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

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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 sesquigignans, 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<sub>2</sub> 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).<sup>1</sup> *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.<sup>2</sup> Modern pharmacological studies have shown that it possesses multiple biological activities, such as anti-inflammatory,<sup>3,4</sup> central and peripheral analgesic effects,<sup>5,6</sup> relieving gastrointestinal smooth muscle spasm, and regulating immune activities.<sup>4</sup> Previous phytochemical investigations of *I. dunnianum* indicated the presence of sesquiterpenes,<sup>7–11</sup> phenylpropanoids,<sup>7,12–15</sup> phenol glycosides,<sup>16</sup> flavonoids,<sup>2,17,18</sup> triterpenes,<sup>2,14</sup> and others.<sup>2,7,17</sup> 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<sub>2</sub> 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<sub>31</sub>H<sub>42</sub>O<sub>14</sub> by analysing the HRESIMS peak at *m/z* 661.2471 [M + Na]<sup>+</sup> (calcd for 661.2472). The <sup>1</sup>H NMR (Table 1) spectrum displayed the presence of one 1,2,4-trisubstituted aromatic ring [ $\delta_H$  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 [ $\delta_H$  6.73 (2H, br s, H-2', H-6')]. Signals for one oxymethine [ $\delta_H$  5.57 (1H, d, *J* = 5.8 Hz, H-7)], two oxymethylenes [ $\delta_H$  3.92 (2H, m, H-9), 3.57 (2H, t, *J* = 6.5 Hz, H-9')], one methine [ $\delta_H$  3.62 (1H, m, H-8)], two methylenes [ $\delta_H$  2.62 (2H, t, *J* = 7.7 Hz, H-7'), 1.82 (2H, m, H-8')], and two methoxys [ $\delta_H$  3.85 (3H, s, 5'-OCH<sub>3</sub>), 3.83 (3H, s, 3-OCH<sub>3</sub>)], along with two anomeric proton signals [ $\delta_H$  4.35 (1H, d, *J* = 6.9 Hz, H-1''), 5.18 (1H, d, *J* = 1.3 Hz, H-1''')] were also observed. The <sup>13</sup>C NMR data with assistance of HSQC spectrum displayed 31 carbon resonances, including two aromatic rings [ $\delta_C$  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 [ $\delta_C$  102.3, 72.3, 72.2, 74.0, 69.9, 17.9] and a xylopyranose group [ $\delta_C$  103.6, 79.2, 78.9, 71.5, 66.9], as well as two methines [ $\delta_C$  89.8, 53.1], four methylenes [ $\delta_C$  72.6, 62.2, 35.8, 32.9] and two methoxys [ $\delta_C$  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*-[ $\alpha$ -L-rhamnopyranosyl (1 → 6)]- $\beta$ -D-glucopyranoside, except that the  $\beta$ -D-glucopyranoside moiety was replaced by a  $\beta$ -D-xylopyranosyl moiety.<sup>19</sup> This was further

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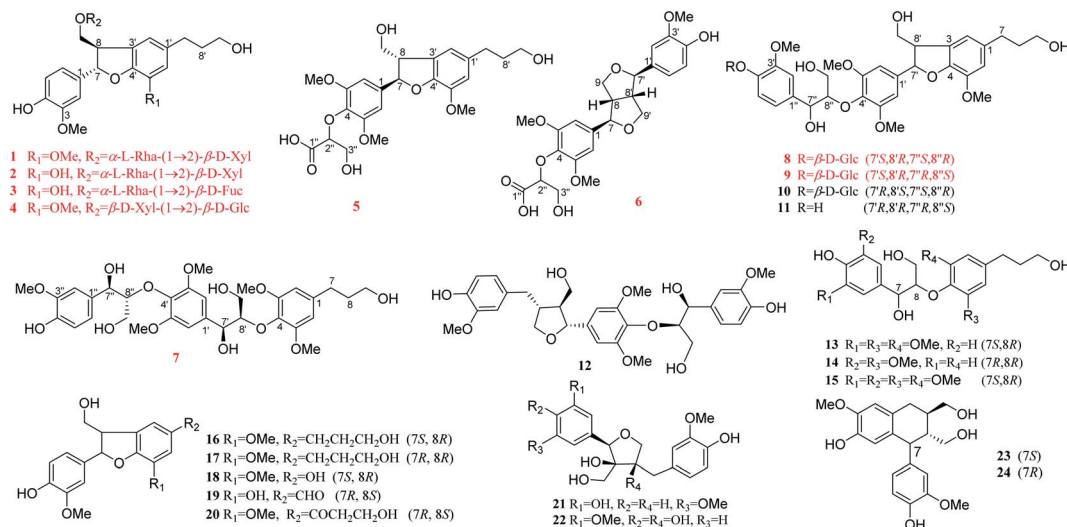


Fig. 1 Chemical structures of compounds 1–24.

