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Pseudoguaianolides and Guaianolides from *Inula hupehensis* as Potential Anti-inflammatory Agents

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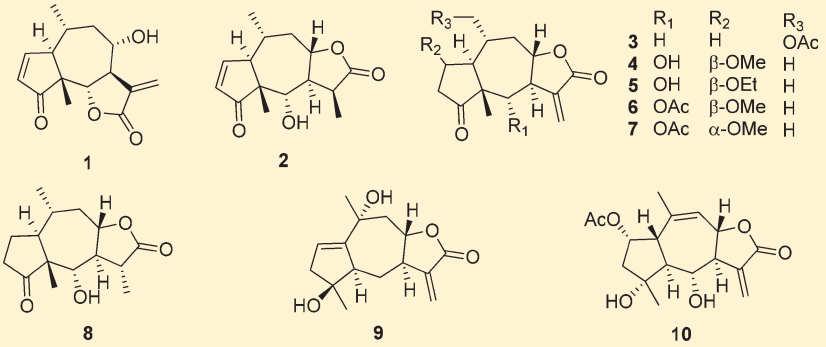
Supporting Information

ABSTRACT:



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Eight new pseudoguaianolides (**1**−**8**), two new guaianolides (**9** and **10**), and 14 known sesquiterpenes were isolated from the aerial parts of *Inula hupehensis*. The structures were elucidated using spectroscopic methods and circular dichroism analysis. All compounds were tested for inhibitory activities against LPS-induced nitric oxide production in RAW264.7 macrophages. Compounds **13** and **22** were found to inhibit nitric oxide production potently, with IC_{50} values of 0.9 and 0.6 μM, respectively. Preliminary structure−activity relationships for these compounds are proposed.

Inula is an important genus that comprises approximately 100 species in the Asteraceae family.[1,2] Plants belonging to this genus show high diversity in their secondary metabolites and pharmacological effects.[3−14] *Inula hupehensis*, commonly referred to as "JinFeiCao" in China, is used in traditional Chinese medicine (TCM) for the treatment of bronchitis, diabetes, and intestinal ulcers.[1] Previously, only two cytotoxic pseudoguaianolides and five antimicrobial thymol derivatives were isolated from this plant.[3,12] As part of an ongoing research program searching for bioactive metabolites from the *Inula* genus, the phytochemical investigation of *I. hupehensis* was undertaken and resulted in the isolation of eight new pseudoguaianolides (**1**−**8**), two new guaianolides (**9** and **10**), and 14 known sesquiterpenes (**11**−**24**). In addition, the inhibitory activities of all 24 compounds against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in the RAW264.7 macrophage are also described here.

RESULTS AND DISCUSSION

The dried aerial parts of *I. hupehensis* were powdered and extracted with 95% aqueous ethanol, and the extract was successively partitioned with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc fraction was subjected to column chromatography over silica gel, macroporous resin MCI, Sephadex LH-20, and preparative HPLC to yield eight new pseudoguaianolides (**1**−**8**), two new guaianolides (**9** and **10**), and 14 known sesquiterpenes: aromaticin (**11**),[15] 8-epihelenalin (**12**),[16] bigelovin (**13**),[17] graveolide (**14**),[18] carpesiolin (**15**),[19] ergolide (**16**),[3] 6α-acetoxyisoinuviscolide (**17**),[20] confertin (**18**),[21] burrodin (**19**),[22] 8-epiinuviscolide (**20**),[23] inuviscolide (**21**),[24] inuchinenolide C (**22**),[25] 4-epiisoinuviscolide (**23**),[25] and inuchinenolide B (**24**).[25]

Compound **1** was obtained as an optically active, amorphous powder. Its molecular formula, $C_{15}H_{18}O_4$, was established by HR-ESIMS (*m*/*z* 285.1097 for $C_{15}H_{18}O_4Na$, calcd *m*/*z* 285.1103), indicating seven degrees of unsaturation. The IR spectrum showed the presence of a hydroxy group (3441 cm^{-1}), an α,β-unsaturated γ-lactone group (1767 cm^{-1}), and a carbonyl group (1726 cm^{-1}). The ^1H NMR spectrum exhibited one methyl singlet [δ_H 1.21 (3H, s, H_3-15)], one methyl doublet [δ_H 1.28 (3H, d, J = 6.6 Hz, H_3-14)], two oxymethine [δ_H 5.58 (1H, d, J = 7.6 Hz, H-6); 4.81 (1H, m, H-8)], and four olefinic protons [δ_H 7.90 (1H, dd, J = 6.0, 1.8 Hz, H-2); 6.06 (1H, dd, J = 6.0, 2.9 Hz, H-3); 6.11 (1H, d, J = 3.5 Hz, H-13a); 5.77 (1H, d, J = 3.2 Hz, H-13b)]. The corresponding

