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Enantiomeric neolignans and sesquineolignans from *Jatropha integerrima* and their absolute configurations†

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Two pairs of new sesquineolignan enantiomers, (±)-jatrointelignans A and B (**1a/1b** and **2a/2b**), one pair of new neolignan enantiomers, (±)-jatrointelignan D (**4a/4b**), and two new neolignans, (+)-jatrointelignan C (**3a**), and (+)-schisphenlignan I (**5a**) together with seven known analogues (**3b**, **5b**, **6a**, **6b**, **7a**, **7b**, and **8**) were isolated from the trunks of *Jatropha integerrima*. The structures were determined by combined spectroscopic and chemical methods, and their absolute configurations were elucidated by the circular dichroism (CD) method, in particular the configurations of the aryl glycerol-7″,8″-yloxy moiety in **1** and **2** were determined *via* the $Rh_2(OCOCF_3)_4$-induced CD analysis. All compounds were examined for their inhibitory effects on nitric oxide (NO) production induced by lipopolysaccharide (LPS) in BV-2 microglial cells, and compounds **5a**, **6a**, and **4b** exhibited pronounced inhibition on NO production with IC_{50} values in the range of 5.9–8.9 μM, being more active than the positive control, quercetin (IC_{50} = 17.0 μM).

Introduction

Neuroinflammation has been considered as one of the pathological factors in neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), stroke, dementia, and amyotrophic lateral sclerosis (ALS).[1,2] Activation of brain microglial cells and consequent overexpression of proinflammatory mediators such as nitric oxide (NO) are involved in the neuroinflammatory process. The production of NO and superoxide also lead to the formation of peroxynitrite, which results in lots of oxidation and potential destruction of host cellular constituents causing dysfunctional critical cellular processes, cell signaling pathway disruption, and brain cell death *via* cell apoptosis and necrosis.[3,4] There is numerous evidence suggesting that suppression of proinflammatory mediators (such as NO) and further inhibition of the neuroinflammatory responses in microglia could attenuate the severity or delay the progress of these neurodegenerative disorders.[5,6] Therefore, suppression of NO production in microglial cells might be an important and attractive therapeutic target for the treatment of neurodegenerative diseases.

Jatropha integerrima Jacq (Euphorbiaceae), is a common ornamental shrub widely distributed in subtropical and tropical areas of the world.[7] Its leaves have been reported to possess a strong purgative effect.[8] Previous chemical investigation of this plant led to the isolation of macrocyclic diterpenes,[8] sesquiterpenes,[9] sesquiterpene-coumarin,[9] and cyclic peptides,[10] some of which showed antimicrobial,[11] antiplasmodial,[9] and antituberculosis activities.[9] In our screening program aimed at the discovery of novel NO inhibitors from natural resource,[12] the EtOAc fraction of the ethanolic extract of *J. integerrima* showed a certain inhibitory activity against the lipopolysaccharide (LPS)-induced NO production in BV-2 microglial cells. Subsequent chemical investigation led to the isolation of seven pairs of lignan enantiomers and a racemic mixture, including eight new compounds. Bioassay verified that 13 compounds were responsible for the NO inhibitory activities of the EtOAc fraction, with IC_{50} values ranging from 5.9 to 38.0 μM. Herein, details of the isolation, structural elucidation, and NO inhibitory activities of these compounds were described.

Results and discussion

The air-dried powder of the trunks of *J. integerrima* was extracted with 95% EtOH at room temperature (rt) to give a crude extract, which was suspended in H_2O and successively partitioned with petroleum ether, EtOAc, and *n*-BuOH. Various column chromatographic separations of the EtOAc extract

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afforded seven pairs of enantiomers **1a**/**1b**–**7a**/**7b** and a racemic mixture (**8**).

Compound **1** (**1a**/**1b**), colorless oil, exhibited a molecular formula of $C_{31}H_{36}O_{11}$ as determined by the ^{13}C NMR data and a HRESIMS ion at *m*/*z* 607.2121 $[M + Na]^+$ (calcd for $C_{31}H_{36}O_{11}Na$, 607.2129). The IR spectrum exhibited absorption bands for OH (3460 cm^{-1}) and aromatic (1595, 1502, and 1464 cm^{-1}) functionalities. The ^1H NMR spectrum revealed the presence of four methoxy groups [δ_H 3.89 (3H, s), 3.82 (3H × 2, s), and 3.81 (3H, s)], three oxygenated methylenes [δ_H 4.19 (2H, dd, J = 6.0, 1.2 Hz, H-9′), 3.86 (1H, m, H-9a), 3.80 (1H, m, H-9b), 3.73 (1H, m, H-9″a), and 3.34 (1H, m, H-9″b)], three oxygenated methines [δ_H 5.58 (1H, d, J = 6.0 Hz, H-7), 4.98 (1H, d, J = 6.9 Hz, H-7″), and 4.08 (1H, ddd, J = 6.9, 5.0, 3.5 Hz, H-8″)], one disubstituted *E* double bond [δ_H 6.54 (1H, d, J = 15.8 Hz, H-7′) and 6.23 (1H, dt, J = 15.8, 6.0 Hz, H-8′)], a symmetrically 1,2,3,5-tetrasubstituted benzene ring (ring A) [δ_H 6.72 (2H, s, H-2/6)], an 1,2,3,5-tetrasubstituted benzene ring (ring B) [δ_H 6.95 (2H, brs, H-2′/6′)], and an 1,2,4-trisubstituted benzene ring (ring C) [δ_H 6.86 (1H, ddd, J = 8.0, 2.0, 2.0 Hz, H-6″), 6.75 (1H, d, J = 8.0 Hz, H-5″), and 6.99 (1H, dd, J = 2.0, 2.0 Hz, H-2″)]. The ^{13}C NMR spectrum, in combination with DEPT experiments, resolved 31 carbon resonances attributable to three benzene rings, one double bond, three oxygenated methylenes, four methines (three oxygenated methines), and four methoxy groups. Above-mentioned data implied that **1** possessed a sesquineolignan feature[13,14] and resembled that of buddlenol B isolated from *Buddleja davidii*.[15] The gross structure of **1** was further established by analysis of its 2D NMR data. The ^1H–^1H COSY spectrum established three C_3 spin systems from C-7 to C-9, C-7′ to C-9′, and C-7″ to C-9″. The HMBC correlations (Fig. 1) from H-7 to C-1, C-2, and C-6, from H-7′ to C-1′, C-2′, and C-6′, and from H-7″ to C-1″, C-2″, and C-6″ connected the three C_3 units to three benzene rings, respectively, thus indicating the presence of three phenylpropanoid units. The connectivities of the three phenylpropanoid units were established by HMBC correlations from both H-8, H-7 to C-3′ and C-4′ and from H-8″ to C-4, which necessitated the presence of a 2,3-dihydrofuran ring. The locations of the

