RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2021, **11**, 30725

Illiciumlignans G–O from the leaves of *Illicium dunnianum* and their anti-inflammatory activities†

Sen-Ju Ma, ‡[a] Hai-Bo Li, ‡[b] Ting Li, [a] Zhen-Zhen Su, [b] Zhen-Zhong Wang, [b] Xin-Sheng Yao, *[a] Wei Xiao *[b] and Yang Yu *[a]

Received 6th May 2021
Accepted 10th June 2021

DOI: 10.1039/d1ra03520g

rsc.li/rsc-advances

Phytochemical investigations on the dry leaves of *Illicium dunnianum* have led to the isolation of 24 lignans. Illiciumlignans G–K (**1–5**) were five undescribed benzofuran lignans, illiciumlignan L (**6**) was one undescribed ditetrahydrofuran lignan, illiciumlignans M–O (**7–9**) were three new sesquilignans, and compounds **10**, **12**, **13**, **15**, and **18–21** were firstly isolated from the genus *Illicium*. Their structures were elucidated by detailed spectroscopic analyses (UV, IR, HR-ESI-MS, and NMR) and CD experiments. All isolates were evaluated by measuring their inhibitory effects on PGE_2, and NO production in LPS-stimulated RAW 264.7 macrophages.

1 Introduction

The genus *Illicium* L. belongs to the family Magnoliaceae. It is commonly distributed in the southwest and east of China, with a total of 28 species (including 2 varieties).[1] *Illicium* plants have a long medical history, which was recorded as early as the Ming Dynasty in Compendium of Materia Medica. *Illicium dunnianum* is a folk plant found throughout Southern China and used as a medicine for relieving pain and treating rheumatism.[2] Modern pharmacological studies have shown that it possesses multiple biological activities, such as anti-inflammatory,[3,4] central and peripheral analgesic effects,[5,6] relieving gastrointestinal smooth muscle spasm, and regulating immune activities.[4] Previous phytochemical investigations of *I. dunnianum* indicated the presence of sesquiterpenes,[7–11] phenylpropanoids,[7,12–15] phenol glycosides,[16] flavonoids,[2,17,18] triterpenes,[2,14] and others.[2,7,17] Up to now, research mainly focused on the fruit and roots of *I. dunnianum* and there is a lack of chemical studies of its leaves. To explore the bioactive constituents, the chemical constituents of the leaves of *I. dunnianum* were investigated, and 24 lignans were isolated (Fig. 1), of which illiciumlignans G–O (**1–9**) were new lignans, and compounds **10**, **12**, **13**, **15**, **18–21** were isolated from the genus *Illicium* for the first time. In addition, all isolates were evaluated for their inhibitory effects on PGE_2 and NO. Herein, the isolation, structure elucidation, and anti-inflammatory activities assay of these compounds are reported.

2 Results and discussion

2.1 Structural elucidation

Compound **1** was obtained as a brown amorphous powder with an optical rotation of $[\alpha]_D^{25}$ −35.2 (*c* 0.75, MeOH). Its molecular formula was determined as $C_{31}H_{42}O_{14}$ by analysing the HRESIMS peak at *m*/*z* 661.2471 $[M + Na]^+$ (calcd for 661.2472). The ^1H NMR (Table 1) spectrum displayed the presence of one 1,2,4-trisubstituted aromatic ring [δ_H 6.96 (1H, d, J = 1.8 Hz, H-2), 6.83 (1H, dd, J = 8.2, 1.8 Hz, H-6), 6.75 (1H, d, J = 8.2 Hz, H-5)] and one 1,2,3,5-tetrasubstituted aromatic ring [δ_H 6.73 (2H, br s, H-2′, H-6′)]. Signals for one oxymethine [δ_H 5.57 (1H, d, J = 5.8 Hz, H-7)], two oxymethylenes [δ_H 3.92 (2H, m, H-9), 3.57 (2H, t, J = 6.5 Hz, H-9′)], one methine [δ_H 3.62 (1H, m, H-8)], two methylenes [δ_H 2.62 (2H, t, J = 7.7 Hz, H-7′), 1.82 (2H, m, H-8′)] and two methoxys [δ_H 3.85 (3H, s, 5′-OCH_3), 3.83 (3H, s, 3-OCH_3)], along with two anomeric proton signals [δ_H 4.35 (1H, d, J = 6.9 Hz, H-1″), 5.18 (1H, d, J = 1.3 Hz, H-1‴)] were also observed. The ^{13}C NMR data with assistance of HSQC spectrum displayed 31 carbon resonances, including two aromatic rings [δ_C 149.0, 147.5, 147.4, 145.2, 136.9, 134.8, 129.0, 119.7, 118.0, 116.1, 114.2, 110.8], together with a rhamnosyl group [δ_C 102.3, 72.3, 72.2, 74.0, 69.9, 17.9] and a xylopyranose group [δ_C 103.6, 79.2, 78.9, 71.5, 66.9], as well as two methines [δ_C 89.8, 53.1], four methylenes [δ_C 72.6, 62.2, 35.8, 32.9] and two methoxys [δ_C 56.8, 56.4]. The NMR spectroscopic data of **1** were highly similar to those of (−)-(7*S*,8*R*)-4,9,9′-trihydroxy-3′,5-dimethoxy-4′,7-epoxy-8,3′-neoligan-9-*O*-[α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranoside, except that the β-D-glucopyranoside moiety was replaced by a β-D-xylopyranosyl moiety.[19] This was further

[a]*Institute of Traditional Chinese Medicine & Natural Products, Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University, Guangzhou 510632, P. R. China. E-mail: 1018yuyang@163.com; tyaoxs@jnu.edu.cn; Fax: +86-20-85221559; Tel: +86-20-85221559*

[b]*Jiangsu Kanion Pharmaceutical Co., Ltd., State Key Laboratory of New-tech for Chinese Medicine Pharmaceutical Process, Lianyungang, Jiangsu, 222001, China. E-mail: xw_kanion@163.com*

† Electronic supplementary information (ESI) available: NMR spectra of all new compounds. See DOI: 10.1039/d1ra03520g

‡ These authors have contributed equally to this work.

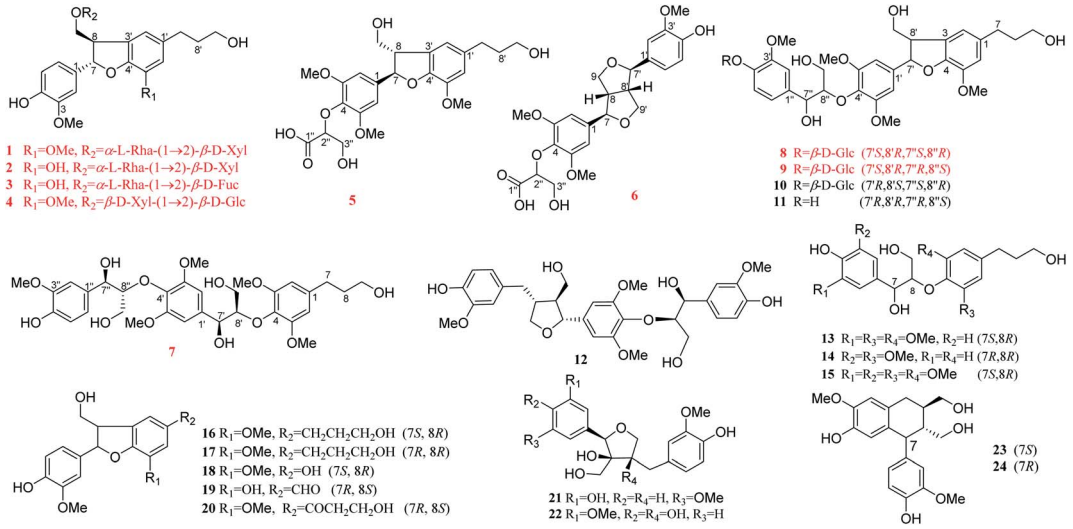

Fig. 1 Chemical structures of compounds **1–24**.

