

Triterpenoids from *Alisma orientale* and their NF- κ B Inhibitory Activity



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ABSTRACT

The rhizome of *Alisma orientale* (sam.) Juz. is used in clinics for eliminating dampness, reducing edema, and promoting diuresis. This study aimed to elucidate the compounds and investigate their nuclear factor-kappa inhibitory activities in human embryonic kidney 293 cells. A new triterpene, alismaketone B (**1**); a new natural nortriterpene, noralisolic acid A (**2**); and 13 known protostane-type triterpenes were isolated from the rhizome of *A. orientale*. The new structures and their absolute configurations were established using HRESIMS, NMR, and electronic circular dichroism experiments. All isolated compounds were evaluated for their inhibitory activity on NF- κ B. The compounds **8**, **9**, **10**, and **14** showed moderate NF- κ B inhibitory activities with their IC₅₀ values being 64.7, 32.3, 47.3, and 37.3 μ M, respectively.

Introduction

Alisma orientale (Sam.) Juz. (Alismataceae) is an aquatic medicinal herb mainly harvested in Fujian, Jiangxi, and Sichuan, China. This plant's dried tubers (*Rhizoma alismatis*), known as "Zexie" in China [1], are often used in clinics for eliminating dampness, reducing edema, and promoting diuresis [2, 3]. Triterpenes [4, 5] and sesquiterpenes [6] are the major components in *A. orientale* tubes. Pharmacological studies showed that this plant's ethanol or water extracts have diuretic [7], lipid-lowering [8], liver-protecting [9],

ABBREVIATIONS

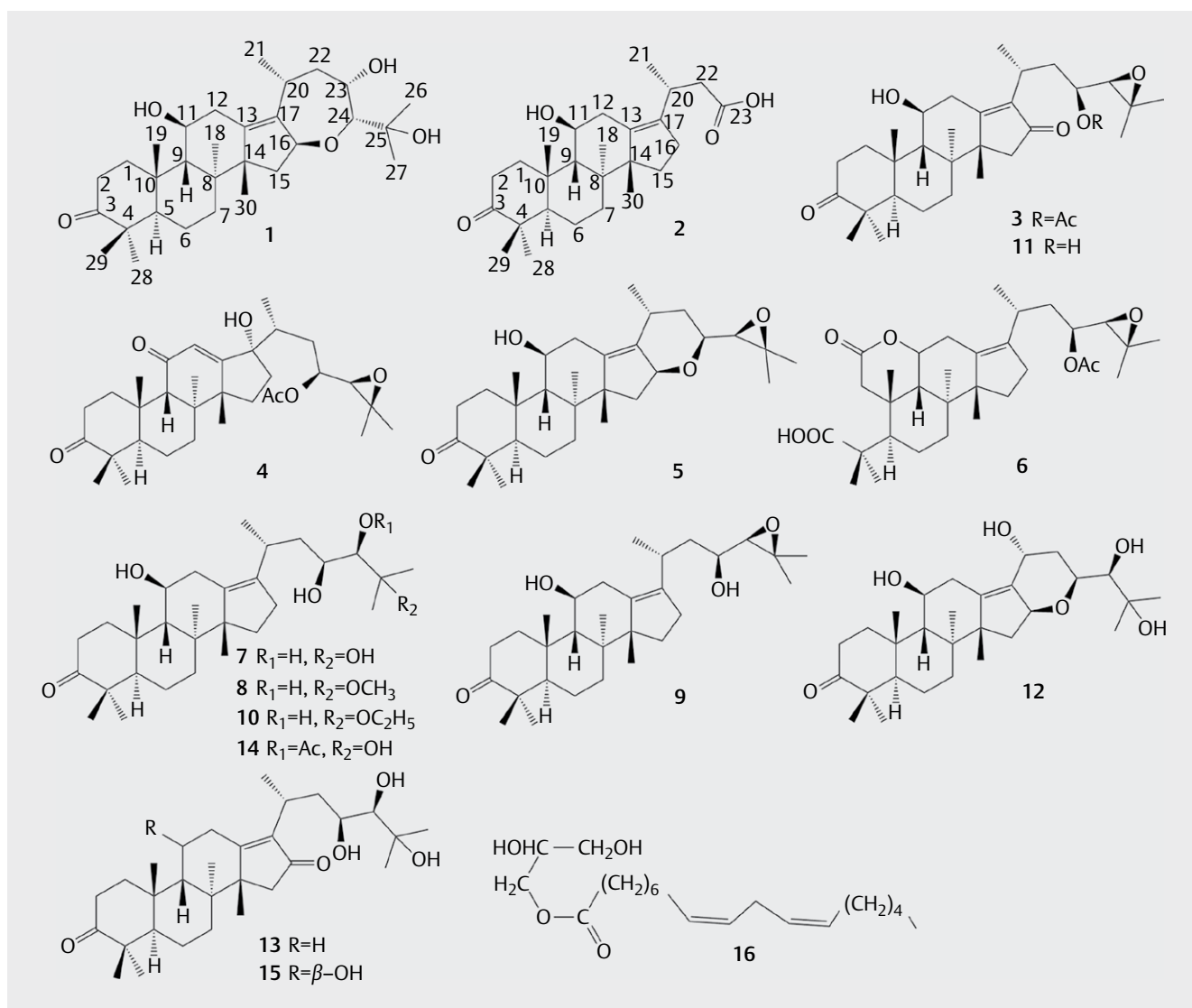
TCM	traditional Chinese medicine
NF- κ B	nuclear factor-kappa B
ECD	electronic circular dichroism
TDDFT	time-dependent density functional theory
CC	column chromatography

