Informatics and Computationally Assisted Discovery of Anti-Inflammatory Diterpenoids from *Isodon rubescens*

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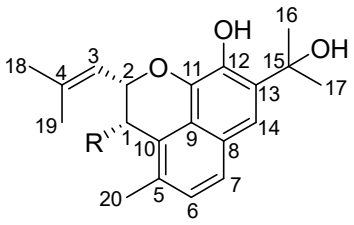
ABSTRACT：Diterpenoids occupy a valuable region of the natural products diversity space with wide ranges of bioactivities and complex structures, providing potential applications for the development of therapeutics. Five new abietane-type diterpenoids salvonitin B (**1**), salvonitin C (**2**), prionoid G (**3**), prionidipene F (**4**), viroxocin B (**5**), one new totarane-type diterpenoid

plebedipene E (**6**) and one new sempervirane-type diterpenoid hispidanol C (**7**) were isolated from *Isodon rubescens* (*I. rubescens*). Their structures were established by spectroscopic analysis, electronic circular dichroism (ECD) calculations, and X-ray diffraction analysis. In the evaluation of bioactivities, compound **4** (10 μM) increased cell viability (0.872 ± 0.157) in lipopolysaccharide-induced RAW 264.7 cells.

INTRODUCTION

The genus *Isodon*, contains approximately 150 species worldwide mainly distributed in tropical and subtropical Asia[1]. Some plants from this genus have been used in traditional Chinese folk medicine, including *I. rubescens*, *I. xerophilus*, *I. serra*, etc[2]. *I. rubescens* also called "Xihuangcao" in Chinese folk medicine has long history to treat jaundice hepatitis, acute cholecystitis, enteritis, and other inflammatory intestinal disorders[3]. It is rich in diterpenoids with diverse structural scaffolds including enmein, spirolactone, C-20-non-oxygenated, and C-20-oxygenated-kauranoids types[4]. The diterpenoids of the genus *Isodon* are of great interest due to not only the complex structure but also their variety and promising bioactivities, such as cytotoxic, anti-inflammatory, antibiotic, and anti-virus activities[5-7]. To date 324 metabolites have been characterized from just this single species.

In order to investigate additional diterpenoids from *I. rubescens*, the whole plant was selected for investigation. The study led to the isolation of purify 12 diterpenoids, including 5 new abietane-type diterpenoids (**1-5**), one new totarane-type diterpenoid (**6**), one new sempervirane-type diterpenoid (**7**), together with 5 known analogs[3, 8, 9] (**8-12**) (**Figure 1**). Structural elucidation using NMR, HR-ESI-MS, ECD and X-ray diffraction. **4** (10 μM) increased cell viability (0.872 ± 0.157) in lipopolysaccharide-induced RAW 264.7 cells.



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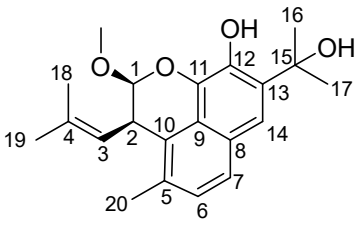
3: R=O



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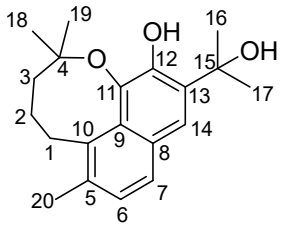

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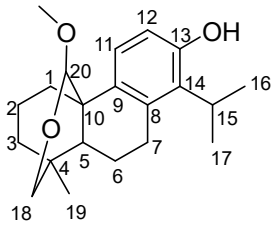

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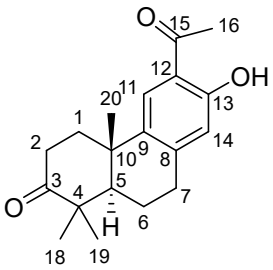

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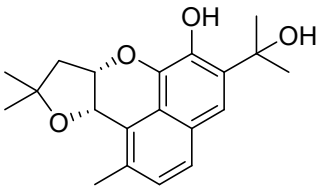
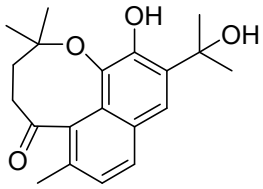
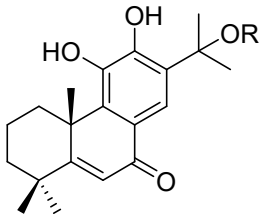


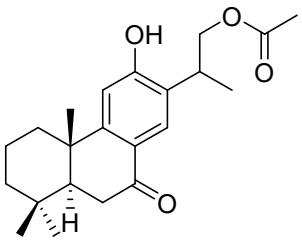
8 15-hydroxyl salprionin



9 Rabdosin D



10 Gerardianin C R= H
11 15-hydroxysalvinolone R=CH_3



12 16-acetoxylsugiol

Figure 1. Structures of compounds **1-12**

Table 1. 1D-NMR data for compounds **1** (in MeOD), **2** and **3** (in $CDCl_3$). [a]

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.88 (d, *J*= 2.0)	66.2	4.57 (d, *J*= 1.8)	73.7		192.0
2	5.32 (dd, *J*= 8.5, 2.0)	77.2	5.57 (dd, *J*= 8.8, 1.8)	73.0	5.42 (d, *J*= 8.7)	78.8
3	4.83 (d, *J*= 8.5)	120.4	4.86 (d, *J*= 8.7)	119.0	5.27 (d, *J*= 8.8)	117.6
4		137.9		138.0		140.8
5		132.8		133.0		139.7
6	7.16 (d, *J*= 8.4)	126.4	7.19 (d, *J*= 8.5)	126.0	7.18 (d, *J*= 8.5)	127.8
7	7.62 (d, *J*= 8.4)	127.5	7.64 (d, *J*= 8.5)	127.1	7.80 (d, *J*= 8.4)	133.1
8		125.8		124.9		124.9
9		120.6		119.5		122.4
10		124.3		121.1		119.3
11		134.5		133.1		133.5
12		139.9		138.6		140.6
13		135.5		133.6		134.1
14	7.39 (s)	115.2	7.32 (s)	114.5	7.36 (s)	116.0
15		73.2		72.5		72.9
16	1.69 (s)	28.5	1.71 (s)	29.0	1.70 (s)	28.9
17	1.69 (s)	28.6	1.75 (s)	28.3	1.72 (s)	28.7
18	1.87 (s)	17.3	1.88 (s)	17.7	1.82 (s)	18.0
19	1.57 (s)	24.4	1.59 (s)	24.7	1.69 (s)	25.0
20	2.49 (s)	16.4	2.50 (s)	17.3	2.74 (s)	21.3
OCH_3			3.46 (s)	55.0		

[a] δ in ppm, *J* in Hz. ^{1}H-NMR: 600 MHz, ^{13}C-NMR: 150 MHz.

