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•Original article•

Anti-inflammatory germacrane-type sesquiterpene lactones from *Vernonia sylvatica*

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[ABSTRACT] Nine new germacranolides, sylvaticalides A−H (**1**–**9**), and three known analogues (**10**–**12**) were isolated from the aerial part of *Vernonia sylvatica*. Their structures were established using comprehensive spectroscopic analysis, including high-resolution electrospray ionization mass spectroscopy (HR-ESI-MS) and 1D and 2D nuclear magnetic resonance (NMR) spectra. Their absolute configurations were determined by X-ray diffraction experiments. The anti-inflammatory activities of all isolated compounds were assessed by evaluating their inhibitory effects on the nuclear factor kappa B (NF-κB) pathway, which was activated by lipopolysaccharide (LPS)-stimulated human THP1-Dual cells, and the interferon-stimulated gene (ISG) pathway, activated by STING agonist MSA-2 in the same cell model. Compounds **1**, **2** and **6** showed inhibitory effects on the NF-κB and ISG signaling pathways, with IC_{50} values ranging from 4.12 to 10.57 μmol·L^{-1}.

[KEY WORDS] *Vernonia sylvatica*; Germacrane-type sesquiterpene lactone; Sylvaticalides A−H; Anti-inflammatory; NF-κB pathway; ISG pathway

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Introduction

Germacranolides, naturally occurring secondary metabolites, are characterized by a 10-membered carbocyclic skeleton featuring multiple oxygenated functionalities. Plants in the Compositae family, particularly within the genera of *Carpesium* [1, 2] and *Vernonia* [3, 4], are abundant sources of bioactive germacranolides. These compounds have demonstrated a range of bioactivities, such as cytotoxic [5], anti-inflammatory [6, 7], antiprotozoal [8, 9] and antiproliferative [10] effects, garnering significant interest in the field of natural product chemistry over the past decades. Among them, a germacrene sesquiterpene lactone named parthenolide displays remarkable anti-inflammatory and antitumor activities, and its dimethylamino-derivative has progressed to a Phase I clinical trial for the treatment of acute myeloid leukemia [11, 12].

The genus *Vernonia*, one of the largest genera in the Compositae family, consists of more than 1000 species and is widely distributed in the tropical and temperate zones of Asia, America, and Africa. There are 27 species of *Vernonia* in China, some of which have long been used as traditional Chinese folk medicines to treat ailments, such as asthma, stomachache, and bronchitis [13]. Sesquiterpenoids, especially germacranolides, are the predominant and characteristic metabolites of this genus, known for their cytotoxic and anti-inflammatory properties [14-16]. *Vernonia sylvatica*, a perennial shrub found in the Yunnan and Guangxi provinces of China, has not been investigated for its chemical constituents and biological activities [17]. In our ongoing search for natural bioactive compounds, an extensive investigation into

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These authors have no conflict of interest to declare.

V. sylvatica was undertaken, leading to the isolation of 12 germacranolides including nine new analogues (Fig. 1). These new structures were elucidated by extensive analysis of 1D and 2D nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectroscopy (HR-ESI-MS) data, as well as X-ray diffraction studies. All isolates were evaluated for their anti-inflammatory activities by testing their ability to inhibit the nuclear factor kappa B (NF-κB) and interferon-stimulated gene (ISG) pathways in human THP1-Dual cells.

Results and Discussion

Sylvaticalide A (**1**) was obtained as colorless crystals. Its HR-ESI-MS peak at *m/z* 437.1818 ($[M + H]^+$, Calcd. for 437.1812) and the ^{13}C NMR data suggested a molecular formula of $C_{22}H_{28}O_9$, indicating nine degrees of unsaturation. The infrared (IR) spectrum displayed absorption bands for hydroxy (3472 cm^{-1}) and carbonyl groups (1766, 1741 and 1721 cm^{-1}). The ^1H NMR data (Table 1) revealed one olefinic proton (δ_H 5.74), three oxygenated methines (δ_H 4.78, 4.76, 3.10), and five methyl groups (δ_H 2.11, 2.04, 1.92, 1.57, 1.46). The ^{13}C NMR data (Table 1) displayed 22 resonances attributed to five methyls (δ_C 27.4, 22.2, 21.2, 20.8, 18.9), four methylenes (δ_C 53.1, 41.6, 33.2, 32.6), three oxygenated tertiary carbons (δ_C 80.8, 63.4, 59.0), two quaternary carbons (δ_C 85.2, 61.4), one olefinic methine (δ_C 114.8), three olefinic quaternary carbons (δ_C 161.0, 159.9, 129.9), three ester carbonyls (δ_C 172.3, 170.1, 165.6), and one ketone carbonyl (δ_C 208.2). Heteronuclear multiple bond correlations (HMBCs) (Fig. 2) from H-2' (δ_H 5.74) to C-4' (δ_C 27.4)/C-5′ (δ_C 20.8) and from H-5' (δ_H 2.11) to C-1′ (δ_C 165.6)/C-2' (δ_C 114.8)/C-3' (δ_C 159.9) indicated the presence of a senecioate group. The methyl carbon (δ_C 21.2) and carbonyl carbon (δ_C 170.1) were identified as part of an acetoxyl group. Apart from these two substituents, the remaining 15 carbon resonances were indicative of a typical sesquiterpene skeleton. The spin systems inferred from the $^1H–^1H$ COSY spectrum (Fig. 2), H_2-2/H_2-3, H-5/H-6 and H-8/H-9, together with the HMBC correlations (Fig. 2) from H_3-14 (δ_H 1.46) to C-1 (δ_C 208.2)/C-9 (δ_C 41.6)/C-10 (δ_C 85.2), from H_2-3 (δ_H 1.52, 2.40) to C-1 (δ_C 208.2), from H_3-15 (δ_H 1.57) to C-3 (δ_C 32.6)/C-4 (δ_C 61.4)/C-5 (δ_C 59.0), from H-6 (δ_H 4.78) to C-7 (δ_C 161.0)/C-11 (δ_C 129.9), and from H-8 (δ_H 4.76) to C-7 (δ_C 161.0)/C-11 (δ_C 129.9) revealed that compound **1** contained a 10-membered ring with Me-14 located at C-10 and Me-15 at C-4. However, an oxygenated methylene (δ_C 53.1) and an ester carbonyl (δ_C 172.3) did not show correlations through the 2D NMR spectrum. Comparing the NMR data with those of the known compound (4*R*,5*R*,6*S*,8*S*,l0*R*)-1-oxo-4,5-epoxy-8-senecioyloxy-10,13-diacetoxygermacr-7(11)-en-6,12-olide (**10**) [18] indicated that the ester carbonyl together with C-6/C-7/C-11 established a lactone moiety and the oxygenated methylene was designated as C-13. The location of the senecioate group at C-8 was confirmed by the HMBC correlation from H-8 (δ_H 4.76) to C-1' (δ_C 165.6). Additionally, the undescribed oxygen atoms were ascribed to a hydroxy group at C-13 and an epoxy moiety between C-4 and C-5 determined by the relevant 1D and 2D NMR data (Fig. 2).