hypoglycemic [10], anti-inflammatory [11], and renal protective activities [12]. Besides, *A. orientale*'s ethanol extract attenuates lung inflammation in LPS-induced acute lung injury mice by suppressing NF- κ B and nuclear factor erythroid-2 related factor 2 activities [13, 14]. However, the *A. orientale*'s triterpenoids' inhibitory activities on NF- κ B are still to be studied.

In this study, 15 protostane-type triterpenoids (**1–15**), including a new triterpenoid, alismaketone B (**1**), and a new natural nortriterpene, noralisolic acid A (**2**), as well as a known fatty acid compound (**16**), were identified from *A. orientale*'s rhizomes (► **Fig. 1**). The new compounds' absolute structures were elucidated by various spectroscopic or spectrometric methods, including 1D and 2D NMR, HRESIMS, and ECD. HEK293/NF- κ B cells were used to investigate the 15 triterpenoids' NF- κ B inhibition potential, and the isolates' IC₅₀ values were evaluated for their activities. These compounds exhibited NF- κ B inhibition properties and could explain the plant's traditional use to treat renal diseases.

Results and Discussion

The rhizomes of *A. orientale* were extracted with 60% EtOH to produce a brown crude extract. The extract was separated using silica gel and ODS-A column chromatography as well as semi-preparative HPLC into a new triterpenoid (**1**), alismaketone B; a new natural nortriterpene, noralisolic acid A (**2**); and 14 known compounds (**3–16**). Compound identification was performed using mass spectrometry and NMR spectroscopy, and spectroscopic data were compared with reference compounds reported in the literature. The known compounds were identified as follows: alisol C 23-acetate (**3**) [15], alisol Q 23-acetate (**4**) [15], 16,23-oxido-alisol B (**5**) [16], alismalactone 23-acetate (**6**) [17], alisol A (**7**) [15], 25-O-methylalisol A (**8**) [18], alisol B (**9**) [15], 25-O-ethylalisol A (**10**) [18], alisol C (**11**) [16], alisol F (**12**) [19], 16-oxo-11-deoxy-alisol A (**13**) [16], alisol A 24-acetate (**14**) [15], 16-oxoalisol A (**15**) [16], and (9Z,12Z)-2,3-dihydroxypropyl octadecadienoate (**16**) [20].



► **Fig. 1** Compounds isolated from *A. orientale*.

Compound **1** was obtained as a yellow amorphous powder. This compound's molecular formula was established as $C_{30}H_{48}O_5$ with a positive HRESIMS (m/z 533.3499, calcd. 533.3478, $[M+HCOO]^-$). The 1H NMR spectrum (► **Table 1**) of **1** showed the presence of 8 methyl groups at δ_H 1.32 (s), 1.27 (s), 1.19 (s), 1.17 (d, $J=7.1$ Hz), 1.07 (s), 1.06 (s), 1.05 (s), and 0.89 (s) and 4 oxygenated methines at δ_H 5.13 (t, $J=6.5$ Hz), 4.10 (m), 3.28 (m), and 3.83 (m). The ^{13}C NMR spectrum displayed 30 signals, including a carbonyl carbon at δ_C 220.0; 2 olefinic carbons at δ_C 142.6 and 135.5; 4 oxygenated methine carbons at δ_C 69.9, 71.7, 76.6, and 83.6; and an oxygenated