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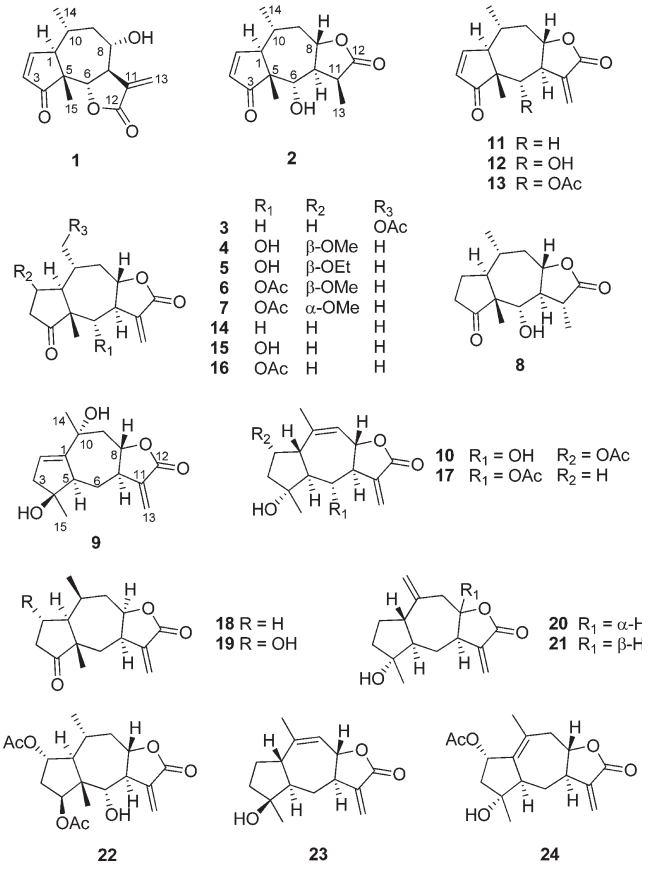

carbons were differentiated by ^{13}C and DEPT spectra (Tables 1 and 2) as two methyl [δ_C 23.4 (C-15) and 20.2 (C-14)], two oxymethine [δ_C 75.3 (C-6) and 78.3 (C-8)], and three olefinic carbons [δ_C 166.3 (C-2), 131.4 (C-3), and 121.3 (C-13)]. The ^{13}C NMR spectrum also indicated three sp^2 quaternary carbons comprising one olefinic, one α,β-unsaturated carbonyl, and one ester carbonyl carbon. The remaining degrees of unsaturation were due to a tricyclic core. The analysis of the $^1H-^1H$ COSY spectrum revealed the long-range spin-system of H-6/H-7/H-8/H_2-9/H-10(H_3-14)/H-1/H-2/H-3, as shown in Figure 1. Additionally, the HMBC correlations of H-2 to C-4 and C-5, H-6 to C-1 and C-8, H_2-13 to C-7, C-11, and C-12, H_3-14 to C-1, C-9, and C-10, and H_3-15 to C-4, C-5, and C-6 and the chemical shift of H-6 (δ_H 5.58) established the planar structure of **1** as 8-hydroxy-4-oxopseudoguai-2(3),11(13)-dien-12,6-olide (Figure 1). According to the NOESY spectrum, H-7 correlated with H-1 and H_3-14, confirming that H-1, H-7, and H_3-14 were cofacial. Further analysis of the NOESY spectrum showed correlations between H-6/H-8, H-6/H_3-15, H-8/H-10, and H-8/H_3-15, which indicated that these protons/methyl groups were cofacial (Figure 1). The coupling constants between H-7 and H_2-13 (3.5 and 3.2 Hz) confirmed the presence of the *trans*-fused lactone ring.[26] In the CD spectrum, a negative Cotton effect at 328 nm ($\Delta\varepsilon = -11.6$) indicated a 1*R* configuration of the α,β-unsaturated cyclopentenone moiety.[17,27−30] Therefore, the structure of **1** was concluded to be (1*R*,5*R*,6*S*,7*R*,8*S*,10*R*)-8-hydroxy-4-oxopseudoguai-2(3),11(13)-dien-12,6-olide.

Compound **2** showed a molecular formula of $C_{15}H_{20}O_4$ by the positive HR-ESIMS ion at m/z 265.1441 $[M + H]^+$. The ^{13}C NMR data of **2** were similar to those of 8-epihelenalin (**12**).[16] Differences included the chemical shifts of C-6 (δ_C 69.7), C-11 (δ_C 38.1), C-12 (δ_C 178.5), and C-13 (δ_C 11.9) in **2**, in contrast to the chemical shifts at δ_C 74.0, 138.7, 169.3, and 121.6 in **12**, respectively (Tables 1 and 2). The absence of exocyclic olefinic protons in the ^1H NMR spectrum of **2** supported hydrogenation of the C-11 double bond in compound **12**. In the NOESY spectrum, correlations between H_3-13/H-6 and H_3-13/H-8 were observed. On the basis of the negative Cotton effect at 330 nm ($\Delta\varepsilon = -5.2$) in the CD spectrum of **2** and the chemical correlation between **2** and **12**, compound **2** was defined as (1*R*,5*R*,6*S*,7*S*,8*S*,10*R*,11*S*)-6-hydroxy-4-oxopseudoguai-2(3)-en-12,8-olide.