The relative configuration of **1** was inferred from the nuclear overhauser enhancement spectroscopy (NOESY)

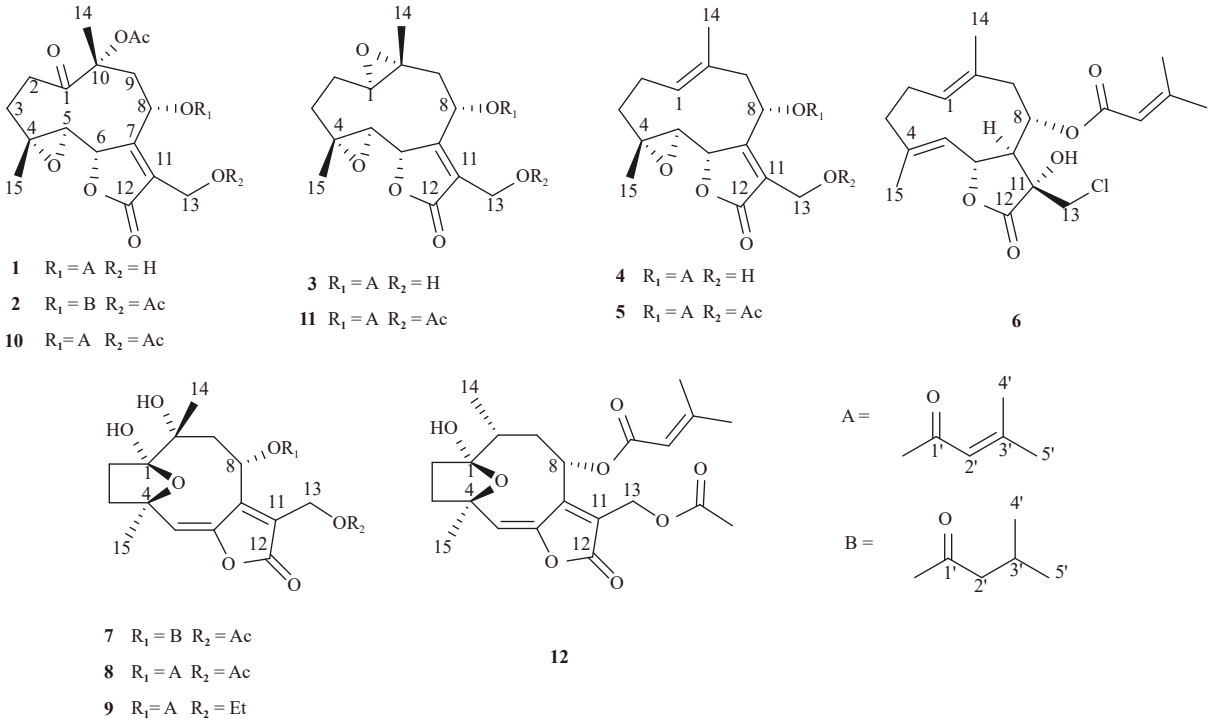


Fig. 1 Chemical structures of compounds 1-12.

Table 1 ^{1}H and ^{13}C NMR Data for compounds 1–3 (δ in ppm, J in Hz)

No.	1 [a, d]		2 [b, c]		3 [b, c]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	208.2		206.0		62.3	2.69, m
2	33.2	3.12, m 2.25, m	32.4	2.92, br s 2.27, m	22.5	1.56, m 2.11, m
3	32.6	1.52, m 2.40, m	31.2	1.67, m 2.60, br s	36.0	1.34, ddd (13.5, 6.1, 5.3) 2.29, ddd (13.5, 5.3, 1.9)
4	61.4		61.0		59.8	
5	59.0	3.10, m	58.3	2.77, br s	65.5	2.58, d (8.7)
6	80.8	4.78, m	80.0	4.88, d (9.6)	82.5	4.97, d (8.7)
7	161.0		162.4		161.0	
8	63.4	4.76, m	63.2	4.77, m	65.4	5.20, dd (9.3, 0)
9	41.6	2.54, m 2.81, dd (16.4, 8.1)	39.9	2.24, dd (15.3, 7.1) 2.76, m	45.8	1.97, m 2.72, m
10	85.2		84.2		58.2	
11	129.9		124.7		131.7	
12	172.3		170.3		172.4	
13	53.1	4.22, m	54.7	4.80, d (12.8) 4.93, d (12.8)	56.0	4.49, s
14	18.9	1.46, s	18.4	1.52, s	17.4	1.47, s
15	22.2	1.57, s	21.9	1.65, s	16.8	1.53, s
1'	165.6		171.8		165.2	
2'	114.8	5.74, s	42.0	2.20, dd (21.2, 7.1)	114.0	5.67, s
3'	159.9		25.0	2.05, m	161.6	
4'	27.4	1.92, s	21.8	0.95, d (6.7)	27.8	1.94, s
5'	20.8	2.11, s	21.8	0.94, d (6.7)	20.7	2.16, s
OAc	170.1 21.2	2.04, s	169.7 20.4	2.13, s		
OAc			169.2 20.5	2.07, s		

[a] recorded at 600 MHz (^{1}H) or 150 MHz (^{13}C), [b] recorded at 500 MHz (^{1}H) or 125 MHz (^{13}C), [c] In $CDCl_3$, [d] In DMSO-d_6 (90 °C).

experiment. The NOESY correlations (Fig. 3) of H-6/H-8, H-6/Me-15, Me-15/H-3β, H-8/Me-14, and Me-14/H-9β suggested that these protons were cofacial and β-oriented, while the cross-peaks of H-5/H-3α and H-5/H-9α indicated that H-5 was on the other face and α-oriented. The absolute configuration of **1** (Fig. 4) was assumed to be the same as that of **10** based on the high similarity of NMR data and biosynthetic considerations, which was further confirmed by a single-crystal X-ray crystallographic diffraction experiment with Cu Kα radiation. Therefore, the structure of compound **1** was defined as (4*R*, 5*R*, 6*S*, 8*S*, 10*R*)-1-oxo-4,5-epoxy-8-senecioyloxy-10-acetoxy-13-hydroxygermacr-7(11)-en-6,12-olide.

Sylvaticalide B (**2**) was obtained as a colorless gum. Its HR-ESI-MS peak at *m/z* 481.2085 ([M + H]$^+$, Calcd. for 481.2074) and the ^{13}C NMR data suggested a molecular formula of $C_{24}H_{32}O_{10}$, indicating nine degrees of unsaturation. A comparison of the NMR data of **2** and **10** suggested that they shared the same skeleton except that the senecioate group in **10** was replaced by a 3-methylbutyryloxy group (δ_C 171.8, 42.0, 25.0, 21.8, 21.8) (Table 1) in **2**, which was verified by the ^{1}H–^{1}H COSY correlations of H_2-2′/H-3′/H_3-4′/H_3-5′, together with the HMBC correlation from H_2-2′ (δ_H 2.20) to C-1′ (δ_C 171.8). The NOESY correlations of H-6/Me-15, H-8/H-6, and H-8/Me-14 suggested that these protons were cofacial and β-oriented. Therefore, based on the biosynthetic consideration, the full structure of **2** was proposed as (4*R*,5*R*,6*S*,8*S*,10*R*)-1-oxo-4,5-epoxy-8-(3-methylbutyryloxy)-10,13-diacetoxy-germacr-7(11)-en-6,12-olide.

