Tetrahedron 66 (2010) 9379–9388

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

New sesquiterpenes from *Inula japonica* Thunb. with their inhibitory activities against LPS-induced NO production in RAW264.7 macrophages

Jiang-Jiang Qin [a], Hui-Zi Jin [a,*], Jia-Xian Zhu [a], Jian-Jun Fu [a], Qi Zeng [a], Xiang-Rong Cheng [a], Yan Zhu [a], Lei Shan [b], Shou-De Zhang [a], Yue-Xing Pan [b], Wei-Dong Zhang [a,b,*]

[a] School of Pharmacy, Shanghai Jiao Tong University, Dongchuan Rd 800, Shanghai 200240, PR China
[b] Department of Phytochemistry, Second Military Medical University, Shanghai 200433, PR China

ARTICLE INFO

Article history:
Received 17 June 2010
Received in revised form 18 September 2010
Accepted 29 September 2010
Available online 7 October 2010

Keywords:
Asteraceae
Inula japonica Thunb.
Sesquiterpenes
Nitric oxide (NO)
RAW264.7 macrophages
Structure–activity relationship (SAR)

ABSTRACT

Twenty-two new sesquiterpenes were isolated from the aerial parts of *Inula japonica* Thunb., together with fifteen known ones. Their structures were determined by detailed spectroscopic analysis, X-ray diffraction studies, and modified Mosher method. All 37 compounds were evaluated for the inhibition of LPS-induced nitric oxide (NO) production in RAW264.7 macrophages, and most of isolates significantly inhibited the NO production with IC_{50} values in the range of 3.5–20 μM. Besides, results obtained in our studies provided a structure–activity relationship that would be used to design anti-inflammatory agents in the future.

1. Introduction

Inula is a very important genus comprising about 100 species in the family Asteraceae.[1,2] Plants belonging to this genus show high diversity in their secondary metabolites as well as in pharmacological effects.[3] *Inula japonica* Thunb. is well known in China as 'Jinfeicao' and its aerial parts are used in traditional Chinese medicine for the treatment of various diseases such as tracheitis, bronchitis, hepatitis, and alimentary tract carcinoma.[1,2] Modern pharmacological studies have exhibited its diverse biological activities, such as anti-inflammatory, antifungal, antibacterial, antidiabetic, and hypolipidemic effects.[3–9] In the previous studies, 12 dimeric sesquiterpenes and 4 diterpenes have been reported.[7–9] As a part of our ongoing research program for bioactive secondary metabolites from *Inula* genus, the phytochemical analysis of *I. japonica* was further progressed and resulted the isolation and identification of 22 new sesquiterpenes (**1**–**22**) together with 15 known ones (**23**–**37**). In this paper, we described the isolation and structure elucidation of these new sesquiterpenes. Moreover, the inhibitory activities of all 37 isolates against LPS-induced NO production in RAW264.7 macrophages were also evaluated.

2. Results and discussion

2.1. Structure elucidation of new sesquiterpenes

The dried aerial parts of *I. japonica* were powdered and extracted with 95% ethanol and the extract was successively partitioned with petroleum ether, CH_2Cl_2, EtOAc, and *n*-BuOH, respectively. The CH_2Cl_2 fraction was subjected to column chromatography over silica gel, Sephadex LH-20 and preparative HPLC to afford 22 new sesquiterpenes (**1**–**22**), together with 15 known ones: 5α-hydroxyasperilin (**23**),[10] 1β-hydroxyalantolactone (**24**),[11] isoivasperin (**25**),[12] ivangustin (**26**),[13] 1,6α-dihydroxyeriolanolide (**27**),[14] 1-acetoxy-6α-hydroxyeriolanolide (**28**),[14] 1β-hydroxy-8β-acetoxycostic acid methyl ester (**29**),[6] 1β-hydroxy-8β-acetoxy-isocostic acid methyl ester (**30**),[6] 4*H*-xanthalongin (**31**),[11] xanthalongin (**32**),[15] eupatolide (**33**),[16] 7-epiloliolide (**34**),[17] vomifoliol (**35**),[18] corchoionol C (**36**),[19] grasshopper ketone (**37**)[20] (Fig. 1).

Compound **1** was obtained as optically active, colorless bulk crystals. The molecular formula $C_{15}H_{22}O_4$, indicating five degrees of unsaturation, was established by HRESIMS (*m*/*z* 289.1411 for $[M+Na]^+$, calcd *m*/*z* 289.1416). The IR spectrum of **1** showed bands characteristic of hydroxyl (3489, 3354 cm^{-1}), carbonyl (1751 cm^{-1}), and olefinic bond (1664 cm^{-1}). These observations were in agreement with the observation of signals in the ^{13}C and DEPT NMR spectra (Table 2) for two oxygenated methines (δ_C 75.4, C-1; δ_C 79.8,

* Corresponding authors. Tel./fax: +86 021 34205989; e-mail addresses: kimhz@sjtu.edu.cn (H.-Z. Jin), wdzhangy@hotmail.com (W.-D. Zhang).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.09.091

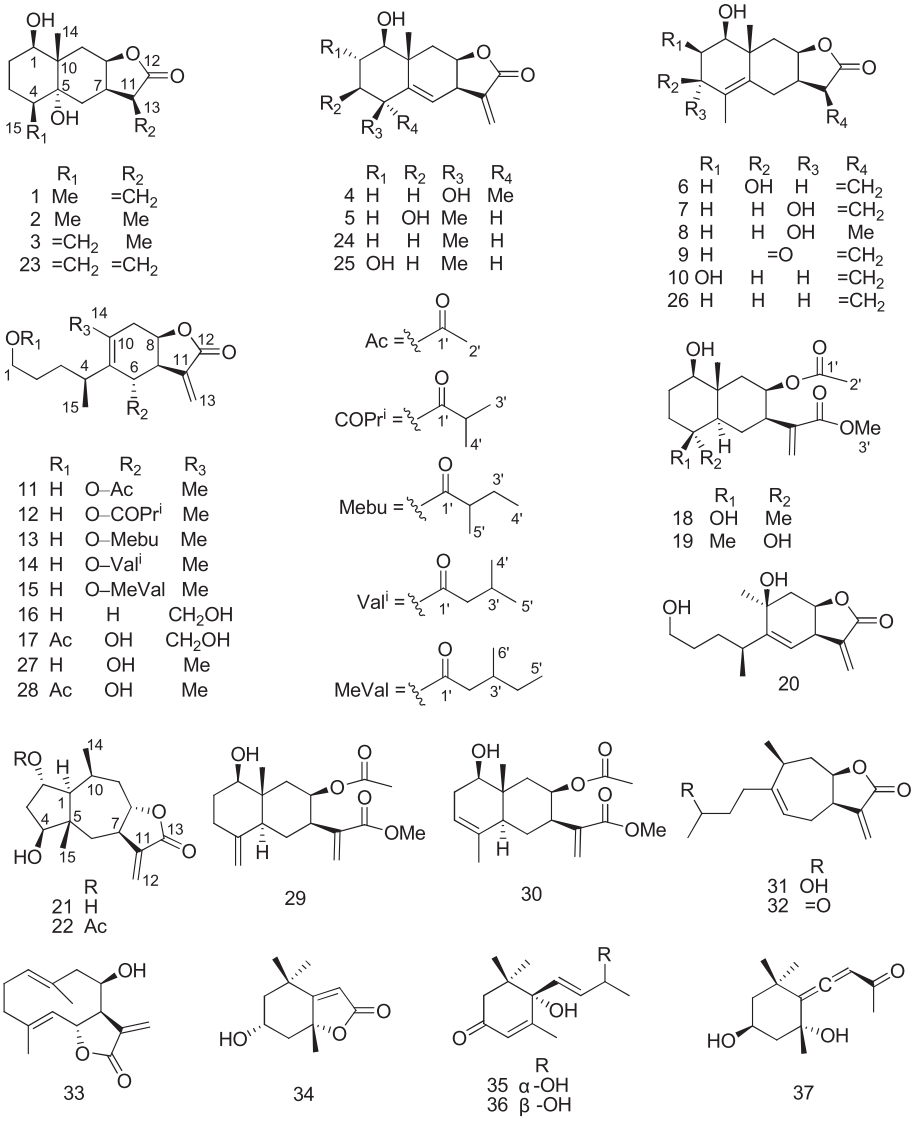

Fig. 1.

C-8), one oxygenated quaternary carbon (δ_C 76.8, C-5), one olefinic group (δ_C 144.5, 120.9; C-11 and C-13), and one ester carbonyl (δ_C 173.2, C-12) accounting for two degrees of unsaturation. The remaining degrees of unsaturation were due to the presence of tricyclic nucleus in the molecule. Furthermore, the ^{1}H NMR spectrum of **1** (Table 1) indicated the presence of one methyl singlet (δ_H 1.02, s, Me-14), one methyl doublet (δ_H 1.01, d, J=7.0 Hz, Me-15), two oxymethines (δ_H 3.81, dd, J=11.8, 4.3 Hz, H-1 and δ_H 4.64, m, H-8), and two disubstituted olefinic protons (δ_H 6.05, s, H-13a and δ_H 5.62, s, H-13b). In the ^{1}H–^{1}H COSY experiments, the correlations of H-1 through H_2-2, H_2-3 and H-4 to H_3-15, and H_2-6 through H-7 and H-8 to H_2-9 established two fragments (Fig. 2). The HMBC correlations traced from the methyls (H_3-14 and H_3-15) and olefinic proton (H_2-13) suggested the presence of a eudesmane sesquiterpene moiety (Fig. 2). Some other key HMBC correlations between H-1/C-9, C-10 and Me-14; H-7/C-6, C-11, C-12 and C-13; and H-8/C-9 and C-10 were also observed. Moreover, the observed correlation of olefinic group (C-11 and 13), the ester carbon (C-12), and exocyclic olefinic protons (H-13a and 13b) authenticated the existence of a characteristic α-methylene lactone functionality. On the basis of above data, compound **1** was 1,5-dihydroxy-substituted eudesmane sesquiterpene lactone.