Table 1 ^{1}H and ^{13}C NMR spectral data of compounds **1–4** (measured at 400 MHz for ^{1}H and 100 MHz for ^{13}C in CD_3OD)

	1		**2**		**3**		**4**	
Pos.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	134.8		135.1		135.2		134.7	
2	110.8	6.96, d (1.8)	110.7	6.99, d (1.5)	110.9	7.02, d (1.3)	110.9	6.98, d (1.5)
3	149.0		148.9		148.9		149.1	
4	147.4		147.2		147.2		147.5	
5	116.1	6.75, d (8.2)	116.1	6.75, d (8.1)	116.0	6.74, d (8.1)	116.2	6.78, d (8.1)
6	119.7	6.83, dd (8.2, 1.8)	119.7	6.86, dd (8.1, 1.5)	119.7	6.87, dd (8.1, 1.3)	120.1	6.86, dd (8.1, 1.5)
7	89.8	5.57, d (5.8)	89.5	5.57, d (5.6)	89.3	5.63, d (5.5)	89.4	5.62, d (6.8)
8	53.1	3.62, m	53.3	3.60, m	53.4	3.60, m	53.0	3.64, dd (12.2, 6.9)
9	72.6	3.92, m	72.8	3.91, m	72.9	3.92, m	71.9	4.06, dd (9.1, 7.9), 3.88, m
1′	136.9		136.7		136.7		137.0	
2′	118.0	6.73, brs	116.7	6.61, brs	116.8	6.63, brs	117.9	6.74, brs
3′	129.0		129.0		129.2		129.5	
4′	147.5		146.5		146.5		147.5	
5′	145.2		141.9		141.9		145.2	
6′	114.2	6.73, brs	117.1	6.57, brs	117.1	6.56, brs	114.2	6.73, brs
7′	32.9	2.62, t (7.7)	32.7	2.56, t (7.7)	32.7	2.56, t (7.6)	32.9	2.63, t (7.6)
8′	35.8	1.82, m	35.8	1.79, m	35.8	1.80, m	35.9	1.82, m
9′	62.2	3.57, t (6.5)	62.3	3.56, t (6.5)	62.3	3.56, t (6.6)	62.3	3.58, t (6.5)
1″	103.6	4.35, d (6.9)	103.6	4.36, d (6.6)	103.4	4.33, d (7.6)	103.6	4.48, d (6.9)
2″	79.2	3.40, d (7.1)	79.3	3.41, d (6.1)	77.1	3.67, m	83.0	3.45, m
3″	78.9	3.44, d (8.8)	78.9	3.45, d (8.8)	76.3	3.60, m	77.0	3.53, m
4″	71.5	3.50, dd (9.3, 5.2)	71.5	3.50, dd (9.1, 5.7)	73.6	3.56, m	71.0	3.53, m
5″	66.9	3.18, dd (11.4, 9.9)	66.9	3.19, dd (10.5, 9.8)	71.9	3.60, m	66.5	3.22, m
		3.87, d (6.6)		3.86, d (5.4)				3.88, m
6″					16.7	1.27, d (6.4)		
1‴	102.3	5.18, d (1.3)	102.3	5.18, brs	102.2	5.18, brs	105.4	4.43, d (7.6)
2‴	72.3	3.92, m	72.3	3.91, m	72.4	3.92, m	76.2	3.22, m
3‴	72.2	3.68, dd (9.5, 3.3)	72.2	3.68, dd (9.5, 2.9)	72.2	3.70, dd (6.1, 3.4)	77.6	3.27, m
4‴	74.0	3.36, m	74.0	3.36, m	74.1	3.35, d (9.6)	71.4	3.27, m
5‴	69.9	3.90, m	69.9	3.90, m	69.8	3.92, m	78.2	3.03, m
6‴	17.9	0.99, d (6.2)	17.9	1.01, d (6.1)	17.9	1.0, d (6.2)	62.6	3.70, dd (12.0, 2.1), 3.58, m
3-OCH_3	56.4	3.83, s	56.4	3.83, s	56.5	3.84, s	56.5	3.83, s
5′-OCH_3	56.8	3.85, s					56.7	3.86, s

confirmed by HPLC analysis after acid hydrolysis and glycosyl derivatization (Fig. S82†), as well as the *J* value of the anomeric proton mentioned above. The β-D-xylopyranosyl moiety was located at C-9 and the α-L-rhamnopyranosyl unit was linked to C-2″ of the β-D-xylopyranosyl moiety, according to the key HMBC correlations from H-1″ to C-9 and from H-1‴ to C-2″ (Fig. 2).

NOESY correlations of H-8/H-2, H-8/H-6 and H-7/H-9 combining with coupling constant ($J_{7,8}$ = 5.8 Hz) indicated that H-7 and H-8 were in relative-*trans* form. The absolute configuration of **1** was assessed to be 7*S* and 8*R*, respectively, based on the positive Cotton effect at 242 and 291 nm and the negative Cotton effect at 226 nm(ref. 19 and 20) (Fig. S9†). Therefore, the structure of **1** was assigned as (−)-(7*S*,8*R*)-4,9,9′-trihydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligan-9-*O*-[α-L-rhamnopyranosyl (1→2)]-β-D-xylopyranoside, and given a trivial name of illiciumlignan G.

Compound **2** was isolated as a yellow amorphous powder. The HRESIMS data showed a sodium adduct molecular ion at *m*/*z* 647.2333 $[M + Na]^+$ (calcd for 647.2316), corresponding to a molecular formula of $C_{30}H_{40}O_{14}$ with eleven degrees of unsaturation. The NMR spectra (Table 1) of **2** were highly similar to **1**, except for the substitution at C-5′. Further analysis indicated that the 5′-OCH_3 in **1** was replaced by a hydroxyl group. The relative-*trans* configuration of **2** was confirmed by NOESY correlations and the coupling value $J_{7,8}$ = 5.6 Hz. The (7*S*,8*R*) absolute configuration of **2** was deduced from the CD data (positive Cotton effects at 239 and 293 nm, and negative Cotton effect at 226 nm) (Fig. S18†). Thus, the structure of **2** was elucidated and named as illiciumlignan H.

Compound **3** was yielded as a brown amorphous powder. Its molecular formula was shown to be $C_{31}H_{42}O_{14}$ based on its $[M + Na]^+$ ion at *m*/*z* 661.2477 in the HRESIMS (calcd for 661.2472). The ^1H and ^{13}C NMR spectra of **3** were very similar to **2** (Table 1), except for the substitution of glycosyl groups at C-9. Acid hydrolysis and subsequent HPLC analysis of hydrolysate of **3** showed that retention time of the saccharide derivative peaks were consistent to D-fucose and L-rhamnose derivatives, respectively. The relative configuration of fucosyl unit were determined to be β, on the basis of coupling constant value [δ_H 4.33 (1H, d, J = 7.6 Hz, H-1″)], while rhamnosyl unit for α [δ_H 5.18 (1H, brs, H-1‴)]. The rhamnosyl group was located at C-2″, according to the HMBC correlation from H-1‴ to C-2″. The absolute configuration was assessed to be 7*S* and 8*R*, respectively, based on the NOESY data and CD spectrum (Fig. S27†) using the same protocol as previously described. Therefore, the structure of **3** was elucidated and named as illiciumlignan I.