Compound **3** exhibited an $[M + H]^+$ ion at m/z 307.1547 in the positive HR-ESIMS, corresponding to the molecular formula $C_{17}H_{22}O_5$. The NMR data of **3** showed a similarity to those of graveolide (**14**),[18] except for the absence of a methyl group and the presence of an oxymethylene and an acetoxy group (Tables 1 and 2). The HMBC correlations of H_2-14 to C-1′ suggested that the acetoxy group was attached to C-14; therefore, the structure of **3** was constructed. In accordance with the positive Cotton effect at 296 nm ($\Delta\varepsilon = +14.5$) of **3**, C-1 of the cyclopentanone moiety was assigned an *S* configuration.[27,28,31,32] Compound **3** was thus identified as (1*S*,5*S*,7*R*,8*S*,10*R*)-14-acetoxy-4-oxopseudoguai-11(13)-en-12,8-olide.

Compound **4** showed a molecular formula of $C_{16}H_{22}O_5$ by the positive HR-ESIMS ion at m/z 295.1551 $[M + H]^+$. The ^1H and ^{13}C NMR spectra were similar to those of carpesiolin (**15**), except for the presence of the C-2 *O*-methyl group of **4**.[19] The coupling constant (4.5 Hz) between H-1 and H-2 suggested a *cis*-relationship of the two protons (Tables 1 and 2). The planar structure and relative configuration were confirmed by 2D NMR spectra. A positive Cotton effect at 295 nm ($\Delta\varepsilon = +6.1$) was found in the CD spectrum of **4**. Therefore, compound **4** was determined to be (1*S*,2*R*,5*R*,6*S*,7*S*,8*S*,10*R*)-6-hydroxy-2-methoxy-4-oxopseudoguai-11(13)-en-12,8-olide.

Compound **5** showed a molecular formula of $C_{17}H_{24}O_5$ by the positive HR-ESIMS ion at m/z 309.1711 $[M + H]^+$. The ^1H and ^{13}C NMR spectra of **5** were all comparable to those of **4**, except for the absence of the C-2 *O*-methyl group of **4** and its replacement by an *O*-ethyl group in **5** (Tables 1 and 2). Compound **5** was determined to be (1*S*,2*R*,5*R*,6*S*,7*S*,8*S*,10*R*)-6-hydroxy-2-ethoxy-4-oxopseudoguai-11(13)-en-12,8-olide.

Compound **6** was assigned the molecular formula $C_{18}H_{24}O_6$ from the positive HR-ESIMS ion at m/z 359.1481 $[M + Na]^+$. The ^1H and ^{13}C NMR spectra were similar to those of **4**, except for an additional acetoxy group. The downfield shift of H-6 from δ_H 3.95 to 5.43 as compared with **4** established the connection of the acetoxy group to C-6 in **6** (Tables 1 and 2). Compound **6** was determined to be (1*S*,2*R*,5*R*,6*S*,7*R*,8*S*,10*R*)-6-acetoxy-2-methoxy-4-oxopseudoguai-11(13)-en-12,8-olide.

Compound **7** had a molecular formula of $C_{18}H_{24}O_6$, which was determined to be the same as compound **6** from the HR-ESIMS ion at m/z 337.1650 $[M + H]^+$. The IR spectrum was similar to that of **6**, and the ^1H and ^{13}C NMR spectra of **7** also resembled those of **6**, suggesting the same skeleton (Tables 1 and 2). Differences were observed in the chemical shifts of C-5 and C-10 in the ^{13}C NMR spectra, which were assumed to result from the orientation of the C-2 *O*-methyl group. Actually, the upfield shifts of C-5 and C-10 in **6** (δ_C 55.0 and 26.2, respectively), with respect to the corresponding shift values in **7** (δ_C 58.1 and 30.1, respectively), were due to a *γ-gauche* effect of the C-2 *O*-methyl group.[33−36] Compound **7** was determined to be (1*S*,2*S*,5*R*,6*S*,7*R*,8*S*,10*R*)-6-acetoxy-2-methoxy-4-oxopseudoguai-11(13)-en-12,8-olide.

Compound **8** had a molecular formula of $C_{15}H_{22}O_4$ by the positive HR-ESIMS ion at m/z 267.1598 $[M + H]^+$. The ^1H and ^{13}C NMR data were similar to those of carpesiolin (**15**), except for the α-methylene lactone functionality (Tables 1 and 2).

Table 1. ^{1}H NMR Data (400 MHz) [δ_H (J in Hz)] for Compounds 1−10

position	1[a]	2[b]	3[b]	4[b]	5[b]
1	3.14, ddd (10.7, 2.4, 2.4)	2.95, m	2.21, m	2.21, m	2.19, m
2	7.90, dd (6.0, 1.8)	7.76, dd (6.0, 1.7)	2.05, 1.67, m	3.96, t (4.5)	4.06, t (4.1)
3	6.06, dd (6.0, 2.9)	6.13, dd (6.0, 2.8)	2.47, 2.23, m	2.66, d (19.0); 2.24, m	2.65, 2.23 m
6	5.58, d (7.6)	4.07, d (8.8)	2.50, m	3.95, d (9.0)	3.96, d (8.5)
			1.53, dd (14.9, 11.9)		
7	3.30, m	2.45, m	2.84, m	2.83, m	2.83, m
8	4.81, m	4.61, ddd (11.2, 11.2, 3.3)	4.29, m	4.42, ddd (12.0, 10.0, 2.5)	4.42, ddd (13.0, 10.0, 2.9)
9	2.49, ddd (12.5, 4.4, 3.0)	2.49, m	2.54, m	2.50, ddd (12.0, 4.5, 3.0)	2.50, ddd (13.0, 3.5, 3.5)
	1.61, q (12.5)	1.41, q (12.2)	1.65, m	1.44, q (12.0)	1.44, m
10	2.09, m	2.00, m	2.18, m	2.17, m	2.21, m
11		2.92, m			
13	6.11, d (3.5)	1.29, d (7.7)	6.20, d (3.5)	6.20, d (3.5)	6.20, d (3.5)
	5.77, d (3.2)		5.52, d (3.2)	5.92, d (3.0)	5.92, d (3.2)
14	1.28, d (6.6)	1.25, d (6.6)	4.26, dd (11.0, 2.8)	1.15, d (6.0)	1.15, d (6.6)
			4.09, dd (11.0, 5.7)		
15	1.21, s	1.13, s	1.06, s	1.22, s	1.26, s
2-OMe				3.24, s	
2-OEt					3.50, 3.24, m
					1.17, t (7.1)
14-OAc			2.10, s		