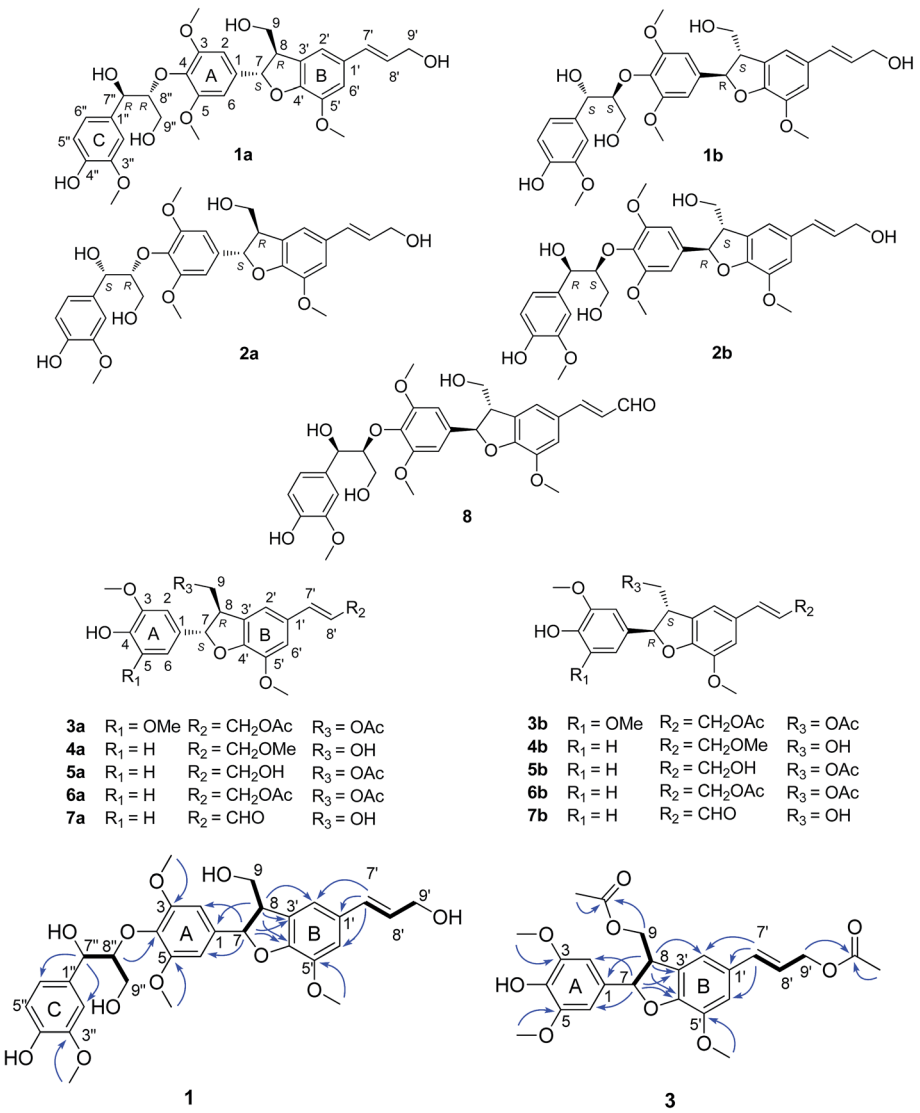

Fig. 1 Selected ^1H–^1H COSY (—) and HMBC (→) correlations of **1** and **3**.