Compound **4** was obtained as a brown amorphous powder. The sodium adduct ion at *m*/*z* 677.2423 $[M + Na]^+$ by HRESIMS demonstrated that the molecular formula of **4** was $C_{31}H_{42}O_{15}$. Comparison of ^1H and ^{13}C NMR data of **4** and **1** (Table 1) indicated that they have the same aglycone except for the substitution of glycosyl groups at C-9. Using the same method as above, it was determined that the two sugar groups were β-D-xylopyranoside and β-D-glucopyranoside. The glucosyl group was located at C-2″, according to the HMBC correlation from H-1‴ to C-2″. In addition, the absolute configuration was also assessed to be 7*S* and 8*R* (Fig. S36†), respectively. Thus, the structure of **4** was defined and named as illiciumlignan J.

Illiciumlignan K (**5**), a yellow oil, gave the molecular formula of $C_{24}H_{30}O_{10}$ based on HRESIMS (*m*/*z* 479.1923 $[M + H]^+$, calcd

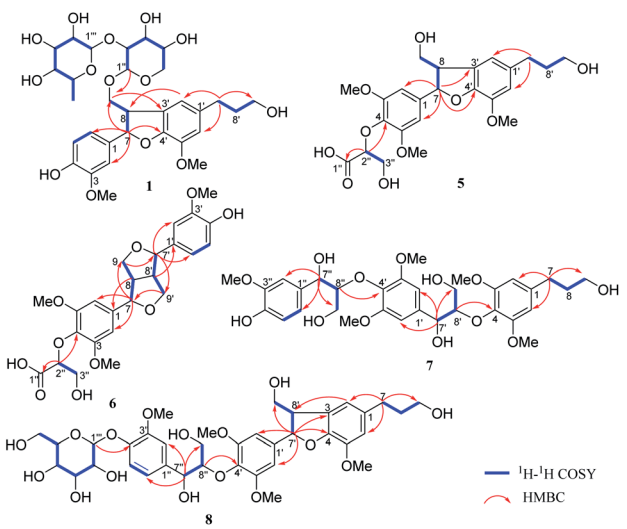

Fig. 2 Key ^1H–^1H COSY and HMBC correlations of new compounds **1**, **5**-**8**.

Table 2 ^1H and ^{13}C NMR spectral data of compounds **5**-**6**[a]

	5		**6**	
Pos.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	140.2		139.5	
2	103.8	6.73, s	104.2	6.71, s
3	154.1		154.1	
4	136.1		135.9	
5	154.1		154.1	
6	103.8	6.73, s	104.2	6.71, s
7	88.5	5.56, d (5.7)	87.2	4.76, d (3.8)
8	55.8	3.46, dd (12.6,5.7)	55.3	3.13, m
9	65.1	3.75, m; 3.90, m	72.7	3.90, m
1′	137.2		133.7	
2′	117.9	6.71, s	111.0	6.95, d (1.5)
3′	129.5		149.1	
4′	147.4		147.3	
5′	145.3		116.1	6.77, d (8.1)
6′	114.3	6.74, s	120.0	6.81, dd (8.1, 1.5)
7′	32.9	2.62, t (7.7)	87.4	4.71, d (4.1)
8′	35.8	1.81, m	55.8	3.13, m
9′	62.2	3.56, t (6.4)	72.9	4.26, dt (6.1, 5.2)
1″	174.1		174.2	
2″	83.9	4.50, t (3.1)	84	4.50, t (3.8)
3″	63.5	3.86, m	63.6	3.85, m
3-OCH_3	56.7	3.81, s	56.7	3.85, s
5-OCH_3	56.7	3.81, s	56.7	3.85, s
3′-OCH_3			56.4	3.85, s
5′-OCH_3	56.8	3.87, s		

[a] Measured at 400 MHz for ^1H and 100 MHz for ^{13}C in CD_3OD.

for 479.1917). The ^{1}H NMR (Table 2) spectrum displayed the presence of a symmetrical 1,2,3,5-tetrasubstituted aromatic ring [δ_H 6.73 (2H, s, H-2 and H-6)] and an asymmetrical 1,2,3,5-tetrasubstituted aromatic ring [δ_H 6.74 (1H, brs, H-6′), 6.71 (1H, brs, H-2′)]. Signals for three methoxys [δ_H 3.81 (6H, s, 3,5-OCH$_3$), 3.87 (3H, s, 5′-OCH$_3$)], three oxymethylenes [δ_H 3.90, (1H, m, H-9a), 3.75, (1H, m, H-9b); 3.56 (2H, t, J = 6.4 Hz, H-9′); 3.86, (2H, m, H-3″)], two methylenes [δ_H 2.62 (2H, t, J = 7.7 Hz, H-7′), 1.81 (2H, m, H-8′)] and three methines [δ_H 5.56 (1H, d, J = 5.7 Hz, H-7), 3.46 (1H, dd, J = 12.6, 5.7 Hz, H-8), 4.50 (1H, t, J = 3.1 Hz, H-2″)]. The ^{13}C NMR spectrum displayed 24 carbon resonances including a carbonyl [δ_C 174.1], twelve sp2 aromatic carbons [δ_C 154.1 × 2, 147.4, 145.3, 140.2, 137.2, 136.1, 129.5, 117.9, 114.3, 103.8 × 2], three methoxys [δ_C 56.8, 56.7 × 2], five methylenes [δ_C 65.1, 63.5, 62.2, 35.8, 32.9] and three methines [δ_C 88.5, 83.9, 55.8]. The NMR spectroscopic data of **5** were very similar to those of dunnianeolignan A,[7] except that the group connected at C-4. Carbon signals at δ 174.1, 83.9 and 63.5, corresponding to a carbonyl, an oxymethine [δ_H 4.50 (1H, t, J = 3.1 Hz, H-2″)] and an oxymethylene [δ_H 3.86 (2H, m, H-3″)]. HMBC correlation from H-2″ to C-4, C-1″ and H-3″ to C-1″, suggested that it was an a glyceric acid moiety located at C-4 (Fig. 2).

A *trans* configuration of H-7 and H-8 of **5** was determined by the $J_{7,8}$ value (5.7 Hz) and the NOESY cross peaks from H-8 to H-2/H-6 and from H-7 to H-9. The optical rotation values of **5** were closed to zero, combined with the results of chiral column analysis, indicating that **5** was racemic mixtures. The peaks of the of **5** (**5a** and **5b**) were observed at t_R 20.0 (**5a**)/23.5 (**5b**) min,

Table 3 ^{1}H and ^{13}C NMR spectral data of compounds **7–9** (measured at 400 MHz for ^{1}H and 100 MHz for ^{13}C in CD$_3$OD)

