1*H*-pyran-1-one **(3)**: colourless crystal; $[\alpha]_D^{20}$ +5.50 (*c* 0.02, MeOH); mp 128.4–129.3 °C; UV (MeOH) λ_{max}: 204, 230, and 281 nm; IRν_{max} 3392.8, 1763.1, 1603.5, and 1518.3 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 2; HR-ESI-MS: *m/z* 291.0854 $[M+Na]^+$ (calcd. for $C_{13}H_{16}NaO_6$, 291.0839).

(1*R*,10*S*)-1-hydroxy-7-(10-hydroxybutyl)-2,3-dihydrobenzofuran-8(6*H*)-one **(4)**: yellowish oil; $[\alpha]_D^{20}$ +4.65 (*c* 0.15, MeOH); UV (MeOH) λ_{max}: 202 and 274 nm; IRν_{max} 3365.1 and 1737.8 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 2; HR-ESI-MS: *m/z* 247.0946 $[M+Na]^+$ (calcd. for $C_{12}H_{16}NaO_4$, 247.0941).

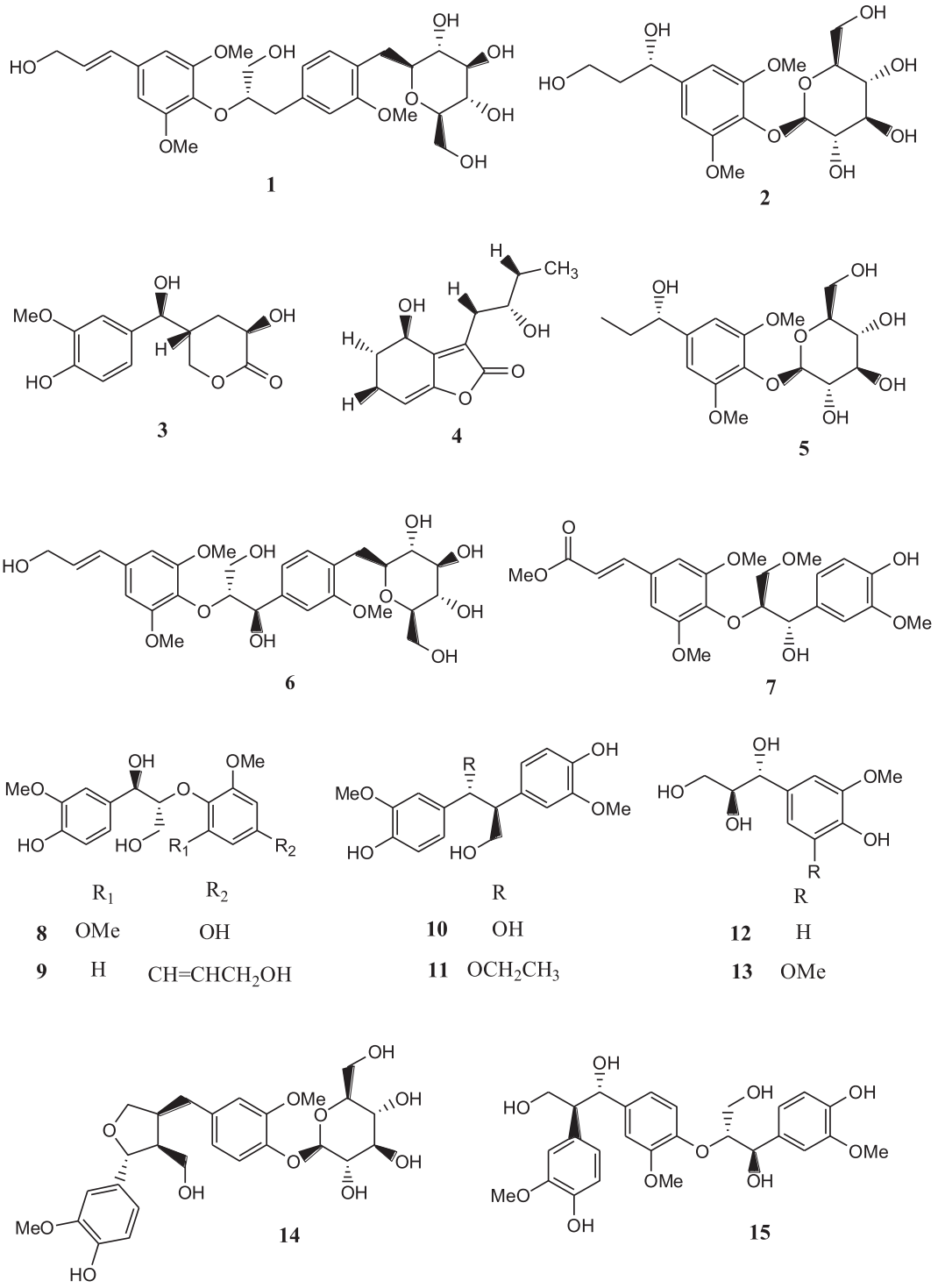

Fig. 1. Structure of compounds **1–15**.

3.2. Structure determination

Compound **1** was isolated as a colorless powder. Its HR-ESI-MS data showed a sodium adduct ion at *m/z* 575.2112 $[M+Na]^+$, establishing the molecular formula as $C_{27}H_{36}O_{12}$, indicating 10 degrees of unsaturation. The IR spectrum showed absorption bands for a hydroxyl group (3372.3 cm^{-1}) and an aromatic ring group (1660.9, 1598.9, 1501.5 cm^{-1}) and its UV spectrum exhibited at λ_{max}: 203 and 274 nm.

The ^1H NMR data of compound **1** indicated the presence of the 1,3,4-trisubstituted benzene system at δ_H 6.72 (1H, d, J = 1.5 Hz, H-2), 6.97 (1H, d, J = 7.5 Hz, H-5), 6.97 (1H, dd, J = 7.5, 1.5 Hz, H-6) and a single peak at δ_H 6.75 (2H, s, H-2′/6′) in the aromatic field (Table 1). Moreover, a set of typical signals at δ_H 6.58 (1H, d, J = 15.9 Hz, H-7′), 6.27 (1H, dt, J = 15.9, 6.0 Hz, H-8′), and 4.22 (2H, d, J = 76.0 Hz, H-9′), which revealed a trans-propenol group in compound **1**, it was connected to aromatic ring indicated the phenylpropanoid skeleton [18] was in compound **1**. A characteristic doublet at δ_H 4.86 (1H, d, J = 7.5 Hz, H-1″) was ascribed to the anomeric proton of glucosyl unit, corresponding to δ_C 105.3 (C-1″) of the ^{13}C NMR and HSQC spectra (Table 1), which indicated the presence of the glucose was in compound **1**. The fragments of compound **1** was determined by the HMBC correlations of H-2/C-4, C-7; H-5/C-1; H-6/C-7; H-7/C-9; H-8/C-4′; H-7′/C-

Table 1
^{1}H NMR (300 MHz, CD_3OD), ^{13}C NMR(125 MHz, CD_3OD), and key HMBC correlations of compounds **1** and **2**.

No.	**1**			**2**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	–	129.9	–	–	135.2	–
2	6.72(d,1.5)	114.2	C-4,C-7	–	154.2	–
3	–	145.6	–	6.73(s)	104.7	C-1
4	–	135.7	–	–	143.5	–
5	6.97(d,7.5)	116.5	C-1	6.73(s)	104.7	C-1
6	6.97(dd,7.5,1.5)	112.2	C-7	–	154.2	–
7	3.42(m)	55.8	C-9	4.78(dd,8.1,3.0)	72.3	C-3,C-5,C-9
8	5.60(dd,9.6,5.7)	88.8	C-4′	1.92(m)	42.9	–
9a	3.86(m)	64.9	–	3.72(m)	60.0	–
9b	3.79(m)	64.9	–	3.63(m)	60.0	–
1′	–	132.8	–	4.84(d,7.5)	105.5	C-1,C-3′
2′	6.75(s)	104.4	–	3.44(m)	75.7	–
3′	–	154.5	–	3.21(m)	78.3	C-5′
4′	–	140.0	–	3.43(m)	71.3	C-6′
5′	–	154.5	–	3.42(m)	77.8	–
6′a	6.75(s)	104.4	–	3.81(m)	62.6	–
6′b	6.75(s)	104.4	–	3.68(m)	62.6	–
7′	6.58(d,15.9)	131.9	C-2′, C-6′, C-9′	–	–	–
8′	6.27(dt,15.9,6.0)	127.7	–	–	–	–
9′	4.22(d,6.0)	63.8	–	–	–	–
1″	4.86(d,7.5)	105.3	C-4,C-3″	–	–	–
2″	3.49(m)	75.7	–	–	–	–
3″	2.27(m)	78.3	C-5″	–	–	–
4″	3.43(m)	71.3	C-6″	–	–	–
5″	3.47(m)	77.8	–	–	–	–
6″a	3.83(m)	62.6	–	–	–	–
6″b	3.68(m)	62.6	–	–	–	–
2-OCH_3	–	–	–	3.87(s)	56.9	–
6-OCH_3	–	–	–	3.87(s)	56.9	–
3′-OCH_3	3.83(s)	57.0	–	–	–	–
5′-OCH_3	3.91(s)	56.8	–		–	–