methoxyl groups were further defined by detailed HMBC analyses (Fig. 1). The relative configuration of **1** was established on the basis of the NOESY experiment and interpretation of ^1H–^1H coupling constants. A coupling constant of 15.8 Hz between H-8′ and H-7′ and strong NOE interaction of H-9′/H-7′ assigned $\Delta^{7'}$ as *E* configuration. A coupling constant of 6.0 Hz between H-7 and H-8 implied the *trans*-relationship between these two protons,[16] which was further supported by NOESY correlations between H-7 and H_2-9 (ESI S48†), while the coupling constant between H-7″ and H-8″ (6.9 Hz) implied a *threo* configuration of these two protons.[17,18]

Compound **1** was primarily obtained with the specific rotation being almost zero and no Cotton effect in its electronic circular dichroism (ECD), indicating a near racemic nature. Subsequent chiral resolution of **1** afforded the anticipated enantiomers **1a** and **1b**, which showed mirror image-like ECD curves (Fig. 2A) and opposite specific rotation ($[\alpha]_D^{20}$ +15 in **1a**, $[\alpha]_D^{20}$ −13 in **1b**). The absolute configurations (ACs) of **1a** and **1b** were determined by CD analysis. According to the reversed helicity rule for the 7-methoxy-2,3-dihydrobenzo[*b*]furan chromophore, *P*/*M* helicity of the heterocyclic ring leads to positive/negative ^1L_b band CD (at 260–310 nm).[19,20] In the CD spectrum of **1a**, a positive Cotton effect at 273 nm ($\Delta\varepsilon$ +2.77) indicated a *P*-helicity and thus assigned the ACs of C-7 and C-8 as 7*S* and 8*R*.[21,22] The absolute configuration of C-7″ was assigned by $Rh_2(OCOCF_3)_4$-induced CD analysis. On the basis of the bulkiness rule for secondary alcohols, a positive Cotton effect at around 350 nm (the E band) in the $Rh_2(OCOCF_3)_4$-induced CD spectrum indicated a *S*-configuration, while negative Cotton effect implied a *R*-configuration.[23,24] Thus, a negative Cotton effect at 353 nm (the E band) in the $Rh_2(OCOCF_3)_4$-induced CD spectrum of **1a** assigned the 7″*R* configuration.[13] (Fig. 2B) Consequently, the 8″*R* configuration was defined by the 7″,8″-*threo* relationship in **1a**. Thus, **1a** was identified as (+)-(7*S*,7″*R*,8*R*,8″*R*,7′*E*)-4″-hydroxy-3,3″,5,5′-tetramethoxy-4′,7-epoxy-8,3′-sesquineolign-7′-en-7″,9,9′,9″-tetraol and was given a trivial name (+)-jatrointelignan A. Consequently, its enantiomer **1b** was named as (−)-jatrointelignan A.

Compound **2** (**2a**/**2b**), colorless oil, had the same molecular formula as that of **1**. The NMR data of **2** was very similar to that of **1**, except for the slight discrepancy arising from the oxygenated methines (C-7″ and C-8″). Detailed 2D analysis revealed that **2** shared the same planar structure as that of **1**, indicating that **2** was a stereoisomer of **1** with variation at configuration at C-7″ or C-8″. An *erythro* configuration for the aryl glycerol-8″-yloxy moiety in **2** was further determined by the coupling constants between H-7″ and H-8″ ($J_{7'',8''}$ = 5.1 Hz).[13,14,17] Compound **2** also had negligible optical activity and CD spectrum. Subsequent chiral resolution of **2** afforded the enantiomers **2a** and **2b**. Using the same methods as described for **1a** and **1b**, the 7*S*,7″*S*,8*R*,8″*R* configuration of **2a** and the 7*R*,7″*R*,8*S*,8″*S* configuration of **2b** were elucidated (ESI, S15 and S16†). Thus, **2a** was assigned as (+)-(7*S*,7″*S*,8*R*,8″*R*,7′*E*)-4″-hydroxy-3,3″,5,5′-tetramethoxy-4′,7-epoxy-8,3′-sesquineolign-7′-en-7″,9,9′,9″-tetraol, and was given a trivial name (+)-jatrointelignan B. Consequently, its enantiomer **2b** was named as (−)-jatrointelignan B.

Compound **3** (**3a**/**3b**) was obtained as a pale gum. The spectroscopic data of **3** were identical to those of (−)-(7′*R*,8′*S*)-5′-methoxyl-(dimeric coniferyl acetate) (**3b**),[25] except for a negligible specific rotation and CD spectrum in **3**, indicating **3** was a racemic mixture. Subsequent chiral resolution of **3** afforded the anticipated enantiomers **3a** and **3b**, which were opposite in terms of ECD curves and optical rotation, respectively. The ACs of **3a** and **3b** were determined by using the same CD methods as described for 7-methoxy-2,3-dihydrobenzo[*b*]furan chromophore in **1a** and **1b**. In the CD spectrum of **3a**, (Fig. 2A) a positive Cotton effect at 273 nm ($\Delta\varepsilon$ +3.58) indicated a 7*S*,8*R* configuration,[21] which was supported by its optical rotation ($[\alpha]_D^{20}$ +102), as neolignans containing a dihydrobenzo[*b*]furan skeleton with 7*S*,8*R* configuration exhibited a positive optical rotation.[22] Thus, **3a** was defined as (+)-(7*S*,8*R*,7′*E*)-4-hydroxy-3,5,5′,-trimethoxy-4′,7-epoxy-8,3′-neolign-7′-en-9,9′-diyil diacetate and was given a trivial name (+)-jatrointelignan C. Consequently, **3b** was identified as the known compound (−)-(7′*R*,8′*S*)-5′-methoxyl-(dimeric coniferyl acetate).