Sylvaticalide C (**3**) was obtained as a colorless gum. Its HR-ESI-MS peak at *m/z* 379.1769 ([M + H]$^+$, Calcd. for 379.1753) and the ^{13}C NMR data suggested a molecular formula of $C_{20}H_{26}O_7$, indicating eight degrees of unsaturation. The absorption bands at 3464, 1763, and 1716 cm^{-1} in the IR spectrum indicated the presence of hydroxy and carbonyl groups. Both the ^{1}H and ^{13}C NMR data of compound **3** were similar to the known compound **11** ((l*R*,4*R*,5*R*,6*S*,8*S*,10*R*)-1(10),4,5-diepoxy-8-senecioyloxy-13-acetoxygermacr-7(11)-en-6,12-olide)[18], suggesting that they processed the same germacrane skeleton with two epoxy moieties located at C-1/C-10, and C-4/C-5, respectively. This inference was confirmed by the correlations of H-1/H_2-2/H_2-3, H-5/H-6, and H-8/H_2-9 in the ^{1}H–^{1}H COSY spectrum (Fig. 2), as well as the HMBC correlations (Fig. 2) from H_3-14 (δ_H 1.47) to C-1 (δ_C 62.3)/C-9 (δ_C 45.8)/C-10 (δ_C 58.2), from H_3-15 (δ_H 1.53) to C-3 (δ_C 36.0)/C-4 (δ_C 59.8)/C-5 (δ_C 65.5), from H-8 (δ_H 5.20) to C-6 (δ_C 82.5)/C-7 (δ_C 161.0)/C-11 (δ_C 131.7), and from H_2-

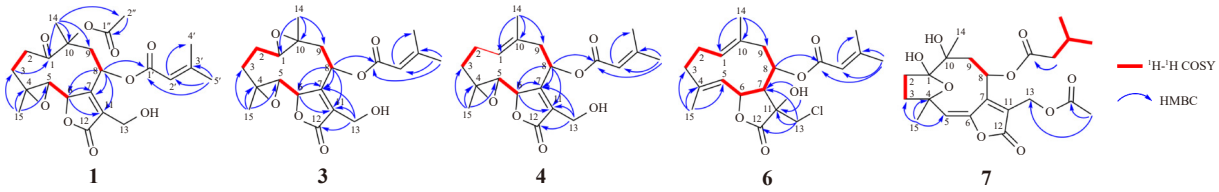


Fig. 2 Key HMBC and ^{1}H-^{1}H COSY correlations of typical compounds 1, 3, 4, 6 and 7.

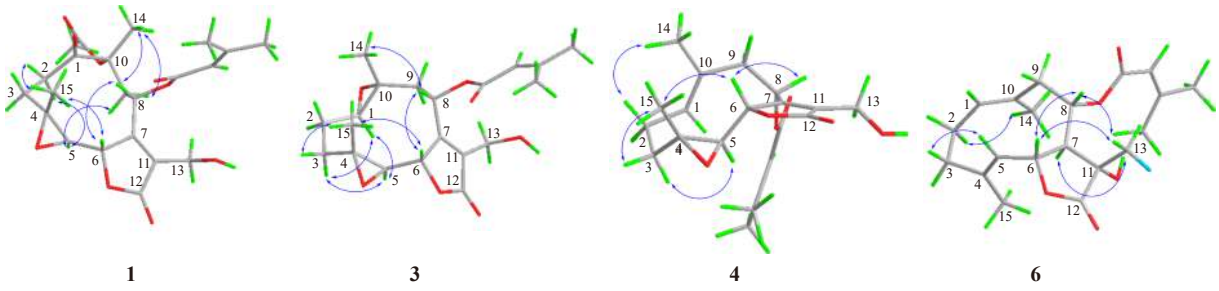


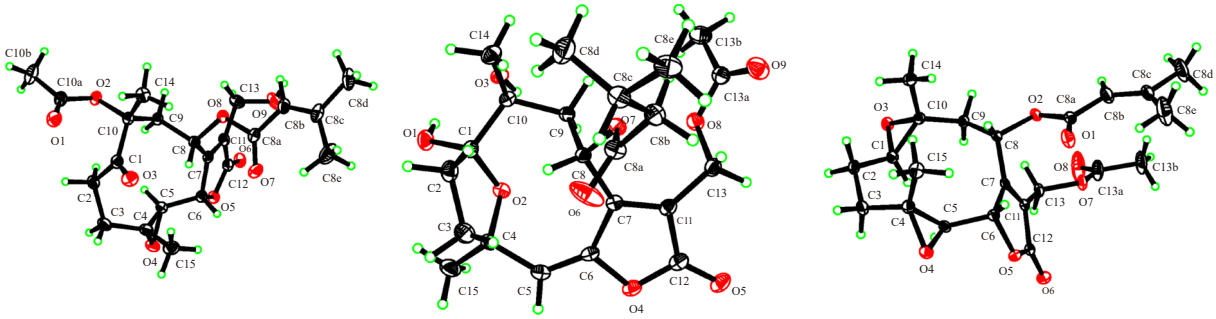
Fig. 3 Key NOESY correlations of compounds 1, 3, 4 and 6.

13 (δ_H 4.49) to C-7 (δ_C 161.0)/C-11 (δ_C 131.7)/C-12 (δ_C 172.4). Detailed NMR data comparison of **3** and **11** revealed the downfield shift of C-11 (from δ_C 128.0 to δ_C 131.7) and C-12 (from δ_C 170.4 to δ_C 172.4), indicating a hydroxy group rather than an acetoxyl was observed in **3**. The NOESY correlations (Fig.3) of H-6/Me-15, Me-15/H-3β, H-6/H-8, and H-8/Me-14 suggested the β-orientations of H-6, H-8, Me-14, and Me-15, while the NOESY correlations (Fig. 3) of H-3α/H-5, H-3α/H-1, and H-5/H-1 indicated that H-1 and H-5 were on the other face and α-oriented. The absolute configuration of compound **11** was further established by a single-crystal X-ray crystallographic diffraction experiment with Cu Kα radiation (Fig. 4). Therefore, based on biosynthetic consideration and the high similarity of the NMR data between compounds **3** and **11**, the full structure of **3** was proposed as (1*R*,4*R*,5*R*,6*S*,8*S*,10*R*)-1(10),4,5-diepoxy-8-senecioyloxy-13-hydroxygermacr-7(11)-en-6,12-olide.