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

	1		2		3		4	
Pos.	$\delta_C$	$\delta_H$ (J in Hz)	$\delta_C$	$\delta_H$ (J in Hz)	$\delta_C$	$\delta_H$ (J in Hz)	$\delta_C$	$\delta_H$ (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) 3.87, d (6.6)	66.9	3.19, dd (10.5, 9.8) 3.86, d (5.4)	71.9	3.60, m	66.5	3.22, m 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 <sub>3</sub>	56.4	3.83, s	56.4	3.83, s	56.5	3.84, s	56.5	3.83, s
5'-OCH <sub>3</sub>	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  $\beta$ -D-xylopyranosyl moiety was located at C-9 and the  $\alpha$ -L-rhamnopyranosyl unit was linked to C-2'' of the  $\beta$ -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-[ $\alpha$ -L-rhamnopyranosyl (1 → 2)]- $\beta$ -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 [ $M + Na$ ]<sup>+</sup> (calcd for 647.2316), corresponding to a molecular formula of C<sub>30</sub>H<sub>40</sub>O<sub>14</sub> 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<sub>3</sub> 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<sub>31</sub>H<sub>42</sub>O<sub>14</sub> based on its [ $M + Na$ ]<sup>+</sup> ion at  $m/z$  661.2477 in the HRESIMS (calcd for 661.2472). The <sup>1</sup>H and <sup>13</sup>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  $\beta$ , on the basis of coupling constant value [ $\delta_H$  4.33 (1H, d,  $J = 7.6$  Hz, H-1'')], while rhamnosyl unit for  $\alpha$  [ $\delta_H$  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 [ $M + Na$ ]<sup>+</sup> by HRESIMS demonstrated that the molecular formula of **4** was C<sub>31</sub>H<sub>42</sub>O<sub>15</sub>. Comparison of <sup>1</sup>H and <sup>13</sup>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  $\beta$ -D-xylopyranoside and  $\beta$ -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<sub>24</sub>H<sub>30</sub>O<sub>10</sub> based on HRESIMS ( $m/z$  479.1923 [ $M + H$ ]<sup>+</sup>, calcd

Table 2 <sup>1</sup>H and <sup>13</sup>C NMR spectral data of compounds 5–6<sup>a</sup>

Pos.	5		6	
	$\delta_C$	$\delta_H$ ( $J$ in Hz)	$\delta_C$	$\delta_H$ ( $J$ 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 <sub>3</sub>	56.7	3.81, s	56.7	3.85, s
5-OCH <sub>3</sub>	56.7	3.81, s	56.7	3.85, s
3'-OCH <sub>3</sub>			56.4	3.85, s
5'-OCH <sub>3</sub>	56.8	3.87, s		

<sup>a</sup> Measured at 400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C in CD<sub>3</sub>OD.

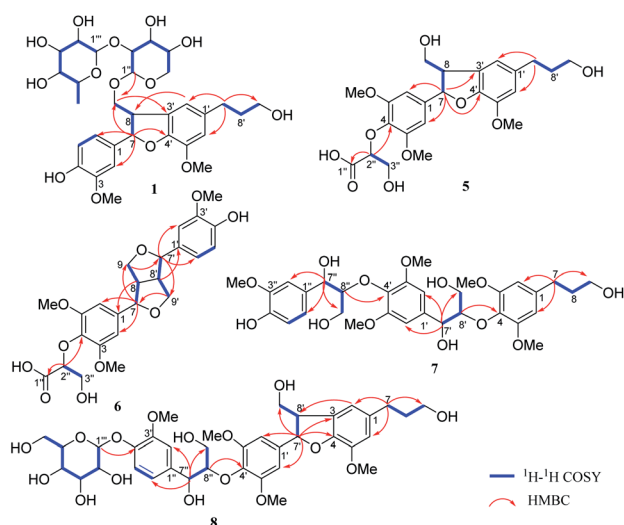


Fig. 2 Key <sup>1</sup>H–<sup>1</sup>H COSY and HMBC correlations of new compounds **1**, **5**–**8**.