Table 2
^{1}H NMR (300 MHz, CD_3COCD_3), ^{13}C NMR(125 MHz, CD_3COCD_3), and key HMBC correlations of compounds **3** and **4.**

No.	**3**			**4**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	–	132.5	–	3.99(m)	70.4	C-7
2	7.04(d,1.8)	110.6	C-4	2.05(m)	24.7	C-6
3	–	148.4	–	2.55(m)	17.7	C-5
4	–	147.3	–	5.39(t,15.6,7.8)	112.3	–
5	6.82(d,8.1)	115.6	C-1	2.22(d,6.0)	153.9	–
6	6.88(dd,8.1,1.8)	119.8	–	–	149.6	–
7	4.68(d,6.3)	87.0	C-2,C-3′	–	126.9	–
8	–	–	–	–	169.2	–
9	–	–	–	2.33(m)	28.6	C-8
10	–	–	–	4.28(m)	65.0	–
11	–	–	–	1.49(dd,14.7,7.2)	22.9	C-12
12	–	–	–	0.95(dd,14.7,7.2)	14.0	C-9
1′	–	179.1	–	–	–	–
2′	3.22(m)	70.6	C-4	–	–	–
3′a	4.36(dd,9.6,2.1)	46.9	–	–	–	–
3′b	4.03(dd,9.0,3.3)	46.9	–	–	–	–
4′	3.54(dd,9.0,3.6)	49.1	–	–	–	–
5′a	4.54(dd,9.6,6.9)	70.8	C-1′	–	–	–
5′b	4.28(m)	70.8	C-1′	–	–	–
3-OCH_3	3.85(s)	56.2	–	–	–	–

2′, C-6′, C-9′; H-1″/C-4, C-3″; H-3″/C-5″; H-4″/C-6″ (Fig. 2) and the ^{1}H-^{1}H COSY correlations of H-5/H-6; H-7/H-8; H-7′/H-8′; H-8′/H-9′ (Fig. 2). The absolute configuration of C-8 in compound **1** was determined by the CD method. Compound **1** was hydrolyzed to afford the aglycone **1**a [19]. The negative cotton effect at 274 nm was observed in the CD spectrum of compound **1**a, which was induced by reagent of $Rh_2(OCOCF_3)_4$. According to the spiral rule and literature [20], the absolute configuration of C-8 was determined as 8*S*. Therefore, compound **1** was identified as (7′*E*,8*S*)-9′-hydroxy-7′-propen-3′,5′-dimethoxyphenyl-3-methoxyphenyl-7,9-propanediol-4-O-*β*-D-glu-copyranoside.

Compound **2** was acquired as a colorless powder with a rotation of $[\alpha]_D^{20}$ −6.10 (*c* 0.12, MeOH). Its molecular formula was determined to be $C_{17}H_{26}O_{10}$ on the basis of the HR-ESI-MS: *m/z* 413.1425 $[M+Na]^+$ (calcd. for $C_{17}H_{26}NaO_{10}$ 413.1418) and NMR spectroscopic data (Table 1). According to the spectroscopic data of UV (MeOH) λ_{max}: 203 nm and IRν_{max} 3369.0, 1642.3, 1597.8, 1509.3 cm^{-1}, it was concluded that compound **2** was a derivative of phenylpropanoid [18].