quaternary carbon at δ_C 73.7. Furthermore, 4 quaternary carbons, 3 methines, 7 methylenes, and 8 methyls were determined using ^{13}C NMR and DEPT experiments. These data closely resembled those of alismaketones B 23-acetate [21], except for the absence of an acetyl group at C-23. Accordingly, compound **1** should have a hydroxyl group at C-23 instead of the 23-acetate of alismaketones B 23-acetate. The structure of compound **1** was confirmed by the correlations of H-26 (δ_H 1.32)/C-27 (δ_C 28.6), H-24 (δ_H 3.28)/C-27 (δ_C 28.6), H-23 (δ_H 4.10)/C-20 (δ_C 28.7), H-21 (δ_H 1.17)/C-22 (δ_C 41.2), and H-16 (δ_H 5.13)/C-24 (δ_C 76.6) observed in the HMBC spectrum, which we have shown in ► **Fig. 2**.

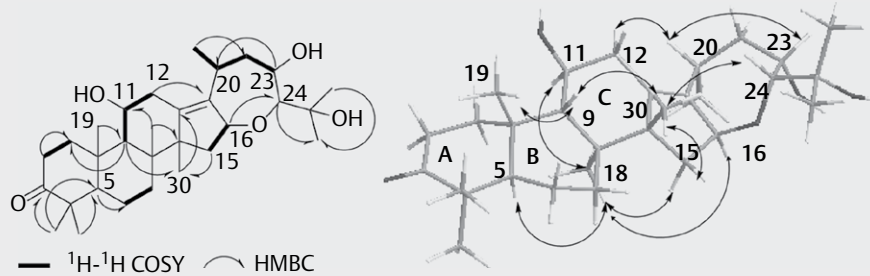
► **Table 1** 1H (400 MHz) and ^{13}C (100 MHz) NMR for compounds **1** and **2** in $CDCl_3$.

Pos.	1		2	
	δ_H, J (Hz)	δ_C	δ_H, J (Hz)	δ_C
1	2.20 m	30.7 (t)	2.23 m	31.0 (t)
	2.06 m		2.10 m	
2	2.67 m	33.5 (t)	2.66 m	33.7 (t)
	2.34 m		2.33 m	
3	–	220.0 (s)	–	220.4 (s)
4	–	46.9 (s)	–	47.0 (s)
5	2.11 m	48.2 (d)	2.08 m	48.5 (d)
6	1.47 m	19.9 (t)	1.45 m	20.0 (t)
	1.28 m		1.29 m	
7	2.02 m	34.4 (t)	2.70 m	34.3 (t)
	1.28 m		1.23 m	
8	–	40.6 (s)	–	40.5 (s)
9	1.75 d (10.7)	49.3 (d)	1.72 d (10.6)	49.6 (d)
10	–	36.9 (s)	–	36.9 (s)
11	3.83 m	69.9 (d)	3.85 m	70.0 (d)
12	2.64 m	34.1 (t)	2.76 m	34.4 (t)
	2.08 m		2.02 m	
13	–	142.6 (s)	–	137.3 (s)
14	–	54.8 (s)	–	57.0 (s)
15	2.32 m	40.0 (t)	1.87 m	30.5 (t)
	1.33 m		1.34 m	
16	5.13 t (6.5)	83.8 (d)	2.29 m	29.3 (t)
			2.17 m	
17	–	135.6 (s)	–	134.5 (s)
18	1.19 s	23.6 (q)	0.97 s	24.1 (q)
19	1.06 s	25.4 (q)	1.05 s	25.6 (q)
20	2.69 m	28.7 (d)	3.07 m	29.4 (d)
21	1.17 d (7.1)	20.8 (q)	1.06 d (7.1)	19.5 (q)
22	1.88 m	41.2 (t)	2.31 m	40.0 (t)
	1.70 m			
23	4.10 m	71.7 (d)	–	177.0 (s)
24	3.28 m	76.6 (d)	–	–
25	–	73.7 (s)	–	–
26	1.32 s	26.1 (q)	–	–
27	1.27 s	28.6 (q)	–	–
28	1.07 s	29.5 (q)	1.07 s	29.6 (q)
29	1.05 s	20.0 (q)	1.05 s	20.1 (q)
30	0.89 s	23.5 (q)	1.10 s	22.8 (q)