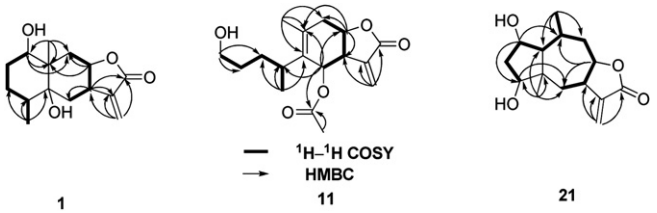

Fig. 2.

The relative stereochemistry of **1** was further confirmed by detailed analysis of NOESY spectra and an X-ray diffraction study (Figs. 3 and 4). In the NOESY spectrum, the correlations of H-1/H-4, H_3-14/H_3-15, and H-7/H-8 were observed, which were in good agreement with the X-ray diffraction study. The absolute configuration was determined by modified Mosher method.[21,22] The (*S*)- and (*R*)-MTPA esters of **1** (**1a** and **1b**, respectively) were prepared using the corresponding (*R*)-(−)- and (*S*)-(+)-MTPA chloride, respectively. The determination of $\Delta\delta$ values ($\delta_S-\delta_R$) for protons neighboring C-1 led to the assignment of the *R* configuration at C-1 in **1**, while the $\Delta\delta$ value for methyl on C-4 was zero, possibly

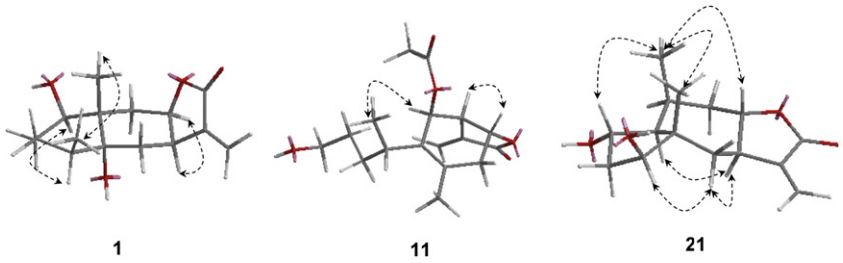

Fig. 3.

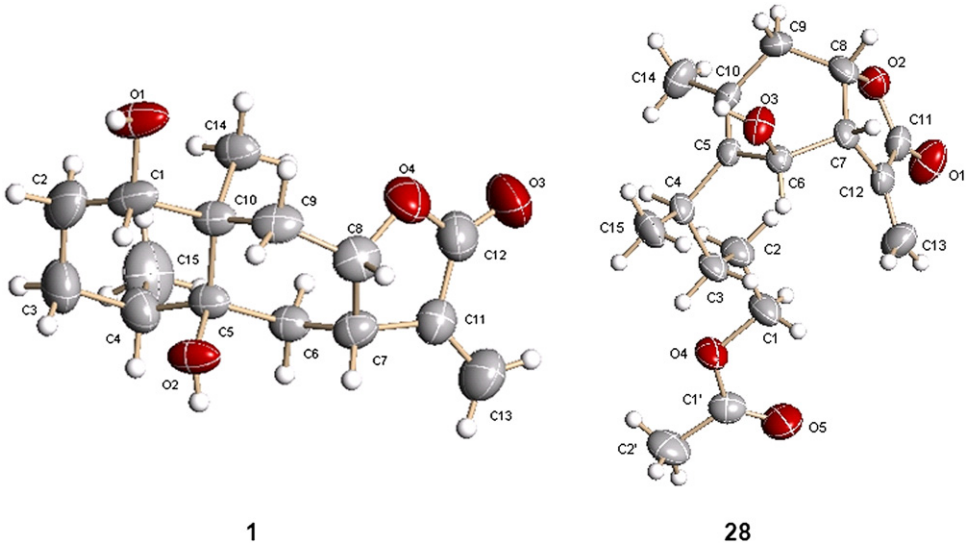

Fig. 4.

because it lay on the MTPA plane (Fig. 5).[22] Therefore, all relevant chiral centers in **1** were assigned as 1*R*, 4*S*, 5*R*, 7*R*, 8*R* and 10*S* configurations on the basis of the $\Delta\delta$ results summarized in Fig. 3. Thus, **1** was elucidated as (1*R*,4*S*,5*R*,7*R*,8*R*,10*S*)-1,5-dihydroxy-eudesma-11(13)-en-12,8-olide.

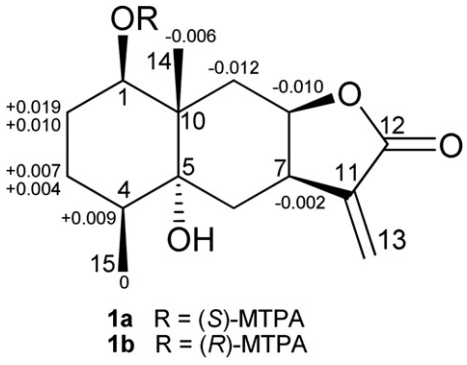

Fig. 5.

Compound **2** was shown to possess a molecular formula of $C_{15}H_{24}O_4$ (HRESIMS $[M+Na]^+$, *m*/*z* 291.1544). The ^1H and ^{13}C NMR data (Tables 1 and 2) of **2** were comparable to those of **1** except for the absence of the signals assigned to the exocyclic olefinic protons H-13a (δ_H 6.05) and H-13b (δ_H 5.62) in **1** and the upfield shifts of the signals corresponding to the C-11 and C-13 protons from δ_C 144.5 and 120.9 in **1** to 42.3 and 9.9 in **2**. Detailed assignments of the ^1H and ^{13}C NMR data were determined on the basis of HSQC, $^1H-^1H$ COSY, and HMBC experiments. The important NOESY correlations of H-11/H-7 and H-8 and other NOESY signals of **2** suggested the same relative configuration than **1**. Therefore, **2** was identified as 1β,5α-dihydroxy-4α,11α*H*-eudesma-12,8β-olide.

Compound **3** gave its molecular formula $C_{15}H_{22}O_4$, as established from HRESIMS (*m*/*z* 267.1587 for $[M+H]^+$). The ^1H NMR data (Table 1) of **3** were very similar to those of 5α-hydroxyasperilin (**23**) except for the absence of exocyclic olefinic protons H-13a (δ_H 6.07) and H-13b (δ_H 5.67) in **23** and the presence of one methyl doublet at δ_H 1.16 in **3**, which supported the hydrogenation of the double bond at C-11 and C-13 in **3**. Detailed analysis of 2D NMR spectra further confirmed the planar structure and relative configuration of **3**. The important NOESY correlations of H-11/H-7 and H-8 were observed. Thus, the structure of **3** was identified as 1β,5α-dihydroxy-11α*H*-eudesma-4(15)-en-12,8β-olide.

Both of compounds **4** and **5** possessed their molecular formula $C_{15}H_{20}O_4$, as shown from their positive HRESIMS at *m*/*z* 265.1446 $[M+H]^+$. The similarities between NMR spectra of **4** and **5** with known compound 1β-hydroxyalantolactone (**24**) suggested the same skeleton.[11] Close comparison of ^{13}C NMR data of **4** and **24** indicated that **4** had an additional hydroxyl substituent at C-4, which was confirmed by analysis of 2D NMR spectra, including HSQC, $^1H-^1H$ COSY, and HMBC. The relative configuration of **4** was established by NOESY experiment, and the significant correlations between H-6/H$_3$-15 and H-7 were observed. Thus, the final structure of **4** was elucidated as 1β,4β-dihydroxy-eudesma-5(6),11(13)-dien-12,8β-olide. Similarly, comparison of the ^1H and ^{13}C NMR data of **5** (Tables 1 and 2) and **24** indicated that **5** has an additional hydroxyl group at C-3.[11] This was confirmed by the $^1H-^1H$ COSY

Table 1
^{1}H NMR spectroscopic data for compounds **1**–**23** (*J* in Hz within parentheses)

No.	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]
1	3.81 dd (11.8, 4.3)	3.83 dd (11.9, 4.5)	3.98 dd (11.9, 4.9)	3.23 dd (12.0, 4.0)	3.27 dd (11.5, 4.5)	3.53 dd (12.7, 3.7)
2	1.71 m; 1.53 m	1.72 m; 1.52 m	1.71 m; 1.52 m	2.07 m; 1.53 m	1.90 m; 1.78 m	2.16 m; 1.80 m
3	2.21 m; 1.33 brd (13.4)	2.22 m; 1.32 m	2.70 m; 2.12 m	1.80 ddd (13.9, 13.9, 3.2); 1.45 ddd (13.7, 13.7, 4.2)	3.72 m	4.08 t (8.4)
4	1.64 m	1.63 m			2.57 m	
6	1.83 m; 1.49 m	1.64 m; 1.26 dd (14.0, 6.3)	1.64 m; 1.44 m	5.62 d (4.0)	5.40 d (4.0)	2.96 dd (12.0, 7.5); 2.02 t (12.1)
7	3.34 m	2.82 m	2.78 m	3.71 m	3.67 m	3.18 m
8	4.64 m	4.62 dd (6.5, 4.1)	4.63 br t (4.7)	4.88 ddd (6.6, 3.2, 3.2)	4.90 ddd (6.0, 3.0, 3.0)	4.54 ddd (11.8, 8.3, 4.3)
9	2.08 m; 2.06 m	2.04 m; 2.00 m	2.19 dd (15.5, 1.5); 1.96 dd (15.5, 2.5)	2.53 dd (15.3, 3.0); 1.59 dd (15.3, 3.3)	2.51 dd (16.0, 3.0); 1.54 dd (15.0, 3.0)	2.25 dd (13.7, 4.6); 1.40 dd (13.7, 11.6)
11		2.92 m	2.97 m			
13	6.05 s; 5.62 s	1.13 d (7.2)	1.16 d (7.2)	6.15 d (1.7); 5.80 d (1.5)	6.15 d (2.0); 5.77 d (1.5)	6.22 d (3.1); 5.75 d (2.8)
14	1.02 s	1.00 s	0.79 s	1.27 s	1.11 s	1.06 s
15	1.01 d (7.0)	1.03 d (7.7)	4.86 s; 4.76 s	1.31 s	1.04 d (7.0)	1.76 s