Compound **4** (**4a**/**4b**), obtained as a pale gum, gave a $[M + Na]^+$ ion at *m*/*z* 395.1458 in the HRESIMS corresponding to a molecular formula of $C_{21}H_{24}O_6$. The spectroscopic data showed similarity to that of co-isolated known compound **6b**.[26] In comparison with **6b**, the major differences of **4** were due to 9′-OCH_3 and 9-OH functionalities. This was supported by the HMBC correlation from the methoxy protons (δ_H 3.39, s) to C-9′ (δ_C 73.2) and the upfield-shifted H_2-9 signals in **4** with respect to those of **6b** [δ_H 3.97 (H-9a), 3.92 (H-9b) in **4**; δ_H 4.42 (H-9a), 4.28

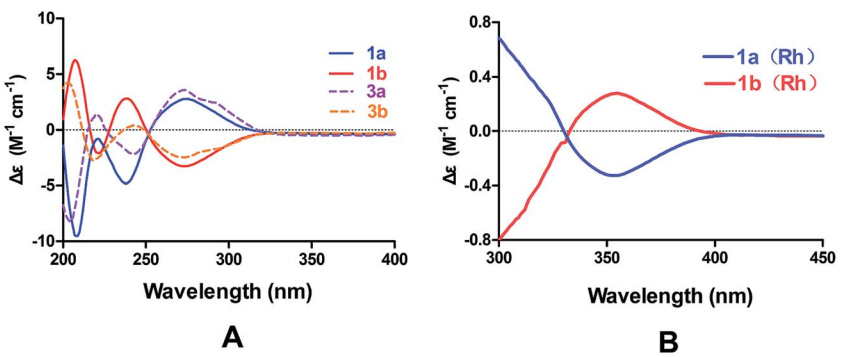

Fig. 2 The CD spectra of **1a**, **1b**, **3a**, and **3b** in MeCN (**A**) and the $Rh_2(OCOCF_3)_4$ induced CD spectra of **1a** and **1b** in CH_2Cl_2 (**B**).

(H-9b) in **6b**]. The relative configuration of **4** was assigned to be the same as that of **3** based on a coupling constant of 7.5 Hz between H-7 and H-8 and NOE correlations between H-7 and H_2-9. Compound **4** also had negligible optical activity and CD spectra. Subsequent chiral resolution of **4** afforded the enantiomers **4a** and **4b**. The ACs of the **4a** and **4b** were determined to be 7*S*,8*R* and 7*R*,8*S*, respectively, by using the same methods as described for those of **3a** and **3b** (ESI, S34†). Thus, **4a** was elucidated the same ACs as **3a**, and was defined as (+)-(7*S*,8*R*,7′*E*)-4-hydroxy-3,5′,9′-trimethoxy-4′,7-epoxy-8,3′-neolign-7′-en-9-ol and was given a trivial name (+)-jatrointelignan D. Consequently, its enantiomer **4b** was named as (−)-jatrointelignan D.

Compound **5** (**5a**/**5b**), obtained as a yellow oil, gave a $[M + Na]^+$ ion at *m*/*z* 423.1404 in the HRESIMS appropriate for the molecular formula of $C_{22}H_{24}O_7$. The NMR data of **5** were identical to those of schisphenlignan I, which was isolated from *Clematis armandii*[27] and *Schisandra sphenanthera*.[28] Chiral resolution of **5** afforded the enantiomers **5a** and **5b**. The specific rotation and CD data of **5b** were almost identical to those of schisphenlignan I, indicating **5b** possessed the same 7*R*,8*S* configuration (ESI, S41†). Thus, compound **5a** was elucidated as (+)-(7*S*,8*R*,7′*E*)-4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligna-7′-en-9-acetyl-9′-ol and was given a trivial name (+)-schisphenlignan I.

Compound **8**, a colorless oil, had a molecular formula $C_{31}H_{34}O_{11}$ as determined by a HRESIMS ion at *m*/*z* 605.1967 $[M + Na]^+$ (calcd for $C_{31}H_{34}O_{11}Na$, 605.1969). The spectroscopic data of **8** were identical to those of buddlenol A[15] with a negligible specific rotation and CD spectrum. Chiral analysis of **8** in HPLC revealed two blunt peaks with poor resolution. Attempts to separate each peak failed in various conditions probably due to its low polarity. To increase the polarity of **8**, a reduction reaction was carried out under $NaBH_4$ to transform the formyl group to alcohol which subsequently generated **2**. Compound **2** was further purified by chiral separation to give **2a** and **2b**. Therefore the buddlenol A (**8**) was confirmed as a racemic mixture.

The known compounds (−)-(7′*R*,8′*S*)-5′-methoxyl-(dimeric coniferyl acetate) (**3b**),[25] schisphenlignan I (**5b**),[27,28] dimeric coniferyl acetate (**6a**),[29] (−)-(7′*E*)-(7*R*,8*S*,7″*E*)-4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neolig-7′-en-9,9′-diyil diacetate (**6b**),[26] (+)-balanophonin (**7a**),[22,30] (−)-balanophonin (**7b**),[31] and buddlenol A (**8**)[15] were identified by comparison of their NMR data, optical rotations, and CD spectra with those in the literature.