	7		**8**		**9**	
Pos.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	140.0		137.2		137.2	
2	106.9	6.54, s	117.9	6.72, brs	118.0	6.72, s
3	154.3		129.6		129.5	
4	134.7		147.5		147.5	
5	154.3		145.3		145.3	
6	106.9	6.54, s	114.2	6.74, brs	114.2	6.74, s
7	33.4	2.64, t (7.7)	32.9	2.63, t (7.6)	32.9	2.63, t (7.6)
8	35.4	1.83, m	35.8	1.82, m	35.8	1.82, m
9	62.2	3.56, t (6.4)	62.2	3.57, t (6.4)	62.2	3.57, t (6.4)
1′	139.0		139.6		139.8	
2′	105.3	6.76, s	104.0	6.70, s	103.9	6.72, s
3′	153.9		154.5		154.6	
4′	136.4		136.2		136.2	
5′	153.9		154.5		154.6	
6′	105.3	6.76, s	104.0	6.70, s	103.9	6.72, s
7′	74.0	4.95, d (5.2)	88.7	5.53, d (6.1)	88.6	5.55, d (5.9)
8′	87.2	4.20, dd (9.0, 4.8)	55.7	3.47, m	55.7	3.46, m
9′	61.4	3.90, m	65.0	3.75, m	65.1	3.75, m
				3.85, m		3.85, d (2.2)
1″	133.4		137.4		137.4	
2″	111.8	7.02, brs	112.3	7.05, d (1.1)	112.4	7.06, d (1.2)
3″	148.8		150.4		150.5	
4″	147.2		147.2		147.2	
5″	115.9	6.75, m	117.4	7.09, d (8.2)	117.6	7.12, d (8.3)
6″	121.0	6.87, dd (8.1, 1.3)	120.9	6.89, dd (8.2, 1.1)	120.7	6.92, dd (8.3, 1.2)
7″	74.6	4.99, d, (7.2)	73.8	4.92, d (5.4)	73.8	4.94, d (5.2)
8″	89.2	4.01, m	87.0	4.27, dd (8.9, 5.1)	87.2	4.24, dd (8.8, 5.0)
9″	61.7	3.29, m	61.6	3.61, m	61.5	3.57, m
		3.76, m		3.90, m		3.90, d (4.9)
1‴			102.8	4.87, d (7.4)	102.8	4.88, d (7.2)
2‴			74.9	3.50, m	74.9	3.49, m
3‴			78.2	3.39, m	78.2	3.40, m
4‴			71.4	3.36, m	71.4	3.40, m
5‴			77.8	3.45, m	77.8	3.46, m
6‴			62.6	3.65, m	62.5	3.69, dd (12.1, 2.7)
				3.84, m		3.85, m
3-OCH$_3$	56.6	3.81, s				
5-OCH$_3$	56.6	3.81, s	56.8	3.87, s	56.8	3.87, s
3′,5′-OCH$_3$	56.6	3.86, s	56.6	3.78, s	56.7	3.78, s
3″-OCH$_3$	56.4	3.84, s	56.7	3.83, s	56.7	3.83, s

respectively, and their relative peak area ratio in the HPLC chromatogram was approximately 1 : 1 (Fig. S79†). Therefore, structure of **5** was elucidated.

Compound **6** was obtained as a yellow oil with molecular formula $C_{24}H_{28}O_{10}$ by HRESIMS (*m*/*z* 477.1762 $[M + H]^+$, calcd for 477.1761). The NMR spectroscopic data (Table 2) of **6** was similar to that of medioresinol,[21] expect that **6** has an additional carbon signals at δ 174.2, 84.0 and 63.6. Compared with compound **5**, it was identified as a glyceric acid moiety. HMBC (Fig. 2) correlation from H-2″ to C-4, 1″, 3″; H-3″ to C-1″, suggested that glyceric acid moiety located at C-4. Glyceric acid moiety was degraded from sesquilignan and the absolute configuration of C-2″ are not stereospecificity.[22] On the basis of the coupling constants of the oxymethine protons [δ 4.76 (1H, d, J = 3.8 Hz, H-7) and 4.71 (1H, d, J = 4.1 Hz, H-7′)] of **6**, two sets of protons (H-7/H-8 and H-7′/H-8′) were indicated as being *trans* oriented. The NOESY correlations between H-8 and H-2, H-6 and between H-8′ and H-2′, H-6′ also confirmed the postulated arrangement. Compound **6** were found to be enantiomers by chiral chromatographic column analysis, and the relative content ratio of the two enantiomers was 20 : 80 (Fig. S80†). Therefore, the structure of **6** was elucidated, and named as illiciumlignan L.

Compound **7** was isolated as a brown amorphous powder. Its molecular formula was $C_{32}H_{42}O_{13}$ indicated by HRESIMS *m*/*z* 657.2526 $[M + Na]^+$, calcd for 657.2523.

The ^{1}H NMR spectrum (Table 3) showed three aromatic proton signals at [δ_H 7.02 (1H, brs, H-2″), 6.75 (1H, m, H-5″), 6.87 (1H, dd, J = 8.1, 1.3 Hz, H-6″)], indicating the presence of an ABX-coupled benzene ring. Two symmetrical 1,2,3,5-tetrasubstituted aromatic ring at [δ_H 6.76 (2H, s, H-2′, 6′); 6.54 (2H, s, H-2, 6)] revealed the other two benzenes. Five methoxy proton signals at [δ_H 3.81 (6H, s, 3,5-OCH$_3$), 3.84 (3H, s, 3″-OCH$_3$), and 3.86 (6H, s, 3′,5′-OCH$_3$)], four methines signals at [δ_H 4.99 (1H, d, J = 7.2 Hz, H-7″), 4.95 (1H, d, J = 5.2 Hz, H-7′), 4.20 (1H, dd, J = 9.0, 4.8 Hz, H-8′), 4.01 (1H, m, H-8″)] and five methylenes signals at [δ_H 3.90 (2H, m, H-9′), 3.76 (1H, m, H-9″a), 3.56 (2H, t, J = 6.4 Hz, H-9), 3.29 (1H, m, H-9″b), 2.64 (2H, t, J = 7.7 Hz, H-7), 1.83 (2H, m, H-8). The ^{13}C NMR spectrum revealed 25 peaks for 32 carbons, including 18 aromatic carbons for three aromatic rings, four oxymethylene carbon signals, five methines and five methoxy carbon signals. The ^{1}H–^{1}H COSY correlations between H-7/H-8 and H-9 and HMBC correlations from H-7 to C-1,2,6,8,9 confirmed the presence of a propanolguaiacol unit. Similarly, the presence of two guaiacylglycerol units were determined based on the COSY correlations of H-7′/H-8′/H-9′, H-7″/H-8″/H-9″, and the HMBC correlations from H-7′ to C-1′,2′,6′,8′,9′ and from H-7″ to C-1″,2″,6″,8″,9″. In addition, the HMBC correlations between H-8″ and C-4′, between H-8′ and C-4 confirmed the oxygen bridge between C-8″ and C-4′ as well as C-8′ and C-4, respectively. The positions of the methoxy groups were also confirmed by HMBC correlations (Fig. 2). These above assignments suggested that the basic skeleton of **7** is an 8-*O*-4′ system sesquineolignan. The 7′,8′-*erythro* and 7″, 8″-*threo* configurations for **7** were substantiated by the $J_{7',8'}$ value (5.2 Hz) and $J_{7'',8''}$ value (7.2 Hz).[23,24] Similar to **6**, compound **7** was determined to be a pair of enantiomers by chiral column analysis, and the results showed that the relative peak area ratio of **7a** and **7b** was 70 : 30 (Fig. S81†). Based on the above data, structure of **7** was elucidated, and it was named as illiciumlignan M.