Table 2. 1D-NMR data for compounds **4** and **5** (in $CDCl_3$). [a]

Position	**4**		**5**	
	δ_H	δ_C	δ_H	δ_C
1	5.25 (d, *J* = 1.5)	101.0	a 3.91 (m)	26.5
			b 2.93(m)	
2	4.11 (dd, *J* = 10.1, 1.5)	39.6	a 2.18 (m)	23.0
			b 1.64 (m)	
3	4.97 (d, *J* = 10.2)	121.3	a 1.59 (m)	33.3
			b 1.25 (m)	
4		131.9		85.4
5		130.8		127.5
6	7.12 (d, *J* = 8.4)	126.6	7.12 (d, *J* = 8.3)	127.3
7	7.51 (d, *J* = 8.4)	124.7	7.49 (d, *J* = 8.3)	126.4
8		125.3		130.3
9		119.2		131.8
10		124.9		133.5
11		132.3		135.2
12		138.5		146.6
13		133.1		133.6
14	7.31 (s)	115.4	7.46 (s)	120.5
15		73.4		72.6
16	1.76 (s)	29.0	1.73 (s)	29.3
17	1.77 (s)	28.8	1.70 (s)	29.5
18	1.89 (s)	17.2	1.16 (s)	27.5
19	1.69 (s)	24.7	1.64 (s)	26.3
20	2.32 (s)	17.2	2.43 (s)	19.9
OCH_3	3.44 (s)	54.6		

[a] δ in ppm, *J* in Hz. ^{1}H-NMR: 600 MHz, ^{13}C-NMR: 150 MHz.

Table 3. 1D-NMR data for compounds **6** and **7** (in $CDCl_3$). [a]

Position	**6**		**7**	
	δ_H	δ_C	δ_H	δ_C
1	a 2.19 (dd, *J* = 13.7, 5.9)	39.4	a 2.51 (m)	37.7
	b 1.38 (m)		b 1.97 (m)	
2	a 2.49 (m)	21.9	a 2.74 (m)	34.6
	b 1.59 (m)		b 2.62 (m)	
3	a 1.72 (m)	40.7		216.5
	b 1.42 (m)			
4		32.7		47.4
5	1.33 (d, *J* = 3.3)	45.7	1.87 (dd, *J* = 12.1, 2.8)	50.6
6	a 2.26 (dtd, *J* = 13.6, 12.3, 5.5)	19.5	1.82 (m)	19.8
	b 1.77 (m)			
7	a 2.94 (ddd, *J* = 16.8, 5.8, 1.8)	28.1	a 2.99 (m)	31.1
	b 2.61 (ddd, *J* = 16.8, 12.1, 6.6)		b 2.87 (m)	
8		136.2		145.4
9		134.5		138.9
10		40.6		36.9
11	6.92 (d, *J* = 8.4)	123.6	7.59 (s)	127.5
12	6.51 (d, *J* = 8.4)	114.2		118.6
13		152.1		159.7
14		130.9	6.68 (s)	117.4
15	3.34 (m)	27.2		203.9
16	1.36 (dd, *J* = 7.1, 4.7)	20.4	2.61 (s)	26.5
17	1.36 (dd, *J* = 7.1, 4.7)	20.5		
18	a 3.75 (dd, *J* = 11.1, 2.7)	66.3	1.18 (s)	26.6
	b 3.30 (dd, *J* = 11.0, 1.4)			
19	0.79 (s)	23.6	1.15 (s)	21.2
20	4.54 (s)	105.9	1.31 (s)	25.0
OCH_3	3.09 (s)	55.3		

[a] δ in ppm, *J* in Hz. ^{1}H-NMR: 600 MHz, ^{13}C-NMR: 150 MHz.

RESULTS AND DISCUSSION

Compound **1** was white powder, and its molecular formula was determined to be $C_{20}H_{24}O_4$ based on the ^{13}C NMR data and a sodium adduct ion at *m/z* 351.1569 ($[M+Na]^+$, calcd 351.1572) in its positive mode HR-ESI-MS, implying nine indices of hydrogen deficiency. In the ^1H-NMR spectrum, five methyls [δ_H 2.49 (3H, s, H-20), 1.87 (3H, s, H-18), 1.69 (3H, s, H-17), 1.69 (3H, s, H-16) and 1.57 (3H, s, H-19)], two oxymethine protons [δ_H 5.32 (1H, dd, *J*= 8.5, 2.0 Hz, H-2), 4.88 (1H, d, *J*= 2.0 Hz, H-1)], and four olefinic protons [δ_H 7.62 (1H, d, *J*= 8.4 Hz, H-7), 7.39 (1H, s, H-14), 7.16 (1H, d, *J*= 8.4 Hz, H-6), 4.83 (1H, d, *J*= 8.5 Hz, H-3)] were observed. The ^{13}C-NMR and HSQC data of **1** showed 20 carbon resonances, consisting of five methyls at δ_C 28.6, 28.5, 24.4, 17.3, 16.4, six methines (two oxygenated at δ_C 77.2, 66.2 and four olefinic at δ_C 127.5, 126.4, 120.4, 115.2), and nine non-protonated carbons at δ_C 139.9, 137.9, 135.5, 134.5, 132.8, 125.8, 124.3, 120.6 and 73.2. The above NMR data was similar to the de-*O*-ethylsalvonitin which isolated from the *Salvia prionitis Hance* (Labiatae)[8]. Unlike this known structure, compound **1** has a hydroxyl at C-15 (δ_C 73.2), which was secured by the key HMBC correlations (**Figure 2**) from H-16 (δ_H 1.69) and H-14 (δ_H 7.39) to C-15 (δ_C 73.2).

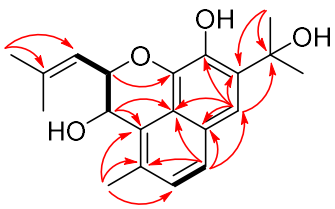


Figure 2. Structure and key HMBC (H→C) and COSY (H—H) correlations of **1**.

The coupling constant ($J_{1,2}$ = 1.8 Hz) between H-1 and H-2 suggested that compound **1** possessed a *cis* relative configuration. The absolute configuration of **1** was defined by its calculated ECD spectrum (

Figure 3) of **1** agrees with the experimental data, showing the 1*S*, 2*S*.

On the basis of the above findings, the structure of compound **1** was characterized as (1*S*,2*S*)-15-hydroxy-*de*-*O*-ethylsalvonitin which named salvonitin B.

