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Monomeric and dimeric sesquiterpene lactones from *Artemisia heptapotamica*

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[ABSTRACT] One new dimeric (**1**) and two monomeric sesquiterpene lactones (**5** and **13**), together with 10 known compounds (**2**–**4**, **6**–**12**), were isolated from *Artemisia heptapotamica* collected in Almaty region of Kazakhstan. All compounds were isolated from this plant for the first time. The structures of the new compounds were mainly achieved by extensive analysis of MS, 1D and 2D NMR spectroscopic data, and ECD spectrum as well. The inhibitory activities of all isolates against activation of NF-κB induced by LPS were assessed on a THP1-Dual cell model. Some of them showed strong inhibitory activity with IC_{50} values ranging from 2 to 25 μmol·L^{-1}.

[KEY WORDS] *Artemisia heptapotamica*; Artemisiane E; Artemdubolide I; Ajaniaolide B; Sesquiterpene lactone; Anti-inflammation

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Introduction

Natural compounds play an important role in treating human diseases, and in recent years have received much attention of scientists and pharmaceutical industries [1], as referring to three clinical drugs, taxol, etoposide and, artemisinin. Artemisinin is a sesquiterpene lactone with unique structure that was firstly isolated from a Chinese herb *Artemisia annua* L, which was recorded as an anti-malarial agent in ancient medicinal books.

The genus *Artemisia* L., usually represented by small herbs and shrubs, is one of the largest genera in the Asteraceae family consisting of more than 500 species, which is predominantly distributed in the northern temperate region of the world. Many species have been used since ancient times as folk remedies in various treatments (reducing phlegm, relieving cough, invigorating blood circulation, and etc) [2]. According to the literature, over 260 *Artemisia* species have been investigated, revealing that they contain many classes of secondary metabolites including sesquiterpene lactones. As reported, some substances from the genus showed antimalarial, antiviral, antitumour, antihemorragic, anticoagulant, antioxidant, and antiulcerogenic activities [3].

Over 6000 plant species grow in territory of the Republic of Kazakhstan, among which 667 are endemic ones. A total of 133 plant species from the Asteraceae family were investigated during the period from 1996 to 2015. As a result, 61 sesquiterpene lactones were isolated and identified, including 32 compounds of guaiane type, 15 germacranes, 11 eudesmanes, and 3 pseudoguaianes. Inclusively, 3 dimeric sesquiterpene lactones were reported. Twelve out of the identified sesquiterpene lactones are previously undescribed [4-5].

Aetemisia heptapotamica is a plant endemic in the northern area of the Tian Shan Mountain, and has not been investigated chemically before. In our continuing effort to seek bioactive secondary metabolites from the *Artemisia* genus, *A. heptaptamica* collected in Almaty region of Kazakhstan was systematically investigated, resulting in the isolation of one

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new dimeric (**1**) and two new monomeric sesquiterpene lactones (**5** and **13**), together with 10 known compounds including three dimers (**2**–**4**), four guaianolides (**6**–**9**), and three *seco*-guianolides (**10**–**12**). All compounds were isolated from this plant for the first time. Here in, we report the isolation and structural elucidation of these new compounds, as well as their inhibitory activities against activation of NF-κB induced by LPS on a THP1-Dual cell model.

Results and Discussion

Compound **1**, obtained as a colorless gum, had the molecular formula of $C_{30}H_{36}O_{11}$ on the basic analysis of HR-ESI-MS data (*m/z* 617.2242, $[M + COOH]^-$, Calcd. for 617.2240). The IR absorptions suggested the presence of hydroxyl (3439 cm^{-1}), γ-lactone (1768 cm^{-1}), and double bonds (1657 cm^{-1}). The ^1H NMR data (Table 1) showed signals of four methyls (δ_H 1.28, 1.40, 1.47, 2.11, each, s), two exocyclic methylene groups (δ_H 6.13, 6.25, each, br d, J = 3.0 Hz; 5.72, 6.22, each, br s), and a *cis*-double bond (δ_H 6.29, 7.35, each, d, J = 5.9 Hz) (Table 1). The ^{13}C and DEPT NMR data (Table 1) displayed 30 carbon resonances ascribed to four methyls (δ_C 21.1, 26.5, 27.3 and 30.0), five methylenes (δ_C 29.6, 41.1, 43.8, 122.2 and 127.5), ten methines (δ_C 38.0, 52.7, 58.4, 62.1, 70.8, 75.8, 79.2, 133.4, 134.7 and 163.9), and eleven quaternary carbons (δ_C 73.0, 74.3, 76.3, 81.8, 138.3, 141.5, 142.0, 164.9, 169.4, 194.6 and 209.8). All these data suggested that compound **1** might be a sesquiterpene lactone dimer constructed by two different monomeric units.