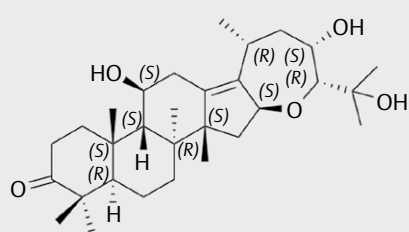
The relative configuration of compound **1** was established from the $^3J_{H,H}$ coupling value as well as NOESY data. A considerable $^3J_{9,11}$ value of 10.7 Hz indicated the axial-axial relationship of H-9 and H-11. As shown in ► **Fig. 2**, the NOESY correlations of Me-18 (δ_H 0.89) with H-5 (δ_H 2.11), H-11 (δ_H 3.83), H-16 (δ_H 5.13), and H-15 α (δ_H 2.32); the correlations of Me-30 (δ_H 1.19) with H-9 (δ_H 1.75) and H-24 (δ_H 3.28); and the correlation of H-24 (δ_H 3.28) with H-15 β (δ_H 1.33) indicated that H-5, H-11, H-16, and Me-18 were α -oriented, whereas H-9, H-24, and Me-30 were β -oriented. Additionally, the correlations of Me-19 (δ_H 1.06)/H-9 (δ_H 1.75), H-20 (δ_H 2.70)/H-23 (δ_H 4.10), H-11 (δ_H 3.83)/Me-21 (δ_H 1.17), and H-16 (δ_H 5.13)/Me-21 (δ_H 1.17) indicated the β -configurations of Me-19, H-20, and H-23. Compound **1**'s experimental ECD spectrum showed a positive cotton effect at 290 nm and a negative cotton effect at 205 nm. The comparison between the TDDFT calculated spectrum (B3LYP/6-311 G * level) and the experimental data showed good agreement with 5R, 8R, 9S, 10S, 11S, 14S, 15S, 20R, 23S, and 24R. Still, the alternative form revealed a curve with the opposite cotton effect (► **Fig. 3**). To the best of our knowledge, **1** was designated as the new protostane-type triterpenoid alismaketone B.

Compound **2** was obtained as a yellow amorphous powder. This compound's molecular formula was assigned as $C_{26}H_{40}O_4$ by negative HRESIMS at m/z 461.2883 $[M+HCOO]^-$ (calcd. 461.2903). The 1H -NMR spectrum (► **Table 1**) showed 6 tertiary methyl groups at δ_H 0.97 (s), 1.05 (s), 1.05 (s), 1.06 (d, $J=7.1$ Hz), 1.07 (s), and 1.10 (s) and an oxygenated proton signal at δ_H 3.85 (1H, m). The ^{13}C -NMR data displayed signals for 2 carbonyls at δ_C 220.4 and 177.0. Compound **2**'s 1H and ^{13}C NMR spectroscopic data were highly similar to those reported for alisol A (**7**) [15], except for the absence of oxygenated carbon signals at C-23, C-24, and C-25, and the presence of an additional carbonyl group (δ_C 177.0). Accordingly, **2** should have a carboxyl group at C-23 instead of the triol unit of alisol A (**7**). This feature is consistent with HMBC correlations observed (► **Fig. 4**) between H-22 (δ_H 2.31) and C-23 (δ_C 177.0), C-21 (δ_C 40.0) and C-17 (δ_C 134.5), H-20 (δ_H 3.07) and C-23 (δ_C 177.0), as well as between H-20 (δ_H 3.07) and C-16 (δ_C 29.3).

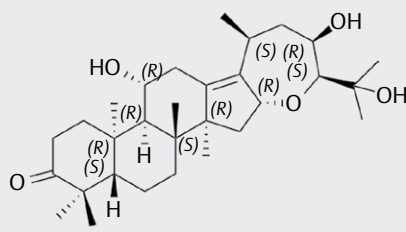
A considerable $^3J_{9,11}$ value of 10.6 Hz indicated the axial-axial relationship of H-9 and H-11. The NOESY correlations of Me-18 (δ_H 0.97)/H-11 (δ_H 3.85), H-11/H-5 (δ_H 2.08), Me-18/H-15 α (δ_H 1.87), Me-30 (δ_H 1.10)/H-9 (δ_H 1.72), Me-30/H-15 β (δ_H 1.34), and H-9/Me-19 (δ_H 1.05) supported the chair, boat, and chair conformations of rings A, B, and C, as well as their ring junction's *trans-cis-trans* relationships (► **Fig. 4**). The experimental ECD spectrum of **2** showed a positive cotton effect at 293 nm and a negative cotton effect at 203 nm. A comparison of the TDDFT-calculated spectrum (B3LYP/6–



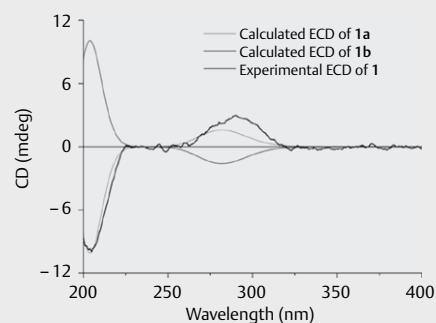
► **Fig. 2** Key ^1H - ^1H COSY, HMBC (H→C) and NOESY (↔) correlations of compound **1**.