The ^{1}H NMR spectrum of compound **2** showed a single peak at δ_H 6.73 (2H, s, H-3/5) in the aromatic field. A characteristic glucosyl unit was found at δ_H 4.84 (1H, d, J = 7.5 Hz, H-1′) and δ_C 105.5 (C-1″) in the ^{1}H NMR and ^{13}C NMR spectra (Table 1), which indicated the presence of the glucose was in compound **2**. In the middle of ^{1}H NMR spectrum, a 1,3-propanediol unit was found at δ_H 4.78 (1H, dd, J = 8.1, 3.0 Hz, H-7), 1.92 (2H, m, H-8), 3.72 (1H, m, H-9a), 3.63 (1H, m, H-9b), corresponding to δ_C 72.3 (C-7), 42.9 (C-8), 60.0 (C-9) of the ^{13}C NMR and HSQC spectra (Table 1). The fragments of compound **2** were connected by some key correlations of H-3/C-1; H-5/C-1; H-7/C-3, C-5, C-9; H-1′/C-1, C-3′; H-3′/C-5′; H-4′/C-6′ in the HMBC spectrum (Fig. 2) and some key correlations of H-7/H-8; H-8/H-9 in the ^{1}H-^{1}H COSY spectrum (Fig. 2). Compound **2** was hydrolyzed to afford the aglycone **2**a [19]. The positive cotton effect at 273 nm and the negative cotton effect at 327 nm were observed in the CD spectrum of compound **2**a. The absolute configuration of C-7 in compound **2** was determined as 7*R* according to the spiral rule and literature [20]. Consequently, compound **2** was identified as (7*R*)-2,6-dimethoxyphenyl-7,9-propanediol-1-O-*β*-D-glucopyranoside.

Compound **3** was obtained as a colorless crystal with a molecular formula of $C_{13}H_{16}O_6$, which was determined based on HR-ESI-MS at *m/z* 291.0854 (calcd for $C_{13}H_{16}O_6Na$, 291.0839), indicating 6 degrees of unsaturation. Its UV spectrum showed absorptions at λ_{max}: 204, 230, 281 nm and IR absorptions indicated the existence of hydroxyl (3392.8 cm^{-1}), carbonyl (1763.1 cm^{-1}), aromatic ring (1603.5, 1518.3 cm^{-1}) functional groups.

The ^{1}H NMR spectrum of compound **3** showed an ABX system at δ_H 7.04 (1H, d, J = 1.8 Hz, H-2), 6.82 (1H, d, J = 8.1 Hz, H-5), 6.88 (1H, dd, J = 8.1, 1.8 Hz, H-6). Moreover, a methoxyl signal at δ_H 3.85 (3H, s) and a double peak at δ_H 4.68 (1H, d, J = 6.3 Hz, H-7) were observed in the middle of ^{1}H NMR spectrum of compound **3** (Table 2). Additionally, a saturated cyclohexanone [δ_C 179.1 (C-1′), 70.6 (C-2′), 46.9 (C-3′), 49.1 (C-4′), 70.8 (C-5′)] was determined by its molecular formula, degrees of unsaturation, and NMR spectrum of compound **3** [21]. The relative structure of compound **3** was determined by the HMBC correlations of H-2/C-4; H-5/C-1; H-7/C-2, C-3′; H-2′/C-4′; H-5′/C-1′ (Fig. 2) and the ^{1}H-^{1}H COSY correlations of H-5/H-6; H-2′/H-3′; H-3′/H-4′; H-4′/H-7 (Fig. 2). The absolute configurations of C-2′, C-4′ in compound **3** were determined by the CD method. The 7-OH of compound **3** was protected by *tert*-butyldimethylsilyl chloride (TBSCl) in order to eliminate the interference of measuring its CD spectrum, and afforded the derivative **3**a. The negative cotton effect at 256 nm was observed in the CD spectrum of compound **3**a, which was induced by reagent of $Rh_2(OCOCF_3)_4$. The absolute configurations of C-2′, C-4′ in compound **3** were confirmed as 2′*R*, 4′*R* according to the octant rule of lactone and reference [22]. Meanwhile, the absolute configuration of C-7 in compound **3** was determined as 7*S* by the correlation between 7-OH/2′-OH and 7-OH/H-4′ in the 2D-NOESY spectrum and the Cahn-Ingold-Prelog order rule [23]. Hence, compound **3** was identified as (2′*R*,4′*R*,7*S*)-2′,4-dihydroxy-3-methoxyphenyl-4′-hydroxymethyl-tetrahydro-1*H*-pyran-1-one.