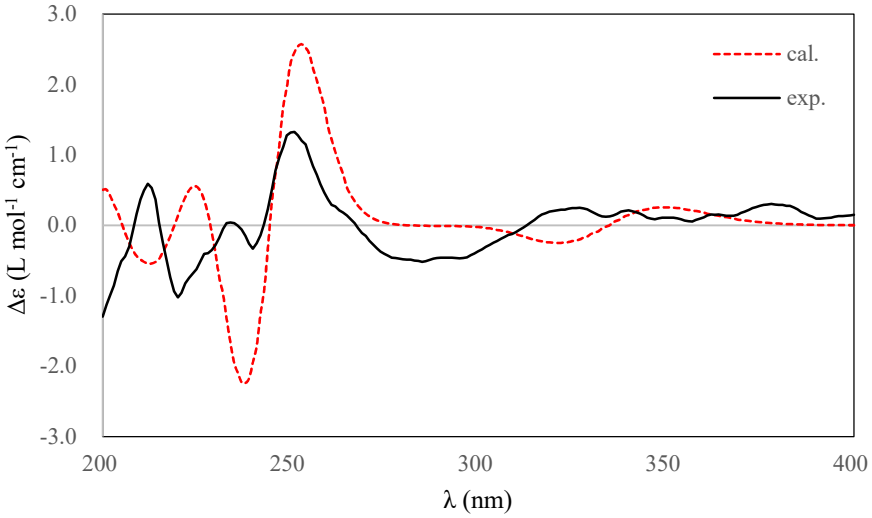


Figure 3. ECD of compound **1**

Compound **2** was obtained as yellow amorphous powder, and had a molecular formula $C_{21}H_{26}O_4$ with nine degrees of unsaturation based on the ^{13}C-NMR data and analysis of its positive ion mode HR-ESI-MS (*m/z* 365.1727 $[M+Na]^+$, calcd 365.1729). The ^{1}H-NMR spectrum of **2** displayed resonances signals for five singlet methyls [δ_H 1.71 (3H, s, H-16), 1.75 (3H, s, H-17), 1.88 (3H, s, H-18), 1.59 (3H, s, H-19) and 2.50 (3H, s, H-20)], a oxymethyl singlet [δ_H 3.46 (3H, s, OMe)], two oxymethine protons [δ_H 4.57 (1H, d, *J*= 1.8 Hz, H-1), 5.57 (1H, dd, *J* = 8.8, 1.8 Hz, H-2)], four olefinic protons [δ_H 7.19 (1H, d, *J* = 8.5 Hz, H-6), 7.64 (1H, d, *J* = 8.5 Hz, H-7), 7.32 (1H, s,

H-14) and 4.86 (1H, d, J = 8.7 Hz, H-3)]. The ^{13}C-NMR and HSQC spectra exhibited 21 carbon signals, including five methyls (δ_C 29.0, 28.3, 24.7, 17.7, 17.3), an oxymethyl (δ_C 55.0), six methines (two oxygenated at δ_C 73.7, 73.0 and four olefinic at δ_C 127.1, 126.0, 119.0, 114.5), and nine non-protonated carbons (δ_C 138.6, 138.0, 133.6, 133.1, 133.0, 124.9, 121.1, 119.5, 72.5). Analysis of the 1D and 2D NMR data for **2** revealed an 4,5-*seco*-abietane diterpenoids substructure in common with *de*-*O*-ethylsalvonitin[10]. Compared with those of *de*-*O*-ethylsalvonitin, ^{13}C-NMR of **2** demonstrated an additional hydroxyl at C-15, which was confirmed by key HMBC correlations (**Figure 4**) from the anomeric H-16 (δ_H 1.71), H-17 (δ_H 1.75) to C-15 (δ_C 72.5).

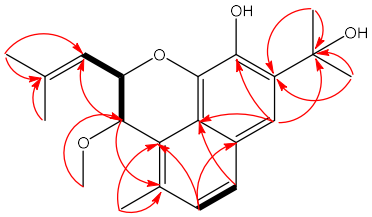


Figure 4. Structure and key HMBC (H→C) and COSY (H—H) correlations of **2**.

The coupling constant ($J_{1,2}$ = 1.8 Hz) between H-1 and H-2 suggested the *cis* relationship of H-1 and H-2. The absolute configurations of **2** was determined by the comparison of experimental and calculated ECD spectra (**Figure 5**), allowing an unambiguous assignment to be 1*S*,2*S*. And **2** was characterized as (1*S*,2*S*)-15-hydroxy-2-methoxyl-*de*-*O*-ethylsalvonitin which named salvonitin C.

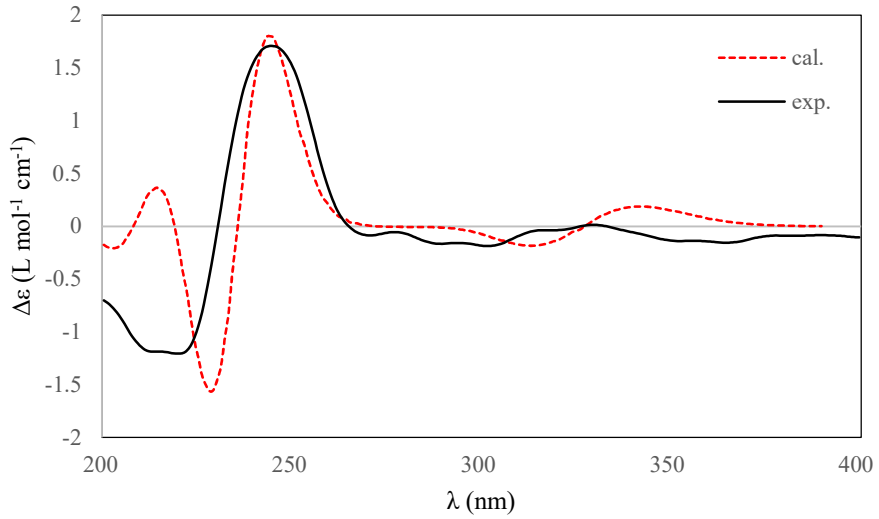


Figure 5. ECD of compound **2**

Compound **3** was obtained as yellow amorphous powder. Its molecular formula was established to be $C_{20}H_{22}O_4$ by positive ion mode HR-ESI-MS (*m/z* 327.1596 $[M+H]^+$, calcd 327.1596) and ^{13}C NMR data. The ^1H-NMR spectrum of **3** displayed resonances signals for five singlet methyls [δ_H 1.70 (3H, s, H-16), 1.72 (3H, s, H-17), 1.82 (3H, s, H-18), 1.69 (3H, s, H-19) and 2.74 (3H, s, H-20)], one oxymethine proton [δ_H 5.42 (1H, d, J = 8.7 Hz, H-2)], four olefinic protons [δ_H 7.18 (1H, d, J = 8.5 Hz, H-6), 7.80 (1H, d, J = 8.4 Hz, H-7), 7.36 (1H, s, H-14) and 5.27 (1H, d, J = 8.8 Hz, H-3)]. The ^{13}C NMR and HSQC spectra exhibited 20 carbon signals, including five methyls (δ_C 28.9, 28.7, 25.0, 21.3, 18.0), five methines (one oxygenated at δ_C 78.8 and four olefinic at δ_C 133.1, 127.8, 117.6, 116.0), ten non-protonated carbons (δ_C 192.0, 140.8, 140.6, 139.7, 134.1, 133.5, 124.9, 122.4, 119.3, 72.9), including one carbonyl group (δ_C 192.0). The 1D and 2D NMR spectrum of **3** suggested an 4,5-*seco*-abietane diterpenoids substructure similar to those of prionoid B[11]. Detailed comparison of the NMR data of **3** with those of the known compound prionoid B revealed a high degree of similarity except for the presence of one additional hydroxyl group in **3**.

This hydroxyl group was placed at C-15, as inferred from the related HMBC correlation (**Figure 6**) of C-15 (δ_C 72.9) with the anomeric H-16 (δ_H 1.70), H-17 (δ_H 1.72).

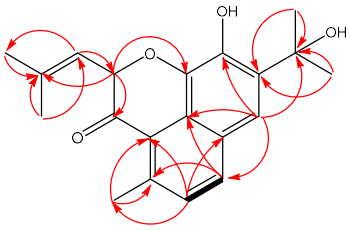


Figure 6. Structure and key HMBC (H→C) and COSY (H—H) correlations of **3**.

Subsequently, the absolute configuration of **3** was determined to be 2*S* by a comparison of the observed and calculated ECD data (**Figure 7**). Consequently, compound **3** was established as (2*S*)-15-hydroxy-prionoid B which named prionoid G.