No.	**7**[a]	**8**[a]	**9**[a]	**10**[a]	**11**[b]	**12**[b]
1	3.79 dd (12.5, 4.0)	3.70 dd (12.4, 4.0)	3.92 dd (10.6, 7.3)	3.40 d (2.8)	3.50 m; 3.42 m	3.53 m; 3.46 m
2	1.97 m; 1.87 m	1.79 m; 1.76 m	2.61 s; 2.59 d (4.3)	3.97 ddd (5.7, 5.7, 2.8)	1.33 m; 1.10 m	1.35 m; 1.10 m
3	4.01 d (4.5)	3.95 m		2.27 m	1.30 m; 1.06 m	1.32 m; 1.07 m
4					2.68 m	2.67 m
6	2.95 dd (13.5, 7.5); 2.00 m	2.51 m; 1.70 m	3.23 dd (11.7, 7.1); 2.38 t (11.7)	2.80 dd (14.7, 7.3); 1.96 dd (14.7, 7.9)	5.18 d (1.5)	5.16 d (1.7)
7	3.18 m	2.40 m	3.30 m	3.08 m	3.48 m	3.47 m
8	4.60 m	4.55 dd (6.3, 4.0)	4.69 m	4.54 dd (11.5, 6.0)	4.97 ddd (7.5, 2.5, 2.5)	4.97 ddd (7.6, 3.2, 2.5)
9	2.23 dd (14.0, 4.5); 1.51 dd (14.0, 11.0)	2.54 dd (12.0, 2.5); 1.54 dd (12.0, 4.2)	2.49 dd (13.8, 4.7); 1.52 dd (13.8, 12.1)	1.97 dd (14.6, 4.9); 1.77 dd (14.6, 5.0)	2.72 dd (12.0, 2.0); 2.50 dd (12.0, 2.0)	2.73 dd (12.0, 2.0); 2.51 dd (12.0, 2.0)
11		2.95 m				
13	6.22 d (3.0); 5.75 d (3.0)	1.18 d (7.2)	6.29 d (3.2); 5.85 d (2.9)	6.13 d (2.1); 5.72 d (1.9)	6.37 d (2.5); 6.00 d (2.0)	6.37 d (2.6); 6.00 d (2.3)
14	0.98 s	1.01 s	1.14 s	1.22 s	1.81 s	1.81 s
15	1.80 s	1.76 s	1.82 s	1.71 s	0.89 d (7.0)	0.87 d (7.0)
2′					2.04 s	2.49 m
3′						1.14 d (6.7)
4′						1.16 d (6.7)

No.	**13**[b]	**14**[b]	**15**[b]	**16**[a]	**17**[a]	**18**[b]
1	3.54 m; 3.47 m	3.54 m; 3.47 m	3.52 m; 3.44 m	3.50 m; 3.47 m	3.95 m; 3.90 m	3.29 dd (11.5, 4.0)
2	1.36 m; 1.10 m	1.36 m; 1.10 m	1.32 m; 1.13 m	1.35 m; 1.20 m	1.36 m; 1.17 m	1.89 m; 1.61 m
3	1.32 m; 1.07 m	1.32 m; 1.07 m	1.32 m; 1.00 m	1.30 m; 1.15 m	1.28 m; 1.01 m	1.73 m; 1.61 m
4	2.67 m	2.67 m	2.70 m	2.76 m	2.76 m	
5						1.17 m
6	5.19 d (1.7)	5.19 d (1.7)	5.23 d (1.7)	2.30 dd (15.0, 6.8); 2.16 dd (15.0, 4.2)	4.21 d (2.0)	1.92 m; 1.61 m
7	3.49 m	3.49 m	3.50 m	3.30 m	3.52 m	2.88 ddd (13.0, 3.0, 3.0)
8	4.96 m	4.96 m	5.01 m	4.96 ddd (8.5, 4.3, 4.3)	5.12 ddd (7.5, 3.5, 2.5)	5.26 m
9	2.71 dd (12.1, 2.0); 2.50 dd (12.1, 2.0)	2.71 dd (12.1, 2.0); 2.50 dd (12.1, 2.0)	2.75 dd (16.2, 2.1); 2.51 dd (16.2, 2.3)	2.55 dd (15.4, 4.2); 2.41 dd (15.4, 4.2)	2.78 m; 2.70 m	2.21 dd (14.5, 2.9); 1.43 dd (14.5, 3.3)
13	6.38 d (2.6); 6.00 d (2.3)	6.38 d (2.6); 6.00 d (2.3)	6.36 d (2.6); 6.02 d (2.3)	6.18 d (2.7); 5.78 d (2.3)	6.23 d (2.5); 5.85 d (2.5)	6.28 s; 5.64 s
14	1.83 s	1.83 s	1.82 s	4.31 d (12.0); 3.83 d (12.0)	4.33 d (12.5); 3.84 d (12.5)	1.20 s
15	0.88 d (7.0)	0.88 d (7.0)	0.90 d (7.0)	1.00 d (7.0)	1.11 d (7.0)	1.20 s
2′	2.35 m	2.17 m	2.32 m; 2.11 m		2.02 s	1.95 s
3′	1.51 m; 1.48 m	2.09 m	1.87 m			3.76 s
4′	0.90 t (7.0)	0.96 d (6.6)	1.37 m; 1.26 m			
5′	1.13 d (7.0)	0.96 d (6.6)	0.92 t (7.5)			
6′			0.94 d (7.0)			

No.	**19**[a]	**19**[b]	**20**[a]	**21**[a]	**22**[b]	**23**[a]
1	3.30 m	3.35 dd (10.9, 4.3)	3.53 m; 3.48 m	1.68 m	1.95 m	3.97 dd (11.8, 4.8)
2	1.67 m; 1.64 m	1.70 m; 1.65 m	1.50 m; 1.44 m	4.07 ddd (15.0, 10.2, 3.0)	5.06 ddd (14.8, 9.8, 3.5)	1.71 m; 1.51 m
3	1.75 m; 1.55 m	1.76 m; 1.60 m	1.46 m; 1.40 m	1.90 m; 1.86 m	2.02 m; 1.96 m	2.70 ddd (13.7, 5.5, 5.5); 2.13 m
4			2.46 m	3.90 m	3.95 t (9.1)	
5	1.41 m	1.45 m				
6	1.78 m; 1.76 m	1.80 m; 1.74 m	5.25 d (4.2)	2.42 m; 1.20 m	2.41 dd (14.9, 4.1); 1.28 dd (14.9, 12.0)	1.85 dd (14.2, 7.2); 1.66 dd (14.2, 12.0)
7	2.81 m	2.85 brt (6.8)	3.68 m	2.85 m	2.78 m	3.31 m
8	5.27 m	5.27 brd (2.6)	4.90 m	4.31 ddd (12.0, 9.0, 3.0)	4.25 ddd (12.1, 9.0, 3.5)	4.64 t (5.2)
9	2.15 dd (14.6, 2.9); 1.44 dd (14.6, 3.0)	2.21 dd (14.5, 3.0); 1.43 dd (14.5, 3.0)	2.18 dd (14.4, 5.0); 2.01 dd (14.3, 3.8)	2.26 dt (12.7, 2.8); 1.75 m	2.30 m; 1.77 ddd (12.5, 12.5, 5.6)	2.22 brd (15.7); 2.03 dd (15.7, 5.1)

Table 1 (*continued*)

No.	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]
10				2.45 m	2.28 m	
13	6.24 s; 5.67 s	6.27 s; 5.61 s	6.14 d (2.2); 5.74 d (2.0)	6.10 d (3.4); 5.55 d (3.1)	6.21 d (3.4); 5.50 d (3.1)	6.07 s; 5.67 s
14	1.02 s	1.02 s	1.26 s	1.12 d (7.0)	1.14 d (7.0)	0.81 s
15	1.16 s	1.21 s	1.11 d (6.8)	0.95 s	1.02 s	4.86 s; 4.72 s
2′	1.92 s	1.95 s			2.06 s	
3′	3.73 s	3.75 s				

[a] Measured at 500 MHz in CD_3OD.
[b] Measured at 500 MHz in $CDCl_3$.