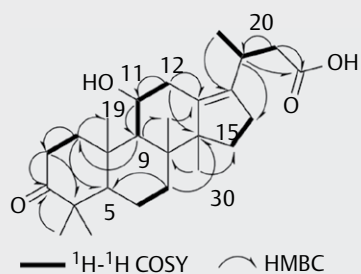
1a



1b



► **Fig. 3** Experimental and calculated ECD spectra of **1** in MeOH.



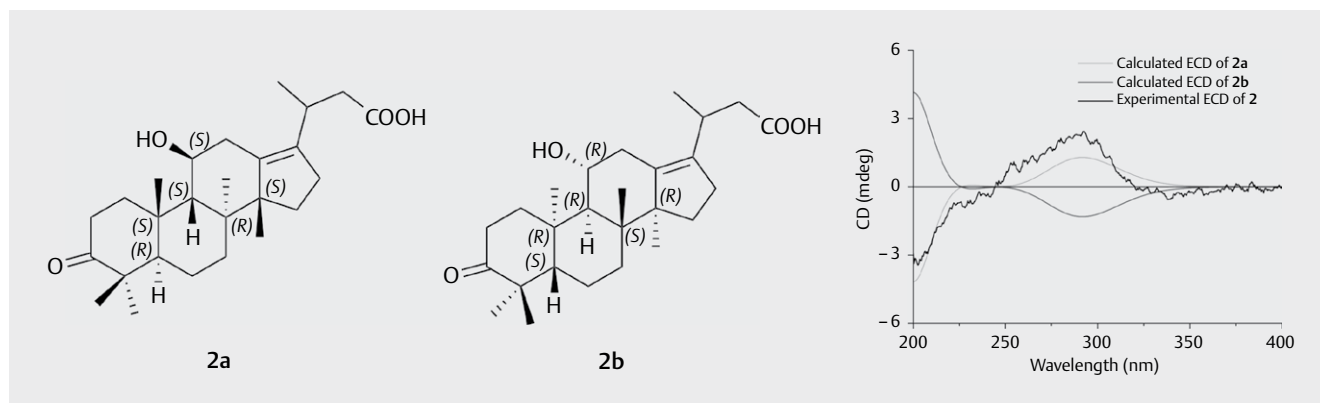
► **Fig. 4** Key ^1H - ^1H COSY, HMBC (H→C) and NOESY (↔) correlations of compound **2**.

311 G* level) and the experimental data showed good agreement with 5*R*, 8*R*, 9*S*, 10*S*, 11*S*, and 14*S*, while the alternative form revealed a curve with the opposite cotton effect (► **Fig. 5**). Besides, the absolute configuration at C-20 was considered to be *R* based on the biogenesis of the protostane skeleton [22]. For the first time, the absolute configuration of **2** was discussed. Compound **2** was assigned as a new natural nortriterpenoid noralisolic acid A.

Lee reported the synthesis of noralisolic acid A, and its structure was assigned based on IR analysis and limited NMR data [23]. The ^{13}C -NMR data of synthetic noralisolic acid A closely resembled those of compound **2**, except for the lower field shift of the signal of C-22 (δ_{C} 57.7). However, this assignment of C-22's chemical shift was considered incorrect based on the following evidence: (1) the

methylene carbon attached to the carboxyl group may give a signal at approximately δ_{C} 38.0 [24]; (2) an HMBC correlation from H-22 (δ_{H} 2.31) to C-23 (δ_{C} 177.0) permitted the carboxyl group of compound **2** to be attached to C-22.

Compounds **1**–**15** were evaluated for their NF- κ B inhibition activity in TNF- α stimulated HEK 293 cell line. Triterpenoids **8**–**10** and **14** showed moderate inhibitory activity with IC_{50} values of 64.7, 32.3, 47.3, and 37.3 μM , respectively. However, the other compounds displayed mild NF- κ B inhibition at a maximum tested concentration of 50 μM . Additionally, the IC_{50} of IMD-0354 was 13.3 μM , which was proved that the test system and calculation method were feasible. The results were shown in ► **Table 2** and ► **Fig. 6**. The results indicated that the sp^3 hybrid C-16 of Alisma



► **Fig. 5** Experimental and calculated ECD spectra of compound **2** in MeOH.