Table 1 ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data for compound 1 in $CDCl_3$

No.	δ_H (multi, J in Hz)	δ_C	No.	δ_H (multi, J in Hz)	δ_C
1	–	73.0 (C)	1′	–	194.6 (C)
2	3.63 (br s)	62.1 (CH)	2′	6.29 (d, J = 6.0 Hz)	133.4 (CH)
3	5.03 (d, J = 1.1 Hz)	79.2 (CH)	3′	7.35 (dd, J = 6.0, 0.9 Hz)	163.9 (CH)
4	–	81.8 (C)	4′	–	76.3 (C)
5	2.77 (d, J = 11.5 Hz)	58.4 (CH)	5′	–	142.0 (C)
6	4.16 (dd, J = 11.5, 9.5 Hz)	75.8 (CH)	6′	6.51 (dd, J = 11.1, 0.9 Hz)	134.7 (CH)
7	3.66 (m)	52.7 (CH)	7′	2.02 (m, 1H) 4.14 (m)	38.0 (CH)
8	4.04 (m)	70.8 (CH)	8′	1.81 (m)	29.6 (CH_2)
9	2.27 (dd, J = 15.9, 1.9 Hz) 2.04 (dd, J = 15.9, 5.1 Hz)	43.8 (CH_2)	9′	2.44 (t, J = 7.0 Hz, 2H)	41.1 (CH_2)
10	–	74.3 (C)	10′	–	209.8 (C)
11	–	138.3 (C)	11′	–	141.5 (C)
12	–	169.4 (C)	12′	–	164.9 (C)
13	6.25 (br d, J = 3.0 Hz); 6.13 (br d, J = 3.0 Hz)	122.2 (CH_2)	13′	6.22 (br s) 5.72 (br s)	127.5 (CH_2)
14	1.28 (s, 3H)	27.3 (CH_3)	14′	1.47 (s, 3H)	26.5 (CH_3)
15	1.40 (s, 3H)	21.1 (CH_3)	15′	2.11 (s, 3H)	30.0 (CH_3)

A comparison of NMR data of **1** and the known compound artemisiane A (**2**), reported from *A. argyi* and also obtained in this study [6], revealed high similarities between these two compounds, except for an oxygenated methane (δ_H 4.04, m; δ_C 70.8) rather than a methylene present in **1**. Detailed analysis of the 2D NMR data of **1** further established the structure. Starting from the proton resonance at δ_H 4.16 (dd, J = 11.5, 9.5 Hz, H-6), the HMBC correlations from H-6 to C-4 and C-8, H_2-13 to C-7 and C-12, H_2-9 to C-14, H-5 to C-2 and C-3, and H-3 to C-1, together with succeeding ^1H–^1H COSY correlations of H-5/H-6/H-7/H-8/H_2-9 specified the moiety of unit A with a hydroxy attached to C-8 (Fig. 2). ^1H–^1H COSY correlations of H-2′/H-3′ and HMBC correlations from H-2′ to C-4′ and C-5′, H-3′ to C-1′, C-5′ and C-14′, H-6′ to C-1′, C-4′, C-8′ and C-11′, H-7′ to C-9′, C-12′ and C-13′, H_2-8′ to C-10′ and C-11′, H_2-9′ to C-15′, H_2-13′ to C-12′, and H_3-14′ to C-5′ constructed the moiety of unit B (Fig. 2). The key HMBC correlation between H-3 and C-12′ further confirmed that these two units were linked through a C-3-*O*-C-12′ ester bond (Fig. 2). Thus, the planar structure of compound **1** was established as an 8-hydroxyl derivative of artemisiane A.