position	6[b]	7[b]	8[b]	9[a]	10[b]
1	2.25, dd (10.8, 4.0)	2.33, dd (10.5, 9.0)	2.19, m		2.45, brd (12.5)
2	3.97, t (4.0)	3.68, dd (16.2, 9.0)	2.14, 1.48, m	5.59, brs	5.25, t (4.2)
3	2.65, d (18.0)	2.94, dd (18.7, 7.3)	2.41, m	2.35, m	2.13, dd (15.7, 5.0)
	2.18, d (18.0, 4.7)	2.25, dd (18.7,8.2)	2.15, m		2.00, dd (15.7, 3.0)
5				2.69, brd (12.0)	2.31, dd (12.7, 10.0)
6	5.43, d (7.6)	5.46, d (7.8)	3.90, d (8.0)	2.09, ddd (12.0, 4.5, 4.5)	4.04, t (10.0)
				1.37, q (12.0)	
7	2.97, m	3.03, m	2.04, m	3.41, m	2.79, m
8	4.52, ddd (13.0, 9.2, 2.8)	4.44, ddd (13.0, 9.7, 2.8)	4.39, ddd (11.0,11.0,3.0)	5.11, ddd (12.0, 8.0, 4.0)	4.42, brd (9.8)
9	2.54, ddd (13.0, 4.3, 2.8)	2.45, ddd (13.0, 4.0, 3.0)	2.42, m	2.17, dd (14.0, 4.0)	5.93, brs
	1.47, dd (13.0, 2.0)	1.54, m	1.38, m	1.79, dd (14.0, 12.0)	
10	2.20, m	1.99, m	1.82, m		
11			2.54, m		
13	6.18, d (3.5)	6.20, d (3.5)	1.36, d (7.5)	6.17, d (3.0)	6.34, m
	5.82, d (3.1)	5.84, d (3.1)		5.70, d (3.0)	6.32, m
14	1.17, d (6.2)	1.20, d (6.8)	1.07, d (6.5)	1.49, s	1.81, s
15	1.26, s	1.08, s	1.02, s	1.29, s	1.45, s
2-OMe	3.24, s	3.34, s			
2-OAc					2.11, s
6-OAc	1.96, s	1.98, s			
6-OH			2.82, brs		

[a] Measured in methanol-d_4. [b] Measured in $CDCl_3$.

The absence of the $\Delta^{11,13}$ exocyclic methylene group was confirmed by the upfield shifts of C-11 and C-13 and a downfield shift of C-12 in **8**, compared with those of **15**.[19] In the NOESY spectrum, a correlation between H-6/H-11 was observed. Therefore, compound **8** was determined to be (1*S*,5*R*,6*S*,7*S*,8*S*,10*R*,11*R*)-6-hydroxy-4-oxopseudoguai-12,8-olide.

Compound **9** showed a molecular formula of $C_{15}H_{20}O_4$, deduced from the positive HR-ESIMS ion at m/z 287.1265 $[M + Na]^+$, indicating six degrees of unsaturation. The IR absorptions at 3425, 1754, and 1629 cm^{-1} indicated the presence of hydroxy, α,β-unsaturated lactone carbonyl, and olefinic groups, respectively. The ^{1}H NMR, ^{13}C NMR, and DEPT spectra showed the signals of two singlet methyl groups [δ_H 1.49 (3H, s, H_3-14) and 1.29 (3H, s, H_3-15); δ_C 30.3 (C-14) and 24.8 (C-15)], one oxymethine group [δ_H 5.11 (1H, ddd, J = 12.0, 8.0, 4.0 Hz, H-8); δ_C 80.0 (C-8)], two oxygenated quaternary carbons [δ_C 82.3 (C-4) and 70.2 (C-10)], four olefinic carbons [δ_H 5.59 (1H, brs, H-2), 6.17 (1H, d, J = 3.0 Hz, H-13a), and 5.70 (1H, d, J = 3.0 Hz, H-13b); δ_C 152.9 (C-1), 123.1 (C-2), 142.6 (C-11), and 122.9 (C-13)], and one ester carbonyl carbon [δ_C 172.5