for 479.1917). The  $^1\text{H}$  NMR (Table 2) spectrum displayed the presence of a symmetrical 1,2,3,5-tetrasubstituted aromatic ring [ $\delta_{\text{H}}$  6.73 (2H, s, H-2 and H-6)] and an asymmetrical 1,2,3,5-tetrasubstituted aromatic ring [ $\delta_{\text{H}}$  6.74 (1H, brs, H-6'), 6.71 (1H, brs, H-2')]. Signals for three methoxys [ $\delta_{\text{H}}$  3.81 (6H, s, 3,5-OCH<sub>3</sub>), 3.87 (3H, s, 5'-OCH<sub>3</sub>)], three oxymethylenes [ $\delta_{\text{H}}$  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 [ $\delta_{\text{H}}$  2.62 (2H, t,  $J = 7.7$  Hz, H-7'), 1.81 (2H, m, H-8'')] and three methines [ $\delta_{\text{H}}$  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  $^{13}\text{C}$  NMR spectrum displayed 24 carbon resonances including a carbonyl [ $\delta_{\text{C}}$  174.1], twelve sp<sup>2</sup> aromatic carbons [ $\delta_{\text{C}}$  154.1  $\times$  2, 147.4, 145.3, 140.2, 137.2, 136.1, 129.5, 117.9, 114.3, 103.8  $\times$  2], three methoxys [ $\delta_{\text{C}}$  56.8, 56.7  $\times$  2], five methylenes

[ $\delta_{\text{C}}$  65.1, 63.5, 62.2, 35.8, 32.9] and three methines [ $\delta_{\text{C}}$  88.5, 83.9, 55.8]. The NMR spectroscopic data of **5** were very similar to those of dunnianeolignan A,<sup>7</sup> except that the group connected at C-4. Carbon signals at  $\delta$  174.1, 83.9 and 63.5, corresponding to a carbonyl, an oxymethine [ $\delta_{\text{H}}$  4.50 (1H, t,  $J = 3.1$  Hz, H-2'')] and an oxymethylene [ $\delta_{\text{H}}$  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  $t_{\text{R}}$  20.0 (**5a**)/23.5 (**5b**) min,

Table 3  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of compounds 7–9 (measured at 400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$  in CD<sub>3</sub>OD)

Pos.	7		8		9	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ ( $J$ in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ ( $J$ in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ ( $J$ 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 <sub>3</sub>	56.6	3.81, s				
5-OCH <sub>3</sub>	56.6	3.81, s	56.8	3.87, s	56.8	3.87, s
3',5'-OCH <sub>3</sub>	56.6	3.86, s	56.6	3.78, s	56.7	3.78, s
3''-OCH <sub>3</sub>	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,<sup>21</sup> except that **6** has an additional carbon signals at  $\delta$  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 sesquignan and the absolute configuration of C-2'' are not stereospecificity.<sup>22</sup> On the basis of the coupling constants of the oxymethine protons [ $\delta$  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  $^1H$  NMR spectrum (Table 3) showed three aromatic proton signals at [ $\delta_H$  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-tetra-substituted aromatic ring at [ $\delta_H$  6.76 (2H, s, H-2', 6'); 6.54 (2H, s, H-2, 6)] revealed the other two benzenes. Five methoxy proton signals at [ $\delta_H$  3.81 (6H, s, 3,5-OCH<sub>3</sub>), 3.84 (3H, s, 3''-OCH<sub>3</sub>), and 3.86 (6H, s, 3',5'-OCH<sub>3</sub>)], four methines signals at [ $\delta_H$  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 [ $\delta_H$  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  $^1H$ - $^1H$  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 propanolguaiaicol 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 sesquignolignan. 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).<sup>23,24</sup> 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  $^1H$  and  $^{13}C$  NMR (Table 3) of **8** showed signals attributed to a 1,2,4-trisubstituted aromatic rings at [ $\delta_H$  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 [ $\delta_H$  6.70 (2H, s, H-2'/6')], an asymmetrical 1,2,3,5-tetrasubstituted aromatic ring at [ $\delta_H$  6.74 (1H, brs, H-6), 6.72 (1H, brs, H-2)], and four methoxy groups at [ $\delta_H$  3.87 (3H, s, 5-OCH<sub>3</sub>), 3.78 (6H, s, 3',5'-OCH<sub>3</sub>), 3.83 (3H, s, 3''-OCH<sub>3</sub>)]. Additionally, an anomeric proton [ $\delta_H$  4.87 (1H, d,  $J = 7.4$  Hz, H-1'')] was indicative of a monosaccharide moiety in **8**, which was identified as  $\beta$ -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 [ $\delta_C$  88.7, 87.0, 73.8, and 55.7] and five methylenes [ $\delta_C$  65.0, 62.2, 61.6, 35.8, and 32.9], attributing to three  $C_3$  moieties, which were confirmed by  $^1H$ - $^1H$  COSY and HMBC correlations. These NMR spectroscopic data supposed **8** to be a sesquignan glycoside and were in good agreement with those of acernikol-4''-O- $\beta$ -D-glucopyranoside.<sup>25</sup> Subsequently, the  $^1H$ - $^1H$  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  $^1H$ ,  $^{13}C$ , 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- $\beta$ -D-glucopyranoside<sup>25</sup> (**10**), acernikol<sup>26</sup> (**11**), seslignanoccidentalol A<sup>27</sup> (**12**), *erythro*-4,7,9,9'-tetrahydroxy-3,3',5'-trimethoxy-8-O-4'-neolignan<sup>28</sup> (**13**), *threo*-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-O-4'-neolignan<sup>29</sup>(**14**), *erythro*-4,7,9,9'-tetrahydroxy-