The relative configuration of compound **1** was confirmed by the NOESY correlations combined with the molecular modeling simulations (Fig. 3). For the unit A, as it was determined as a guaianolide moiety, H-5 is supposed generally to be *α*-oriented, thereof H-6 *β*-oriented and H-7 *α*-oriented, appropriately, in consequence of their large coupling constants ($J_{H\text{-}5,\,H\text{-}6}$ = 11.5 Hz, $J_{H\text{-}6,\,H\text{-}7}$ = 9.5 Hz). The orientation of H-5 and H-7 can be further verified by the observed NOESY correlations between them (Fig. 3). The correlations of H-6/H-8, H-6/H-9*β*, and H-6/H_3-15 indicated that H-8, H-9, and CH_3-4 were also *β*-oriented, which ambiguously set a conformer as shown in Fig. 3. Consequently, the correlation of H-9/H-14 and H-15/H-3 defined the orientation of H-14 and H-3 as *β*, too. The relative configuration of H-2 remained unclear because only a correlation of H-2 and H-14 were found, which could not be used to fix its configuration due to the fact that CH_3-10 was located at a flexible seven-membered ring. According to the generated modeling, we finally

placed H-2 at the α orientation based on the very small coupling constant between H-2 and H-3 (1.1 Hz), which results from their dihedral angle approaching to 90. Therefore, the epoxide located at C-1 and C-2 was established as β-oriented. In unit B, the ROESY correlations of H-7/H-14' and H-6'/H-13' assigned a 5'*E* geometry. The relative configuration of unit B was confirmed to be the same as the counterpart of artemisiane A (**2**), inferred not only from the mostly overlapping spectroscopic data, but also the identical Cotton effects in their ECD spectra (Fig. 4) [6, 7]. Therefore, the full structure of compound **1** was constructed and named artemisiane E.