All compounds were evaluated for their inhibitory effects on the NO production in LPS-induced BV-2 microglial cells using the Griess assay. Compounds **1a** and **1b**, were inactive (<50% inhibition at 50 μM), while compounds **2a**, **2b**, **3a**, **3b**, **4a**, **5b**, **6b**, **7a**, **7b**, and **8** showed moderate inhibitory activities with IC_{50} values ranging from 12.5 to 38.0 μM. Compounds **4b**, **6a**, and **5a** showed remarkable inhibitory activities with IC_{50} values of 8.9, 7.9, and 5.9 μM, respectively, more active than the positive control quercetin (IC_{50} = 17.0 μM), a well-known NO inhibitor (Table 3). The inhibitory curves of **4b**, **6a**, **5a**, and quercetin were represented in Fig. 3. To investigate whether the inhibitory activities of the active compounds

Table 1 ^{1}H NMR data of compounds **1**–**5**[a]

Position	**1**[b]	**2**[b]	**3**[c]	**4**[c]	**5**[c]
2	6.72, s	6.70, s	6.62, s	6.91, s	6.89, s
5				6.88, s	6.87, s
6	6.72, s	6.70, s	6.62, s	6.89, s	6.87, s
7	5.58, d (6.0)	5.57, d (6.0)	5.45, d (7.5)	5.58, d (7.5)	5.46, d (7.5)
8	3.47, ddd (6.5, 6.0, 5.7)	3.48, ddd (6.5, 6.0, 5.7)	3.78, ddd (7.5, 7.3, 5.5)	3.62, ddd (7.5, 6.0, 5.0)	3.76, ddd (7.5, 7.3, 5.5)
9a	3.86, m	3.90, m	4.45, dd (11.2, 5.5)	3.97, dd (11.2, 6.0)	4.43, dd (11.2, 5.5)
9b	3.80, m	3.87, m	4.32, dd (11.2, 7.3)	3.92, dd (11.2, 5.0)	4.30, m
2′	6.95, brs	6.95, brs	6.89, s	6.89, s	6.87, s
6′	6.95, brs	6.95, brs	6.89, s	6.89, s	6.87, s
7′	6.54, d (15.8)	6.54, d (15.8)	6.60, d (15.8)	6.55, d (15.8)	6.55, d (15.8)
8′	6.23, dt (15.8, 6.0)	6.23, dt (15.8, 6.0)	6.16, dt (15.8, 6.0)	6.15, dt (15.8, 6.0)	6.24, dt (15.8, 6.0)
9′	4.19, dd (2H, 6.0, 1.2)	4.19, dd (2H, 6.0, 1.2)	4.72, dd (2H, 6.0, 1.2)	4.07, dd (2H, 6.0, 1.2)	4.30, (2H, m)
2″	6.99, dd (2.0, 2.0)	6.96, brs			
5″	6.75, d (8.0)	6.74, d (8.0)			
6″	6.86, ddd (8.0, 2.0, 2.0)	6.79, dd (8.0, 1.6)			
7″	4.98, d (6.9)	4.90, d (5.1)			
8″	4.08, ddd (6.9, 5.0, 3.5)	4.25, ddd (5.1, 4.7, 3.5)			
9″a	3.73, m	3.90, m			
9″b	3.34, m	3.58, dd (12.0, 3.5)			
3-OCH_3	3.82, s	3.78, s	3.87, s	3.86, s	3.85, s
5-OCH_3	3.82, s	3.78, s	3.87, s		
5′-OCH_3	3.89, s	3.89, s	3.91, s	3.90, s	3.90, s
9′-OCH_3				3.39, s	
3″-OCH_3	3.81, s	3.80, s			
9-OAc			2.04, s		2.02, s
9′-OAc			2.10, s		

[a] Data were recorded at 400 MHz, chemical shifts are in ppm, coupling constant *J* is in Hz. [b] In CD_3OD. [c] In $CDCl_3$.