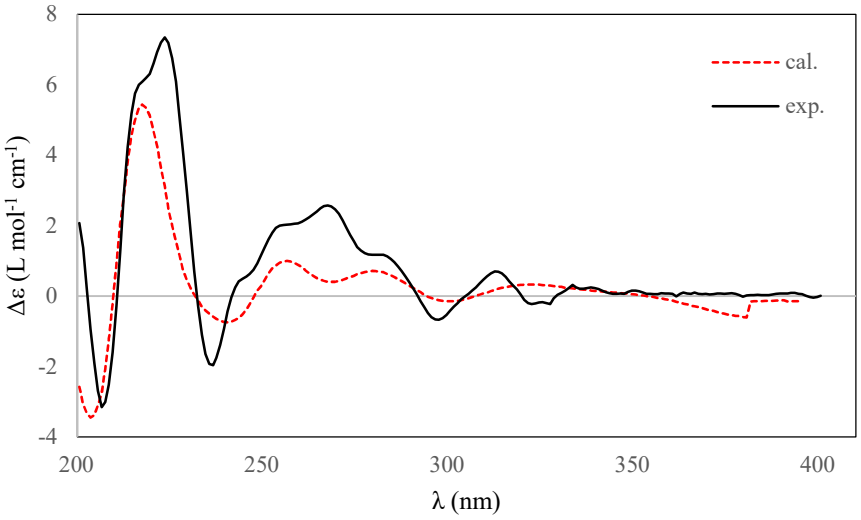


Figure 7. ECD of compound **3**

Compound **4** was isolated as yellow powder. The molecular formula ($C_{21}H_{26}O_4$), with nine indices of hydrogen deficiency, was deduced by the HR-ESI-MS peak at *m/z* 365.1725 $[M+Na]^+$, (calcd 365.1729) and ^{13}C NMR data. The ^1H-NMR spectrum of **4** displayed resonances signals for

five singlet methyls [δ_H 1.76 (3H, s, H-16), 1.77 (3H, s, H-17), 1.89 (3H, s, H-18), 1.69 (3H, s, H-19) and 2.32 (3H, s, H-20)], a singlet oxymethyl [δ_H 3.44 (3H, s, OMe)], a methine [δ_H 4.11 (1H, dd, J = 10.1, 1.5 Hz, H-2)], one oxymethine proton [δ_H 5.25 (1H, d, J = 1.5 Hz, H-1)], four aromatic protons [δ_H 7.12 (1H, d, J = 8.4 Hz, H-6), 7.51 (1H, d, J = 8.4 Hz, H-7), 7.31 (1H, s, H-14) and 4.97 (1H, d, J = 10.2 Hz, H-3)]. A total of 21 carbon signals were observed in the ^{13}C NMR and HSQC spectra of **4**: five methyls (δ_C 29.0, 28.8, 24.7, 17.2, 17.2), an oxymethyl (δ_C 54.6), six methines (one at δ_C 39.6, one oxygenated at δ_C 101.0 and four olefinic at δ_C 126.6, 124.7, 121.3, 115.4), and nine non-protonated carbons (δ_C 138.5, 133.1, 132.3, 131.9, 130.8, 125.3, 124.9, 119.2, 73.4). The 1D and 2D NMR data of **4** were similar to those of prionidipene B except for the existence of an additional hydroxyl[10]. Key HMBC correlations (**Figure 8**) from the anomeric H-16 (δ_H 1.76), H-17 (δ_H 1.77) to C-15 (δ_C 73.4) established the additional hydroxyl at C-15 in **4**.

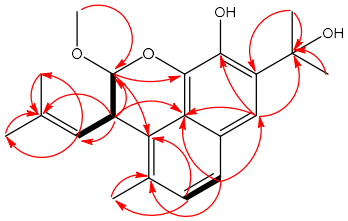


Figure 8. Structure and key HMBC (H→C) and COSY (H—H) correlations of **4**.

The relative configuration of **4** was determined on the coupling constant ($J_{1,2}$ = 1.5 Hz) revealed the *cis* relationship between H-1 and H-2. The absolute configuration of **4** was established by the ECD spectra (**Figure 9**). The computed calculated ECD spectra agreed with the experimental spectrum of the 1*S*,2*R* absolute configuration.

On the basis of the above findings, the structure of compound **4** was characterized as (1*S*,2*R*)-15-hydroxy-prionidipene B which named prionidipene F.

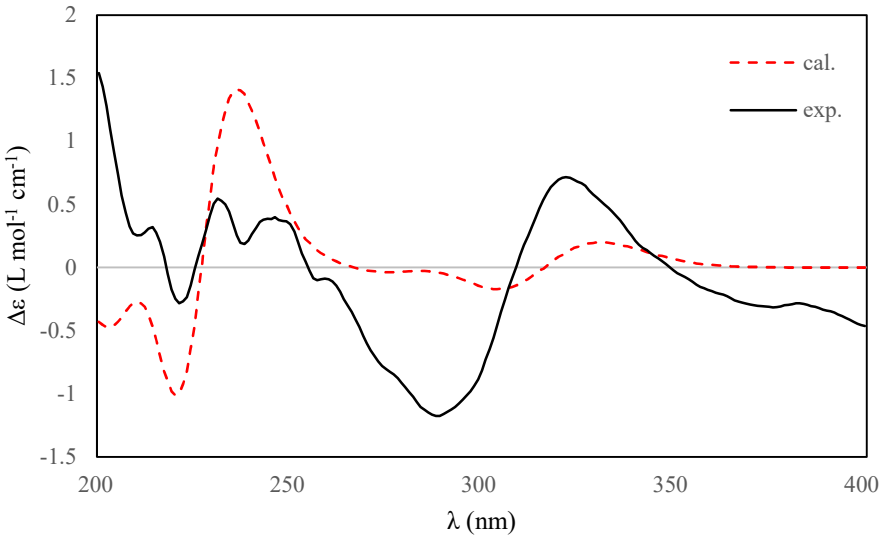


Figure 9. ECD of compound **4**

Compound **5** was obtained as yellow powder. The molecular formula of compound **5** was determined as $C_{20}H_{26}O_3$ based on ^{13}C-NMR date and the protonated molecular ion peak at m/z 297.1869 $[M+H-H_2O]^+$, (calcd for $C_{20}H_{26}O_3$, 297.1854) in the HR-ESI-MS, implying eight indices of hydrogen deficiency. The ^{1}H-NMR spectrum exhibited signals characteristic of five methyl groups [δ_H 2.43 (3H, s, H-20), 1.73 (3H, s, H-16), 1.70 (3H, s, H-17), 1.64 (3H, s, H-19) and 1.16 (3H, s, H-18)]. The ^{13}C-NMR and HSQC spectrum suggested the presence of five methyls at δ_C 29.5, 29.3, 27.5, 26.3 and 19.9. In addition, there were, three methylenes at δ_C 33.3, 26.5 and 23.0, three olefinic methines at δ_C 127.3, 126.4 and 120.5, nine non protonated carbons at δ_C 146.6, 135.2, 133.6, 133.5, 131.8, 130.3, 127.5, 85.4 and 72.6. The above NMR data was similar to a recently described natural product, 1-deoxyviroxocin (10-isopropyl-2,2,6-trimethyl-2,3,4,5-tetrahydronaphtho [1,8-*bc*] oxocin-11-ol) from *Taxodium distichum*[12]. Unlike the known structure, compound **5** had a hydroxyl group at C-15 (δ_C 72.6), which was confirmed by the key HMBC correlations (**Figure 10**) from H-14 (δ_H 7.46), H-16 (δ_H 1.73) and H-17 (δ_H 1.70) to C-15 (δ_C 72.6).