► **Table 2** NF- κ B cell line HEK293 inhibitory activities of active compounds.

compound	inhibition rate (%)	IC ₅₀ (μ M)
8	58.4 \pm 0.0 ^a	64.7 (C.I. 33.4–139.2)
9	94.8 \pm 0.4 ^a	47.3 (C.I. 29.2–78.3)
10	51.7 \pm 3.7 ^a	47.3 (C.I. 29.2–78.3)
14	64.8 \pm 3.8 ^a	37.3 (C.I. 24.3–59.5)
IMD0354	57.6 \pm 2.7 ^b	13.3 (C.I. 10.2–17.6)

^a Measured in 50 μ M; ^b Measured in 30 μ M. IC₅₀ was afforded with confidence interval (n = 2); C.I.: 95% confidence interval. Positive control: IMD-0354.

triterpenoids (**8–10** vs. **3**, **11**, **13**, and **15**) is essential for NF- κ B inhibition. The side-chain cyclization at C-17 depleted the NF- κ B inhibition (**9** vs. **5**). The hydroxyl groups at C-23 and the less hydrophilic groups at C-24/25 (-OAc or epoxy group) contributed to the NF- κ B inhibitory activity (**9** and **14** vs. **3**, **4**, **6**, **8**, **10**, **13**, and **15**) (► **Fig. 7**).

It has been reported that ethanol extract of *A. orientale* tuber could suppress LPS-induced NF- κ B activity and NF- κ B dependent gene expression in RAW 264.7 cells. Alisol B (**9**) was detected in EEAO by HPLC analysis but was not tested for its NF- κ B inhibitory activity [13]. Our results confirmed that not only alisol B (**9**) but also 25-O-methylalisol A (**8**), 25-O-ethylalisol A (**10**), and alisol A 24-acetate (**14**) were involved in NF- κ B activity. Compounds **9** and **14** were also reported to show anti-inflammatory and anti-allergic activities *via* inhibiting CD147 and MMP-9 secretion and leukotriene production [25–27].

In addition, compounds **1–15** were also tested for their antihuman dihydroorotate dehydrogenase activity by the reported method [28]. Nonetheless, they did not show any benefit (Data not shown).

Materials and Methods

General experimental procedures

IR spectra were recorded on Chiral IR-2X vibrational circular dichroism spectrometer in deuterated chloroform. The optical rotation

was determined on JASCO P-2000 automatic polarimeter in MeOH. NMR spectra were measured on Bruker Avance DRX-400. HRESIMS was performed on Waters Xevo G2-XS Q-TOF mass spectrometer, and the semi-preparative HPLC was performed on Chuangxin Tongheng 3050 N HPLC system with a C₁₈ column (YMC-Pack ODS-A, 5 μ m, 10.0 \times 250 mm). The ECD spectra were acquired in MeOH using JASCO J-815 circular dichroism spectrometer. Chemiluminescence measurements for the NF- κ B cell line HEK293 inhibition assay were recorded on Envision (PerkinElmer).

Solvents and chemicals

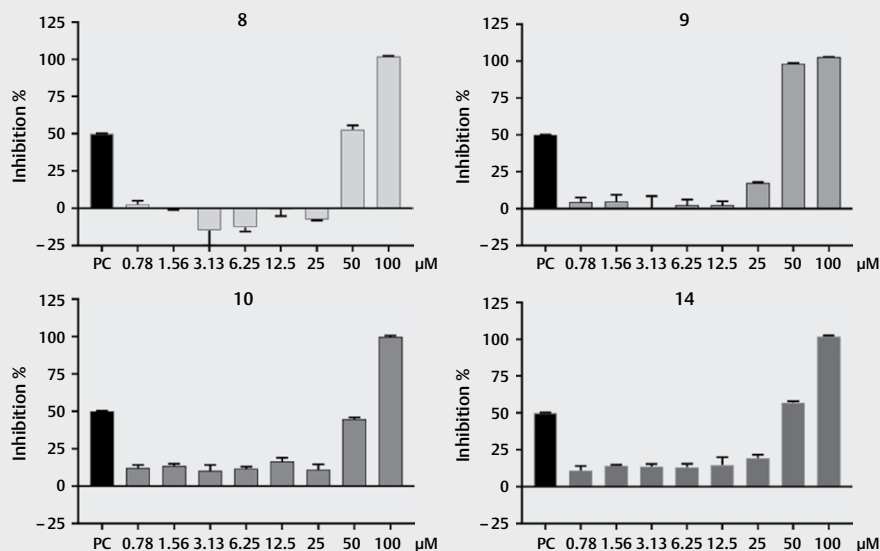
IMD-0354 (purity \geq 99%) was purchased from Aladdin. DMEM was acquired from BI and FBS from Gibco. Penicillin and streptomycin (P/S) were obtained from Procell. Bright-Glo was gifted by Promega. TNF- α was purchased from Peprotech. DMSO was acquired from Sigma. The solvents used for extraction and chromatographic separation were of analytical purity.