Sylvaticalide D (**4**) was obtained as a colorless gum, and its molecular formula was determined to be $C_{20}H_{26}O_6$ by HR-ESI-MS and ^{13}C NMR data, indicating eight degrees of unsaturation. The IR spectrum showed absorption bands for hydroxy (3477 cm^{-1}) and carbonyl groups (1760 and 1715 cm^{-1}). The ^{1}H–^{1}H COSY correlations (Fig. 2) of H-1/H_2-2/H_2-3, H-5/H-6, and H-8/H_2-9, together with the HMBC correlations (Fig. 2) from H_3-14 (δ_H 1.71) to C-1 (δ_C 126.6)/C-9 (δ_C 42.7)/C-10 (δ_C 129.8), from H_3-15 (δ_H 1.38) to C-3 (δ_C 35.0)/C-4 (δ_C 60.0)/C-5 (δ_C 64.5), from H-6 (δ_H 4.84) to C-7 (δ_C 161.7)/C-11 (δ_C 131.4)/C-12 (δ_C 171.2), from H-8 (δ_H 5.10) to C-6 (δ_C 81.4)/C-7 (δ_C 161.7), H_2-13 (δ_H 4.28, 4.35) to C-7 (δ_C 161.7)/C-11 (δ_C 131.4)/C-12 (δ_C 171.2) indicated that compound **4** processed a typical germacrane skeleton. The remaining signals in ^{13}C NMR spectrum (δ_C 164.3, 157.4, 114.5, 26.3, 19.5) (Table 2) were ascribed to a senecioate group, which could be confirmed by the related ^{1}H-^{1}H COSY and HMBC correlations (Fig. 2). The NOESY correlations (Fig. 3) of H-6/Me-15, H-6/H-8, and H-3β/Me-15 suggested the β-orientations of H-6, H-8, and Me-15, while the NOESY correlation (Fig. 3) of H-3α/H-5 indicated that H-5 was α-oriented. In addition, the NOESY correlation (Fig. 3) of H_2-2/Me-14 suggested an *E*-configuration of the $\Delta^{(1,10)}$ olefinic bond. Therefore, based on biosynthetic consideration, the structure and absolute configuration of compound **4** was proposed as (4*R*, 5*R*, 6*S*, 8*S*)-4,5-epoxy-8-senecioyloxy-13-hydroxy-1*E*,7(11)-germacradien-6,12-olide.

Sylvaticalide E (**5**) was isolated as colorless gum and its molecular formula was determined to be $C_{22}H_{28}O_7$ by HR-ESI-MS and ^{13}C NMR data, indicating nine degrees of unsaturation. The NMR data of **5** resembled those of **4** (Table 2), except that the hydroxy of **4** was replaced by an acetoxyl group at C-13, which could be proved by the downfield shift of C-13 (from δ_C 52.5 to δ_C 55.0) and a upfield shift of C-11 (from δ_C 131.4 to δ_C 126.6). It was also confirmed by the HMBC correlation observed from H_2-13 (δ_H 4.86, 4.91) to $COCH_3$ (δ_C 169.3). The NOESY correlations of H-6/Me-15, H-6/H-8, and H-8/Me-15 suggested the β-orientations of H-6, H-8, and Me-15. In addition, the NOESY correlation of H-2/Me-14 suggested the *E*-configuration of the $\Delta^{(1, 10)}$ olefinic bond. Therefore, based on biosynthetic consideration, the structure and absolute configuration of compound **5** was proposed as (4*R* ,5*R*,6*S*,8*S*)-4,5-epoxy-8-senecioyloxy-13-acetoxy-1*E*, 7(11)-germacradien-6,12-olide.

Sylvaticalide F (**6**) was obtained as a colorless gum. Its HR-ESI-MS peaks at *m/z* 405.1428 ([M + Na]$^+$, Calcd. for 405.1445) and *m/z* 407.1400, with a ratio being 3 : 1, indicated the presence of a chlorine atom and a molecular formula of $C_{20}H_{27}O_5Cl$. The ^{13}C NMR data (δ_C 165.2, 160.4, 115.2, 27.8, 20.7) (Table 2) indicated the presence of a senecioate group which was assigned to C-8 based on the HMBC correlation (Fig. 2) from H-8 (δ_H 5.45) to C-1' (δ_C 165.2). The ^{1}H–^{1}H COSY correlations (Fig. 2) revealed the presence of two spin systems of H-1/H_2-2/H_2-3 and H-5/H-6/H-7/H-8/H_2-9. The HMBC correlations (Fig. 2) from H_3-14 (δ_H 1.48) to C-



1 **7** **11**

Fig. 4 Perspective ORTEP drawings of compounds 1, 7 and 11.

Table 2 ^{1}H and ^{13}C NMR data for compounds 4–6 (δ in ppm, J in Hz)

No.	4 [a, d]		5 [a, d]		6 [b, c]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	126.6	5.44, dd (8.3, 8.3)	127.1	5.44, dd (8.4, 8.4)	130.0	4.8, dd (8.6, 8.6)
2	21.8	2.26, m	22.1	2.26, m	26.0	2.18, m
3	35.0	1.27, dt (13.5, 9.2) 2.07, ddd(13.5, 6.4, 3.8)	35.0	1.26, dt(12.9, 9.3) 2.08, ddd(12.9, 7.0, 2.0)	39.2	1.99, m 2.35, m
4	60.0		60.3		142.2	
5	64.5	2.38, d (8.9)	64.2	2.46, d (9.4)	127.0	4.58, d (9.7)
6	81.4	4.84, d (8.9)	82.0	4.93, d (9.4)	76.4	5.10, dd(10.2, 9.7)
7	161.7		164.8		58.9	2.80, dd (10.5, 10.2)
8	69.3	5.10, dd (10.9, 3.3)	69.1	5.15, m	69.8	5.45, ddd (10.5, 2.8, 2.8)
9	42.7	2.68, m 2.81, m	43.0	2.70, m	48.8	2.35, m 2.72, br d (12.8)
10	129.8		129.3		133.4	
11	131.4		126.6		78.2	
12	171.2		170.2		175.4	
13	52.5	4.28, dd (12.4, 3.9) 4.35, dd (12.4, 4.9)	55.0	4.86, d (13.2) 4.91, d (13.2)	43.9	3.66, d (5.2)
14	17.6	1.71, s	17.4	1.72, s	16.7	1.48, s
15	17.0	1.38, s	16.9	1.38, s	17.8	1.73, s
1'	164.3		164.2		165.2	
2'	114.5	5.72, t (2.0)	114.2	5.67, s	115.2	5.70, s
3'	157.4		158.3		160.4	
4'	26.3	1.90, d (1.3)	26.4	1.91, s	27.8	1.95, s
5'	19.5	2.1, d (1.3)	19.6	2.11, s	20.7	2.23, s
OAc			20.0 169.3	2.02, s		
OH						3.15, s

[a] recorded at 600 MHz (^{1}H) or 150 MHz (^{13}C); [b] recorded at 500 MHz (^{1}H) or 125 MHz (^{13}C); [c] In $CDCl_3$; [d] In DMSO-d_6 (90 °C).

1 (δ_C 130.0)/C-9 (δ_C 48.8)/C-10 (δ_C 133.4), and from H_3-15 (δ_H 1.73) to C-3 (δ_C 39.2)/C-4 (δ_C 142.2)/C-5 (δ_C 127.0) supported a 10-membered ring of a typical germacranolide, with Me-14 and Me-15 located at C-10 and C-4, respectively. The HMBC correlations (Fig. 2) from H_2-13 (δ_H 3.66) to C-7 (δ_C 58.9)/C-11 (δ_C 78.2)/C-12 (δ_C 175.4) indicated a 5-membered lactone ring. The HMBC correlations (Fig. 2) from OH-11 (δ_H 3.15) to C-7 (δ_C 58.9)/C-11 (δ_C 78.2)/C-12 (δ_C 175.4) indicated a hydroxy group connecting at C-11, so the chlorine atom was assumed to locate to the remaining carbon C-13 (δ_C 43.9). The relative stereochemistry of **6** was assigned according to the NOESY experiment and ^{1}H-^{1}H coupling constant value. The coupling constant value between H-6 and H-7 (10.2 Hz) indicated a *trans*-axial relationship between H-6 and H-7. The NOESY correlations (Fig. 3) of H-6/H_2-13 and H-6/H-8 suggested the β-orientation of H-6, H-8, and H_2-13, while the NOESY correlation (Fig. 3) of H-7/OH-11 indicated that H-7 and OH-11 were α-oriented. Besides, the NOESY correlations of H_2-2/Me-14 and H_2-3/H-5 suggested the *E*-configurations of the $\Delta^{(1, 10)}$ and $\Delta^{(4, 5)}$ olefinic bonds. Therefore, based on biosynthetic consideration, the full structure of **6** was proposed as (6*R*,7*R*,8*S*, 11*S*)-8-senecioyloxy-11-hydroxy-13-chlorine-1*E*,5*E*-germacradien-6,12-olide.