Compound **5** was assigned as 1-deoxyviroxocin (10-isopropyl-2,2,6-trimethyl-2,3,4,5-tetrahydronaphtho [1,8-*bc*] oxocin-11,15-diol), which named as viroxocin B.

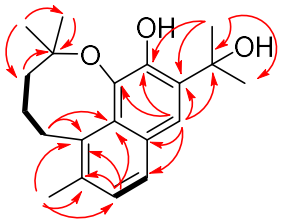


Figure 10. Structure and key HMBC (H→C) and COSY (H—H) correlations of **5**.

Compound **6** was obtained as yellow powder, and its molecular formula was determined to be $C_{21}H_{30}O_3$ based on the ^{13}C-NMR data and a sodium adduct ion at *m/z* 353.2090 $[M+Na]^+$ (calcd for $C_{21}H_{30}O_3$, 353.2093) in its positive mode HR-ESI-MS, implying seven indices of hydrogen deficiency. The ^{1}H-NMR spectrum of **6** displayed resonances signals for a singlet methyls [δ_H 0.79 (3H, s, H-19)], an isopropyl [δ_H 3.34 (1H, m, H-15), 1.36 (6H, dd, J = 7.1, 4.7 Hz, H-16, H-17)], a singlet oxymethyl [δ_H 3.09 (3H, s, OMe)], two oxymethylenes protons [δ_H 3.75 (1H, d, J = 11.1, 2.7 Hz, H-18a) and 3.30 (1H, d, J = 11.0, 1.4 Hz, H-18b)], a oxymethines proton [δ_H 4.54 (1H, s, H-20)], two aromatic protons [δ_H 6.92 (1H, d, J = 8.4 Hz, H-11) and 6.51 (1H, d, J = 8.4 Hz, H-12)]. The ^{13}C-NMR and HSQC spectra in $CDCl_3$ exhibited 21 carbon signals including three methyl (δ_C 23.6, 20.5, 20.4), an oxymethyl (δ_C 55.3), six methylenes (δ_C 40.7, 39.4, 28.1, 21.9, 19.5 and one oxygenated at δ_C 66.3), five methines (δ_C 123.6, 114.2, 105.9, 45.7, 27.2), six non-protonated carbons at δ_C 152.1, 136.2, 134.5, 130.9, 40.6 and δ_C 32.7. The above NMR data was similar to the (-)-(4*S*,5*S*,10*R*,20*R*)-12,18-dihydroxyabieta-8,11,13-trien-20-aldehyde 18,20-methyl acetal which isolated from the *Fraxinus sieboldiana* Blume (Oleaceae)[13]. Unlike this known structure, compound **6** was totarane-type rather than abietane-type diterpenoid, which was confirmed by the key COSY correlations from H-11 (δ_H 6.92) to H-12 (δ_H 6.51) and HMBC

correlations (**Figure 11**) from H-7 (δ_H 2.94, 2.61), H-15 (δ_H 3.34), H-16/17 (δ_H 1.36) to C-14 (δ_C 130.9).

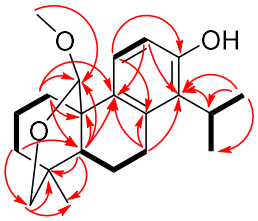


Figure 11. Structure and key HMBC (H→C) and COSY (H—H) correlations of **6**.

The relative configuration of **6** was defined by NOESY (**Figure 12**). NOESY correlations (**Figure 12**) between H-1a, H-11, H-18a and H-20 suggested these protons to be assigned randomly as *β*-oriented. The NOESY cross-peaks between H-6b and H-5 indicated H-5 to be assigned randomly as *β*-oriented. The absolute configuration of **6** was determined to be ECD spectra (**Figure 13**). The computed calculated ECD spectra agreed with the experimental spectrum of the *4X*, 5*X, 10X, 20X* absolute configuration.

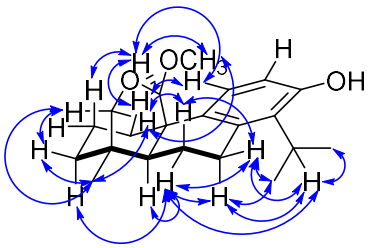


Figure 12. Structure and key NOESY (H↔H) correlations of **6**.

On the basis of the above findings, the structure of compound **6** was characterized as (*4X*, 5*X, 10X, 20X*)-13,18-dihydroxytotara-8,11,13-trien-20-aldehyde 18,20-methyl acetal. It was a new diterpenoid with a rearranged totarane skeleton which named plebedipene E.

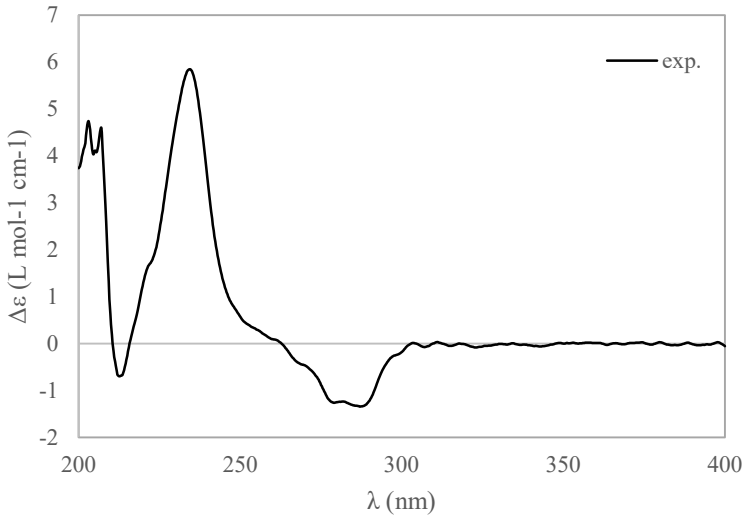


Figure 13. ECD of compound **6**

Compound **7** was isolated as colorless crystals (from MeOH). The molecular formula was established as $C_{19}H_{24}O_3$ (eight degrees of unsaturation) according to HREIMS (*m/z* 301.1802 ($[M+H]^+$, calcd 301.1804) and ^{13}C NMR data. The ^1H-NMR spectrum of **7** displayed signals of four methyls [δ_H 2.61 (3H, s, H-16), 1.18 (3H, s, H-18), 1.15 (3H, s, H-19), 1.31 (3H, s, H-20)], two aromatic protons [δ_H 7.59 (1H, s, H-11) and 6.68 (1H, s, H-14)]. Moreover, analysis of the ^{13}C-NMR and HSQC spectrum revealed the presence of four methyls (δ_C 26.6, 26.5, 25.0, 21.2), four methylenes (δ_C 37.7, 34.6, 31.1, 19.8), three methines (one at δ_C 50.6 and two olefinic at δ_C 127.5, 117.4), and six non-protonated carbons (δ_C 159.7, 145.4, 138.9, 118.6, 47.4, 36.9) and two carbonyl groups (δ_C 216.5, 203.9). Analysis of the 1D and 2D NMR spectroscopic data suggested **7** to be a sempervirane-type diterpenoids closely related to hispidanol A[14]. Compared with those of hispidanol A, ^{13}C-NMR of **7** demonstrated an additional carbonyl group (δ_C 216.5), instead of a hydroxy group at C-3 position in hispidanol A. In the HMBC correlations (**Figure 14**) from the anomeric H-1 (δ_H 2.51), H-18 (δ_H 1.18) and H-19 (δ_H 1.15) to C-3 (δ_C 216.5) established the additional carbonyl group in **7**.