Compound **4** was obtained as a yellowish oil, the molecular formula

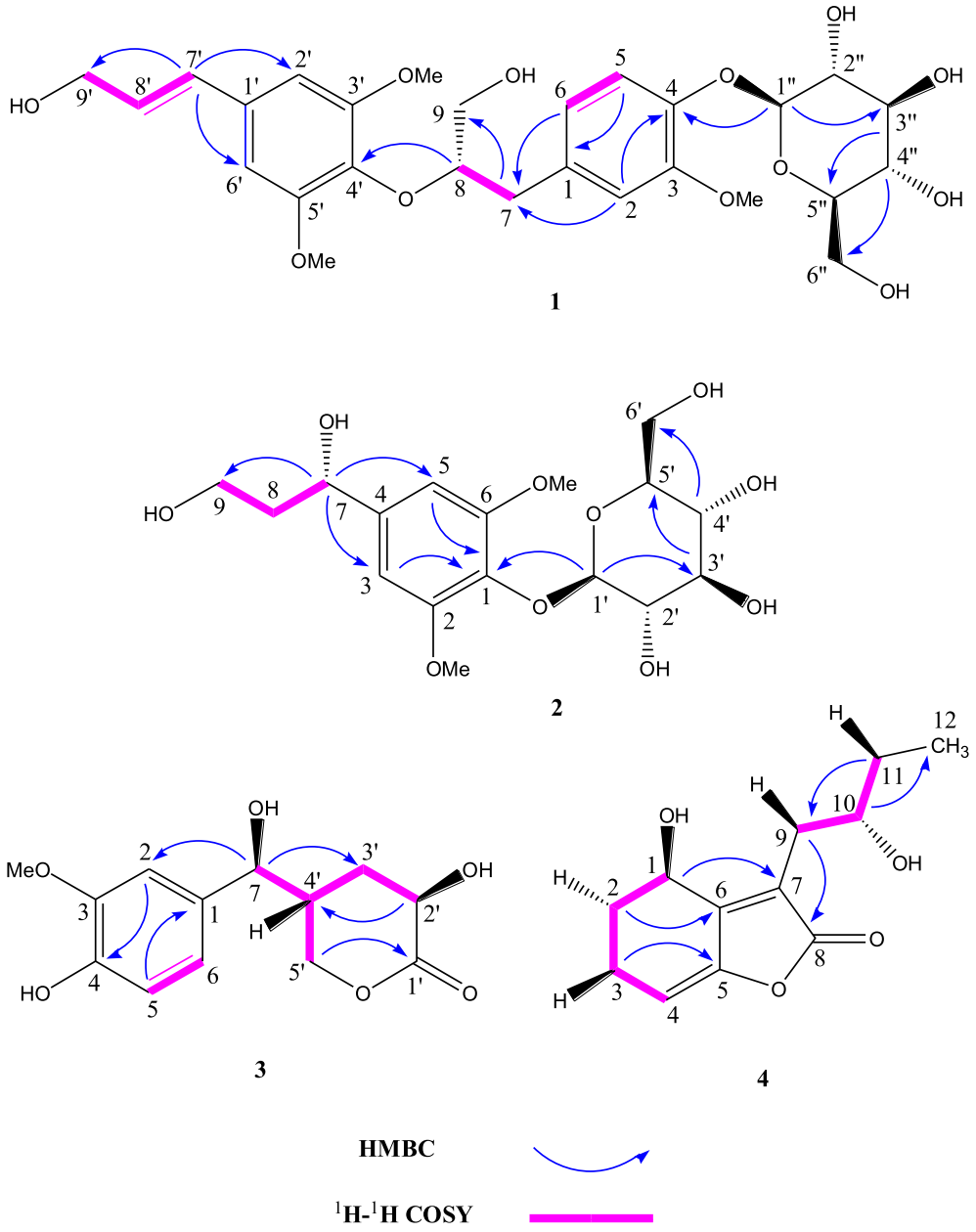

Fig. 2. Key HMBC and ^{1}H-^{1}H COSY correlations of compounds **1**–**4**.

of compound **4** was determined as $C_{12}H_{16}O_4$ by HR-ESI-MS showed $[M+Na]^+$ at *m/z* 247.0946 $[M+Na]^+$ (calcd. for $C_{12}H_{16}NaO_4$, 247.0941), indicating 5 degrees of unsaturation. Its UV spectrum displayed absorbance at λ_{max}: 202 and 274 nm, and its IR spectrum showed the absorption bands of hydroxyl (3365.1 cm^{-1}) and carbonyl (1737.8 cm^{-1}) functionalities.