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Table 2. ^{13}C NMR Data (100 MHz) (δ_C, mult.) for Compounds 1−10

position	1[a]	2[b]	3[b]	4[b]	5[b]	6[b]	7[b]	8[b]	9[a]	10[b]
1	53.7, CH	50.8, CH	44.8, CH	49.5, CH	49.5, CH	50.9, CH	50.8, CH	44.9, CH	152.9, C	45.8, CH
2	166.3, CH	164.9, CH	23.3, CH_2	77.7, CH	75.9, CH	77.5, CH	78.4, CH	24.5, CH_2	123.1, CH	75.2, CH
3	131.4, CH	130.9, CH	35.1, CH_2	43.6, CH_2	44.3, CH_2	43.9, CH_2	44.0, CH_2	37.7, CH_2	47.5, CH_2	48.3, CH_2
4	212.4, C	213.9, C	221.5, C	222.6, C	222.9, C	217.4, C	214.2, C	223.8, C	82.3, C	79.9, C
5	57.9, C	57.4, C	49.8, C	56.8, C	56.8, C	55.0, C	58.1, C	57.7, C	56.5, CH	55.6, CH
6	75.3, CH	69.7, CH	34.4, CH_2	76.3, CH	76.3, CH	75.6, CH	74.4, CH	76.9, CH	33.6, CH_2	73.5, CH_2
7	53.7, CH	53.1, CH	44.7, CH	52.1, CH	52.1, CH	52.7, CH	52.5, CH	55.7, CH	43.8, CH	50.8, CH
8	78.3, CH	76.6, CH	80.5, CH	76.1, CH	76.1, CH	76.3, CH	76.1, CH	76.2, CH	80.0, CH	76.8, CH
9	45.3, CH_2	44.8, CH_2	38.5, CH_2	43.7, CH_2	43.8, CH_2	43.9, CH_2	44.4, CH_2	44.4, CH_2	43.9, CH_2	128.4, CH
10	28.8, CH	27.0, CH	34.1, CH	26.2, CH	26.2, CH	26.2, CH	30.1, CH	30.0, CH	70.2, C	134.7, C
11	140.2, C	38.1, CH	139.8, C	138.9, C	139.1, C	137.3, C	137.1, C	43.8, CH	142.6, C	137.0, C
12	171.3, C	178.5, C	169.6, C	169.6, C	169.4, C	169.1, C	169.3, C	178.2, C	172.5, C	170.2, C
13	121.3, CH_2	11.9, CH_3	120.4, CH_2	121.5, CH_2	121.5, CH_2	121.8, CH_2	122.1, CH_2	15.2, CH_3	122.9, CH_2	125.0, CH_2
14	20.2, CH_3	19.9, CH_3	66.3, CH_2	19.7, CH_3	19.6, CH_3	19.5, CH_3	20.3, CH_3	20.1, CH_3	30.3, CH_3	22.1, CH_3
15	23.4, CH_3	23.7, CH_3	21.9, CH_3	22.0, CH_3	22.1, CH_3	21.2, CH_3	20.3, CH_3	19.0, CH_3	24.8, CH_3	26.5, CH_3
2-OMe				56.5, CH_3		56.4, CH_3	57.1, CH_3			
2-OEt					64.4, CH_2					
					15.4, CH_3					
2-OAc										170.3, C
										21.5, CH_3
6-OAc						169.1, C	169.9, C			
						21.0, CH_3	21.1, CH_3			
14-OAc			171.4, C							
			20.9, CH_3							

[a] Measured in methanol-d_4. [b] Measured in $CDCl_3$.

(C-12)] (Tables 1 and 2). Analysis of the ^{1}H−^{1}H COSY spectrum established two spin-systems of H-2/H_2-3 and H-5/H_2-6/H-7/H-8/H_2-9. The HMBC correlations were observed from H-2 to C-4 and C-5, H_2-13 to C-7, C-11, and C-12, H_3-14 to C-1, C-9, and C-10, and H_3-15 to C-3, C-4, and C-5. These data allowed the planar structure of **9** to be defined as 4,10-dihydroxyguai-1(2),11(13)-dien-12,8-olide (Figure 2). The NOESY correlations between H-5/H-6α (δ_H 2.09), H-5/H-7, and H-6α/H_3-15 indicated they were cofacial and were arbitrarily assigned as α-oriented. As a consequence, the NOESY correlations between H-8/H-9β (δ_H 2.17) and H-9β/H_3-14 revealed that they were β-oriented (Figure 2). The coupling constant between H-7 and H-8 (8.0 Hz) and the coupling constants between H-7 and H_2-13 (3.0 and 3.0 Hz) confirmed the *trans*-fused lactone ring.[26] Therefore, compound **9** was identified as 4β,10α-dihydroxy-5αH-guai-1(2),11(13)-dien-12,8α-olide.

Compound **10** had a molecular formula of $C_{17}H_{22}O_6$, as established from the positive HR-ESIMS ion at m/z 345.1317 $[M + Na]^+$. The ^{1}H and ^{13}C NMR spectra were similar to those of gaillardin, except for an additional hydroxy group at C-6.[37] In the NOESY spectrum, correlations between H-6/H-8 and H-6/H_3-15 were observed. Compound **10** was determined to be 2α-acetoxy-4α,6α-dihydroxy-1β,5αH-guai-9(10),11(13)-dien-12,8α-olide.