Sylvaticalide G (**7**) was obtained as colorless crystals. Its

HR-ESI-MS peak at *m/z* 461.1788 ([M + Na]$^+$, Calcd. for 461.1788) and the ^{13}C NMR data suggested a molecular formula of $C_{22}H_{30}O_9$, containing eight degrees of unsaturation. The IR spectrum showed absorption bands for hydroxy (3460 cm^{-1}) and carbonyl (1766 and 1743 cm^{-1}) groups. The 3-methylbutyryloxy group was identified on the basis of the ^{1}H–^{1}H COSY correlations (Fig. 2) of H_2-2′/H-3′/H_3-4′/H_3-5 and the HMBC correlations (Fig. 2) from H_2-2′ (δ_H 2.22) to C-1′ (δ_C 171.7), C-4′ (δ_C 22.6), and C-5′ (δ_C 22.4). The HMBC correlations (Fig. 2) from $COCH_3$ (δ_H 2.06) to $COCH_3$ (δ_C 170.5) indicated the presence of an acetoxyl. Apart from these two substituents, the remaining 15 carbon resonances were indicative of a sesquiterpene skeleton. The ^{1}H–^{1}H COSY correlations (Fig. 2) revealed the presence of two spin systems of H_2-2/H_2-3 and H-8/H_2-9. Comparison of its ^{1}H and ^{13}C NMR data with those of a known compound named piptocarphin A [19], revealed a strong resemblance except that the methacrylate signals of piptocarphin A were replaced by those of a 3-methylbutyryloxy group in **7**. Based on the above analyses, compound **7** was assumed as a germacrane-type sesquiterpene with an oxygen atom connecting C-1 and C-4. Finally, a single crystal of **7** was obtained and the X-ray crystallographic data using Cu Kα radiation confirmed its absolute configuration (Fig. 4). Therefore, the structure of **7** was defined as (1*S*,4*R*,8*S*,10*R*)-1,4-epoxy-1,10-dihydroxy-8-(3-methylbutyryloxy)-13-acetoxy-5*E*,7(11)-germacradien-6,12-olide.

Sylvaticalide H (**8**) was obtained as a white amorphous powder. Its HR-ESI-MS peak at *m/z* 459.1642 ([M + Na]$^+$, Calcd. for 459.1636) and the ^{13}C NMR data suggested a molecular formula of $C_{22}H_{28}O_9$, containing nine degrees of unsaturation. Comparison with the NMR data of **7** and **8** indicated that they might share the same skeleton, except that the 3-methylpropanoyloxy group of **7** was replaced by a senecioate group, which was supported by the ^{1}H–^{1}H COSY correlations of H-2′/H_3-4′/H_3-5′ and HMBC correlations from H-5′ (δ_H 2.14) to C-1′ (δ_C 164.8)/C-2′ (δ_C 114.5)/C-3′ (δ_C 160.9). Therefore, based on biosynthetic consideration, the full structure of **8** was proposed as (1*S*,4*R*,8*S*,10*R*)-1,4-epoxy-1,10-dihydroxy-8-senecioyloxy-13-acetoxy-5*E*,7(11)-germacradien-6,12-olide.

Sylvaticalide I (**9**) was obtained as a colorless gum, and its molecular formula was determined to be $C_{22}H_{30}O_8$ by HR-ESI-MS and ^{13}C NMR data, containing eight degrees of unsaturation. Analysis of 1D and 2D NMR data of **9** and **7** suggested the high similarities of their structures, except for the differences observed for two substituents at C-8 and C-13. An ethoxy was observed from ^{1}H–^{1}H COSY correlation of OCH_2CH_3/OCH_2CH_3, and was placed at C-13 through the HMBC correlation from H_2-13 (δ_H 4.31, 4.59) to OCH_2CH_3 (δ_C 66.7). In addition, a senecioate group (δ_C 164.9, 160.2, 114.8, 27.6, 20.6) rather than the 3-methylbutyryloxy in **7** was observed in **9**, and placed at C-8. Therefore, based on biosynthetic consideration, the full structure of **9** was proposed as (1*S*,4*R*,8*S*,10*R*)-1,4-epoxy-1,10-dihydroxy-8-senecioyloxy-13-ethyl-5*E*,7(11)-germacradien-6,12-olide.

In addition to the above new compounds, three known compounds were also isolated from *V. sylvatica* and identified as (4*R*,5*R*,6*S*,8*S*,l0*R*)-1-oxo-4,5-epoxy-8-senecioyloxy-10,13-diacetoxygermacr-7(11)-en-6,12-olide (**10**)[18], (l*R*,4*R*,5*R*,6*S*,8*S*,10*R*)-1(10),4,5-diepoxy-8-senecioyloxy-13-acetoxygermacr-7(11)-en-6,12-olide (**11**)[18], and (1*S*, 4*R*, 8*S*, 10*R*)-1,8-dihydroxy-1,4-epoxy-13-acetoxy-5*E*, 7(11)-germacradien-6,12-olide (**12**) [20] by comparing their NMR data with those in literature.

All isolates were evaluated for their anti-inflammatory activities by assessing their inhibitory effect against the activation of NF-κB induced by lipopolysaccharide (LPS) [21] and the ISG pathway induced by MSA-2 [22] in THP1-Dual cells which contain both ISG and NF-κB reporters. Results showed that compounds **1**, **2**, and **6** inhibited the NF-κB pathway induced by LPS, with IC_{50} of 9.64 μmol·L^{-1}, 7.08 μmol·L^{-1}, and 4.12 μmol·L^{-1}, respectively, displaying potency comparable to that of the positive control, NF-κB inhibitor SC75741 (IC_{50} 3.4 μmol·L^{-1}) (Fig. S1A). Moreover, compounds **1**, **2**, and **6** could also inhibit the activation of ISG induced by MSA-2, with IC_{50} of 10.57 μmol·L^{-1}, 6.57 μmol·L^{-1}, 9.64 μmol·L^{-1}, respectively, which were slightly less potent than that of SC75741 (IC_{50} 2.15 μmol·L^{-1}) (Fig. S1B). Additionally, compounds **2** and **6** did not show notable cytotoxicity since their IC_{50} values were over 20 μmol·L^{-1}, while compound **1** exhibited low cytotoxicity with IC_{50} of 18.89 μmol·L^{-1} (Fig. S1C).