A typical triplet at δ_H 5.39 (1H, t, *J* = 15.6, 7.8 Hz, H-4) and three saturated carbon signals at δ_C 70.4 (C-1), 24.7 (C-2), 17.7 (C-3) were found in the NMR spectra of compound **4**, which revealed the typical characteristic of the cyclohexene skeleton [24]. In the middle of ^{1}H NMR spectrum, two typical multiple peaks at δ_H 3.99 (1H, m, H-1), 4.28 (1H, m, H-10), corresponding to δ_C 70.4 (C-1), 65.0 (C-10) of the ^{13}C NMR and HSQC spectra (Table 2), which indicated two oxygenate carbons were in compound **4**. The fragment of $-CH_2CH_3$ at δ_H 1.49 (2H, dd, *J* = 14.7, 7.2 Hz), 0.95 (3H, t, *J* = 14.7, 7.2 Hz) were observed in the high of ^{1}H NMR spectrum, corresponding to δ_C 22.9 (C-11), 14.0 (C-12) of the ^{13}C NMR and HSQC spectra (Table 2). The fragments of compound **4** were connected by some key correlations of H-1/C-7; H-2/C-6; H-3/C-5; H-9/C-8; H-10/C-12; H-11/C-9 in the HMBC spectrum (Fig. 2) and some key correlations of H-1/H-2; H-2/H-3; H-3/H-4; H-9/H-10; H-10/H-11 in the ^{1}H-^{1}H COSY spectrum (Fig. 2). The absolute configurations of C-1 in compound **4** was determined as 1*R* on the basis of biosynthetic pathway and reference [25]. In the same way, the 1-OH of compound **4** was protected by *tert*-butyldimethylsilyl chloride (TBSCl) in order to eliminate the interference of measuring CD spectrum of C-10, and afforded the derivative **4**a. The positive cotton effect at 232 nm and the negative Cotton effect at 277 nm were observed in the CD spectrum of compound **4**a, which were induced by reagent of $Rh_2(OCOCF_3)_4$. The absolute configurations of C-10 in compound **4** were confirmed as 10*S* according to the octant rule and reference [25]. So, compound **4** was determined as (1*R*,10*S*)-1-hydroxy-7-(10-hydroxybutyl)-2,3-dihydrobenzofuran-8(6*H*)-one.

Additionally, eleven known compounds (**5**–**15**) belonging to phenylpropanoid derivatives, which were isolated and identified as (1′*R*)-4-O-*β*-D-glucopyranoside-3,5-dimethoxyphenyl-1′-propanol (**5**) [26], citrusin B (**6**) [27], (7′*R*,8′*R*,8*E*)-4′,7′-dihydroxy-3′-methoxyphenyl-8′-hydroxymethyl-ethoxy-3,5-dimethoxyphenyl-8-propenoic acid methyl ester (**7**) [28], (7*S*,8*S*)-1′-hydroxy-3′,5′-dimethoxyphenoxy-4-hydroxy-

Table 3
Inhibitory effects of compounds (**1–15**) on IL-6-induced STAT3 activation.[a]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	11.5 ± 1.1	**9**	> 100
2	18.7 ± 3.8	**10**	> 100
3	> 100	**11**	> 100
4	> 100	**12**	> 100
5	> 100	**13**	> 100
6	8.9 ± 6.8	**14**	> 100
7	> 100	**15**	22.7 ± 5.4
8	> 100	**Genistein**[b]	24.5 ± 1.9

[a] Values are mean ± SD of three experiments, with each data point done in triplicate.
[b] Genistein was used as the positive control.