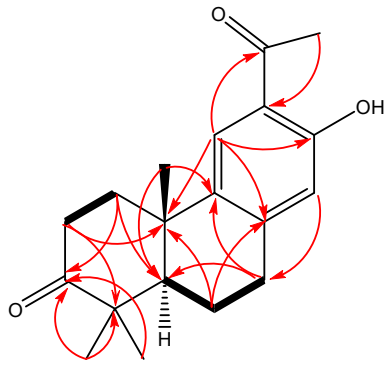


Figure 14. Structure and key HMBC (H→C) and COSY (H—H) correlations of **7**.

Finally, the planar structure and absolute configuration of **7** were further confirmed by the following X-ray diffraction data analysis (**Figure 15**). The final refinement of the Cu Kα data resulted in a small Flack parameter of −0.06 (12), allowing the assignment of the absolute configuration of **7**. Therefore, the absolute configuration of **7** was determined to be 5*R*,10*S*, which was named hispidanol C.

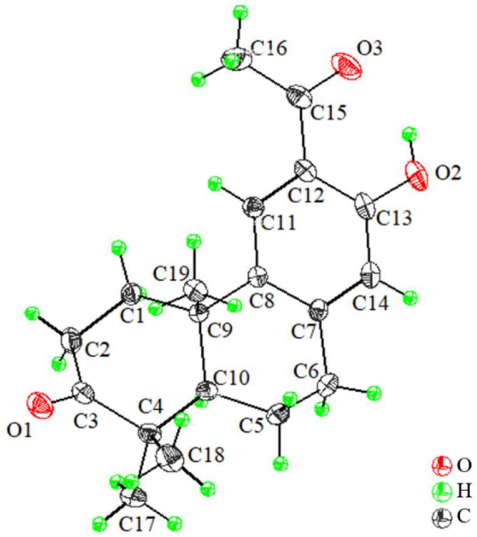


Figure 15. X-Ray structure of compound **7**

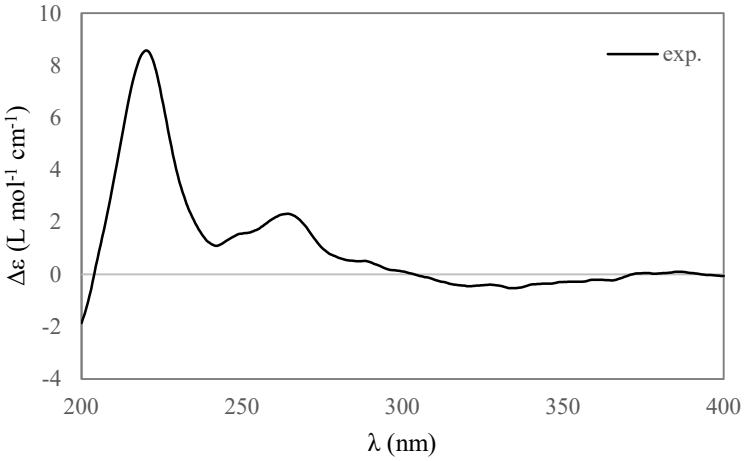


Figure 16. ECD of compound **7**

Table 4. The Effects of Compounds on LPS-induced RAW 264.7 cells ($\bar{x}$ ±*sd*, n = 4).

Group	Dose	Cell viability
Ctrl	-	1.000 ± 0.087
LPS	-	0.867 ± 0.029
1	10 μM	0.180 ± 0.032
3	10 μM	0.304 ± 0.039
4	10 μM	0.872 ± 0.157
5	10 μM	0.058 ± 0.004
7	10 μM	0.144 ± 0.025

The isolated compounds were evaluated *in vitro* for their anti-inflammatory activities by using lipopolysaccharide (LPS)-induced RAW 264.7 cells. As shown in **Table 4**, Treatment with compound **4** at a dose of 10 μM protected the cells and increased cell viability (0.872 ± 0.157).

EXPERIMENTAL SECTION

General Experimental Procedures. The CD spectra were recorded on a JASCO-J1500 spectropolarimeter. The NMR spectra were obtained on a Bruker Avance NEO 600 MHz spectrometer. HRESIMS data were obtained on an Agilent 6560 mass spectrometer. Semipreparative and preparative HPLCs were performed using SEP LC-52 series HPLC instruments with corresponding detectors, fraction collectors, and software. Column chromatography (CC) was performed using silica gel (Yu-Ming-Yuan Silysia Chemical Ltd., Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). TLC was conducted on precoated silica gel GF254 plates. Spots were visualized under UV light (254nm). X-ray data were collected on a ROD, Synergy Custom system, HyPix diffractometer equipped with graphite-monochromatized Cu *Kα* radiation.

Plant Material. Whole plants of *I. rubescens* were collected by Dr. Shengpeng Wang (State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau. Macau, China) in August 2020 from the Qingyaun in the Guangdong Province of China. The plant was identified by Dr. Xiaojuan Wang (School of Pharmacy, Lanzhou University of China).

Extraction and Isolation. The dried whole plant of *I. rubescens* (15 kg) was extracted with EthOH (3 × 20 L) at room temperature. After filtration, the solvents were removed under vacuum to give a crude extract (650 g), which was suspended in H_2O (2 L) and partitioned with EthOAc (3 × 1.5 L). The EtOAc extract (650 g) was subjected to silica gel (200-300 mesh) CC with Petroleum ether (PE)/EthOAc gradients (from 10:1 to 0:1, v/v) and CH_2Cl_2/MeOH gradients (from 10:1 to 0:1, v/v) to afford fifteen fractions (A1-A15) monitored by TLC. Fr. A10 (19 g) was further isolated by silica gel (200-300 mesh) CC with PE/EthOAc gradients (from 5:1 to 0:1, v/v) and CH_2Cl_2/MeOH

gradients (from 10:1 to 0:1, v/v) to afford four fractions (B1-B4) monitored by TLC. Fr. B4 was separated by an Sephadex LH-20 eluted with isocratic of MeOH/H_2O (80%, v/v) and purified using semipreparative HPLC (MeOH/H_2O, 65:35, v/v, 3.0 mL/min) to yield compounds **1** (3.0 mg, t_R = 20.7 min). Fr. A9 was performed by silica gel with PE/EthOAc (from 5:1 to 1:1, v/v) and MeOH (100%) to afford 15 sub-fractions (Z1-Z15). Fr. Z4 applied repeated silica gel and purified using semipreparative HPLC (MeOH/H_2O, 65:35, v/v, 3.0 mL/min) to yield compounds **7** (3.0 mg, t_R = 50.1 min). Fr. Z6 was subjected to Sephadex LH-20 and further purified by semipreparative HPLC with MeOH/H_2O (60:40, v/v) to yield compounds **3** (1.6 mg, t_R = 74.0 min). Fr. Z7 was isolated by Sephadex LH-20 and further purified using semipreparative HPLC (MeOH/H_2O, 70:30, v/v, 3.0 mL/min) to yield compounds **2** (3.0 mg, t_R = 23.9 min) and **4** (2.7 mg, t_R = 42.4 min). Fr. A8 (20.5 g) was eluted by an Sephadex LH-20 [CH_2Cl_2/MeOH/H_2O (5:5:1, v/v)] and purified using semipreparative HPLC (MeOH/H_2O, 80:20, v/v, 3.0 mL/min) to yield compounds **5** (5.0 mg, t_R = 21.0 min). Fr. A12 was separated by repeated silica gel and purified using semipreparative HPLC (MeOH/H_2O, 80:20, v/v, 3.0 mL/min) to yield compounds **6** (3 mg, t_R= 17.8 min).