Compound **8** was obtained as a yellowish oil and its molecular formula was confirmed as $C_{37}H_{48}O_{16}$ by HRESIMS at *m*/*z* 771.2847 $[M + Na]^+$ ($C_{37}H_{48}O_{16}Na$, calcd for 771.2840), possessing 14 degrees of unsaturation. The ^{1}H and ^{13}C NMR (Table 3) of **8** showed signals attributed to a 1,2,4-trisubstituted aromatic rings at [δ_H 7.05 (1H, d, J = 1.1 Hz, H-2″), 7.09 (1H, d, J = 8.2 Hz, H-5″), and 6.89 (1H, dd, J = 8.2, 1.1 Hz, H-6″)], a symmetrical 1,2,3,5-tetrasubstituted aromatic ring at [δ_H 6.70 (2H, s, H-2′/6′)], an asymmetrical 1,2,3,5-tetrasubstituted aromatic ring at [δ_H 6.74 (1H, brs, H-6), 6.72 (1H, brs, H-2)], and four methoxy groups at [δ_H 3.87 (3H, s, 5-OCH$_3$), 3.78 (6H, s, 3′,5′-OCH$_3$), 3.83 (3H, s, 3″-OCH$_3$)]. Additionally, an anomeric proton [δ_H 4.87 (1H, d, J = 7.4 Hz, H-1‴)] was indicative of a monosaccharide moiety in **8**, which was identified as β-D-glucosyl residue by HPLC analysis after acid hydrolysis and glycosyl derivatization. The ^{13}C NMR gave 37 carbon signals, except for eighteen aromatic carbons, six sugar carbons and four methoxy carbons, the remaining carbons were four oxymethines [(δ_C 88.7, 87.0, 73.8, and 55.7)] and five methylenes [(δ_C 65.0, 62.2, 61.6, 35.8, and 32.9)], attributing to three C_3 moieties, which were confirmed by ^{1}H–^{1}H COSY and HMBC correlations. These NMR spectroscopic data supposed **8** to be a sesquilignan glycoside and were in good agreement with those of acernikol-4″-*O*-β-D-glucopyranoside.[25] Subsequently, the ^{1}H–^{1}H COSY, HSQC and HMBC data (Fig. 2) confirm that they shared the same planar structure. The absolute configuration of the dihydrofuran ring was determined to be 7′*S*,8′*R*, based on the NOESY correlations of H-8′/H-2′, H-6′ and H-7′/H-9′, coupling constant ($J_{7',8'}$ = 6.1 Hz), and the negative Cotton effect at 225 nm. The relative configuration of H-7″ and H-8″ was determined to be *erythro* based on the small coupling constant ($J_{7'',8''}$ = 5.4 Hz) and the absolute configuration was assigned as (7″*S*,8″*R*) based on the negative Cotton effect at 242 nm (Fig. S69†). Thus, structure of **8** was elucidated and named as illiciumlignan N.

Compound **9** was obtained as white amorphous powder with an optical rotation of $[\alpha]_D^{25}$ −15.43 (*c* 0.7, MeOH). The HRESIMS data of **9** indicated that it possessed the same molecular formula as **8**. A comparison of the ^{1}H, ^{13}C, and 2D NMR data (Table 3) of **9** and **8** suggested that the two compounds had the same planar structures. The 7′,8′-*trans* and 7″,8″-*erythro* configuration of **9** was identical to **8**, which was confirmed by NOSEY correlations of H-8′/H-2′, H-6′, H-7′/H-9′ and coupling constant values of $J_{7',8'}$ = 5.9 Hz, and $J_{7'',8''}$ = 5.2 Hz. The absolute configuration was assigned as (7′*S*,8′*R*,7″*R*,8″*S*) on the basis of a negative Cotton effect at 228 nm and a positive Cotton effect at 243 nm observed in the CD spectrum (Fig. S78†). Based on the above data, structure of **9** was elucidated, and named as illiciumlignan O.

The other fifteen known compounds were identified as acernikol-4″-*O*-β-D-glucopyranoside[25] (**10**), acernikol[26] (**11**), sesliganoccidentaliol A[27] (**12**), *erythro*-4,7,9,9′-tetrahydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan[28] (**13**), *threo*-4,7,9,9′-tetra-hydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan[29](**14**), *erythro*-4,7,9,9′-tetrahydroxy-

3,5,3′,5′-tetramethoxy-8,4′-oxyneolignan[30] (**15**), (7*S**,8*R**)-dihydrodehydrodiconiferyl alcohol[31] (**16**), (7*R**,8*R**)-dihydrodehydrodiconiferyl alcohol[32] (**17**), samwirin A[33] (**18**), hierochin C[34] (**19**), prunustosanan AI[35] (**20**), (7′*R**,8*S**,8′*S**)-3,5′-dimethoxy-3′,4,8′,9′-tetrahydroxy-7′,9-epoxy-8,8′-lignan[36] (**21**), massoniresinol[37] (**22**), isolariciresinol[28] (**23**), and burselignan[28] (**24**).

2.2 The activity of anti-inflammatory

PGE_2 and NO levels of LPS-stimulated RAW264.7 cells were tested to evaluate the anti-inflammatory effects of the isolated compounds. The microscopic observation showed that all compounds performed no obvious cytotoxicity to RAW264.7 cells at the maximum concentrations of 100 μM, indicated that the anti-inflammatory effects of the tested compounds was not caused by cytotoxicity. Results showed that compounds **16** and **17** exhibited inhibitory effects on the production of PGE_2 with the IC_{50} values of 18.41 μM and 10.84 μM, respectively (Fig. 3). Compound **20** had a moderate inhibitory effect with an IC_{50} value of 50.58 μM (Fig. S83†), while other compounds showed no effect at dosage up to 100 μM. Besides, compounds **16** and **17** can decrease amount of NO release of the cells with an IC_{50} values of 53.09 μM and 53.70 μM, respectively (Fig. S84†).

The observed PGE_2 and NO inhibitory activities appear to be somewhat correlated with their structures. For example, with regard to the results for **5**, **16** and **17**, it appeared that the 4-OH might be important for higher activities.[38] Comparing the structures and inhibitory activities of **16–20**, it appeared that the carbonyl group at C-7′ and shortening of the side chain may cause a reduction in the inhibition of PGE_2 and NO production.[39] Interestingly, consideration of the structures of **1–4** *versus* **16-17** suggested that 9-OH was replaced by glycosyl groups, resulting in a decrease in the inhibitory activities of those dihydrobenzofuran neolignans.[40,41] In addition, the inflammatory activity of one pair of diastereoisomers, **16** and **17**, was similar, which led us to conclude that the absolute configuration of the compounds might have little or no inhibitory effect on PGE_2 and NO production.[42] These were not sufficient to clarify the accurate structure–activity relationship between the lignan derivatives and/or other components. More research may be required to clarify their potential selective NO and PGE_2 inhibitory activity.

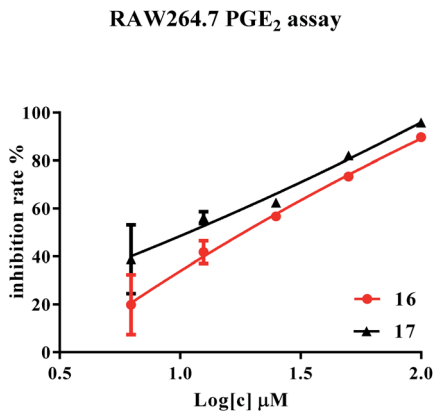

Fig. 3 Effect of compounds **16** and **17** on PGE_2 production in LPS-stimulated RAW264.7 cells.

3 Conclusions

In the present research, nine undescribed compounds (**1–9**), including five benzofuran lignans, one ditetrahydrofuran lignan and three sesquilignans, together with fifteen know analogues (**10–24**) were isolated from the leaves of *I. dunnianum.* Their structures were established on the basis of extensive spectroscopic analysis (MS, UV, IR, NMR), in combination with CD spectrum and chemical methods (acidic hydrolysis and sugar analysis). Anti-inflammatory evaluation of the isolates suggested that compounds **16** and **17** had a moderate inhibitory effect on NO and PGE_2 production in LPS-stimulated RAW264.7 cells. This study not only enriched the chemical diversity of ligans in *I. dunnianum*, but also provided an experimental basic for its anti-inflammatory activity.

4 Experimental

4.1 General experimental procedures

Optical rotations were recorded using a JASCO P1020 digital polarimeter. Circular dichroism (CD) spectra were tested by JASCO J-810 circular dichroism spectrometer. Ultraviolet spectra (UV) were recorded on a JASCO V550 UV spectrometer. Infrared spectra (IR) were measured using a JASCO FT/IR-480 plus spectrometer. NMR spectra were acquired on Bruker AV 600 MHz or 400 MHz using solvent signal (CD_3OD: δ_H 3.30/δ_C 49.0) as internal reference. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (Saint Louis, MO, US). High-resolution electrospray ionization mass (HR-ESI-MS) spectra were obtained using a Waters Synapt G2 mass spectrometer.