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**24**, and **26**

No.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[a]	**6**[b]	**7**[a]	**8**[b]	**9**[b]	**10**[b]	**11**[c]	**12**[c]	**13**[c]
1	75.4 d	75.4 d	74.2 d	81.0 d	78.6 d	70.6 d	68.5 d	74.4 d	69.7 d	76.4 d	62.7 t	62.7 t	62.8 t
2	26.8 t	26.9 t	31.4 t	27.0 t	34.8 t	38.3 t	37.3 t	36.9 t	43.6 t	69.3 d	31.2 t	31.2 t	31.3 t
3	27.9 t	27.9 t	31.1 t	39.6 t	70.1 d	71.3 d	70.4 d	71.1 d	199.8 s	38.9 t	31.1 t	31.1 t	31.1 t
4	41.7 d	42.2 d	152.1 s	71.7 s	46.1 d	131.4 s	129.3 s	129.4 s	131.9 s	125.2 s	33.3 d	33.3 d	33.3 d
5	76.8 s	76.9 s	75.4 s	147.5 s	147.2 s	135.5 s	136.4 s	136.5 s	162.1 s	131.7 s	132.0 s	132.2 s	132.2 s
6	37.6 t	31.2 t	28.7 t	122.6 d	124.5 d	29.2 t	28.8 t	23.5 t	31.0 t	29.4 t	69.5 d	69.3 d	69.2 d
7	39.2 d	38.7 d	38.3 d	41.2 d	40.9 d	41.3 d	41.4 d	41.8 d	40.4 d	43.1 d	43.2 d	43.3 d	43.3 d
8	79.8 d	80.7 d	80.2 d	77.8 d	77.8 d	77.7 d	77.5 d	79.4 d	76.7 d	78.9 d	75.6 d	75.6 d	75.6 d
9	37.0 t	37.4 t	34.0 t	40.8 t	40.6 t	38.7 t	38.6 t	41.0 t	37.8 t	41.1 t	34.4 t	34.5 t	34.5 t
10	42.1 s	42.4 s	42.6 s	39.7 s	38.7 s	41.6 s	41.0 s	40.6 s	43.4 s	40.8 s	133.9 s	133.7 s	133.9 s
11	144.5 s	42.3 d	42.5 d	141.7 s	141.5 s	141.5 s	141.4 s	43.4 d	140.6 s	142.9 s	136.1 s	136.3 s	136.3 s
12	173.2 s	182.7 s	182.5 s	172.8 s	172.3 s	173.0 s	172.7 s	182.3 s	172.4 s	173.0 s	170.5 s	170.5 s	170.5 s
13	120.9 t	9.9 q	9.9 q	123.2 t	122.6 t	123.2 t	122.8 t	9.9 q	124.2 t	122.2 t	125.7 t	125.6 t	125.7 t
14	17.4 q	17.6 q	15.1 q	22.4 q	22.9 q	21.4 q	19.7 q	18.6 q	20.0 q	22.4 q	20.6 q	20.6 q	20.7 q
15	17.5 q	17.4 q	109.4 t	29.6 q	15.9 q	15.4 q	16.9 q	17.6 q	11.6 q	19.6 q	18.5 q	18.8 q	18.8 q
1′											170.9 s	176.9 s	176.6 s
2′											21.2 q	34.1 d	41.4 d
3′												18.7 q	26.5 t
4′												18.7 q	11.6 q
5′													16.5 q

No.	**14**[c]	**15**[d]	**16**[b]	**17**[a]	**18**[c]	**19**[b]	**19**[d]	**20**[a]	**21**[b]	**22**[c]	**23**[a]	**24**[c]	**26**[c]
1	62.8 t	61.7 t	63.2 t	65.4 t	79.8 d	80.7 d	79.5 d	63.4 t	56.7 d	51.7 d	74.1 d	80.3 d	72.1 d
2	31.3 t	30.7 t	32.2 t	27.8 t	26.4 t	29.2 t	28.1 t	32.4 t	71.3 d	73.0 d	31.5 t	29.7 t	27.1 t
3	31.1 t	30.3 t	32.2 t	32.3 t	39.5 t	42.0 t	39.0 t	35.5 t	40.2 t	37.5 t	31.2 t	26.0 t	30.9 t
4	33.3 d	32.9 d	35.3 d	34.0 d	71.2 s	72.4 s	71.4 s	33.8 d	79.3 d	78.5 d	151.6 s	37.4 d	126.5 s
5	132.2 s	131.9 s	140.9 s	140.3 s	51.0 d	54.7 d	53.5 d	152.0 s	47.1 s	45.3 s	75.2 s	148.0 s	130.3 s
6	69.2 d	68.8 d	30.0 t	68.7 d	20.9 t	21.7 t	20.4 t	120.1 d	39.0 t	37.8 t	35.0 t	120.6 d	27.8 t
7	43.3 d	42.7 d	38.9 d	47.1 d	42.7 d	44.5 d	42.6 d	41.0 d	46.1 d	44.8 d	38.8 d	39.5 d	40.4 d
8	75.6 d	75.6 d	79.9 d	78.3 d	69.3 d	71.4 d	69.5 d	77.2 d	84.4 d	81.8 d	79.3 d	75.8 d	75.8 d
9	34.5 t	34.1 t	32.9 t	30.7 t	43.1 t	45.7 t	44.0 t	42.6 t	42.9 t	41.3 t	33.7 t	39.3 t	37.5 t
10	133.9 s	133.3 s	130.4 s	134.4 s	39.0 s	40.5 s	41.0 s	70.2 s	29.4 d	28.0 d	42.2 s	38.1 s	39.1 s
11	136.3 s	136.0 s	142.3 s	138.8 s	141.2 s	143.0 s	140.9 s	141.3 s	142.8 s	140.0 s	144.5 s	139.7 s	139.7 s
12	170.5 s	170.5 s	173.3 s	172.2 s	167.1 s	168.9 s	167.1 s	172.6 s	172.4 s	170.0 s	173.1 s	170.2 s	170.7 s
13	125.7 t	125.1 t	124.0 t	124.8 t	125.4 t	126.2 t	125.3 t	123.0 t	120.4 t	120.1 t	121.1 t	121.8 t	122.0 t
14	20.7 q	19.9 q	62.0 t	62.1 t	14.6 q	16.2 q	15.0 q	29.3 q	17.1 q	16.6 q	15.1 q	21.8 q	20.3 q
15	18.8 q	18.1 q	19.9 q	19.4 q	30.1 q	23.0 q	22.8 q	23.8 q	20.7 q	19.7 q	109.6 t	22.5 q	18.9 q
1′	173.0 s	173.4 s		173.0 s	170.1 s	172.4 s	170.2 s			171.2 s			
2′	43.6 t	41.3 t		20.8 q	21.2 q	21.4 q	21.1 q			21.1 q			
3′	25.8 d	31.6 d			52.0 q	52.7 q	52.0 q						
4′	22.4 q	28.8 t											
5′	22.4 q	10.6 q											
6′		18.7 q											

[a] Measured at 125 MHz in CD_3OD.
[b] Measured at 100 MHz in CD_3OD.
[c] Measured at 125 MHz in $CDCl_3$.
[d] Measured at 100 MHz in $CDCl_3$.

spectrum of the correlations from H-1 through H_2-2, H-3, and H-4 to H_3-15, as well as the long-range correlations of H-3 with C-1 and Me-15 in the HMBC spectrum. Based on the NOESY correlation of H-1/H-3, **5** was concluded to be 1β,3β-dihydroxy-4α*H*-eudesma-5(6),11(13)-dien-12,8β-olide.

Both of **6** and **7** gave their molecular formula $C_{15}H_{20}O_4$ as established from their HRESIMS at *m*/*z* 263.1299 $[M-H]^-$, and *m*/*z* 265.1459 $[M+H]^+$, respectively. Their NMR data were very similar to those of known compound ivangustin (**26**) except for an additional hydroxyl group at C-3.[13] The main difference was observed in the NMR resonances mainly from C-1 through C-2, C-3 and C-4 to C-15 (Tables 1 and 2). In particular, the triplet of H-3 in **6** (δ_H 4.08, t, *J*=8.4 Hz) was replaced by a doublet in **7** (δ_H 4.01, d, *J*=4.5 Hz). Obviously, the upfield shift of C-1 in **7** (δ_C 68.5), in contrast to the corresponding shift value in **6** (δ_C 70.6), was due to a γ-*gauche* effect of α-OH at C-3.[23–26] The key NOESY correlation of H-1/H-3 was observed in **6**, whereas the crucial NOESY correlations of H-3/H-2β (δ_H 1.97) and H_3-14/H-2β (δ_H 1.97) were observed in **7**.

Thus, **6** was determined to be 1β,3β-dihydroxy-eudesma-4(5),11(13)-dien-12,8β-olide, and **7** was assigned as the C-3 epimer of **6** and named as 1β,3α-dihydroxy-eudesma-4(5),11(13)-dien-12,8β-olide.

Compound **8** was assigned the molecular formula of $C_{15}H_{22}O_4$, as established from HRESIMS at *m*/*z* 267.1597 $[M+H]^+$. Comparison of the NMR spectroscopic data of **8** with those of **7** showed these to be different in the characteristic α-methylene lactone functionality (Tables 1 and 2). The most significant features of NMR spectra of **8** were upfield shifted as exhibited by C-11, C-13, and H-7, and downfield shifted as exhibited by C-12, which indicated the absence of $\Delta^{11,13}$ exocyclic methylene group. In NOESY experiment, the crucial correlations of H-11/H-7 and H-8 were observed. On the basis of these data, **8** was concluded as 1β,3α-dihydroxy-11α*H*-eudesma-4(5)-en-12,8β-olide.

Compounds **9** and **10** had the molecular formula $C_{15}H_{18}O_4$ and $C_{15}H_{20}O_4$ as established from their HRESIMS *m*/*z* 263.1289 $[M+H]^+$, and *m*/*z* 287.1239 $[M+Na]^+$, respectively. The ^1H and ^{13}C NMR spectra of **9** and **10** were very similar to those of **6** (Tables 1 and 2), except that the hydroxymethine group at C-3 in **6** was replaced by a ketone carbonyl in **9**, and one hydroxyl group at C-3 was replaced at C-2 in **10**. Moreover, the important NOESY correlations of H-2/H-1, H-9α/H-2, and H-8 in **10** showed that these two hydroxyl groups both have the β-configuration. Thus, **9** was elucidated as 1β-hydroxy-3-oxo-eudesma-4(5),11(13)-dien-12,8β-olide, and **10** was 1β,2β-dihydroxy-eudesma-4(5),11(13)-dien-12,8β-olide.

Compound **11** was assigned the molecular formula of $C_{17}H_{24}O_5$, as established from its HRESIMS at *m*/*z* 331.1516 $[M+Na]^+$, accounting for six degrees of unsaturation. Absorption of hydroxyl (3537, 3350 cm^{-1}), carbonyl (1757, 1718 cm^{-1}), and olefinic bond (1656 cm^{-1}) was observed in its IR spectrum. A comparison of the ^1H and ^{13}C NMR spectra of **11** with those of 1-acetoxy-6α-hydroxyeriolanolide (**28**), an isomer isolated from this study.[14] Detailed analysis of the 1D and 2D NMR spectra of **11** and **28** showed that they possessed the same skeleton except for the positional change of one acetoxyl group from C-1 for **28** to C-6 for **11** (Fig. 2). The HMBC correlation of H-6 to C-1′ and the chemical shift of C-1 (δ_C 62.7) confirmed that the acetoxyl group and a hydroxyl group were attached to C-6 and C-1 of **11**, respectively.