3-methoxyphenyl-7,9-propanediol (**8**) [29], (7′*E*,7*S*,8*S*)-9′-hydroxy-7′-propen-3′-methoxyphenyl-4-hydroxy-3-methoxyphenyl-7,9-propanediol (**9**) [30], (1*S*,2*R*)-4,4′-hydroxy-3,3′-methoxyphenyl-1,3-propanediol (**10**) [31], (7*S*,8*R*)-4,4′-dihydroxy-3,3′-dimethoxyphenyl-7-ethoxy-9-propanol (**11**) [32], (7*S*,8*R*)-4-hydroxy-3-methoxyphenyl-7,8,9-propanetriol (**12**) [33], (7*S*,8*R*)-4-hydroxy-3,5-dimethoxyphenyl-7,8,9-propanetriol (**13**) [34], lariciresinol-4-O-*β*-D-glucopyranoside (**14**) [35], and (7*S*,7′*S*,8*S*,8′*R*)-4,4″,7′,9′-Tetrahydroxy-3,3′,3″-trimethoxyphenyl-7,9-propanediol (**15**) [36] by comparison of their physical and spectroscopic data with those reported in the references. To the best of our knowledge, the known compounds (**5–15**) were isolated from this plant for the first time.

3.3. Acid hydrolysis of compounds *1, 2, 5, 6, and 14*

Compounds **1**, **2**, **5**, **6**, and **14** (5 mg each) were treated in 5% HCl (0.5 mL) and heated at 90 °C for 2 h, respectively [37]. After cooling, each reaction mixture was extracted with EtOAc, and the aqueous layer was neutralised with 0.1 M NaOH. As a result, the glucoses were obtained from compounds **1**, **2**, **5**, **6**, and **14**, which were detected by thin layer chromatography (TLC) with authentic sugars. The type of glucose was identified by TLC method with authentic sugar [4].

3.4. Statistical analysis of biological activities

Compounds (**1–15**) were evaluated for their ability to inhibit the activation of IL-6-induced STAT3 in HepG2 cells with genistein as the positive control. The HepG2 cells stably expressing pSTAT3-luciferase were excited with IL-6 (10 ng/mL) for 6 h in the presence of the test compounds (**1–15**), and pSTAT3-inducible luciferase activity was then measured [16]. Compounds **1**, **2**, **6**, and **15** displayed important inhibitory effects, with IC_{50} values of 11.5, 18.7, 8.9, and 22.7 μM, respectively, and genistein was used as the positive control (IC_{50} = 24.5 μM). The other compounds exhibited weak or no inhibitory effects (IC_{50} > 100 μM) (Table 3).

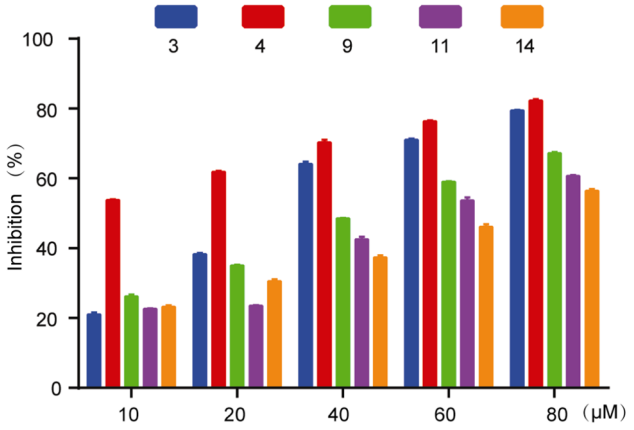

Fig. 3. Inhibitory activities of selected compounds against LPS-induced NO production.

The inhibitory activities of compounds (**1–15**) were screened against NO production in lipopolysaccharide (LPS)-induced RAW264.7 macrophage cells [38]. As shown in Table 4, compounds **3**, **4**, **9**, **11**, and **14** showed certain inhibitions against LPS-induced NO production in RAW264.7 macrophages, with IC_{50} values of 32.7, 7.9, 42.1, 58.9, and 62.4 μM, respectively. Dexameth was used as positive control with an IC_{50} value of 8.5 μM. Among them, compound **4** showed obvious inhibitions against LPS-induced NO production in RAW264.7 macrophages, and compound **3**, **9**, **11**, and **14** showed moderate inhibitions against LPS-induced NO production in RAW264.7 macrophages. However, the other compounds exhibited no inhibitions against LPS-induced NO production in RAW264.7 macrophages with IC_{50} > 100 μM (Fig. 3).