Table 2 ^{13}C NMR (100 MHz) data (δ) of compounds 1–5

Position	1[a]	2[a]	3[b]	4[b]	5[b]
1	139.5, C	139.4, C	134.9, C	132.9, C	132.3, C
2	103.9, CH	104.0, CH	103.1, CH	108.8, CH	108.6, CH
3	154.4, C	154.6, C	147.2, C	146.7, C	146.7, C
4	136.9, C	136.4, C	148.2, C	145.7, C	145.8, C
5	154.4, C	154.6, C	147.2, C	114.3, CH	114.3, CH
6	103.9, CH	103.9, CH	103.1, CH	119.4, CH	119.9, CH
7	88.9, CH	88.9, CH	89.0, CH	88.2, CH	88.7, CH
8	55.4, CH	55.4, CH	50.4, CH	53.5, CH	50.3, CH
9	64.9, CH_2	64.9, CH_2	65.3, CH_2	64.0, CH_2	65.3, CH_2
1′	132.8, C	132.8, C	130.6, C	130.9, C	131.0, C
2′	116.5, CH	116.5, CH	115.3, CH	114.8, CH	115.0, CH
3′	130.0, C	130.0, C	127.7, C	128.0, C	127.7, C
4′	149.1, C	149.1, C	148.2, C	148.3, C	147.9, C
5′	145.5, C	145.5, C	144.4, C	144.4, C	144.4, C
6′	112.2, CH	112.2, CH	110.6, CH	110.5, CH	110.5, CH
7′	131.9, CH	131.9, CH	134.3, CH	132.5, CH	131.3, CH
8′	127.8, CH	127.7, CH	121.2, CH	123.9, CH	126.5, CH
9′	63.8, CH_2	63.8, CH_2	65.2, CH_2	73.2, CH_2	63.7, CH_2
1″	133.4, C	133.8, C			
2″	111.7, CH	111.7, CH			
3″	148.7, C	148.6, C			
4″	147.1, C	146.8, C			
5″	115.8, CH	115.7, CH			
6″	120.9, CH	120.7, CH			
7″	74.5, CH	74.0, CH			
8″	88.9, CH	87.3, CH			
9″	61.8, CH_2	61.6, CH_2			
3-OCH_3	56.7, CH_3	56.6, CH_3	56.4, CH_3	56.0, CH_3	56.0, CH_3
5-OCH_3	56.7, CH_3	56.6, CH_3	56.4, CH_3		
5′-OCH_3	56.8, CH_3	56.8, CH_3	56.0, CH_3	56.0, CH_3	55.9, CH_3
3″-OCH_3	56.4, CH_3	56.3, CH_3			
9′-OCH_3				57.9, CH_3	
9-OAc			170.7, C		170.8, C
			21.0, CH_3		20.7, CH_3
9′-OAc			170.9, C		
			20.8, CH_3		

[a] In CD_3OD. [b] In $CDCl_3$.

Table 3 IC_{50} values of active compounds on LPS-induced NO production in BV-2 cells

Compound	IC_{50}[a] (μM)	Compound	IC_{50}[a] (μM)
2a	12.5 ± 1.2	**5b**	14.7 ± 1.2
2b	38.0 ± 2.1	**6a**	7.9 ± 2.5
3a	32.7 ± 2.3	**6b**	35.5 ± 3.1
3b	25.9 ± 1.7	**7a**	24.9 ± 2.1
4a	26.4 ± 2.0	**7b**	29.8 ± 1.3
4b	8.9 ± 0.9	**8**	12.8 ± 1.3
5a	5.9 ± 0.8	Quercetin[b]	17.0 ± 2.2

[a] Values are represented as means ± SD based on three independent experiments. [b] Positive control.

were generated from their cytotoxicity, the effects of compounds **2a**, **3b**, **4a**, **4b**, **5a**, **6a**, **6b**, and **8** on LPS-induced BV-2 microglial cell viability were measured using the MTT method. These eight compounds (up to 80 μM) did not show any significant cytotoxicity with LPS treatment for 24 h (ESI, S49†).

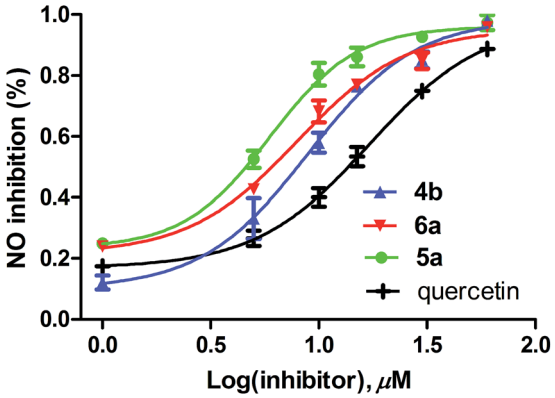

Fig. 3 The inhibitory curves of compounds **4b**, **5a**, **6a**, and quercetin (positive control) on LPS-induced NO production in BV-2 cells.

Conclusions

In summary, four new sesquineolignans, four new neolignans, and seven known analogues were isolated from the trunks of *J. integerrima*. The structures were determined by combined spectroscopic and chemical methods, and their ACs were elucidated by circular dichroism (CD) method, particularly the configurations of the aryl glycerol-7″,8″-yloxy moiety in **1** and **2** were determined *via* the $Rh_2(OCOCF_3)_4$-induced CD analysis. All compounds were examined for their inhibitory effects on the nitric oxide (NO) production induced by lipopolysaccharide (LPS) in BV-2 microglial cells, and compounds **5a**, **6a**, and **4b** exhibited pronounced inhibition on NO production with IC_{50} values in the range of 5.9–8.9 μM, being more active than the positive control, quercetin (IC_{50} = 17.0 μM). *J. integerrima* is known as a rich source of macrocyclic diterpenoids. The current study not only enriched the chemodiversity of this spices by the isolation of a series of bioactive lignans, but also dig out the potential usage of this plant as anti-neuroinflammatory therapy. However, the mechanism of inhibition against NO production of these compounds requires further investigation.

Experimental section

General experimental procedures

Optical rotations were measured on a Rudolph Autopol I automatic polarimeter. CD spectra were obtained on an Applied Photophysics Chirascan spectrometer. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer. IR spectra were determined on a Bruker Tensor 37 infrared spectrophotometer. NMR spectra were measured on a Bruker AM-400 spectrometer at 25 °C. HRESIMS was performed on a Waters Micromass Q-TOF spectrometer. A Shimadzu LC-20 AT equipped with a SPD-M20A PDA detector was used for HPLC. A chiral column (Phenomenex Lux, cellulose-2, 250 × 10 mm, 5 μm) was used for semipreparative HPLC separation. Semipreparative HPLC was performed on a YMC-pack ODS-A (250 × 10 mm, S-5 μm, 12 nm). Silica gel (300–400 mesh, Qingdao Haiyang Chemical Co., Ltd.), and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) were used for column

chromatography (CC). All solvents were of analytical grade (Guangzhou Chemical Reagents Company, Ltd.).