The relative configuration of **11** was determined by NOESY experiment and coupling constants. The small coupling constant (1.5 Hz) between H-6 and H-7 implied a trans-configuration for these protons.[14] Moreover, the allylic coupling observed between H-7 and H_2-13 (2.5 and 2.0 Hz) suggested a *cis*-fused lactone ring of **11**.[27] The strong NOESY correlations of H-6/H_3-15 and H-7/H-8 were observed and gave a relative configuration of **11** (Fig. 3). Furthermore, a single crystal X-ray crystallographic measurement of **28** was also in agreement with the relative configuration of **11** (Fig. 4). Thus, the structure of **11** was determined as 6α-acetoxy-1-hydroxy-4α*H*-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide.

Compound **12** exhibited a $[M+Na]^+$ ion peak at *m*/*z* 359.1832 in the positive HRESIMS, corresponding to the molecular formula, $C_{19}H_{28}O_5$. The ^1H and ^{13}C NMR spectra of **12** were all comparable to those of **28** except for absence of an acetoxyl group attributed to C-1 of **28**, and the presence of an isobutyryl group [δ_H 2.49 (m, H-2′), 1.14 (d, *J*=6.7 Hz, H-3′), and 1.16 (d, *J*=6.7 Hz, H-4′); δ_C 176.9 (C-1′), 34.1 (C-2′), 18.7 (C-3′), and 18.7 (C-4′)] attributed to C-6 of **12** (Tables 1 and 2). Thus, compound **12** was established as 6α-isobutyryloxy-1-hydroxy-4α*H*-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide.

Both of **13** and **14** shared the same molecular formula $C_{20}H_{30}O_5$, and their ion peaks were at *m*/*z* 373.1987 $[M+Na]^+$. Comparison of their 1D and 2D NMR data with those of **28** enabled the structure determination of both **13** and **14**. The ^1H and ^{13}C NMR spectroscopic data of **13** and **14** were very similar to those of **28** except for the presence of a 2-methybutyryl group [δ_H 2.35 (m, H-2′), 1.51 (m, H-3′a), 1.48 (m, H-3′b), 0.90 (t, *J*=7.0 Hz, H-4′), and 1.13 (d, *J*=7.0 Hz, H-5′); δ_C 176.6 (C-1′), 41.4 (C-2′), 26.5 (C-3′), 11.6 (C-4′), and 16.5 (C-5′)] for **13** and an isovaleryl group [δ_H 2.17 (m, H-2′), 2.09 (m, H-3′), 0.96 (d, *J*=6.6 Hz, H-4′), and 0.96 (d, *J*=6.6 Hz, H-5′); δ_C 173.0 (C-1′), 43.6 (C-2′), 25.8 (C-3′), 22.4 (C-4′), and 22.4 (C-5′)] for **14** at C-6 of them, instead of the acetoxyl group, which attached to C-1 of **28** (Tables 1 and 2). Therefore, the structures of compounds **13** and **14** were determined as 6α-(2-methybutyryloxy)-1-hydroxy-4α*H*-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide and 6α-isovaleryloxy-1-hydroxy-4α*H*-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide, respectively.

Compound **15** gave a molecular formula $C_{21}H_{32}O_5$ from its HRESIMS at 365.2339 $[M+H]^+$, and exhibited very similar physical and spectroscopic data to those of **28** except for an additional 3-methyvaleryl group [δ_H 2.32 (m, H-2′a), 2.11 (m, H-2′b), 1.87 (m, H-3′), 1.37 (m, H-4′a), 1.26 (m, H-4′b), 0.92 (t, *J*=7.5 Hz, H-5′), and 0.94 (d, *J*=7.0 Hz, H-6′); δ_C 173.4 (C-1′), 41.3 (C-2′), 31.6 (C-3′), 28.8 (C-4′), 10.6 (C-5′) and 18.7 (C-6′)] for **15** at C-6, instead of the acetoxyl group, which attached to C-1 of **28** (Tables 1 and 2). Thus, compound **15** was elucidated as 6α-(3-methylvaleryloxy)-1-hydroxy-4α*H*-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide.

Compounds **16** and **17** were assigned the molecular formula $C_{15}H_{22}O_4$ and $C_{17}H_{24}O_6$ from their positive HRESIMS *m*/*z* 267.1604 $[M+H]^+$ and 325.1665 $[M+H]^+$, respectively. The ^{13}C NMR spectroscopic data of **16** were similar to those of 1,6α-dihydroxyeriolanolide (**27**) except that a methyl (δ_C 20.3, Me-14) and an oxygenated methine (δ_C 68.8, C-6) in **27** were replaced by an oxygenated methylene (δ_C 62.0, C-14) and a methylene (δ_C 30.0, C-6), respectively, in **16** (Tables 1 and 2).[14] Compound **16** was characterized as 1,14-dihydroxy-4α*H*-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide. The NMR data of **17** compared with those of **28** showed that the only difference was the presence of an oxymethylene (δ_H 4.33 and 3.84; δ_C 62.1) in **17** instead of a methyl (δ_H 1.76, s; δ_C 20.3) in **28** (Tables 1 and 2).[14] Hence, **17** was elucidated as 1-acetoxy-6α,14-dihydroxy-4α*H*-1,10-secoeudesma-5(10),11(13)-dien-12,8β-olide.

Compounds **18** and **19** had the same molecular formula $C_{18}H_{28}O_6$, established from their HRESIMS at *m*/*z* 341.1978 $[M+H]^+$. Both of the NMR data of **18** and **19** showed a great similarity with those of 1β-hydroxy-8β-acetoxycostic acid methyl ester (**29**). In **18**, one oxygenated quaternary carbon (δ_C 71.2, C-4) and a methyl (δ_H 1.20, s; δ_C 30.1) were appeared instead of the $\Delta^{4,15}$ exocyclic methylene group (δ_H 4.82, s and 4.61, s; δ_C 147.9 and 107.1) (Tables 1 and 2).[6] The similar relative configuration of **18** with **29** was deduced by a NOESY experiment, in which the key correlations of H_3-15/H-1 and H-7 were observed. Therefore, **18** was determined as 1β,4β-dihydroxy-8β-acetoxy-5α*H*-eudesma-11(13)-en-12-oic acid methyl ester. Furthermore, the same planar structure of **19** as **18** was also found by analysis of 2D NMR spectra, including HSQC, $^1H-^1H$ COSY, and HMBC. By comparison of the related ^1H and ^{13}C NMR spectroscopic data of **19** with those of **18**, **19** was found to be the C-4 epimer of **18**. This was further confirmed by analysis of the NOESY correlations of **19**, which revealed that H_3-14 was correlated with H_3-15. Thus, **19** was determined as 1β,4α-dihydroxy-8β-acetoxy-5α*H*-eudesma-11(13)-en-12-oic acid methyl ester.

Compound **20** exhibited its molecular formula $C_{15}H_{22}O_4$, as deduced from its positive HRESIMS *m*/*z* 267.1594 $[M+H]^+$, was the same as that of **27**.[14] A comparison of the ^1H NMR spectra of **20** with those of **27** revealed a great similarity except for the presence of a vinyl doublet (δ_H 5.25, d, *J*=4.2) in **20** and the absence of an oxymethines (δ_H 4.16, d, *J*=2.0) in **27** (Table 1), implied the positional changes of a double bond and a hydroxyl group.[14] This presumption above was confirmed by the $^1H-^1H$ COSY correlations from H-6 through H-7 and H-8 to H_2-9, as well as the key correlations of H_3-14 with C-5, C-9, and C-10 in the HMBC spectrum. Compared with **27**, the similar NOESY correlations of **20** determined its relative stereochemistry. The NOESY correlations of H-4/H_3-14 placed Me-14 at the α-configuration and OH-10 at the

β-configuration. Thus, compound **20** was determined as 1,10β-dihydroxy-4α*H*-1,10-secoeudesma-5(6),11(13)-dien-12,8β-olide.

Compounds **21** and **22** had the molecular formula of $C_{15}H_{22}O_4$ and $C_{17}H_{24}O_5$ as established from their HRESIMS at *m*/*z* 267.1597 $[M+H]^+$ and *m*/*z* 309.1696 $[M+H]^+$, respectively. NMR spectroscopic data of **21** were very similar to those of **22**, except for an additional acetoxyl group (δ_C 171.2 and 21.1; δ_H 2.06) in **22**. The characteristic signals of **21** [δ_H 6.10 (d, *J*=3.4, H-13a) and 5.55 (d, *J*=3.1, H-13b); δ_C 142.8 (C-11), 172.4 (C-12), and 120.4 (C-13)] in the ^1H and ^{13}C NMR (Tables 1 and 2) along with an IR absorption bands at 1660 cm^{-1} and 1739 cm^{-1} indicated the existence of an α-methylene lactone functionality. A long-range spin-system of $CH_2CHCHCH_2CH$ $(CH_3)CHCHCH_2CH$ [C-6/C-7/C-8/C-9/C-10 (C-14)/C-1/C-2/C-3/C-4], combined with the significant HMBC correlations of H_3-14 to C-1, 9, and 10, H_3-15 to C-4, 5, and 6, and H_2-13 to C-7, 11, and 12, indicated the presence of a pseudoguaianolide moiety (Fig. 2). In addition, the chemical shifts of C-2 (δ_C 71.3) and C-4 (δ_C 79.3) implied that two hydroxyl groups were attached to C-2 and C-4 of the pseudoguaianolide moiety, respectively. Hence, the planar structure of **21** was constructed as 2,4-dihydroxy-pseudoguaianolide. The relative stereochemistry of **21** was mainly deduced from NOESY correlations of H-4/H-6α, H-7/H-1, and H-6α, and H_3-14/H-2, H-8, and H_3-15 (Fig. 3). In the bargain, the large coupling constant (9.0 Hz) between H-7 and H-8 and the coupling constants between H-7 and H_2-13 (3.4 and 3.1 Hz) further confirmed the *trans*-fused lactone ring.[27] Thus, compound **21** was elucidated as 2α,4β-dihydroxy-1α*H* 10α*H*-pseudoguai-11(13)-en-12,8α-olide. Furthermore, The HMBC correlation between δ_H 5.06 (H-2) and δ_C 171.2 (C-1′) established the connection of the acetoxyl group to C-2 in **22** (Tables 1 and 2). Compound **22** was then concluded as 2α-acetoxy-4β-hydroxy-1α*H*,10α*H*-pseudoguai-11(13)-en-12,8α-olide.