In summary, the phytochemical investigation of *V. sylvatica* led to the isolation of nine new germacrane-type sesquiterpene lactones and three known analogues. Among them, compounds **1**–**6**, **10**, **11** showed different degrees of oxidation at C-1, 10 and C-4, 5, while compounds **7**–**9**, **12** possessed a germacrane skeleton with a 1,4-epoxy moiety. Compounds **1**, **2** and **6** demonstrated inhibitory effects against the activation of NF-κB and ISG signaling pathways, with IC_{50} values ranging from 4.12 to 10.57 μmol·L^{-1}, suggesting that these sesquiterpenoids might contribute to its traditional usage for the treatment of asthma, stomachache, bronchitis.

Experimental

General experimental procedures

Optical rotation values were recorded on a Rudolph Research Analytical Autopol VI 90079 polarimeter (Hackettstown, NJ). IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrophotometer using KBr disks. HR-ESI-MS data were recorded on the Waters Synapt G2-Si Q-Tof mass spectrometers. NMR spectra were collected on a Bruker AVANCE III 500 or 600 MHz instrument (Bruker Biospin AG, Switzerland). Single-crystal X-ray diffraction measurements were conducted on a Bruker D8 Venture diffractometer or a Bruker Apex-II CCD diffractometer. LC-ESI-MS data were recorded on a Waters 2695 instrument with a 2998 PDA detector equipped with a Waters Acquity ELSD, and a Waters 3100 SQDMS detector. Preparative HPLC was per-

formed on a Waters 2545 Binary Gradient Module instrument with a Waters 2489 UV/visible detector with a SunFire column (Prep C_{18} OBD, 5 μm, 30 mm × 150 mm). Semi-preparative HPLC was performed on a Waters 1525 instrument with a Waters 2489 UV/Visible detector using a SunFire column (Prep C_{18} OBD, 5 μm, 19 mm × 150 mm). MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Industries, Tokyo, Japan), silica gel (100–200, 200–300, and 300–400 mesh, Qingdao Marine Chemical Industrials, Qingdao, China), ODS gel AAG12S50 (12 nm, S-50 μm, YMC, Japan), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), Toyopearl HW-40F (Tosoh Corporation, Tokyo, Japan) was used for column chromatography. TLC was carried out on precoated silica gel GF254 plates (Yantai Chemical Industrials, Yantai, China), and the TLC spots were observed at 254 nm and visualized with 5% H_2SO_4 in EtOH containing 10 mg·mL^{-1} vanillin by heating. All solvents used for CC were of analytical grade (Shanghai Chemical Reagents Co., Ltd., Shanghai, China), and solvents used for analytic and preparative HPLC were of HPLC grade (Merck KGaA, Darmstadt, Germany; Ourchem, Shanghai, China).

Plant material

The aerial part of *V. sylvatica* was collected from Sipsongpanna, Yunnan Province, China, in August 2020, and identified by Zhang Jun from the Kunming Biotechnology Co., Ltd.. A voucher specimen (No. 20200822) has been deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation

The air-dried aerial part of *V. sylvatica* (30.0 kg) was extracted with 95% ethanol at room temperature. After the evaporation of the solvent, the obtained residue (1.3 kg) was suspended in water and partitioned with petroleum ether (PE), CH_2Cl_2 and EtOAc, successively. The CH_2Cl_2-soluble partition (181.2 g) was fractionated over MCI gel (EtOH/H_2O, from 30% to 95%) to obtain eight fractions (Frs. A−H). Fr. F (13.8 g) underwent further separation by Sephadex LH-20 chromatography ($CHCl_3$/MeOH 1 : 1), producing subfractions Frs. F1−F4. Fr. F2 (5.4 g) was separated by Sephadex LH-20 column eluting with MeOH to afford subfractions Frs. F2A–F2E. Fr. F2B (981.4 mg) was chromatographed over a Toyopearl HW-40F column (MeOH)l, yielding subfractions Frs. F2B1–F2B5. Fr. F2B3 (370.5 mg) was separated by CC over a silica gel column eluted with PE/EtOAc (8 : 1, 5 : 1, 2 : 1) to afford subfractions Frs. F2B3A–F2B3G. Fr. F2B3F (91.3 mg) was purified by preparative HPLC (MeCN/H_2O, 0−60 min, from 30% to 60%) to afford compounds **1** (52.9 mg), and **2** (5.2 mg). Fr. F2B3C (38.1 mg) was purified by preparative HPLC (MeCN/H_2O, 0−35 min, from 35% to 55%) to afford compound **3** (5.9 mg). Fr. G (14.3 g) was separated by Sephadex LH-20 column chromatography, with $CHCl_3$/MeOH (1 : 1) as mobile phase, to give four subfractions Frs. G1−G4. Fr. G2 (1.2 g) was subjected to a Sephadex LH-20 column (MeOH), yielding four subfractions Frs. G2A–G2D. Fr. G2D3 (623.3 mg) was separated using a silica gel column eluted with CH_2Cl_2/Acetone (100 : 1, 80 : 1, 50 : 1, 15 : 1) to afford subfractions Frs. G2D3A–Fr. G2D3H. Fr. G2D3B (12.1 mg) was purified by semi-preparative HPLC (MeCN/H_2O, 0−35 min, from 55% to 75%) to afford compound **6** (2.2 mg). Fr. G2D3E (56.0 mg) was purified by preparative HPLC (MeCN/H_2O, 0−60 min, from 35% to 65%) to afford compounds **4** (26.2 mg) and **5** (11.2 mg). Fr. D (7.6 g) was separated into seven additional fractions (Frs. D1−D6) by Sephadex LH-20 column (MeOH). Fr. D3 (1.5 g) was subjected to Sephadex LH-20 column ($CHCl_3$/MeOH 1 : 1) to afford seven subfractions Frs. D3A-D3G. Fr. D3G (567.0 mg) was separated using a silica gel column (200−300 mesh) eluted with PE/EtOAc (5 : 1, 3 : 1, 1 : 1) to afford nine subfractions Frs. D3G1−D3G9. Compounds **7** (56.6 mg) and **8** (109.9 mg) were obtained from Fr. D3G4 (182.2 mg) by preparative HPLC, respectively (MeCN/H_2O, 0−35 min, from 35% to 55%). Compounds **9** (19.2 mg) and **12** (4.7 mg) were obtained from Fr. D3G5 (67.3 mg) by preparative HPLC (MeCN/H_2O, 0−35 min, from 35% to 55%). Compounds **10** (50.6 mg) and **11** (22.7 mg) were obtained from Fr. D3D (291 mg) by CC over silica gel (PE/EtOAc, 5 : 1 to 1 : 1), and then preparative HPLC (MeCN/H_2O, 0−35 min, from 30% to 50%)

Sylvaticalide A (**1**): colorless orthorhombic crystal; $[\alpha]_D^{20}$−79 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3472, 1766, 1741, 1721, 1246, 1140, 1077, 1026 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); HR-ESI-MS *m/z* 437.1818 $[M + H]^+$ (Calcd. for $C_{22}H_{29}O_9$, 437.1812).

Sylvaticalide B (**2**): colorless gum; $[\alpha]_D^{20}$−34 (*c* 0.1, MeOH); IR (KBr) ν_{max} 1773, 1741, 1243, 1029cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); HR-ESI-MS *m/z* 481.2085 $[M + H]^+$ (Calcd. for $C_{24}H_{33}O_{10}$, 481.2074).