Plant material

Trunks of *J. integerrima* were collected in the East campus of Sun Yat-sen University in the Guangzhou city, P. R. China, in November 2013, and were authenticated by Associate Professor Lin Jiang of Sun Yat-sen University. A voucher specimen (accession number: YJIZ-201311) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

Extraction and isolation

The air-dried powder of the trunks of *J. integerrima* (6 kg) was extracted with 95% EtOH (3 × 10 L) at rt to give 462 g of crude extract. The extract was suspended in H_2O (2 L) and successively partitioned with petroleum ether (PE, 3 × 2 L), EtOAc (3 × 2 L), and *n*-BuOH (3 × 2 L) to yield three corresponding portions. The EtOAc extract (34 g) was subjected to MCI gel CC eluted with a MeOH/H_2O gradient (2 : 8 → 10 : 0) to afford five fractions (I–V). Fr. I (17 g) was subjected to silica gel chromatography using hexane/EtOAc mixtures (v/v 1 : 0 → 0 : 1) to afford six fractions Ia–If. Fr. Ib (3.4 g) was subjected to silica gel chromatography using PE–acetone mixtures (v/v 1 : 0, 15 : 1, 10 : 1, 1 : 1) to yield four fractions, Fr. Ib (1–4). Fr. Ib3 was purified using semi-preparative HPLC with a YMC-pack ODS-A column (MeOH/H_2O, 7 : 3, 3 mL min^{-1}), followed by semipreparative chiral HPLC (CH_3OH/H_2O, 9 : 1, 3 mL min^{-1}) to give **4a** (3 mg, t_R 11 min) and **4b** (2.5 mg, t_R 12 min). Fr. Ic (1.5 g) was loaded on a Sephadex LH-20 column and eluted with CH_2Cl_2–MeOH (1 : 1) to yield four fractions, Fr. Ic (1–4). Fr. Ic2 was further purified by semi-preparative chiral HPLC (MeOH/H_2O, 8 : 2, 3 mL min^{-1}) to give **5a** (4 mg, t_R 15 min) and **5b** (5 mg, t_R 17 min). Fr. Ic3 was followed by semipreparative chiral HPLC (CH_3CN/H_2O, 4.5 : 5.5, 3 mL min^{-1}) to give **7a** (9 mg, t_R 10 min) and **7b** (11 mg, t_R 12 min). Fr. Ie (1.2 g) was loaded on a Sephadex LH-20 column and eluted with CH_2Cl_2–MeOH (1 : 1) to yield four fractions, Fr. Ie (1–4). Fr. Ie3 was purified using semipreparative HPLC with a YMC-pack ODS-A column (MeOH/H_2O, 6 : 4, 3 mL min^{-1}) to give **8** (17 mg). Fr. If (962 mg) was separated by a Sephadex LH-20 column ($CHCl_3$/MeOH, 1 : 1), followed by semipreparative HPLC with a YMC-pack ODS-A column (MeOH/H_2O, 5.5 : 4.5, 3 mL min^{-1}) to give Fr. If1 and Fr. If2, which were further purified by semipreparative chiral HPLC (CH_3CN/H_2O, 4 : 6, 3 mL min^{-1}) to give **2b** (7 mg, t_R 22 min) and **2a** (8 mg, t_R 23 min), **1b** (8 mg, t_R 23 min) and **1a** (10 mg, t_R 25 min), respectively. Fr. III (4 g) was subjected to silica gel eluted with hexane/EtOAc mixtures (v/v 1 : 0, 10 : 1, 5 : 1, 0 : 1) to afford six fractions, Fr. III (a–f). Fr. IIIe (900 mg) was loaded on a Sephadex LH-20 column and eluted with CH_2Cl_2–MeOH (1 : 1) to yield four fractions, Fr. IIIe (1–4). Fr. IIIe2 and Fr. IIIe3 were further purified using semipreparative chiral HPLC (CH_3OH/H_2O, 9 : 1, 3 mL min^{-1}) to give **3a** (1.5 mg) and **3b** (1.6 mg), **6a** (10 mg) and **6b** (11 mg), respectively.

(+)-Jatrointelignan A (1a). Colorless oil; $[\alpha]_D^{20}$ +15 (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 276 (4.00), 225 (4.46) nm; CD (*c* 2.74 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 208 (−9.54), 222 (+0.80), 240 (−4.65), 273 (+2.77) nm; IR (KBr) ν_{max} 3460, 2918, 1595, 1502, 1464, 1125 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 607.2121 $[M + Na]^+$ (calcd for $C_{31}H_{36}O_{11}Na$, 607.2129).

(−)-Jatrointelignan A (1b). Colorless oil; $[\alpha]_D^{20}$ −13 (*c* 0.19, $CHCl_3$); CD (*c* 3.25 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 208 (+6.21), 222 (−2.02), 240 (+2.78), 273 (−3.28) nm; UV, IR, NMR, and HRESIMS were the same with those of **1a**.