2.2. Assay for inhibitory activities against NO production

As one of the largest groups of secondary plant metabolites, sesquiterpene lactones were reported to be the active components of many medicinal plants from the Asteraceae family and showed various biological activities such as anti-inflammatory, antiproliferative, and bactericidal effects.[28–30] In particular, their potent anti-inflammatory property has received considerable attention and been reported to be mediated chemically by α,β-unsaturated carbonyl structures, such as an α-methylene-γ-lactone or an α,β-unsubstituted cyclopentenone.[31] Therefore, it was meaningful to investigate the anti-inflammatory effects for these sesquiterpenes isolated from *I. japonica*. In this study, all 37 compounds were tested on their cytotoxic activities on RAW264.7 macrophages and showed no toxic at the dose evaluated (50 μM), and then tested for inhibitory activities against LPS-induced NO production in this cell line under the concentration range from 1 to 50 μM. The IC_{50} values obtained suggested that most of these compounds significantly inhibited the NO production with IC_{50} values in the range of 3.5–20 μM (Table 3) except compounds **2**, **3**, **8**, and **34**–**37**, which was attributed to the absence of α-methylene-γ-lactone. Interestingly, the lactone rings of compounds **18**, **19**, **29**, **30** were broken; nevertheless they exhibited their IC_{50} values under the concentration of 20 μM. These unforeseen results were supposed to arise from the common propenoic methyl ester chain of these four sesquiterpenes. On the other hand, compound **24** showed stronger inhibitory effect than compounds **4**, **5**, and **25** because of the presence of an additional hydroxyl group at C-4, C-3, and C-2 in the latter ones, respectively, reduced cellular penetration of the compounds across the phospholipid bilayers surrounding the cells, and consequently decreased the anti-inflammatory activity.[32] Similarly, the occurrence of an additional hydroxyl group at C-2 or C-3 (compounds **6**, **7**, and **10**) clearly reduced the inhibitory effects on NO production compared to compounds **26** in which non-hydroxyl groups appeared at both C-2 and C-3. Moreover, the acylation of compound **27** at C-1 or C-6 (compounds **11**–**15** and **28**) obviously augmented its activity and compound **15** exhibited the strongest activity with IC_{50} value of 4.3 μM due to its longest lipophilic chain 3-methyvaleryl group at C-6, which further verified the hypothesis above. In conclusion, the α-methylene-γ-lactone and the propenoic methyl ester chain were proposed to be the key chemical characteristic responsible for the above mentioned activities, and the lipophilicity of these compounds also was an important factor for their potential anti-inflammatory activities.

Table 3
Inhibitory effects of compounds isolated from *I. japonica* against LPS-induced NO production in RAW264.7 macrophages (*n*=4)

Compounds	IC_{50}[a] (μM)	Compounds	IC_{50}[a] (μM)
1	7.1	**20**	7.3
2	20.3	**21**	9.6
3	20.5	**22**	3.5
4	8.7	**23**	9.2
5	6.0	**24**	5.1
6	8.8	**25**	12.7
7	9.0	**26**	5.0
8	22.1	**27**	18.6
9	7.3	**28**	10.9
10	12.6	**29**	18.6
11	8.1	**30**	17.4
12	8.8	**31**	6.9
13	4.8	**32**	18.3
14	4.8	**33**	3.5
15	4.3	**34**	49.7
16	17.2	**35**	25.0
17	15.7	**36**	22.6
18	18.9	**37**	33.5
19	13.1	AG[b]	0.6

[a] Inhibitory effects of compounds **1**–**37** against LPS-induced NO production in RAW264.7 macrophages.
[b] Positve control (≥98.0%, Sigma); AG: aminoguanidine.

3. Conclusion

In summary, we have fully described the isolation and structure elucidation of 22 new sesquiterpene derivatives and 15 known ones from the aerial parts of *I. japonica*. Sesquiterpenes show various interesting biological activities, including anti-inflammatory activities,[28–30] therefore, the inhibitory activities of all 37 isolates on LPS-induced NO production in RAW264.7 macrophages were also evaluated. The obtained IC_{50} values demonstrated significant inhibitory activities of most of sesquiterpenes for NO production and a structure–activity relationship analysis had been discussed. These findings would provide information for the future design of anti-inflammatory agents.

4. Experimental

4.1. General procedures

Optical rotations were obtained with a JASCO P-2000 polarimeter. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. 1D and 2D NMR spectra were recorded on a Bruker Avance-400 or Avance-500 spectrometers in $CDCl_3$ or CD_3OD with TMS as internal standard. ESIMS spectra were recorded on an Agilent LC/MSD Trap XCT spectrometer (Waters, USA), and HRESIMS on a Q-TOF micro mass spectrometer (Waters, USA). A preparative column (Shimadzu PRC-ODS EV0233) was used for preparative HPLC (Shimadzu LC-6AD). TLC analysis was run on $HSGF_{254}$ silica gel plates (10–40 μm, Yantai, China). Column chromatography was performed on silica gel (100–200, 200–300 mesh,

Yantai, China), silica gel H (10–40 μm, Qingdao, China), and Sephadex LH-20 (Pharmacia Co. Ltd.).

4.2. Plant material

The aerial parts of *I. japonica* were collected in Anhui province, PR China, in October, 2006, and were authenticated by Professor Bao Kang Huang, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University. A voucher specimen (No. 2007XFH1) was deposited at School of Pharmacy, Shanghai Jiao Tong University.

4.3. Extraction and isolation

The dried aerial parts of *I. japonica* (20.0 kg) were powdered and extracted with 95% ethanol (3×10 L) for three times (48 h, 24 h, and 24 h) at room temperature. The ethanolic extract was successively partitioned with petroleum ether (30 L), CH_2Cl_2 (40 L), EtOAc (30 L), and *n*-BuOH (30 L), respectively. The CH_2Cl_2 fraction (84.5 g) was chromatographed on a silica gel column eluting with a step gradient of CH_2Cl_2/MeOH (100:0, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1) to give 11 fractions (Fr1–Fr11). Fr1 (5.6 g) and Fr2 (8.4 g) were combined and subjected to CC over macroporous resin MCI, Sephadex LH-20, and silica gel to give **24** (820.0 mg), **26** (311.1 mg), **28** (708.5 mg), **32** (15.0 mg), and **33** (23.8 mg). Fr3 (16.3 g) was subjected to a silica gel CC with mixtures of PE/EtOAc (20:1, 10:1, 5:1, 2:1, 1:1, EtOAc) as eluents in a stepwise gradient mode to obtain nine fractions (Fr3-1–Fr3-9). Compounds **12** (27.0 mg), **13** (73.0 mg), **14** (73.0 mg), **15** (224.7 mg), **29** (7.0 mg), and **30** (26.9 mg) were isolated after CC over macroporous resin MCI followed by preparative HPLC (CH_3CN/H_2O, 50:50) from subfraction Fr3-4. From subfraction Fr3-8, compounds **11** (67.0 mg) and **34** (7.0 mg) were obtained after CC over Sephadex LH-20 (MeOH/CH_2Cl_2, 1:1) and preparative HPLC (CH_3CN/H_2O, 35:65). By the same procedures, compound **22** (6.7 mg) was obtained from subfraction Fr3-9. Fr4 (5.2 g) was subjected to silica gel CC eluted with PE/EtOAc (15:1, 10:1, 5:1, 2:1, 1:1, EtOAc) to give seven fractions (Fr4-1–Fr4-7). Subfraction Fr4-1 was subjected to CC over Sephadex LH-20 (MeOH) and followed by preparative HPLC (MeOH/H_2O, 40:60) led to the isolation of **27** (204.5 mg) and **31** (16.7 mg). Similarly, **9** (9.0 mg), **18** (8.0 mg), **23** (20.1 mg), **35** (11.2 mg), and **36** (5.9 mg) were obtained from Fr4-2, while **1** (91.5 mg), **2** (4.6 mg), **3** (3.8 mg), **4** (1.1 mg), **10** (4.6 mg), **20** (9.9 mg), **25** (4.0 mg), and **37** (3.4 mg) from Fr4-3. Fr6 (3.5 g) was subjected to CC over macroporous resin MCI, Sephadex LH-20 (MeOH), and preparative HPLC (MeOH/H_2O, 35:65) to give **5** (226.0 mg), **6** (4.7 mg), **16** (1.0 mg), and **17** (22.5 mg). By the same procedures, **19** (90.4 mg) was isolated from Fr5 (1.3 g), while **7** (36.8 mg), **8** (11.4 mg), and **21** (11.3 mg) from Fr7 (3.2 g). The purities of these compounds were ranging from 95.5 to 99.8% determined by HPLC.

4.3.1. Compound ***1***. Colorless bulk crystals; $[\alpha]_D^{20}$ +153.3 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3489, 3354, 2936, 1751, 1664, 1269, 1165, 1011, 967, 947 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 289 $[M+Na]^+$, 555 $[2M+Na]^+$; ESIMS (negative) *m/z* 265 $[M-H]^-$, 531 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 289.1411 (calcd for $C_{15}H_{22}O_4Na$, 289.1416).