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

## 2.2 The activity of anti-inflammatory

PGE<sub>2</sub> 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<sub>2</sub> with the IC<sub>50</sub> values of 18.41 μM and 10.84 μM, respectively (Fig. 3). Compound **20** had a moderate inhibitory effect with an IC<sub>50</sub> 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<sub>50</sub> values of 53.09 μM and 53.70 μM, respectively (Fig. S84†).

The observed PGE<sub>2</sub> 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.<sup>38</sup> 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<sub>2</sub> and NO production.<sup>39</sup> 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.<sup>40,41</sup> 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<sub>2</sub> and NO production.<sup>42</sup> 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<sub>2</sub> inhibitory activity.

## 3 Conclusions

In the present research, nine undescribed compounds (**1–9**), including five benzofuran lignans, one ditetrahydrofuran lignan and three sesquileignans, together with fifteen known 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<sub>2</sub> production in LPS-stimulated RAW264.7 cells. This study not only enriched the chemical diversity of ligands in *I. dunnianum*, but also provided an experimental basis 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<sub>3</sub>OD: δ<sub>H</sub> 3.30/δ<sub>C</sub> 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<sub>18</sub> 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 C<sub>18</sub> column (5 μm, ϕ 10.0 × 250 mm; Phenomenex Inc., Los Angeles, USA) and preparative HPLC using a Cosmosil Packed C<sub>18</sub> 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

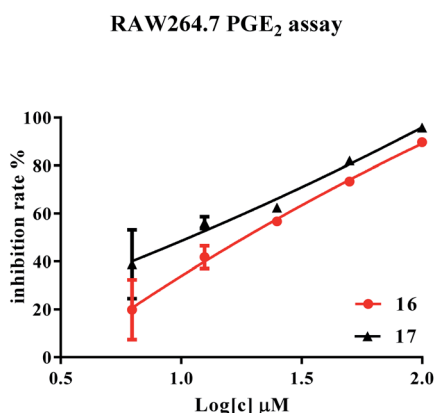


Fig. 3 Effect of compounds **16** and **17** on PGE<sub>2</sub> production in LPS-stimulated RAW264.7 cells.