Sylvaticalide C (**3**): colorless gum; $[\alpha]_D^{20}$ −134 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3464, 1763, 1716, 1225, 1142, 1078, 1014cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); HR-ESI-MS *m/z* 379.1769 $[M + H]^+$ (Calcd. for $C_{20}H_{27}O_7$, 379.1753).

Sylvaticalide D (**4**): colorless gum; $[\alpha]_D^{20}$ −71 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3477, 1760, 1715, 1226, 1140, 1074. 1018 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); HR-ESI-MS *m/z* 363.1787 $[M + H]^+$ (Calcd. for $C_{22}H_{27}O_6$, 363.1808).

Sylvaticalide E (**5**): colorless gum; $[\alpha]_D^{20}$ −85 (*c* 0.1, MeOH); IR (KBr) ν_{max} 1768, 1746, 1716, 1226, 1139, 1076 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); HR-ESI-MS *m/z* 405.1921 $[M + H]^+$ (Calcd. for $C_{22}H_{29}O_7$, 405.1913).

Sylvaticalide F (**6**): colorless gum; $[\alpha]_D^{20}$ +181 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3449, 1779, 1717, 1143, 1079 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 3); HR-ESI-MS *m/z* $[M + Na]^+$ 405.1428 (Calcd. for, $C_{20}H_{27}O_5ClNa$, 405.1445).

Sylvaticalide G (**7**): colorless orthorhombic crystal; $[\alpha]_D^{20}$ +2 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3460, 1766, 1743, 1228, 1079, 1020 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 3); HR-ESI-MS *m/z* $[M + Na]^+$ 461.1788 (Calcd. for $C_{22}H_{30}O_9Na$, 461.1788).

Sylvaticalide H (**8**): white amorphous powder; $[\alpha]_D^{20}$ +47 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3462, 1766, 1748, 1717, 1225, 1137, 1076, 1020 cm^{-1}; ^1H and ^{13}C NMR data (Table 3); HR-ESI-MS *m/z* 459.1642 $[M + Na]^+$ (Calcd. for $C_{22}H_{28}O_9Na$, 459.1636).

Sylvaticalide I (**9**): colorless gum; $[\alpha]_D^{20}$ +27 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3454, 1766, 1718, 1646, 1383, 1226, 1140, 1077, 1019 cm^{-1}; ^1H and ^{13}C NMR data (Table 3); HR-ESI-MS *m/z* 445.1836 $[M + Na]^+$ (Calcd. for $C_{22}H_{30}O_8Na$, 445.1838).

X-ray crystallographic analysis of compounds 1, 7, and 11.

Crystals suitable for X-ray crystallographic analysis were obtained from their MeOH solutions. Selected crystals were analyzed using the Bruker SHELXTL software package, where the structure were determined and refined. Copies of the crystallographic data of every crystal can be obtained free of charge *via* the internet at www.ccdc.cam.ac.uk/conts or upon application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [tel: (+44) 1223-336-408; fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk].

Crystal data for sylvaticalide A (**1**). $C_{22}H_{28}O_9$, (M = 436.44 $g \cdot mol^{-1}$), orthorhombic, space group $P2_12_12_1$, *a* = 10.3274(5) Å, *b* = 12.2296(6) Å, *c* = 17.3560(8) Å, β = 90°, *V* = 2192.06(18) Å3, *Z* = 4, *T* = 170.0 K, μ(CuKα) = 0.864 mm^{-1}, D_{calc} = 1.322 g/cm^3, 15813 reflections measured (9.966° ≤ 2σ ≤ 149.13°), 4340 unique (R_{int} = 0.0333, R_{sigma} = 0.0280), which were used in all calculations. The final R_1 was 0.0349 ($I > 2\sigma(I)$), and wR_2 was 0.0866 (all data). Flack parameter = 0.00(6). Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre with deposit No. CCDC 2241440.

Crystal data for sylvaticalide G (**7**). $C_{22}H_{30}O_9$, (M = 438.46 $g \cdot mol^{-1}$), orthorhombic, space group $P2_12_12_1$, *a* = 8.2819(3) Å, *b* = 9.7415(3) Å, *c* = 27.2946(9) Å, β = 90°, *V* = 2202.08(13) Å3, *Z* = 4, *T* = 170.0 K, μ(CuKα) = 0.860 mm^{-1}, D_{calc} = 1.323 $g \cdot cm^{-3}$, 24737 reflections measured (6.476° ≤ 2σ ≤ 149.372°), 4424 unique (R_{int} = 0.0513, R_{sigma} = 0.0327), which were used in all calculations. The final R_1 was 0.0351 ($I > 2\sigma(I)$), and wR_2 was 0.0823 (all data). Flack parameter = 0.06(9). Crystallographic data for **7** have been deposited at the Cambridge Crystallographic Data Centre with deposit No. CCDC 2241446.

Crystal data for **11**. $C_{22}H_{28}O_8$, (M = 420.44 $g \cdot mol^{-1}$), orthorhombic, space group $P2_12_12_1$, *a* = 8.4559(2) Å, *b* =

Table 3 ^1H and ^{13}C NMR Data for compounds 7–9 in $CDCl_3$ (δ in ppm, *J* in Hz)

No.	7 [a]		8 [a]		9 [a]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	108.8		108.7		108.8	
2	31.9	1.81, br s 1.90, m	32.0	1.57, m 1.79, br s	31.9	1.81, br s
3	38.2	2.02, m 2.40, m	38.1	2.05, m 2.40, m	38.4	2.07, m 2.40, m
4	82.2		82.2		82.1	
5	127.0	5.89, br s	127.0	5.96, br s	125.6	5.82, br s
6	144.2		144.2		144.4	
7	150.1		150.8		151.2	
8	66.4	6.46, br s	65.3	6.51, br s	65.4	6.54, br s
9	37.6	2.05, m 2.54, m	37.6	2.05, m 2.53, br s	38.0	2.07, m 2.51, m
10	78.2		78.1		78.1	
11	131.2		131.0		133.2	
12	167.2		167.2		167.7	
13	55.9	4.85, br s 5.25, br s	55.9	4.91, d (12.2) 5.29, br s	61.8	4.31, br s 4.59, br s
14	25.5	1.22, s	25.5	1.25, s	25.4	1.19, s
15	29.2	1.55, s	29.2	1.57, s	29.2	1.56, s
1'	171.7		164.8		164.9	
2'	43.6	2.22, m	114.5	5.64, s	114.8	5.65, s
3'	25.8	2.06, m	160.9		160.2	
4'	22.6	0.92, dd (6.8, 2.9)	27.7	1.91, s	27.6	1.91, s
5'	22.4	0.92, dd (6.8, 2.9)	20.7	2.14, s	20.6	2.14, s
OAc	170.5 20.9	2.06, s	170.5 20.9	2.05, s		
OEt					66.7 15.3	3.56, m 1.20, br s

[a] recorded at 500 MHz (^1H) or 125 MHz (^{13}C)

9.3297(2) Å, c = 27.4027(6) Å, β = 90°, V = 2161.83(8) Å3, Z = 4, T = 170.0 K, μ(CuKα) = 0.819 mm^{-1}, D_{calc} = 1.292 g·cm^{-3}, 21712 reflections measured (6.45° ≤ 2σ ≤ 149.244°), 4384 unique (R_{int} = 0.0578, R_{sigma} = 0.0363), which were used in all calculations. The final R_1 was 0.0380 (I > 2σ(I)), and wR_2 was 0.0963 (all data). Flack parameter = 0.06(8). Crystallographic data for **11** have been deposited at the Cambridge Crystallographic Data Centre with deposit No. CCDC 2241447.