*(1S,2S)-Salvonitin B (**1**).* ^{1}H-NMR (600 MHz, MeOD): δ: 7.62 (1H, d, J = 8.4 Hz, H-7), 7.39 (1H, s, H-14), 7.16 (1H, d, J =8.4 Hz, H-6), 5.32 (1H, dd, J = 8.5, 2.0 Hz, H-2), 4.88 (1H, d, J = 2.0 Hz, H-1), 4.83 (1H, d, J = 8.5 Hz, H-3), 2.49 (3H, s, H-20), 1.87 (3H, s, H-18), 1.69 (3H, s, H-17), 1.69 (3H, s, H-16), 1.57 (3H, s, H-19). ^{13}C-NMR (150 MHz, MeOD) δ: 139.9 (C-12), 137.9 (C-4), 135.5 (C-13), 134.5 (C-11), 132.8 (C-5), 127.5 (C-7), 126.4 (C-6), 125.8 (C-8), 124.3 (C-10), 120.6 (C-9), 120.4 (C-3), 115.2 (C-14), 77.2 (C-2), 73.2 (C-15), 66.2 (C-1), 28.6 (C-17), 28.5 (C-16), 24.4 (C-19), 17.3 (C-18), 16.4 (C-20).

*(1S,2S)-Salvonitin C (**2**).* ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.64 (1H, d, J = 8.5 Hz, H-7), 7.32 (1H, s, H-14), 7.19 (1H, d, J = 8.5 Hz, H-6), 5.57 (1H, dd, J = 8.8, 1.8 Hz, H-2), 4.86 (1H, d, J = 8.7

Hz, H-3), 4.57 (1H, d, *J* = 1.8 Hz, H-1), 3.46 (3H, s, OCH_3), 2.50 (3H, s, H-20), 1.88 (3H, s, H-18), 1.75 (3H, s, H-17), 1.71 (3H, s, H-16), 1.59 (3H, s, H-19). ^{13}C-NMR (150 MHz, $CDCl_3$) *δ*: 138.6 (C-12), 138.0 (C-4), 133.6 (C-13), 133.1 (C-11), 133.0 (C-5), 127.1 (C-7), 126.0 (C-6), 124.9 (C-8), 121.1 (C-10), 119.5 (C-9), 119.0 (C-3), 114.5 (C-14), 73.7 (C-1), 73.0 (C-2), 72.5 (C-15), 55.0 (OCH_3), 29.0 (C-16), 28.3 (C-17), 24.7 (C-19), 17.7 (C-18), 17.3 (C-20).

*(2S)-Prionoid G (**3**).* ^{1}H-NMR (600 MHz, $CDCl_3$) *δ*: 7.80 (1H, d, *J* = 8.4 Hz, H-7), 7.36 (1H, s, H-14), 7.18 (1H, d, *J* = 8.5 Hz, H-6), 5.42 (1H, d, *J* = 8.7 Hz, H-2), 5.27 (1H, d, *J* = 8.8 Hz, H-3), 2.74 (3H, s, H-20), 1.82 (3H, s, H-18), 1.72 (3H, s, H-17), 1.70 (3H, s, H-16), 1.69 (3H, s, H-19). ^{13}C-NMR (150 MHz, $CDCl_3$) *δ*: 192.0 (C-1), 140.8 (C-4), 140.6 (C-12), 139.7 (C-5), 134.1 (C-13), 133.5 (C-11), 133.1 (C-7), 127.8 (C-6), 124.9 (C-8), 122.4 (C-9), 119.3 (C-10), 117.6 (C-3), 116.0 (C-14), 78.8 (C-2), 72.9 (C-15), 28.9 (C-16), 28.7 (C-17), 25.0 (C-19), 21.3 (C-20), 18.0 (C-18).

*(1S,2R)-Prionidipene F (**4**).* ^{1}H-NMR (600 MHz, $CDCl_3$) *δ*: 7.51 (1H, d, *J* = 8.4 Hz, H-7), 7.31 (1H, s, H-14), 7.12 (1H, d, *J* = 8.4 Hz, H-6), 5.25 (1H, d, *J*= 1.5 Hz, H-1), 4.97 (1H, d, *J* = 10.2 Hz, H-3), 4.11 (1H, dd, *J* = 10.1, 1.5 Hz, H-2), 3.44 (3H, s, OCH_3), 2.32 (3H, s, H-20), 1.89 (3H, s, H-18), 1.77 (3H, s, H-17), 1.76 (3H, s, H-16), 1.69 (3H, s, H-19). ^{13}C-NMR (150 MHz, $CDCl_3$) *δ*: 138.5 (C-12), 133.1 (C-13), 132.3 (C-11), 131.9 (C-4), 130.8 (C-5), 126.6 (C-6), 125.3 (C-8), 124.9 (C-10), 124.7 (C-7), 121.3 (C-3), 119.2 (C-9), 115.4 (C-14), 101.0 (C-1), 73.4 (C-15), 54.6 (OCH_3), 39.6 (C-2), 29.0 (C-16), 28.8 (C-17), 24.7 (C-19), 17.2 (C-18), 17.2 (C-20).

*Viroxocin B (**5**).* ^{1}H-NMR (600 MHz, $CDCl_3$) *δ*: 7.49 (1H, d, *J* = 8.3 Hz, H-7), 7.46 (1H, s, H-14), 7.12 (1H, d, *J* = 8.3 Hz, H-6), 3.91 (1H, m, H-1a), 2.93 (1H, m, H-1b), 2.43 (3H, s, H-20), 2.18 (1H, m, H-2a), 1.73 (3H, s, H-16), 1.70 (3H, s, H-17), 1.64 (3H, s, H-19), 1.64 (1H, m, H-2b), 1.59 (1H, m, H-3a), 1.25 (1H, m, H-3b), 1.16 (3H, s, H-18). ^{13}C-NMR (150 MHz, $CDCl_3$) *δ*:

146.6 (C-12), 135.2 (C-11), 133.6 (C-13), 133.5 (C-10), 131.8 (C-9), 130.3 (C-8), 127.5 (C-5), 127.4 (C-6), 126.4 (C-7), 120.5 (C-14), 85.4 (C-4), 72.6 (C-15), 33.3 (C-3), 29.5 (C-17), 29.3 (C-16), 27.5 (C-18), 26.5 (C-1), 26.3 (C-19), 23.0 (C-2), 19.9 (C-20).