In previous investigations, sesquiterpene lactones (SLs) have been shown to be a rich natural source of potential anti-inflammatory, anticancer, and bactericidal agents.[38,39] The α-methylene-γ-lactone is a chemical characteristic of the SL class and has been reported to be an integral building block for the reported compounds; the compounds also exhibit diverse biological properties.[39,40] Specifically, their potent anti-inflammatory activities have received notable attention.[41,42]

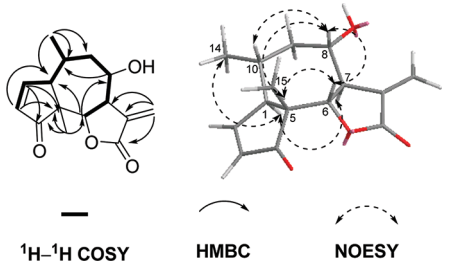

Figure 1. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations for **1**.

NO plays an important part in the inflammatory process, and an inhibitor of NO production may be considered as a potential anti-inflammatory agent.[43] Therefore, compounds **1**−**24** were tested for their inhibitory activities against LPS-induced NO production in RAW264.7 macrophages within the concentration range 0.1 to 50.0 μM. The IC_{50} values obtained (Table 3) suggested that most of the compounds exhibited potent inhibitory activities against NO production (IC_{50} 0.6 to 10.0 μM), as expected for SLs containing α-methylene-γ-lactone. Additionally, a few SLs without the α-methylene-γ-lactone functionality (such as compound **2**) also exhibited moderate inhibition of NO production, which was attributed to the α,β-unsubstituted cyclopentenone moiety.[44,45] Further comparison of SLs with or without the α,β-unsubstituted cyclopentenone moiety (compounds **11** to **16**) indicated that the presence of this moiety in SLs is beneficial for the NO production inhibitory activity.

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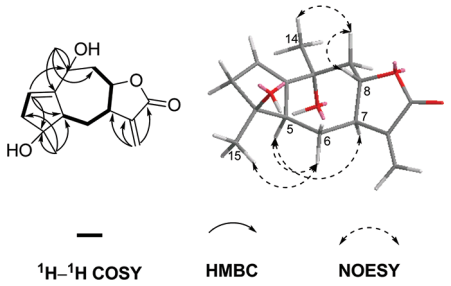

Figure 2. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations for **9**.

Comparison of the potency of inhibitory effects between the pseudoguaianolides revealed that the presence of a hydroxy group at C-2 or C-6 was of pivotal importance. Compound **12** showed a stronger inhibitory effect than compound **11** due to the presence of an additional hydroxy group at C-6 in **12**. A similar phenomenon was also observed between other compounds (see compounds **14** and **18** versus **15** and **19**, in Table 3, respectively). According to multiple references, the acylation or esterification of SLs can enhance the lipophilicity, increase cellular penetration across the phospholipid bilayers surrounding the cells, and augment their NO production inhibitory activities.[13,46] The corresponding traits were also found in the presented compounds (see compounds **3** versus **14**, **4** and **5** versus **15**, **4** versus **6**, **6** and **7** versus **16**, **12** versus **13**, and **15** versus **16**, in Table 3). Moreover, the cytotoxic activities of these compounds against RAW264.7 macrophages were also evaluated by the MTT assay; none of the compounds exhibited significant cytotoxicity at their effective concentrations for the inhibition of NO production.

In conclusion, we have reported the isolation and structure elucidation of eight new pseudoguaianolides, two new guaianolides, and 14 known sesquiterpenes from the aerial parts of *I. hupehensis*. According to previous investigations on SLs, we have evaluated the inhibitory activities of all 24 compounds against LPS-induced NO production in RAW264.7 macrophages; the preliminary structure−activity relationships are proposed. The results provide a potential explanation for the use of this plant as a Chinese herbal medicine in the treatment of inflammatory diseases, and they may be potentially useful in developing new anti-inflammatory agents.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-2000 polarimeter. CD spectra were determined on a JACSO J-815 spectrometer. IR spectra were recorded with a Bruker FTIR Vector 22 spectrometer with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker Avance-400 or Avance-500 spectrometers in $CDCl_3$ or CD_3OD with TMS as an internal standard. ESIMS spectra were recorded on an Agilent LC/MSD Trap XCT spectrometer (Waters, USA), and HR-ESIMS were performed on a Q-Tof micro YA019 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 using silica gel (100−200, 200−300 mesh, Yantai, China), silica gel H (10−40 μm, Qingdao, China), and Sephadex LH-20 (Pharmacia Co. Ltd.).

Plant Material. The aerial parts of *I. hupehensis* were collected in Enshi, in Hubei Province, PR China, in August 2007, and were authenticated by Prof. Bao Kang Huang, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University. A voucher specimen (No. 200708XFHHB) is deposited at the School of Pharmacy, Shanghai Jiao Tong University.