Analytical HPLC was conducted on a Shimadzu HPLC system with an LC-20AB solvent delivery system and an SPD-20A UV/vis detector using a Phenomenex Gemini C_{18} column (5 μm, *Φ* 4.6 × 250 mm; Phenomenex Inc., Los Angeles, USA). Semi-preparative HPLC was carried out on a Shimadzu LC-6AD liquid chromatography system equipped with a SPD-20A detector on a Phenomenex Gemini C18 column (5 μm, *Φ* 10.0 × 250 mm; Phenomenex Inc., Los Angeles, USA) and preparative HPLC using a Cosmosil Packed C18 column (5 μm, *Φ* 20.0 × 250 mm, Nacalai Tesque Inc., Kyoto, Japan). Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), silica gel 200–300 mesh and polyamide 50–100 mesh (Qingdao Haiyang Chemical Co., Ltd., Shandong, China), octadecyl silane (ODS) silica gel (12 nm, S-50 μm, YMC Ltd., Tokyo, Japan) were used for column chromatography (CC). Precoated silica gel GF254 plates for thin-layer chromatography (TLC) were from Qingdao Haiyang Chemical Co., Ltd. HPLC-grade methanol and acetonitrile were bought from Oceanpack Alexative Chemicals Co. Ltd. (Gothenburg, Sweden). All analytical grade reagents were from Concord Chemicals Co. Ltd., (Tianjin, China).

High glucose Dulbecco's modified Eagle's medium (DMEM) and 0.25% trypsin-EDTA were purchased from Gibco BRL Co. (New York, US). Fetal bovine serum (FBS) was purchased from

Lonsera Bio. Tech. (Shanghai, China). Lipopolysaccharide (LPS) was purchased from Nanjing Da Zhi Biological Technology Co., Ltd. (Nanjing, China). DMSO was purchased from Aladdin Reagent (Shanghai, China). PGE2 ELISA kit was purchased from Enzo Life Sciences (Farmingdale, US). The 24-well plates were purchased from JET company. Murine macrophage cell line RAW264.7 was obtained from Chinese Academy of Traditional Chinese Medicines (Beijing, China).

5 Plant material

The leaves of *I. dunnianum* were purchased from Ji'an County, Jiangxi, China, in 2018, and were identified by Prof. Zhou Wu (Jiangsu Kanion Pharmaceutical Co. Ltd.). A sample (2018ID101) was deposited in Institute of Traditional Chinese Medicine & Natural Products, college of pharmacy, Jinan University, Guangzhou, China.

5.1 Extraction and isolation

The air-dried leaves of *I. dunnianum* (ID, 15.5 kg) were extracted with 50% EtOH by heat reflux for 3 times (2 h each). Total extracts (IDEs, 2 kg) were yielded by evaporation under reduced pressure. IDEs were separated by HP-20 resin column chromatography (CC) eluted with EtOH–H_2O (0 : 100, 30 : 70, 50 : 50, 95 : 5) gradient to afford 4 fractions (ID-1 to ID-4). Fr. ID-3 (180 g) was separated over a silica gel column eluting with a CH_2Cl_2–MeOH gradient (100 : 0, 98 : 2, 95 : 5, 90 : 10, 85 : 15, 80 : 20, 70 : 30, 60 : 40, 50 : 50, 0 : 100) to afford 15 fractions (Fr. 3A to 3O). Fr. 3L (17.4 g) was chromatographed by ODS CC using a CH_3OH–H_2O gradient elution (30 : 70–70 : 30, 100 : 0) to give 9 subfractions (Fr. 3L1–3L9). Fr. 3L4 was further chromatographed by polyamide CC using a EtOH–H_2O gradient elution (10 : 90–40 : 60, 95 : 5) to give 7 subfractions (Fr. 3L4A–3L4G). Fr. 3 L4A was isolated using preparative HPLC [35% CH_3OH–H_2O (containing 0.1% HCOOH), 8 mL min^{-1}] to yield 2 (10 mg) and fractions 3L4A2–3L4A9. Fr. 3L4A5 was isolated using semipreparative HPLC [22% CH_3CN–H_2O (0.1% HCOOH), 3 mL min^{-1}] to yield 3 (5.5 mg) and 8 (13 mg). Fr. 3L4A6 was isolated using semipreparative HPLC [16% CH_3CN–H_2O (0.1% HCOOH), 3 mL min^{-1}] to yield 9 (7.9 mg) and 10 (15.3 mg). Fr. 3L4A7 was isolated using semipreparative HPLC [18% CH_3CN–H_2O (0.1% HCOOH), 3 mL min^{-1}] to yield 4 (4.3 mg). Fr. 3L4A8 was isolated using semipreparative HPLC [22% CH_3CN–H_2O (0.1% HCOOH), 3 mL min^{-1}] to yield 5 (11.8 mg). Fr. 3L4A9 was isolated using semipreparative HPLC [23% CH_3CN–H_2O (0.1% HCOOH), 3 mL min^{-1}] to yield 1 (45.4 mg). Fr. 3 G (7.1 g) was chromatographed by ODS CC using a CH_3OH–H_2O gradient elution (25 : 75–65 : 35, 100 : 0) to give 16 subfractions (Fr. 3G1–3G16). Fr.3G8 was further purified by semipreparative HPLC [40% CH_3OH–H_2O (0.1% HCOOH), 3 mL min^{-1}] to yield 6 (5.6 mg). Fr. 3G9 was further purified by semipreparative HPLC [20% CH_3CN–H_2O (0.1% HCOOH), 3 mL min^{-1}] to yield 11 (21 mg). Fr. 3F (5.7 g) was chromatographed by ODS CC using a CH_3OH–H_2O gradient elution (30 : 70–60 : 40, 100 : 0) to give 16 subfractions (Fr. 3F1–3F15). Compound **13** (9.3 mg) was obtained from Fr. 3F6 by semipreparative HPLC with 14% CH_3CN–H_2O (0.1% HCOOH, 3.0 mL min^{-1}). Compound **22** (3.5 mg) was obtained from Fr. 3F6B by semipreparative HPLC with 30% CH_3OH–H_2O (0.1% HCOOH, 3.0 mL min^{-1}). Fr. 3F6D was further purified by semipreparative HPLC [30% CH_3OH–H_2O (0.1% HCOOH), 3 mL min^{-1}] to yield 24 (3.2 mg). Compounds **14** (6.8 mg), 18 (1.1 mg) and 19 (1.1 mg) were obtained from Fr. 3F6E by semipreparative HPLC with 25% CH_3OH–H_2O (0.1% HCOOH, 3.0 mL min^{-1}). Compound **15** (2.6 mg), compound **20** (3 mg), compound **21** (3.8 mg) and compound **23** (22 mg) were obtained from Fr. 3F by semipreparative HPLC with 20% CH_3CN–H_2O, 30% CH_3OH–H_2O, 25% CH_3OH–H_2O and 30% CH_3OH–H_2O (0.1% HCOOH, 3.0 mL min^{-1}), respectively. Compound **16** (1.1 g) was obtained from Fr. 3F8 by silica gel column eluting with a cyclohexane–ethyl acetate gradient. Compound **12** (6.4 mg) was obtained from Fr. 3F9 by semipreparative HPLC with 19% CH_3CN–H_2O (0.1% HCOOH, 3.0 mL min^{-1}). Fr. 3F9E and Fr. 3F9F was further purified by semipreparative HPLC [40% CH_3OH–H_2O, 25% CH_3CN–H_2O (0.1% HCOOH), 3 mL min^{-1}] to yield 17 (9.6 mg) and 7 (1.6 mg), respectively. The chiral HPLC analysis of compounds 5 and 6 were using EnantioPak OD column (4.6 × 250 mm) with 20% CH_3CN–H_2O and 30% CH_3CN–H_2O (0.1% HCOOH, 1 mL min^{-1}, 35 °C), respectively. Compound **7** was using EnantioPak OZ-3 column (4.6 × 250 mm) with 35% CH_3CN–H_2O.