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<sub>2</sub>O (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<sub>2</sub>Cl<sub>2</sub>-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<sub>3</sub>OH-H<sub>2</sub>O 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<sub>2</sub>O 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<sub>3</sub>OH-H<sub>2</sub>O (containing 0.1% HCOOH), 8 mL min<sup>-1</sup>] to yield 2 (10 mg) and fractions 3L4A2-3L4A9. Fr. 3L4A5 was isolated using semipreparative HPLC [22% CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH), 3 mL min<sup>-1</sup>] to yield 3 (5.5 mg) and 8 (13 mg). Fr. 3L4A6 was isolated using semipreparative HPLC [16% CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH), 3 mL min<sup>-1</sup>] to yield 9 (7.9 mg) and 10 (15.3 mg). Fr. 3L4A7 was isolated using semipreparative HPLC [18% CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH), 3 mL min<sup>-1</sup>] to yield 4 (4.3 mg). Fr. 3L4A8 was isolated using semipreparative HPLC [22% CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH), 3 mL min<sup>-1</sup>] to yield 5 (11.8 mg). Fr. 3L4A9 was isolated using semipreparative HPLC [23% CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH), 3 mL min<sup>-1</sup>] to yield 1 (45.4 mg). Fr. 3 G (7.1 g) was chromatographed by ODS CC using a CH<sub>3</sub>OH-H<sub>2</sub>O 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<sub>3</sub>OH-H<sub>2</sub>O (0.1% HCOOH), 3 mL min<sup>-1</sup>] to yield 6 (5.6 mg). Fr. 3G9 was further purified by semipreparative HPLC [20% CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH), 3 mL min<sup>-1</sup>] to yield 11 (21 mg). Fr. 3F (5.7 g) was chromatographed by ODS CC using a CH<sub>3</sub>OH-H<sub>2</sub>O 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<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH, 3.0 mL min<sup>-1</sup>). Compound 22 (3.5 mg) was obtained from Fr. 3F6B by semipreparative HPLC with 30% CH<sub>3</sub>OH-H<sub>2</sub>O (0.1% HCOOH, 3.0 mL min<sup>-1</sup>). Fr. 3F6D was further purified by semipreparative HPLC [30% CH<sub>3</sub>OH-H<sub>2</sub>O (0.1% HCOOH), 3 mL min<sup>-1</sup>] 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<sub>3</sub>OH-H<sub>2</sub>O (0.1% HCOOH, 3.0 mL min<sup>-1</sup>). 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<sub>3</sub>CN-H<sub>2</sub>O, 30% CH<sub>3</sub>OH-H<sub>2</sub>O, 25% CH<sub>3</sub>OH-H<sub>2</sub>O and 30% CH<sub>3</sub>OH-H<sub>2</sub>O (0.1% HCOOH, 3.0 mL min<sup>-1</sup>), 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<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH, 3.0 mL min<sup>-1</sup>). Fr. 3F9E and Fr. 3F9F was further purified by semipreparative HPLC [40% CH<sub>3</sub>OH-H<sub>2</sub>O, 25% CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH), 3 mL min<sup>-1</sup>] 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<sub>3</sub>CN-H<sub>2</sub>O and 30% CH<sub>3</sub>CN-H<sub>2</sub>O (0.1% HCOOH, 1 mL min<sup>-1</sup>, 35 °C), respectively. Compound 7 was using EnantioPak OZ-3 column (4.6 × 250 mm) with 35% CH<sub>3</sub>CN-H<sub>2</sub>O.

### 5.2 Structural characterization of undescribed compounds

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

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

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

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

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



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

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

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

Illiciumlignan O (9): white amorphous powder;  $[\alpha]_D^{25}$   $-15.43$  ( $c$  0.7, MeOH); UV(MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 206 (5.03), 235 (4.51), 277 (4.14) nm; IR (KBr)  $\nu_{\max}$  3374, 2936, 2884, 1722, 1614, 1504, 1461, 1427, 1328, 1212, 1127, 1036  $\text{cm}^{-1}$ ; the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra data see Table 3; HR-ESI-MS  $m/z$ : 771.2833  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{37}\text{H}_{48}\text{O}_{16}\text{Na}$ , 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  $\mu\text{L}$  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  $\mu\text{m}$  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<sub>2</sub> production RAW264.7 cells were plated in 24-well plates at  $1 \times 10^5$  cells per mL (400  $\mu\text{L}$  per well) and cultured for 24 h in a 37 °C 5% CO<sub>2</sub> incubator, then the supernatants were removed. 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  $\mu\text{L}$  per well). After one hour later, 5  $\mu\text{L}$  of LPS at 100  $\mu\text{g mL}^{-1}$  was added into each well, the control/model wells was given 5  $\mu\text{L}$  0.1% DMSO in DMEM. Then the cells were cultured under normal condition for 18 h. Then the supernatants were collected and PGE<sub>2</sub> levels were determined.

NO suppression RAW264.7 cells were plated in 96-well plates at  $2 \times 10^6$  cells per mL (100  $\mu\text{L}$  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.

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