Table 3. Inhibitory Effects of Compounds Isolated from *I. hupehensis* against LPS-Induced NO Production in RAW264.7 Macrophages (n = 4, mean ± SD)

compound	IC_{50}[a] (μM)	compound	IC_{50}[a] (μM)
1	3.5 ± 0.38	**14**	9.2 ± 0.55
2	10.5 ± 1.23	**15**	8.4 ± 1.18
3	6.6 ± 0.45	**16**	3.9 ± 0.22
4	5.1 ± 0.53	**17**	17.1 ± 1.32
5	3.8 ± 0.33	**18**	9.8 ± 0.76
6	2.2 ± 0.12	**19**	9.5 ± 0.75
7	1.5 ± 0.18	**20**	8.0 ± 0.57
8	19.8 ± 1.75	**21**	21.9 ± 1.15
9	26.8 ± 2.48	**22**	0.6 ± 0.05
10	9.2 ± 1.16	**23**	6.3 ± 0.56
11	8.2 ± 1.08	**24**	9.9 ± 0.85
12	1.1 ± 0.13	aminoguanidine[b]	7.9 ± 0.35
13	0.9 ± 0.05		

[a] Inhibitory effects of compounds **1**−**24** against LPS-induced NO production in RAW264.7 macrophages. [b] Positive control (≥98.0%, Sigma).

Extraction and Isolation. The dried aerial parts of *I. hupehensis* (25.0 kg) were powdered and extracted with 95% aqueous EtOH (3 × 10 L; 48, 24, and 24 h) at room temperature. The extract was successively partitioned with petroleum ether (40 L), EtOAc (40 L), and *n*-BuOH (40 L). The EtOAc fraction (98.8 g) was chromatographed on a silica gel column (1 kg, 100−200 mesh, 10 × 70 cm) eluting with a step gradient of CH_2Cl_2−MeOH (100:0, 100:1, 50:1, 20:1, 10:1, 5:1, 2:1, 0:1, each 15 L) to give 12 fractions (Fr1−Fr12). Fr3 (9.5 g) was subjected to column chromatography (CC) over macroporous resin MCI [4.5 × 40 cm, MeOH−H_2O (4:1), 5 L], Sephadex LH-20 [4.0 × 150 cm, MeOH−$CHCl_3$ (1:1), 1.5 L], and silica gel [200 g, 200−300 mesh, 4.5 × 40 cm, PE−EtOAc (5:1), 12 L] to give **1** (3.5 mg), **6** (14.3 mg), **11** (132.9 mg), **13** (950.0 mg), **14** (70.7 mg), **16** (753.0 mg), and **18** (11.6 mg). Using the same procedures, compounds **10** (2.0 mg) and **19** (13.5 mg) were purified from Fr7 (4.5 g). Fr5 (13.4 g) was subjected to silica gel CC (260 g, 200−300 mesh, 4.5 × 40 cm) with mixtures of PE−EtOAc (7:1, 8 L) as eluents to obtain five fractions (Fr5-1−Fr5-5). Compounds **5** (9.3 mg), **7** (2.0 mg), **8** (42.7 mg), **12** (692.0 mg), and **15** (38.8 mg) were isolated after CC over macroporous resin MCI [4.5 × 40 cm, MeOH−H_2O (4:1), 3 L] followed by preparative HPLC (CH_3CN−H_2O, 35:65) from subfraction Fr5-2 (4.5 g). Following the same procedures, compounds **3** (8.9 mg) and **22** (9.8 mg) were obtained from subfraction Fr5-4 (1.6 g). Subfraction Fr5-3 (2.9 g) was subjected to CC over macroporous resin MCI followed by preparative HPLC (CH_3CN−H_2O, 25:75), leading to the isolation of **2** (6.7 mg), **4** (11.9 mg), **20** (1.5 mg), and **21** (12.0 mg). Similarly, **9** (3.0 mg), **17** (15.1 mg), **23** (3.8 mg), and **24** (37.4 mg) were obtained from Fr5-5 (3.4 g).

(1*R*,5*R*,6*S*,7*R*,8*S*,10*R*)-8-Hydroxy-4-oxopseudoguai-2(3),11-(13)-dien-12,6-olide (1): amorphous powder; $[\alpha]_D^{20}$ +37.7 (*c* 0.07, MeOH); CD (*c* 1.34 × 10^{-4} M, MeOH) λ ($\Delta\varepsilon$) 208 (−35.5), 226 (60.1), 328 (−11.6); IR (KBr) ν_{max} 3441, 2934, 1767, 1726, 1584, 1158, 995 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 285.1097 $[M + Na]^+$ (calcd for $C_{15}H_{18}O_4Na$, 285.1103).

(1*R*,5*R*,6*S*,7*S*,8*S*,10*R*,11*S*)-6-Hydroxy-4-oxopseudoguai-2(3)-en-12,8-olide (2): amorphous powder; $[\alpha]_D^{20}$ −46.2 (*c* 0.16, CH_2Cl_2); CD (*c* 1.14 × 10^{-4} M, MeOH) λ ($\Delta\varepsilon$) 209 (16.3), 235 (−13.6), 330 (−5.2); IR (KBr) ν_{max} 3422, 2959, 2926, 1743, 1699, 988 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 265.1441 $[M + H]^+$ (calcd for $C_{15}H_{21}O_4$, 265.1440).