Plant material

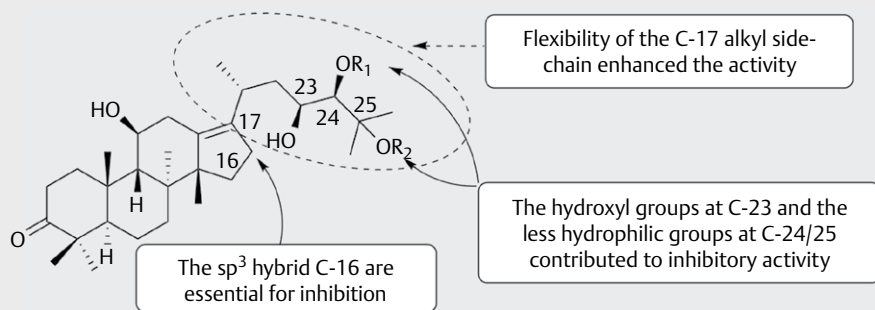
The rhizomes of *A. orientale* were sampled from Pengshan County, Sichuan Province in China, in November 2018 and authenticated by Prof. Tong Wu from Pharmacognosy Department. A voucher specimen (20190409) was deposited in the Shanghai Institute of Pharmaceutical Industry.

Extraction and isolation

The air-dried and powdered rhizomes (5.0 kg) of *A. orientale* were extracted with 60% EtOH (15 L \times 3) for 3 times (2 h each time) under reflux. The combined solution was concentrated to crudeness *in vacuo*, and the crude extract was suspended in water (3 L), sequentially partitioning it with petroleum ether (60–90 $^{\circ}$ C) and dichloromethane (3 \times 3 L each), respectively. The dichloromethane extract (120.4 g) was chromatographed on a silica gel column (CC) (6 \times 72 cm; 200–300 mesh) and eluted by petroleum ether/ethyl acetate (50:1, 20:1, 10:1, 4:1, 2:1, 1:1, v/v) to give 8 fractions (Fr.1–8) based on TLC analyses. Fr.4 (11.17 g) was subjected to an ODS-A gel CC (6.6 \times 20 cm; S-50 μ m, 12 nm; YMC) and eluted by MeOH/H₂O (1:9, 3:7, 1:1, 7:3, 9:1, and 1:0 v/v) to yield 9 fractions (Fr.4a–4i). Fr.4b (1.73 g) was purified using semi-preparative HPLC (60% CH₃CN in water; 4.0 mL/min; UV detection at λ 210 nm) to isolate



► **Fig. 6** Inhibitory activity of compounds **8**, **9**, **10**, and **14** in TNF- α stimulated HEK293 cells. HEK293 cells were treated with indicated doses of constituents for 24 h, and then chemiluminescence was determined. IMD-0354 was used as a positive control and possessed an inhibitory rate of approximately 50% at 15 μ M. Experiments were performed in duplicate, and data were represented as means \pm SD.



► **Fig. 7** Protostane-type triterpenoids' structure-activity relationships.

compound **3** (326.0 mg, t_R = 21 min). Fr.4d (0.79 g) was purified *via* semi-preparative HPLC (50% CH₃CN in water; 4.0 mL/min; UV detection at λ 210 nm) to afford compounds **4** (16.5 mg, t_R = 37 min) and **5** (10.2 mg, t_R = 42 min). Compounds **6** (62.4 mg, t_R = 65.2 min) and **2** (22.9 mg, t_R = 27.9 min; 97% purity by HPLC) were obtained from Fr.4f (0.44 g) through semi-preparative HPLC eluted using CH₃CN/H₂O (78:22, v/v; 3.0 mL/min; UV detection at λ 210 nm). Fr.4h (2.96 g) was subjected to semi-preparative HPLC with CH₃CN/H₂O (60:40–80:20, v/v, 90 min; 4.0 mL/min; UV detection at λ 210 nm) to obtain compounds **7–10** (62.5, 85.8, 228.5, and 34.5 mg, respectively; t_R = 14.7, 28.2, 31.6, and 41.2 min). Compound **16** (17.3 mg, t_R = 21.3 min) was purified from Fr.4i (0.32 g) by semi-preparative HPLC (80% CH₃CN in water; 4.0 mL/min; UV detection at λ 210 nm). Fr.6 (6.45 g) was subjected to an ODS-A gel CC eluted with MeOH/H₂O (1:9, 3:7, 1:1, 7:3, 9:1, and 1:0, v/v) to obtain 6 fractions (Fr.6a–6f). Fr.6b (1.17 g) was further purified *via* semi-preparative HPLC (40% CH₃CN in water; 3.0 mL/min; UV de-