Anti-inflammation assay

THP1-Dual™ cells (Cat. code: thpd-nfis, InvivoGen, USA) at a density of 1 × 10^5 cells/well were suspended in 180 μL of medium and seeded into 96-well plates. Subsequently, 20 μL of the test compounds at indicated concentrations were added to the cells. After 0.5 h, LPS-B5 (Cat. code: tlrl-b5lps, InvivoGen, USA,) at a concentration of 1 μg·mL^{-1} or MSA-2 (Cat. code: 129425-81-6, MCE, China) at 10 μmol·L^{-1} was added to the cells, respectively. SC75741 (Cat. code: S7273, Selleck, China) was used as positive control. After 24 h, 20 μL of the supernatant was combined with 180 μL of QUANTI-Blue™ (Cat. code: rep-qlc2, InvivoGen, USA,) or QUANTI-Luc™ (Cat. code: rep-qbs2, InvivoGen, USA) detection reagent and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 1 h, the absorbance was measured at 650 nm using a multi-well spectrophotometer (SpetraMAX PLUS, Molecular Devices). The IC_{50} values were calculated using a four-parameter logistic model in Graphpad Prism 7.00.

Cell viability assay

THP1-Dual cells (1 × 10^5 cells/well) were plated in 90 μL of medium in 96-well plates. Then, 10 μL of the test compounds at specified concentrations were added to the cells. After 24 h of incubation, 10 μL of Cell Counting Kit-8 (CCK-8) reagent (Cat. code: A311-01, Vazyme, China) was added to each well. The plates were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 2 h, the absorbance was measured at 450 nm using a multi-well spectrophotometer (SpetraMAX PLUS, Molecular Devices). The IC_{50} values were calculated using a four-parameter logistic model in Graphpad Prism 7.00.

References

[1] Zhu L, Liu XQ, Lin YL, *et al*. Cytotoxic germacranolides from the whole plant of *Carpesium minus* [J]. *J Nat Prod*, 2020, **83**(11): 3230-3238.

[2] Zhu NL, Tang C, Xu C, *et al*. Cytotoxic germacrane-Ttype sesquiterpene lactones from the whole plant of *Carpesium lipskyi* [J]. *J Nat Prod*, 2019, **82**(4): 919-927.

[3] Thongnest S, Chawengrum P, Keeratichamroen S, *et al*. Vernodalidimer L, a sesquiterpene lactone dimer from *Vernonia extensa* and anti-tumor effects of vernodalin, vernolepin, and vernolide on HepG2 liver cancer cells [J]. *Bioorg Chem*, 2019, **92**: 103197.

[4] Kuo LY, Tseng PY, Lin YC, *et al*. New hirsutinolide-type sesquiterpenoids from *Vernonia cinerea* inhibit nitric oxide production in LPS-stimulated RAW264.7 cells [J]. *Planta Med*, 2018, **84**(18): 1348-1354.

[5] Wu JW, Tang CP, Cai YY, *et al*. Cytotoxic germacrane-type sesquiterpene lactones from the whole plant of *Inula cappa* [J]. *Chinese Chem Lett*, 2017, **28**(5): 927-930.

[6] Xu W, Bai M, Liu DF, *et al*. MS/MS-based molecular networking accelerated discovery of germacrane-type sesquiterpene lactones from *Elephantopus scaber* L. [J]. *Phytochemistry*, 2022, **198**: 113136.

[7] Jang H, Lee JW, Kim JG, *et al*. Nitric oxide inhibitory constituents from *Siegesbeckia pubescens* [J]. *Bioorg Chem*, 2018, **80**: 81-85.

[8] Mofidi TS, Nejad ES, Salehi P, *et al*. Antiprotozoal germacranolide sesquiterpene lactones from *Tanacetum sonbolii* [J]. *Planta Med*, 2019, **85**(5): 424-430.

[9] Kimani NM, Matasyoh JC, Kaiser M, *et al*. Antiprotozoal sesquiterpene lactones and other constituents from *Tarchonanthus camphoratus* and *Schkuhria pinnata* [J]. *J Nat Prod*, 2018, **81**(1): 124-130.

[10] Yu X, Zhang Q, Tian L, *et al*. Germacrane-type sesquiterpenoids with antiproliferative activities from *Eupatorium chinense* [J]. *J Nat Prod*, 2018, **81**(1): 85-91.

[11] Freund RRA, Gobrecht P, Fischer D, *et al*. Advances in chemistry and bioactivity of parthenolide [J]. *Nat Prod Rep*, 2020, **37**(4): 541-565.

[12] Long J, Zhang SF, Wang PP, *et al*. Total syntheses of parthenolide and its analogues with macrocyclic stereocontrol [J]. *J Med Chem*, 2014, **57**(16): 7098-7112.

[13] Dogra NK, Kumar S, Kumar D. *Vernonia anthelmintica* (L.) Willd. : an ethnomedicinal, phytochemical, pharmacological and toxicological review [J]. *J Ethnopharmacol*, 2020, **256**: 112777.

[14] Zhang M, Yang X, Wei Y, *et al*. Bioactive sesquiterpene lactones isolated from the whole plants of *Vernonia cinerea* [J]. *J Nat Prod*, 2019, **82**(8): 2124-2131.

[15] Sinisi A, Millan E, Abay SM, *et al*. Poly-electrophilic sesquiterpene lactones from *Vernonia amygdalina*: new members and differences in their mechanism of thiol trapping and in bioactivity [J]. *J Nat Prod*, 2015, **78**(7): 1618-1623.

[16] Youn UJ, Miklossy G, Chai X, *et al*. Bioactive sesquiterpene lactones and other compounds isolated from *Vernonia cinerea* [J]. *Fitoterapia*, 2014, **93**: 194-200.

[17] Editor committee. *Flora of China* [M]. Science Press, Beijing, 1985: 18.

[18] Alicia B, Cesar A, Alicia B, *et al*. Glaucolides and related sesquiterpene lactones from *Vernonia incana* [J]. *Phytochemistry*, 1990, **29**: 313-315.

[19] Patricia LC, John MC, Ching C, *et al*. Isolation and structure determination of Piptocarphins A–F, cytotoxic germacranolide lactones from *Piptocarpha chontalensis* [J]. *J Org Chem*, 1981, **46**: 1108-1114.

[20] Susana B, Alicia B, Cesar AN, *et al*. Glaucolides, hirsutinolides and other sesquiterpene lactones from *Vernonanthura pinguis* [J]. *Phytochemistry*, 1997, **44**: 465-470.

[21] Guha M, Mackman N. LPS induction of gene expression in human monocytes [J]. *Cell Signal*, 2001, **13**: 85-94.

[22] Pan BS, Perera SA, Piesvaux JA, *et al*. An orally available non-nucleotide STING agonist with antitumor activity [J]. *Science*, 2020, **369**: 935.

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