5.2 Structural characterization of undescribed compounds

Illiciumlignan G (**1**): brown amorphous powder; $[\alpha]_D^{25}$ −35.2 (*c* 0.75, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.90), 236 (4.34), 284 (4.04) nm; IR (KBr) ν_{max} 3340, 2930, 2882, 1611, 1506, 1455, 1362, 1274, 1212, 1138, 1042 cm^{-1}; the ^1H and ^{13}C NMR spectra data see Table 1; HR-ESI-MS *m*/*z*: 661.2471 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{14}Na$, 661.2472).

Illiciumlignan H (**2**): yellow amorphous powder; $[\alpha]_D^{25}$ −23.8 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.66), 236 (4.10), 285 (3.88) nm; IR (KBr) ν_{max} 3436, 2946, 2873, 1602, 1514, 1450, 1390, 1356, 1271, 1132, 1045 cm^{-1}; the ^1H and ^{13}C NMR spectra data see Table 1; HR-ESI-MS *m*/*z*: 647.2333 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_{14}Na$, 647.2316).

Illiciumlignan I (**3**): brown amorphous powder; $[\alpha]_D^{25}$ −18 (*c* 0.55, MeOH); UV (MeOH) λ_{max} nm (log ε): 205 (4.65), 235 (4.10), 285 (3.86); IR (KBr) ν_{max} 3371, 2927, 2879, 1614, 1511, 1450, 1378, 1333, 1277, 1130, 1056 cm^{-1}; the ^1H and ^{13}C NMR spectra data see Table 1; HR-ESI-MS *m*/*z*: 661.2477 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{14}Na$, 661.2472).

Illiciumlignan J (**4**): brown amorphous powder; $[\alpha]_D^{25}$ −14.75 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.7), 236 (4.16), 284 (3.89) nm; IR (KBr) ν_{max} 3289, 2927, 2876, 1735, 1611, 1504, 1455, 1424, 1271, 1212, 1127, 1073, 1039 cm^{-1}; the ^1H and ^{13}C NMR spectra data see Table 1; HR-ESI-MS *m*/*z*: 677.2423 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{15}Na$, 677.2421).

Illiciumlignan K (**5**): yellow oil; $[\alpha]_D^{25}$ −6.7 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε): 209 (4.77), 236 (4.19), 285 (3.71) nm; IR (KBr) ν_{max} 3462, 3408, 2933, 2867, 1738, 1608, 1501, 1458, 1424, 1331, 1214, 1127, 1053 cm^{-1}; the ^1H and ^{13}C NMR spectra data see Table 2; HR-ESI-MS *m*/*z*: 479.1923 $[M + H]^+$ (calcd for $C_{24}H_{31}O_{10}$, 479.1917).

Illiciumlignan L (**6**): yellow oil; $[\alpha]_D^{25}$ −38.24 (*c* 0.51, MeOH); UV(MeOH) λ_{max} (log ε): 207 (5.26), 233 (4.63), 280 (4.01) nm; IR (KBr) ν_{max} 3420, 2938, 2861, 1750, 1599, 1511, 1461, 1424, 1371, 1336, 1271, 1232, 1124, 1053 cm^{-1}; the ^1H and ^{13}C NMR spectra data see Table 2; HR-ESI-MS *m*/*z*: 477.1762 $[M + H]^+$ (calcd for $C_{24}H_{29}O_{10}$, 477.1761).

Illiciumlignan M (**7**): brown amorphous powder; $[\alpha]_D^{25}$ +10.13 (*c* 0.8, MeOH); UV(MeOH) λ_{max} (log ε): 206 (4.81), 236 (4.23), 275 (3.79) nm; IR (KBr) ν_{max} 3369, 2944, 2854, 1599, 1509, 1458, 1419, 1381, 1333, 1235, 1127, 1036 cm^{-1}; the ^1H and ^{13}C NMR spectra data see Table 3; HR-ESI-MS *m*/*z*: 657.2526 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_{13}Na$, 657.2523).

Illiciumlignan N (**8**): yellowish oil; $[\alpha]_D^{25}$ −52.89 (*c* 0.45, MeOH); UV (MeOH) λ_{max} (log ε): 206 (5.18), 236 (4.64), 277 (4.23) nm; IR (KBr) ν_{max} 3303, 2933, 2873, 1602, 1506, 1458, 1424, 1375, 1331, 1266, 1223, 1130, 1068 cm^{-1}; the ^1H and ^{13}C NMR spectra data see Table 3; HR-ESI-MS *m*/*z*: 771.2847 $[M + Na]^+$ (calcd for $C_{37}H_{48}O_{16}Na$, 771.2840).

Illiciumlignan O (**9**): white amorphous powder; $[\alpha]_D^{25}$ −15.43 (*c* 0.7, MeOH); UV(MeOH) λ_{max} (log ε): 206 (5.03), 235 (4.51), 277 (4.14) nm; IR (KBr) ν_{max} 3374, 2936, 2884, 1722, 1614, 1504, 1461, 1427, 1328, 1212, 1127, 1036 cm^{-1}; the ^1H and ^{13}C NMR spectra data see Table 3; HR-ESI-MS *m*/*z*: 771.2833 $[M + Na]^+$ (calcd for $C_{37}H_{48}O_{16}Na$, 771.2840).

5.3 Acid hydrolysis and sugar analysis

The compounds (1.0 mg) were hydrolyzed with 2 mL of 2 M HCl for 2 h at 90 °C. The hydrolysates were extracted with equal volume of ethyl acetate twice. The aqueous layer was dried, and then reacted with 2.5 mg L-cysteine methyl ester hydrochloride in 1 mL of pyridine for 1 h at 60 °C. After 1 h, a total of 5 μL of *o*-tolyl isothiocyanate was added to the reaction mixture and further reacted at 60 °C for 1 h. The reaction products were filtered by a 0.45 μm filter membrane for HPLC analysis, detected by a UV detector at 250 nm. Authentic samples of D-Glc, L-Glc, D-Xyl, L-Xyl, D-Rha, L-Rha and D-Fuc, L-Fuc were treated following same procedure.

5.4 Anti-inflammatory activity assays

Inhibition of PGE_2 production RAW264.7 cells were plated in 24-well plates at 1 × 105 cells per mL (400 μL per well) and cultured for 24 h in a 37 °C 5% CO_2 incubator, then the supernatants were remove. Compounds were first dissolved in DMSO to prepare the stock solution at a concentration of 100 mM, and then the dilution of the compounds in DMEM (0.1% DMSO) were added in the sample wells (495 μL per well). After one hour later, 5 μL of LPS at 100 μg mL^{-1} was added into each well, the control/model wells was given 5 μL 0.1% DMSO in DMEM. Then the cells were cultured under normal condition for 18 h. Then the supernatants were collected and PGE_2 levels were determined.

NO suppression RAW264.7 cells were plated in 96-well plates at 2 × 10^6 cells per mL (100 μL per well). Then the cells were treated by LPS stimulation following the above method. After the cells had been treated with a series of compounds for 24 h, the production of NO in each supernatant was determined based on the Griess reaction, and the absorbance was measured at 540 nm in a microplate reader.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (program No. 81630097). The authors are grateful to State Key Laboratory of New-Tech for Chinese Medicine Pharmaceutical Process, Jiangsu Kanion Pharmaceutical Co. Ltd. for their assistance of activity test.