tection at λ 210 nm) to obtain compounds **11**, **12**, **13** (150.0, 30.0, and 26.0 mg, t_R = 24, 56 and 31 min) and **1** (16.1 mg, t_R = 33 min; 93% purity by HPLC). Compound **14** (12.1 mg, t_R = 29 min) was obtained from Fr.6d (0.37 g) and purified using semi-preparative HPLC eluted with CH₃CN/H₂O (57:43, v/v; 3.0 mL/min; UV detection at λ 210 nm). Finally, Fr.6e (0.25 g) was subjected to semi-preparative HPLC with CH₃CN/H₂O (35:65, v/v; 3.0 mL/min; UV detection at λ 210 nm) to obtain compound **15** (40.1 mg, t_R = 21.1 min).

Alismaketone B (1): yellow amorphous powder; $[\alpha]_D^{24}$ + 53.4 (c 0.02, MeOH); ECD (MeOH) λ_{max} ($\Delta\epsilon$) 290 (+ 2.98), 205 (– 9.96); IR (CDCl₃) ν_{max} 3390, 2941, 1695 cm⁻¹; ¹H and ¹³C NMR spectroscopic data (see ► **Tables 1**); HRESIMS at m/z 533.3499 [M + HCOO]⁻ (calcd. for C₃₁H₄₉O₇, 533.3478).

Noralisolic acid A (2): yellow amorphous powder; $[\alpha]_D^{24}$ + 57.0 (c 0.04, MeOH); ECD (MeOH) λ_{max} ($\Delta\epsilon$) 293 (+ 2.44), 203 (– 3.43); IR (CDCl₃) ν_{max} 3444, 2941, 1747, 1699, 1654 cm⁻¹; ¹H and ¹³C NMR spectroscopic data (see ► **Tables 1**); HRESIMS at m/z 415.2817

$[M-H]^-$, 831.5751 $[2M-H]^-$, 461.2883 $[M+HCOO]^-$ (calcd. for $C_{27}H_{41}O_6$, 461.2903).

ECD calculation

Computational calculations were performed using the Gaussian 09 software. Conformers were generated by the MMFF94 force field and were obtained at 6 kcal/mol. Conformational analyses and geometry optimizations were performed at the B3LYP/6-311 G * level in MeOH. ECD calculations were performed by TDDFT at the B3LYP/6-311 G * level in MeOH. ECD spectra were obtained by weighing each geometric conformation's Boltzmann distribution rate with a bandwidth σ of 0.3 eV (**1**) or 0.4 eV (**2**).

Inhibitory effects on NF- κ B cell line HEK293

The NF- κ B activity in the TNF- α stimulated HEK293 cell line was used to evaluate the inhibitory effects of all these isolates using the modified Bright-Glo method [29]. Compounds **1–15** were dissolved in DMSO to prepare a stock solution, and then each stock solution was serially diluted 2-fold to different concentrations using a culture medium. HEK293 cells were cultured in a DMEM medium plus 10% FBS and 1% P/S were seeded in 96-well plates at a density of 40,000 cells/well with 80 μ L. After incubating at 37 °C under 5% CO₂ overnight, the tested compounds (10 μ L) were added to the wells. They were incubated at the same conditions for 2 h, and TNF- α (10 μ L, 20 ng/mL) was then added to each well and incubated in the darkness for 24 h. Chemiluminescence was measured on Envision after the addition of 50 μ L Bright-Glo Luciferase Assay Reagent to each well.

$$\text{Inhibition (\%)} = 100 - (B/A \times 100)$$

Where, B was the luminescence value of tested compounds, and A was luminescence of the negative control group (with cells, medium, DMSO, and TNF- α).

Data were analyzed by GraphPad Prism v.7.0 and were presented as a geometric means with 95% confidence intervals of 2 independent experiments. IMD-0354 was used as a positive control and DMSO as a negative control.

Supporting Information

The 1D/2D NMR and HREIMS spectra of compounds **1** and **2**, their optimized geometries, compounds **1** and **2**'s calculated conformers' ECD spectra, the triterpenoid's NF- κ B cell line HEK293 inhibitory activities, as well as the compounds **3–16** experimental NMR data, are available as Supporting Information.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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