(+)-Jatrointelignan B (2a). Colorless oil; $[\alpha]_D^{20}$ +38 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 276 (4.14), 225 (4.61) nm; CD (*c* 2.57 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 208 (−6.53), 222 (+0.55), 240 (−4.35), 268 (+2.73) nm; IR (KBr) ν_{max} 3446, 2918, 1595, 1502, 1464 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 607.2134 $[M + Na]^+$ (calcd for $C_{31}H_{36}O_{11}Na$, 607.2140).

(−)-Jatrointelignan B (2b). Colorless oil; $[\alpha]_D^{20}$ −42 (*c* 0.12, $CHCl_3$); CD (*c* 2.05 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 208 (+9.82), 222 (−1.67), 240 (+4.38), 268 (−5.18) nm; UV, IR, NMR, and HRESIMS were the same with those of **2a**.

(+)-Jatrointelignan C (3a). Pale gum; $[\alpha]_D^{20}$ +102 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 275 (4.15), 213 (4.46) nm; CD (*c* 2.12 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 204 (−8.24), 220 (+1.43), 242 (−2.15), 273 (+3.58) nm; IR (KBr) ν_{max} 3410, 2928, 2214, 1735, 1605, 1501, 1454, 1236, 1113 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 495.1613 $[M + Na]^+$ (calcd for $C_{25}H_{28}O_9Na$, 495.1616).

(+)-Jatrointelignan D (4a). Pale gum; $[\alpha]_D^{20}$ +74 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 276 (3.76), 215 (4.14) nm; CD (*c* 3.49 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 208 (−6.43), 221 (+0.56), 239 (−3.25), 273 (+2.42) nm; IR (KBr) ν_{max} 3473, 2923, 1662, 1516, 1094 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 395.1458 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_6Na$ 395.1465).

(−)-Jatrointelignan D (4b). Pale gum; $[\alpha]_D^{20}$ −63 (*c* 0.15, $CHCl_3$); CD (*c* 3.32 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 204 (+6.61), 220 (−2.35), 242 (+2.90), 273 (−3.34) nm; UV, IR, NMR, and HRESIMS were the same with those of **4a**.

(+)-Schisphenlignan I (5a). Yellow oil; $[\alpha]_D^{20}$ +72 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 276 (3.73), 215 (3.94) nm; CD (*c* 2.69 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 215 (+0.49), 236 (−1.76), 268 (+2.19), 285 (+2.65) nm; IR (KBr) ν_{max} 3442, 2931, 1734, 1603, 1506, 1234 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 423.1404 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_7Na$, 423.1414).

Determination of the absolute configuration of the secondary alcohol units in 1 (1a/1b) and 2 (2a/2b)

Following the reported procedure,[23,24] a 1 : 2 mixture of secondary alcohol–$Rh_2(OCOCF_3)_4$ for **1a** was subjected to CD measurements at a concentration of 0.1 mg mL^{-1} in anhydrous CH_2Cl_2. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the band at around 350 nm in the induced CD spectrum was correlated to the absolute configuration of the secondary alcohol. Compounds **1b**, **2a**, and **2b** were measured by the same methods as **1a**.

Chemical transformation of 8 to 2

$NaBH_4$ (2 mg) was added to a stirred solution of **8** (4 mg) in MeOH (2 mL), and the reaction was stirred for 15 min at rt. The

mixture was then purified by HPLC on a semi-preparative YMC-pack ODS-A column (MeOH/H_2O, 5.5 : 4.5, 3 mL min^{-1}) to afford **2**. Compound **2** was further purified by semipreparative chiral HPLC (CH_3CN/H_2O, 4 : 6, 3 mL min^{-1}) to give **2a** and **2b**. Those compounds were identified by their ^1H NMR spectra, MS, and CD data.

Cell culture and viability assay

BV-2 microglial cells were obtained from Southern Medical University (SMU) Cell Bank (Guangzhou, People's Republic of China). Cells were plated into a 96-well plate (2×10^4 cells per well). After 24 h, they were pretreated with samples for 30 min and stimulated with 1 μg mL^{-1} LPS for another 24 h. The cell viability of the cultured cells was assessed by MTT assay. Briefly, BV-2 cells were incubated with 200 μL MTT solution (0.5 mg mL^{-1} in medium) for 4 h at 37 °C, and then the supernatants were removed and residues were dissolved in 200 μL DMSO. The absorbance was detected at 570 nm using a microplate reader (Molecular Devices, USA) and analyzed using a SoftMax Pro 5 software (Molecular Devices, USA).

Measurement of NO production

The NO concentration was measured by the Griess reaction. Briefly, BV-2 cells were treated with LPS (1.0 μg mL^{-1}) and compounds for 24 h. After that, 100 μL of culture supernatant was allowed to react with 100 μL of Griess reagent (1% sulfanilamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid) for 10 min at rt in the dark. Then, the optical density (100 μL per well) was measured at 540 nm using a microplate reader (Molecular Devices, USA). Sodium nitrite was used as a standard to calculate the nitrite concentration. Inhibition (%) = $(1 - (A_{LPS+sample} - A_{untreated})/(A_{LPS} - A_{untreated})) \times 100$. The experiments were performed in triplicates, and the data were expressed as the mean ± standard deviation (SD) values. Quercetin was used as a positive control.

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