Notes and references

1 H. P. Deng, L. Sun, Z. X. Xi, X. Li, C. Chen and L. N. Sun, *J. Pharm. Pract.*, 2012, **30**, 8–13.
2 J. W. Zhang, *Zhongguo Zhongyao Zazhi*, 1989, **14**, 36–37.
3 L. F. Zhang, K. Z. Lin, W. D. Yang, J. L. Wei, G. H. Yang and X. W. Lu, *Zhongguo Zhongyao Zazhi*, 1995, 717–718.
4 W. L. Zeng, J. Y. Song, Y. F. Cen, Y. Z. Fang, L. S. Qu and S. Z. Li, *Journal of Guiyang College of Traditional Chinese Medicine*, 1992, 60–62.
5 Q. Lin, *Chin. Tradit. Herb. Drugs*, 2002, **33**, 654–657.
6 Y. Z. Fang, J. Y. Song, Y. F. Cen, W. L. Zeng and D. S. Xie, *Journal of Guiyang College of Traditional Chinese Medicine*, 1989, **1**, 59–62.
7 J. Bai, H. Chen, Z. F. Fang, S. S. Yu, S. G. Ma, Y. Li, J. Qu, S. Xu, J. H. Ren, H. N. Lu and X. Chen, *J. Asian Nat. Prod. Res.*, 2012, **14**, 940–949.
8 J. Bai, H. Chen, Z. F. Fang, S. S. Yu, W. J. Wang, Y. Liu, S. G. Ma, Y. Li, J. Qu, S. Xu, J. H. Liu, F. Zhao and N. Zhao, *Phytochemistry*, 2012, **80**, 137–147.
9 L. Sy and G. Brown, *Phytochemistry*, 1998, **47**, 301–302.
10 J. Huang, J. Wang and C. Yang, *Phytochemistry*, 1997, **46**, 777–780.
11 I. Kouno, N. Kawano and C. S. Yang, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1537–1539.
12 J. Li, D. Geng, J. Xu, L. J. Weng, Q. Liu and L. T. Yi, *Eur. J. Pharmacol.*, 2013, **707**, 112–119.
13 L. Sy and G. Brown, *Phytochemistry*, 1996, **43**, 1417–1419.
14 L. Sy, R. Saunders and G. Brown, *Phytochemistry*, 1997, **44**, 1099–1108.
15 C. Y. IsaoKouno, *Chem. Pharm. Bull.*, 1991, **39**, 2606–2608.
16 J. Bai, Z. F. Fang, H. Chen, S. S. Yu, D. Zhang, H. L. Wei, S. G. Ma, Y. Li, J. Qu, S. Xu, J. H. Ren, F. Zhao, N. Zhao and J. H. Liu, *Carbohydr. Res.*, 2012, **361**, 206–211.
17 D. Geng, L. J. Weng, Y. Y. Han and X. Yang, *Zhongguo Zhongyao Zazhi*, 2012, **1914**, 1586–1589.
18 D. Geng, L. J. Weng, Y. Y. Han and X. Yang, *Zhongguo Zhongyao Zazhi*, 2013, **2115**, 3375–3377.
19 L. Y. Wang, M. H. Chen, J. Wu, H. Sun, W. Liu, Y. H. Qu, Y. C. Li, Y. Z. Wu, R. Li, D. Zhang, S. Wang and S. Lin, *J. Nat. Prod.*, 2017, **80**, 1808–1818.

20 C. S. Kim, L. Subedi, S. Y. Kim, S. U. Choi, K. H. Kim and K. R. Lee, *J. Nat. Prod.*, 2015, **78**, 1174–1178.
21 H. H. Xie, J. Su, X. L. Ge, T. T. Dong, X. Li, H. M. Wen and B. H. Sun, *Nat. Prod. Res.*, 2018, **32**, 418–424.
22 Y. Kamaya, F. Nakatsubo and T. Higuchi, *Agric. Biol. Chem.*, 1983, **47**, 299–308.
23 L. Xiong, C. Zhu, Y. Li, Y. Tian, S. Lin, S. Yuan, J. F. Hu, Q. Hou, N. H. Chen, Y. C. Yang and J. Shi, *J. Nat. Prod.*, 2011, **74**, 1188–1200.
24 S. S. Li, Z. L. Hou, G. D. Yao, R. Guo, Y. X. Wang, B. Lin, X. X. Huang and S. J. Song, *Phytochemistry*, 2020, **178**, 112461.
25 P. Y. Gao, L. Z. Li, Y. Peng, F. F. Li, C. Niu, X. X. Huang, M. Ming and S. J. Song, *Biochem. Syst. Ecol.*, 2010, **38**, 988–992.
26 T. Morikawa, J. Tao, K. Ueda, H. Matsuda and M. Yoshikawa, *Chem. Pharm. Bull.*, 2003, **51**, 62–67.
27 S. F. Li, Y. T. Di, Y. H. Wang, C. J. Tan, X. Fang, Y. Z. Zhang, Y. T. Zheng, L. Li, H. P. He, L. S. Li and X. J. Hao, *Helv. Chim. Acta*, 2010, **93**, 1795–1802.
28 A. Jutiviboonsuk, H. Zhang, G. T. Tan, C. Ma, N. Van Hung, N. M. Cuong, N. Bunyapraphatsara, D. D. Soejarto and H. H. Fong, *Phytochemistry*, 2005, **66**, 2745–2751.
29 A. Ahmed, W. Li, J. S. Zhang, P. H. Sam, Y. H. Zou, G. H. Tang and S. Yin, *Nat. Prod. Res.*, 2018, **32**, 175–181.
30 W. H. Cai, K. Matsunami, H. Otsuka and Y. Takeda, *Am. J. Plant Sci.*, 2011, **2**, 609.
31 H. X. Kuang, Y. G. Xia, B. Y. Yang, Q. H. Wang and S. W. Lü, *Arch. Pharmacal Res.*, 2009, **32**, 329–334.
32 R. X. Tan, J. Jakupovic and Z. J. Jia, *Planta Med.*, 1990, **56**, 475–477.
33 H. H. Xiao, Y. Dai, M. S. Wong and X. S. Yao, *J. Asian Nat. Prod. Res.*, 2015, **17**, 625–632.
34 M. Yoshikawa, T. Morikawa and F. Xu, *Heterocycles*, 2003, **60**, 1787–1792.
35 Q. B. Liu, X. X. Huang, M. Bai, X. B. Chang, X. J. Yan, T. Zhu, W. Zhao, Y. Peng and S. J. Song, *J. Agric. Food Chem.*, 2014, **62**, 7796–7803.
36 S. J. In, K. H. Seo, N. Y. Song, D. S. Lee, Y. C. Kim and N. I. Baek, *Arch. Pharmacal Res.*, 2015, **38**, 26–34.
37 Z. Shen and O. Theander, *Phytochemistry*, 1985, **24**, 364–365.
38 X. X. Huang, M. Bai, L. Zhou, L. L. Lou, Q. B. Liu, Y. Zhang, L. L. Li and S. J. Song, *J. Agric. Food Chem.*, 2015, **63**, 7252–7260.
39 L. Xiao, Y. Huang, Y. Wang, J. Xu and X. He, *Fitoterapia*, 2020, **140**, 104440.
40 N. Cho, H. Yang, J. W. Kim, Y. C. Kim and S. H. Sung, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 5675–5678.
41 B. T. Zhao, N. D. Hung, J. H. Lee, B. S. Min and M. H. Woo, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 524–529.
42 Q. B. Liu, X. X. Huang, M. Bai, X. B. Chang, X. J. Yan, T. Zhu, W. Zhao, Y. Peng and S. J. Song, *J. Agric. Food Chem.*, 2014, **62**, 7796–7803.