4.3.2. Compound ***2***. Amorphous powder; $[\alpha]_D^{20}$ +21.8 (*c* 0.13, MeOH); IR (KBr) ν_{max} 3461, 2933, 1749, 1410, 1374, 1270, 1169, 1025, 979 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 291 $[M+Na]^+$; ESIMS (negative) *m/z* 267 $[M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 291.1544 (calcd for $C_{15}H_{24}O_4Na$, 291.1567).

4.3.3. Compound ***3***. Amorphous powder; $[\alpha]_D^{20}$ +67.7 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3484, 2942, 2862, 1754, 1662, 1458, 1262, 1145, 1037, 1001 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 267 $[M+H]^+$; ESIMS (negative) *m/z* 265 $[M-H]^-$; HRESIMS (positive) $[M+H]^+$ *m/z* 267.1587 (calcd for $C_{15}H_{23}O_4$, 267.1596).

4.3.4. Compound ***4***. Amorphous powder; $[\alpha]_D^{20}$ +315.1 (*c* 0.03, MeOH); IR (KBr) ν_{max} 3461, 3315, 2933, 1749, 1662, 1410, 1337, 1270, 1169, 1025, 979 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 551 $[2M+Na]^+$; ESIMS (negative) *m/z* 263 $[M-H]^-$, 527 $[2\,M-H]^-$; HRESIMS (positive) $[M+H]^+$ *m/z* 265.1446 (calcd for $C_{15}H_{21}O_4$, 265.1440).

4.3.5. Compound ***5***. Amorphous powder; $[\alpha]_D^{20}$ +152.8 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3552, 3240, 2943, 2614, 2568, 2362, 1758, 1660, 1466, 1340, 1266, 1155, 1034, 974 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 287 $[M+Na]^+$; ESIMS (negative) *m/z* 263 $[M-H]^-$, 527 $[2\,M-H]^-$; HRESIMS (positive) $[M+H]^+$ *m/z* 265.1446 (calcd for $C_{15}H_{21}O_4$, 265.1440).

4.3.6. Compound ***6***. Amorphous powder; $[\alpha]_D^{20}$ +36.4 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3331, 2973, 2927, 2881, 1924, 1758, 1662, 1453, 1420, 1379, 1088, 1046, 880, 804 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 287 $[M+Na]^+$, 551 $[2M+Na]^+$; ESIMS (negative) *m/z* 263 $[M-H]^-$, 527 $[2\,M-H]^-$; HRESIMS (negative) $[M-H]^-$ *m/z* 263.1299 (calcd for $C_{15}H_{19}O_4$, 263.1283).

4.3.7. Compound ***7***. Amorphous powder; $[\alpha]_D^{20}$ +80.6 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3330, 2973, 2882, 2545, 2350, 2257, 1925, 1753, 1663, 1452, 1379, 1088, 1046, 880 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 287 $[M+Na]^+$, 551 $[2M+Na]^+$; ESIMS (negative) *m/z* 263 $[M-H]^-$; HRESIMS (positive) $[M+H]^+$ *m/z* 265.1459 (calcd for $C_{15}H_{21}O_4$, 265.1440).

4.3.8. Compound ***8***. Amorphous powder; $[\alpha]_D^{20}$ +205.4 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3322, 2960, 2530, 2257, 1925, 1760, 1652, 1421, 1329, 1088, 1046, 880 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 289 $[M+Na]^+$; ESIMS (negative) *m/z* 265 $[M-H]^-$; HRESIMS (positive) $[M+H]^+$ *m/z* 267.1597 (calcd for $C_{15}H_{23}O_4$, 267.1596).

4.3.9. Compound ***9***. Amorphous powder; $[\alpha]_D^{20}$ +20.6 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3484, 2942, 2862, 2571, 1754, 1662, 1458, 1397, 1322, 1262, 1145, 1001, 812 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 285 $[M+Na]^+$; HRESIMS (positive) $[M+H]^+$ *m/z* 263.1289 (calcd for $C_{15}H_{19}O_4$, 263.1283).

4.3.10. Compound ***10***. Amorphous powder; $[\alpha]_D^{20}$ +90.6 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3317, 2973, 2926, 2881, 1925, 1754, 1658, 1454, 1420, 1379, 1328, 1274, 1088, 1046, 880, 803 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 287 $[M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 287.1239 (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

4.3.11. Compound ***11***. Amorphous powder; $[\alpha]_D^{20}$ −22.3 (*c* 0.22, CH_2Cl_2); IR (KBr) ν_{max} 3537, 3350, 2932, 2866, 2133, 1757, 1718, 1656, 1412, 1376, 1277, 1250, 1150, 1019, 979 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 331 $[M+Na]^+$; ESIMS (negative) *m/z* 307 $[M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 331.1516 (calcd for $C_{17}H_{24}O_5Na$, 331.1521).

4.3.12. Compound ***12***. Amorphous powder; $[\alpha]_D^{20}$ −28.0 (*c* 0.21, CH_2Cl_2); IR (KBr) ν_{max} 3534, 2930, 1757, 1720, 1660, 1375, 1321, 1248, 1043, 983 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 359 $[M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 359.1832 (calcd for $C_{19}H_{28}O_5Na$, 359.1834).

4.3.13. Compound ***13***. Amorphous powder; $[\alpha]_D^{20}$ −20.9 (*c* 0.33, CH_2Cl_2); IR (KBr) ν_{max} 3537, 3352, 2932, 2866, 2133, 1757, 1718, 1657,

1376, 1277, 1250, 1151, 1069, 1019, 980, 963 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 373 $[M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 373.1987 (calcd for $C_{20}H_{30}O_5Na$, 373.1991).

4.3.14. Compound ***14***. Amorphous powder; $[\alpha]_D^{20}$ −21.1 (*c* 0.33, CH_2Cl_2); IR (KBr) ν_{max} 3540, 2933, 2860, 1760, 1720, 1652, 1375, 1340, 1277, 1250, 1129, 1020, 979, 923 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 373 $[M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 373.1897 (calcd for $C_{20}H_{30}O_5Na$, 373.1991).

4.3.15. Compound ***15***. Amorphous powder; $[\alpha]_D^{20}$ −14.4 (*c* 0.13, CH_2Cl_2); IR (KBr) ν_{max} 3450, 2935, 1755, 1729, 1660, 1377, 1229, 1129, 980, 920 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 387 $[M+Na]^+$, 751 $[M+Na]^+$; HRESIMS (positive) $[M+H]^+$ *m/z* 365.2339 (calcd for $C_{21}H_{33}O_5$, 365.2328).

4.3.16. Compound ***16***. Amorphous powder; $[\alpha]_D^{20}$ +136.3 (*c* 0.02, MeOH); IR (KBr) ν_{max} 3523, 3090, 2958, 2925, 2869, 1727, 1654, 1418, 1357, 1282, 1073, 1031, 973 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 289 $[M+Na]^+$; HRESIMS (positive) $[M+H]^+$ *m/z* 267.1604 (calcd for $C_{15}H_{23}O_4$, 267.1596).

4.3.17. Compound ***17***. Amorphous powder; $[\alpha]_D^{20}$ +61.8 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3494, 2937, 2552, 2361, 1736, 1654, 1457, 1406, 1364, 1260, 1160, 1031, 952 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 347 $[M+Na]^+$; HRESIMS (positive) $[M+H]^+$ *m/z* 325.1665 (calcd for $C_{17}H_{25}O_6$, 325.1651).

4.3.18. Compound ***18***. Amorphous powder; $[\alpha]_D^{20}$ −18.4 (*c* 0.22, CH_2Cl_2); IR (KBr) ν_{max} 3519, 3423, 3361, 2858, 2638, 2595, 2362, 1716, 1669, 1458, 1392, 1270, 1139, 1032 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 363 $[M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 363.1778 (calcd for $C_{18}H_{28}O_6Na$, 363.1784).

4.3.19. Compound ***19***. Amorphous powder; $[\alpha]_D^{20}$ −25.0 (*c* 0.18, CH_2Cl_2); IR (KBr) ν_{max} 3523, 3422, 3359, 2934, 2637, 2590, 2370, 1750, 1715, 1660, 1457, 1390, 1076, 945, 912 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 363 $[M+Na]^+$; HRESIMS (positive) $[M+H]^+$ *m/z* 341.1975 (calcd for $C_{18}H_{29}O_6$, 341.1964).

4.3.20. Compound ***20***. Amorphous powder; $[\alpha]_D^{20}$ +81.1 (*c* 0.26, MeOH); IR (KBr) ν_{max} 3523, 3090, 2925, 2869, 2361, 1727, 1654, 1418, 1357, 1319, 1282, 1238, 1164, 1031 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 267 $[M+H]^+$; HRESIMS (positive) $[M+H]^+$ *m/z* 267.1594 (calcd for $C_{15}H_{23}O_4$, 267.1596).

4.3.21. Compound ***21***. Amorphous powder; $[\alpha]_D^{20}$ +105.2 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3552, 3239, 2942, 2567, 2361, 1739, 1660, 1466, 1340, 1266, 1155, 1034, 973, 883 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 267 $[M+H]^+$; HRESIMS (positive) $[M+H]^+$ *m/z* 267.1597 (calcd for $C_{15}H_{23}O_4$, 267.1596).

4.3.22. Compound ***22***. Amorphous powder; $[\alpha]_D^{20}$ +54.0 (*c* 0.16, CH_2Cl_2); IR (KBr) ν_{max} 3439, 2930, 2872, 2546, 1766, 1737, 1660, 1455, 1370, 1252, 1156, 1031, 998 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 331 $[M+Na]^+$; HRESIMS (positive) $[M+H]^+$ *m/z* 309.1696 (calcd for $C_{17}H_{25}O_5$, 309.1702).