Table 4
Inhibitory activities of (**1–15**) against LPS-induced NO production in RAW264.7 macrophages.[a]

Compound	Inhibition (%)					IC_{50}(μM)
	80 μM	60 μM	40 μM	20 μM	10 μM	
dexamethasone[b]	–	–	–	–	–	8.5
1	41.37 ± 0.38	30.12 ± 0.17	26.24 ± 0.73	23.36 ± 0.35	17.02 ± 0.52	> 100
2	38.59 ± 0.24	28.10 ± 0.19	21.77 ± 0.13	19.29 ± 0.32	12.38 ± 0.49	> 100
3	79.47 ± 0.12	71.08 ± 0.30	64.11 ± 0.65	38.28 ± 0.33	21.05 ± 0.54	32.7
4	82.33 ± 0.36	76.39 ± 0.24	70.26 ± 0.79	61.85 ± 0.29	53.76 ± 0.29	7.9
5	36.76 ± 0.15	33.28 ± 0.38	27.55 ± 0.84	20.23 ± 0.33	19.22 ± 0.66	> 100
6	46.25 ± 0.82	36.73 ± 0.50	31.64 ± 0.34	24.65 ± 0.31	22.60 ± 0.14	> 100
7	50.15 ± 0.63	34.22 ± 0.58	30.88 ± 0.60	26.35 ± 0.16	24.19 ± 0.25	> 100
8	39.08 ± 0.22	31.21 ± 0.13	28.42 ± 0.54	20.25 ± 0.33	16.62 ± 0.11	> 100
9	67.24 ± 0.28	59.08 ± 0.16	48.50 ± 0.13	35.07 ± 0.23	26.21 ± 0.53	42.1
10	41.19 ± 0.15	34.10 ± 0.17	29.44 ± 0.18	22.25 ± 0.55	20.77 ± 0.58	> 100
11	60.74 ± 0.24	53.71 ± 0.84	42.56 ± 0.71	23.54 ± 0.14	22.59 ± 0.16	58.9
12	37.09 ± 0.33	33.23 ± 0.17	26.44 ± 0.52	21.30 ± 0.45	18.09 ± 0.53	> 100
13	42.22 ± 0.10	39.48 ± 0.13	28.80 ± 0.36	24.62 ± 0.17	19.44 ± 0.33	> 100
14	56.44 ± 0.48	46.13 ± 0.77	37.35 ± 0.64	30.54 ± 0.57	23.18 ± 0.42	62.4
15	42.16 ± 0.52	37.13 ± 0.44	33.27 ± 0.47	28.42 ± 0.35	20.41 ± 0.27	> 100

[a] NO concentration of control group: 2.12 ± 0.11 μM, NO concentration of LPS-treated group: 42.30 ± 0.22 μM.
[b] Positive control.

4. Conclusion

In this work, four new phenylpropanoid derivatives (**1–4**), named (7′*E*,8*S*)-9′-hydroxy-7′-propen-3′,5′-dimethoxyphenyl-3-methoxyphenyl-7,9-propanediol-4-O-*β*-D-glu-copyranoside (**1**), (7*R*)-2,6-dimethoxyphenyl-7,9-propanediol-1-O-*β*-D-glucopyranoside (**2**), (2′*R*,4′*R*,7*S*)-2′,4-dihydroxy-3-methoxyphenyl-4′-hydroxymethyl-tetrahydro-1*H*-pyran-1-one (**3**), (1*R*,10*S*)-1-hydroxy-7-(10-hydroxybutyl)-2,3-dihydrobenzofuran-8(6*H*)-one (**4**), together with analogues (**5–15**) were isolated and identified by spectroscopic analysis and references from the active site (EtOAc soluble fraction) from *M. koenigii* for the first time. Compounds (**1–15**) were evaluated for their inhibitory activities by measuring IL-6-induced STAT3 promoter activities in HepG2 cells, and found compounds **1**, **2**, **6**, and **15** showed inhibitory effects with IC_{50} values of 11.9, 12.2, 14.0, 7.0, and 6.9 μM, respectively. The inhibitory activities of compounds (**1–15**) were screened against NO production in lipopolysaccharide (LPS)-induced RAW264.7 macrophage cells, and found compounds **3**, **4**, **9**, **11**, and **14** exhibited inhibitions against LPS-induced NO production in RAW264.7 macrophages, with IC_{50} values of 32.7, 7.9, 42.1, 58.9, and 62.4 μM, respectively.

Conflict of interest statement

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with the new compounds (**1–4**). Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.038.

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