*(XXX)-plebedipene E (**6**).* ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 6.92 (1H, d, J = 8.4 Hz, H-11), 6.51 (1H, d, J = 8.4 Hz, H-12), 4.54 (1H, s, H-20), 3.75 (1H, dd, J = 11.1, 2.7 Hz, H-18a), 3.34 (1H, m, H-15), 3.30 (1H, dd, J = 11.0, 1.4 Hz, H-18b), 3.09 (3H, s, OCH_3), 2.94 (1H, ddd, J = 16.8, 5.8, 1.8 Hz, H-7a), 2.61 (1H, ddd, J = 16.8, 12.1, 6.6 Hz, H-7b), 2.49 (1H, m, H-2a), 2.26 (1H, dtd, J = 13.6, 12.3, 5.5 Hz, H-6a), 2.19 (1H, dd, J = 13.7, 5.9 Hz, H-1), 1.77 (1H, m, H-6b), 1.72 (1H, m, H-3a), 1.59 (1H, m, H-2b), 1.42 (1H, m, H-3b), 1.38 (1H, m, H-1b), 1.36 (6H, dd, J = 7.1, 4.7 Hz, H-16, H-17), 1.33 (1H, d, J = 3.3 Hz, H-5), 0.79 (3H, s, H-19). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 152.1 (C-13), 136.2 (C-8), 134.5 (C-9), 130.9 (C-14), 123.6 (C-11), 114.2 (C-12), 105.9 (C-20), 66.3 (C-18), 55.3 (OCH_3), 45.7 (C-5), 40.7 (C-3), 40.6 (C-10), 39.4 (C-1), 32.7 (C-4), 28.1 (C-7), 27.2 (C-15), 23.6 (C-19), 21.9 (C-2), 20.5 (C-17), 20.4 (C-16), 19.5 (C-6).

(5R,10S)-Hispidanol C (7). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.59 (1H, s, H-11), 6.68 (1H, s, H-14), 2.99 (1H, m, H-7a), 2.87 (1H, m, H-7b), 2.74 (1H, m, H-2a), 2.62 (1H, m, H-2b), 2.61 (3H, s, H-16), 2.51 (1H, m, H-1a), 1.97 (1H, m, H-1b), 1.87 (1H, dd, J = 12.1, 2.8 Hz, H-5), 1.82 (2H, m, H-6), 1.31 (3H, s, H-20), 1.18 (3H, s, H-18), 1.15 (3H, s, H-19). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 216.5 (C-3), 203.9 (C-15), 159.7 (C-13), 145.4 (C-8), 138.9 (C-9), 127.5 (C-11), 118.6 (C-12), 117.4 (C-14), 50.6 (C-5), 47.4 (C-4), 37.7 (C-1), 36.9 (C-10), 34.6 (C-2), 31.1 (C-7), 26.6 (C-18), 26.5 (C-16), 25.0 (C-20), 21.2 (C-19), 19.8 (C-6).

X-ray Crystallographic Experiment Details. The crystal structures of compounds **7** was solved by direct methods using SHELXS-97. Refinements were performed with SHELXL-2013 using full-matrix least-squares calculations on F with anisotropic displacement parameters for all

the non-hydrogen atoms. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms.

Cell Culture, Viability Assay. RAW 264.7 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). RAW 264.7 cells were cultured in DMEM supplemented with 10% FBS, 100 UmL^{-1} penicillin, and 100 μgmL^{-1} streptomycin in a humidified incubator containing 5 % CO_2 and maintained at 37℃. The cells were seeded in 96-well plates for 1×10^4 cells per well. After cell attachment, the cells were treated with compounds (10 μM) and LPS (1 μg/mL) for 24 h. The cell morphology was observed and the cell vitality was detected using the MTT assay.

ASSOCIATED CONTENT

Supporting Information.

^{1}H NMR, ^{13}C NMR, HMBC, HSQC, ^{1}H-^{1}H COSY, ECD, and HR-ESI-MS data for compounds **1**−**7**, computational detaiSls and the detailed experimental conditions for the anti-inflammatory assay are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

1. Wang, W. Q.; Xuan, L. J. *Phytochemistry* **2016**, *122*, 119-125. DOI: 10.1016/j.phytochem.2015.11.014

2. Li, H. W. *J. Arnold Arbor.* **1988**, *69*, 289-400.

3. Li, C.-Y.; Gao, H.; Jiao, W.-H.; Zhang, L.; Zhou, G.-X.; Yao, X.-S. *Helv. Chim. Acta* **2010**, *93*, 450-456. DOI: 10.1002/hlca.200900219

4. Bartonkova, I.; Dvorak, Z. *Toxicol Lett.* **2018**, *296*, 1-9. DOI: 10.1016/j.toxlet.2018.07.023

5. Hu, X.-P.; Guo, Y.-P.; Chen, T.; Chen, E.-J.; Du, Y.; Zhang, Q.; Yi, P.; Yu, R.-R.; Zhi, Y.-S.; Li, C.-Y. *Phytochem. Lett.* **2022**, *52*, 76-81. DOI: 10.1016/j.phytol.2022.09.009

6. Lin, L.; Gao, Q.; Cui, C.; Zhao, H.; Fu, L.; Chen, L.; Yang, B.; Luo, W.; Zhao, M. *Food Chem.* **2012**, *131*, 1009-1014. DOI: 10.1016/j.foodchem.2011.09.105

7. Bai, N.; He, K.; Zhou, Z.; Lai, C.-S.; Zhang, L.; Quan, Z.; Shao, X.; Pan, M.-H.; Ho, C.-T. *Food Chem.* **2010**, *122*, 831-835. DOI: 10.1016/j.foodchem.2010.03.071

8. Lin, L-Z.; Cordell G. A.; Lin P. *Phytochemistry* **1995**, *40*, 1469-1471. DOI: 10.1016/0031-9422(95)00462-G

9. Lin, C. Z.; Zhao, W.; Feng, X. L.; Liu, F. L.; Zhu, C. C. *Fitoterapia* **2016**, *109*, 14-19. DOI: 10.1016/j.fitote.2015.11.015

10. Li, L.; Zhou, M.; Xue, G.; Wang, W.; Zhou, X.; Wang, X.; Kong, L.; Luo, J. *Bioorg. Chem.* **2018**, *81*, 454-460. DOI: 10.1016/j.bioorg.2018.08.040

11. Chang, J.; Xu, J.; Li, M.; Zhao, M.; Ding, J.; Zhang, J.-S. *Planta Med.* **2005**, *71*, 861-866. DOI: 10.1055/s-2005-871279

12. Naman, C. B.; Gromovsky, A. D.; Vela, C. M.; Fletcher, J. N.; Gupta, G.; Varikuti, S.; Zhu, X.; Zywot, E. M.; Chai, H.; Werbovetz, K. A.; Satoskar, A. R.; Kinghorn, A. D. *J. Nat. Prod.* **2016**, *79*, 598-606. DOI: 10.1021/acs.jnatprod.5b01131

13. Lin, S.; Zhang, Y.; Liu, M.; Yang, S.; Shi, J. *J. Nat. Prod.* **2010**, *73*, 1914-1921. DOI: 10.1021/np100583u

14. Huang, B.; Huang, Z. U.; Xiao, C. I.; Dong, X.; Jiang, B. *Helv. Chim. Acta* **2015**, *98*, 527-533. DOI: 10.1002/hlca.201400235