4.4. Preparation of (*S*)-MTPA ester (1a) and (*R*)-MTPA ester (1b)

Compound **1** (3 mg) was transferred into a clean NMR tube, deuterated pyridine (0.5 mL), small amount of DMAP (dimethyl amino pyridine), and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenyl acetyl chloride (5 μL) were added into the NMR tube immediately under a N_2 gas stream, and then the NMR tube was shaken carefully to mix the sample and MTPA chloride evenly. The reaction NMR tube was permitted to stand in a water bath at 50 °C for 4 h to afford the (*S*)-MTPA ester derivative (**1a**). In the manner described for **1a**, another portion of compound **1** (3 mg) was reacted in a second NMR tube with (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenyl acetyl chloride (5 μL) at 50 °C for 4 h using deuterated pyridine (0.5 mL) as solvent, small amount of DMAP was added, to afford the (*R*)-MTPA derivative (**1b**). The ^1H NMR data of the *S*-MTPA ester derivative (**1a**) and *R*-MTPA ester derivative (**1b**) were obtained directly on the reaction mixture (pyridine-d_5, 400 MHz):

4.4.1. Compound ***1a***. δ 4.458 (1H, dd, *J*=11.6, 4.4 Hz, H-1), 2.026 (1H, m, H-2a), 1.893 (1H, m, H-2b), 2.625 (1H, m, H-3a), 1.861 (1H, m, H-3b), 1.972 (1H, m, H-4), 2.076 (1H, dd, *J*=14.0, 11.6 Hz, H-6a), 1.740 (1H, dd, *J*=14.0, 7.6 Hz, H-6b), 3.633 (1H, m, H-7), 4.763 (1H, ddd, *J*=5.2, 1.2, 1.2 Hz, H-8), 2.588 (2H, d, *J*=4.8 Hz, H-9), 6.217 (1H, s, H-13a), 5.522 (1H, s, H-13b), 1.423 (3H, s, H_3-14), 1.015 (3H, d, *J*=7.6 Hz, H_3-15).

4.4.2. Compound ***1b***. δ 4.458 (1H, dd, *J*=11.6, 4.4 Hz, H-1), 2.007 (1H, m, H-2a), 1.883 (1H, m, H-2b), 2.618 (1H, m, H-3a), 1.857 (1H, m, H-3b), 1.963 (1H, m, H-4), 2.076 (1H, dd, *J*=14.0, 11.6 Hz, H-6a), 1.740 (1H, dd, *J*=14.0, 7.6 Hz, H-6b), 3.635 (1H, m, H-7), 4.773 (1H, ddd, *J*=4.8, 1.2, 1.2 Hz, H-8), 2.600 (2H, d, *J*=4.8 Hz, H-9), 6.217 (1H, s, H-13a), 5.522 (1H, s, H-13b), 1.429 (3H, s, H_3-14), 1.015 (3H, d, *J*=7.6 Hz, H_3-15).

4.5. Crystallographic data of compound 1 and compound 28

Crystallographic data of compound **1** $C_{15}H_{22}O_4$, *M*=266, tetragonal, space group *P*4(1)2(1)2, *a*=8.8042 (7) Å, *α*=90°; *b*=8.8042 (7) Å, *β*=90°; *c*=39.739 (4) Å, γ=90°; *V*=3080.3(5) $Å^3$, *Z*=8, D_{calcd}=1.226 mg/m^3, crystal size 0.369×0.344×0.267 mm^3. Mo Kα (0.71073 Å), *F*(000)=1232, *T*=293(2) K. The final *R* values were *R*=0.0448, and R_w=0.1195, for 1633 observed reflections [$I>2\sigma(I)$].

Crystallographic data of compound **28** $C_{17}H_{24}O_5$, *M*=308, orthorhombic, space group *P*2(1)2(1)2(1), *a*=7.9947 (8) Å, *α*=90°; *b*=12.3402 (12) Å, *β*=90°; *c*=16.8306 (17) Å, γ=90°; *V*=1660.4(3) $Å^3$, *Z*=4, D_{calcd}=1.234 mg/m^3, crystal size 0.432×0.320×0.205 mm^3. Mo Kα (0.71073 Å), *F*(000)=664, *T*=293(2) K. The final *R* values were *R*=0.0413, and R_w=0.1034, for 1864 observed reflections [$I>2\sigma(I)$].

Crystallographic data for **1** and **28** have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 776071 and 776072). Copies of these data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.ac.uk).

4.6. In vitro anti-inflammatory assay and cytotoxicity testing

These two experiments were carried out as previously described.[8,9,33] Briefly, RAW264.7 cells grown on 100 mm culture dish were harvested and seeded in 96-well plates at 2×10^5 cells/well for NO production. The plates were pretreated with various concentrations of samples for 30 min and then incubated for 24 h with or without 1 μg/mL of LPS. The nitrite concentration in the culture supernatant was measured by the Griess reaction. Cell viability was measured by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Sigma–Aldrich).

Acknowledgements

The work was supported by NSFC (30725045), the Special Program for New Drug Innovation of the Ministry of Science and

Technology, China (2009ZX09311-001, 2008ZX09101-Z-029, 2009ZX09103-375), National comprehensive technology platforms for innovative drug R&D, 2009ZX09301-007, Shanghai Leading Academic Discipline Project (B906), and in part by the Scientific Foundation of Shanghai Committee of Science and Technology (08DZ1971302, 09DZ1975700, 09DZ1971500, 09DZ1972200).

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.09.091. These data include MOL files and InChIKeys of the most important compounds described in this article.

References and notes

1. Chinese Academy of Medicine. Institute of Pharmacy. *Traditional Chinese Medicine*; People's Health: Beijing, 1984, pp 476.
2. Lin, R.; Yu, D. J.; Wu, Z. Y. *Flora of China*; Science: Beijing, 1989, pp 263.
3. Zhao, Y. M.; Zhang, M. L.; Shi, Q. W.; Kiyota, H. *Chem. Biodivers.* **2006**, *3*, 371.
4. Shan, J. J.; Yang, M.; Ren, J. W. *Biol. Pharm. Bull.* **2006**, *29*, 455.
5. Wang, C. M.; Jia, Z. J.; Zheng, R. L. *Planta Med.* **2007**, *73*, 180.
6. Yang, C.; Wang, C. M.; Jia, Z. J. *Planta Med.* **2003**, *69*, 662.
7. Qin, J. J.; Jin, H. Z.; Fu, J. J.; Hu, X. J.; Wang, Y.; Yan, S. K.; Zhang, W. D. *Bioorg. Med. Chem. Lett.* **2008**, *19*, 710.
8. Qin, J. J.; Zhu, J. X.; Zhang, W. D.; Zhu, Y.; Fu, J. J.; Liu, X. H.; Jin, H. Z. *Arch. Pharm. Res.* **2009**, *32*, 1369.
9. Qin, J. J.; Jin, H. Z.; Zhu, J. X.; Fu, J. J.; Hu, X. J.; Liu, X. H.; Zhu, Y.; Yan, S. K.; Zhang, W. D. *Planta Med.* **2010**, *76*, 278.
10. Bohmann, F.; Jakupovic, J.; Schuster, A. *Phytochemistry* **1981**, *20*, 1891.
11. Bohlmann, F.; Mahanta, P. K.; Jakupovic, J.; Rastogi, R. C.; Natu, A. *Phytochemistry* **1978**, *17*, 1165.
12. De Trimacro, J. T.; De Riscala, E. C.; Catalán, C. A. N.; Griffin, C. L.; Herz, W. *Biochem. Syst. Ecol.* **2004**, *32*, 1063.
13. Herz, W.; Sumi, Y.; Sudarsanam, V.; Raulais, D. *J. Org. Chem.* **1967**, *32*, 3658.
14. Jeske, F.; Huneck, S.; Jakupovic, J. *Phytochemistry* **1993**, *34*, 1647.
15. Marcinek-Hüpen-Bestendonk, C.; Willuhn, G.; Steigel, A. *Planta Med.* **1990**, *56*, 104.
16. Uchiyama, T.; Miyase, T.; Ueno, A.; Usmanghani, K. *Phytochemistry* **1989**, *28*, 3369.
17. Borkosky, S.; Valdés, D. A.; Bardón, A.; Dőaz, J. G.; Herz, W. *Phytochemistry* **1996**, *42*, 1637.
18. Otsuka, H.; Yao, M.; Kamada, K.; Takeda, Y. *Chem. Pharm. Bull.* **1995**, *43*, 754.
19. Yoshikawa, M.; Shimada, H.; Saka, M.; Yoshizumi, S.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1997**, *45*, 464.
20. Jiang, H. X.; Li, Y.; Pan, J.; Gao, K. *Helv. Chim. Acta* **2006**, *89*, 558.
21. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.
22. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
23. Grant, D. M.; Cheney, B. V. *J. Am. Chem. Soc.* **1967**, *89*, 5315.
24. Seidman, K.; Maciel, G. E. *J. Am. Chem. Soc.* **1977**, *99*, 659.
25. Li, S.; Chesnut, D. B. *Magn. Reson. Chem.* **1985**, *23*, 625.
26. Barfield, M.; Yamamura, S. H. *J. Am. Chem. Soc.* **1990**, *112*, 4747.
27. Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H. *Phytochemistry* **1984**, *23*, 1979.
28. Han, J. W.; Lee, B. G.; Kim, Y. K.; Yoon, J. W.; Jin, H. K.; Hong, S.; Lee, H. Y.; Lee, K. R.; Lee, H. W. *Br. J. Pharmacol.* **2001**, *133*, 503.
29. Jin, H. Z.; Lee, D.; Lee, J. H.; Lee, K. *Planta Med.* **2006**, *72*, 40.
30. Liu, P. L.; Wen, J. K.; Wu, Y. B.; Zhang, J.; Zheng, B.; Zhang, D. Q.; Han, M. *Phytomedicine* **2009**, *16*, 156.
31. Lyb, G.; Knorre, A.; Schmidt, T. J.; Pahl, H. L.; Merfort, I. *J. Biol. Chem.* **1998**, *273*, 33508.
32. Choi, B. G.; Kawk, E. Y.; Chung, B. H. *Arch. Pharm. Res.* **1999**, *22*, 575.
33. Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271.