(1*S*,5*S*,7*R*,8*S*,10*R*)-14-Acetoxy-4-oxopseudoguai-11(13)-en-12,8-olide (3): amorphous powder; $[\alpha]_D^{20}$ +61.4 (*c* 0.11, CH_2Cl_2); CD (*c* 1.63 × 10^{-4} M, MeOH) λ ($\Delta\varepsilon$) 203 (−7.6), 221 (4.4), 296 (14.5); IR (KBr) ν_{max} 3450, 2927, 1737, 1640, 1377, 1239 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 307.1547 $[M + H]^+$ (calcd for $C_{17}H_{23}O_5$, 307.1545).

(1*S*,2*R*,5*R*,6*S*,7*S*,8*S*,10*R*)-6-Hydroxy-2-methoxy-4-oxopseudoguai-11(13)-en-12,8-olide (4): amorphous powder; $[\alpha]_D^{20}$ +57.5 (*c* 0.27, CH_2Cl_2); CD (*c* 1.19 × 10^{-4} M, MeOH) λ ($\Delta\varepsilon$) 203 (−15.8), 226 (5.2), 296 (6.1); IR (KBr) ν_{max} 3462, 2934, 1768, 1741, 1662 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 295.1551 $[M + H]^+$ (calcd for $C_{16}H_{23}O_5$, 295.1545).

(1*S*,2*R*,5*R*,6*S*,7*S*,8*S*,10*R*)-6-Hydroxy-2-ethoxy-4-oxopseudoguai-11(13)-en-12,8-olide (5): amorphous powder; $[\alpha]_D^{20}$ + 105.6 (*c* 0.09, CH_2Cl_2); CD (*c* 1.30 × 10^{-4} M, MeOH) λ ($\Delta\varepsilon$) 203 (−29.7), 225 (17.3), 294 (21.3); IR (KBr) ν_{max} 3460, 2931, 1767, 1741, 1661, 1268, 1155, 1073 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 309.1711 $[M + H]^+$ (calcd for $C_{17}H_{25}O_5$, 309.1702).

(1*S*,2*R*,5*R*,6*S*,7*R*,8*S*,10*R*)-6-Acetoxy-2-methoxy-4-oxopseudoguai-11(13)-en-12,8-olide (6): amorphous powder; $[\alpha]_D^{20}$ + 109.9 (*c* 0.05, CH_2Cl_2); CD (*c* 1.30 × 10^{-4} M, MeOH) λ ($\Delta\varepsilon$) 203 (−15.0), 220 (21.5), 295 (12.1); IR (KBr) ν_{max} 3462, 2936, 2829, 1773, 1749, 1634, 1246 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 359.1481 $[M + Na]^+$ (calcd for $C_{18}H_{24}O_6Na$, 359.1471).

(1*S*,2*S*,5*R*,6*S*,7*R*,8*S*,10*R*)-6-Acetoxy-2-methoxy-4-oxopseudoguai-11(13)-en-12,8-olide (7): amorphous powder; $[\alpha]_D^{20}$ + 207.3 (*c* 0.03, CH_2Cl_2); CD (*c* 1.30 × 10^{-4} M, MeOH) λ ($\Delta\varepsilon$) 204 (−9.3), 222 (12.6), 297 (7.8); IR (KBr) ν_{max} 3449, 2924, 1749, 1633, 1240 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 337.1650 $[M + H]^+$ (calcd for $C_{18}H_{25}O_6$, 337.1651).

(1*S*,5*R*,6*S*,7*S*,8*S*,10*R*,11*R*)-6-Hydroxy-4-oxopseudoguai-12,8-olide (8): amorphous powder; $[\alpha]_D^{20}$ +165.9 (*c* 0.48, CH_2Cl_2); CD (*c* 1.30 × 10^{-4} M, MeOH) λ ($\Delta\varepsilon$) 217 (−13.3), 296 (16.7); IR (KBr) ν_{max} 3492, 2969, 1771, 1733, 1458, 1000 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 267.1598 $[M + H]^+$ (calcd. for $C_{15}H_{23}O_4$, 267.1596).

4*β*,10*α*-Dihydroxy-5*α*H-guai-1(2),11(13)-dien-12,8*α*-olide (9): amorphous powder; $[\alpha]_D^{20}$ +72.5 (*c* 0.03, MeOH); IR (KBr) ν_{max} 3425, 2931, 1754, 1629, 1381, 1269 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 287.1265 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

2*α*-Acetoxy-4*α*,6*α*-dihydroxy-1*β*,5*α*H-guai-9(10),11(13)-dien-12,8*α*-olide (10): amorphous powder; $[\alpha]_D^{20}$ +58.5 (*c* 0.07, MeOH); IR (KBr) ν_{max} 3431, 2971, 1764, 1655, 1381, 1246 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HR-ESIMS (positive) *m*/*z* 345.1317 $[M + Na]^+$ (calcd for $C_{17}H_{22}O_6Na$, 345.1314).

Assay for Inhibitory Activities against LPS-Induced NO Production in RAW264.7 Macrophages. The assay was carried out as previously described.[13,37,47] Briefly, RAW264.7 macrophages were harvested and seeded in 96-well plates (2 × 10^5 cells/well) for NO production. The plates were pretreated with various concentrations of samples for 30 min and incubated with LPS (1 μg/mL) for 24 h. The amount of NO was determined by the nitrite concentration in the cultured RAW264.7 macrophage supernatants with the Griess reagent. The cell viability was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich] reduction.[48]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. 1D and 2D NMR spectra for compounds **1**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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