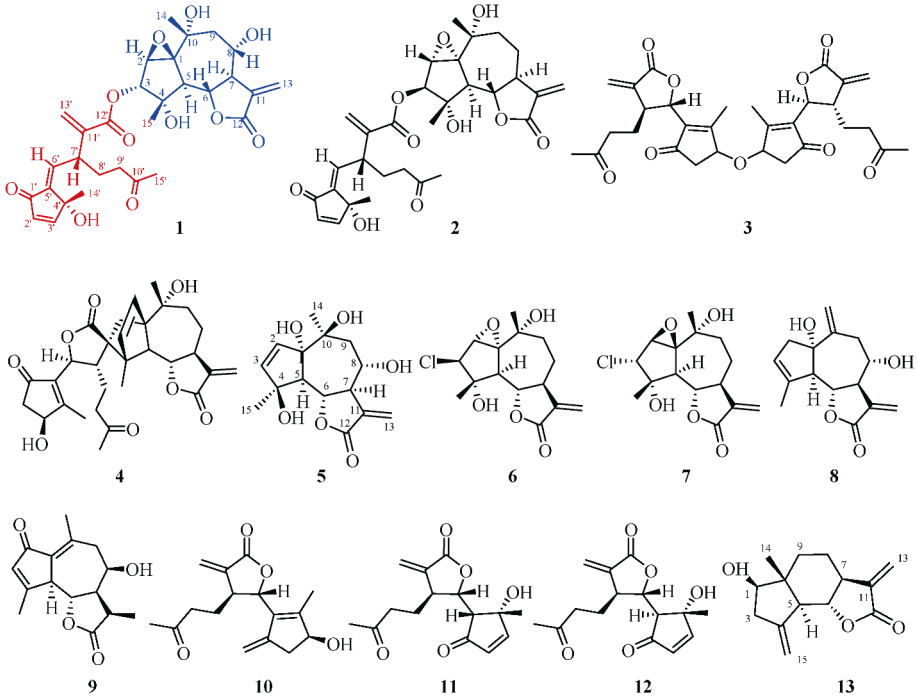

Fig. 1 Structures of compounds 1–13 isolated from *A. heptapotamica*

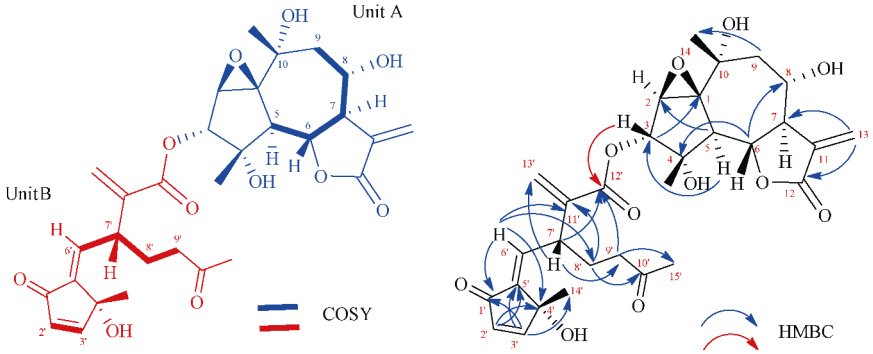

Fig. 2 Key ^{1}H-^{1}H COSY (bold line) and HMBC correlations (H→C) of compound 1

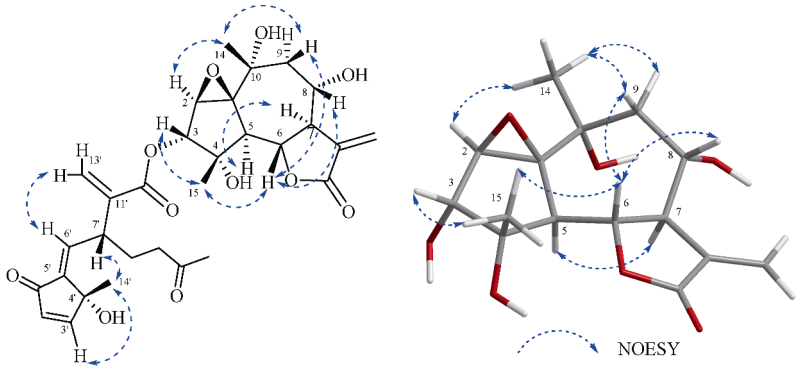

Fig. 3 The NOESY correlations of compound 1 and 3D NOESY correlations of unit A of compound 1

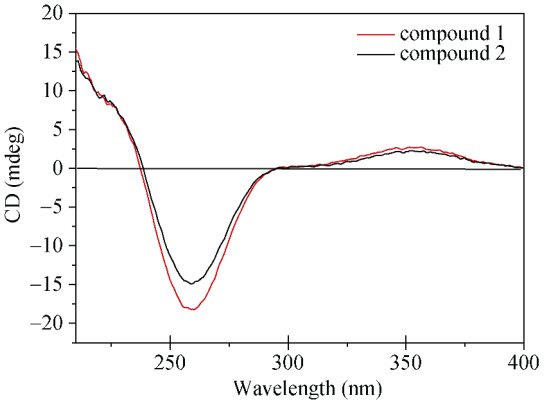

Fig. 4 Comparison of the experimental ECD spectra for compounds 1 and 2 in MeOH

Compound **5**, obtained as a colorless gum, had the molecular formula of $C_{15}H_{20}O_6$ on the basis analysis of HR-ESI-MS data. The IR absorption suggested the presence of hydroxyl (3444 cm^{-1}), γ-lactone (1742 cm^{-1}) signals. The ^{1}H-NMR spectrum results (Table 2) indicated presence of two methyls (δ_H 1.66, 1.78, each s), one exocyclic methylene group (δ_H 6.56, 6.85, each dd, J = 2.8 Hz) and characteristic signals of a *cis*-double bond (6.17, 6.21, each d, J = 5.8 Hz). The ^{13}C and DEPT NMR spectra displayed 15 carbon resonances (Table 2) including two methyls (δ_C 27.2 and 29.3), two methylenes (δ_C 48.7 and 124.5), six methines (δ_C 48.3, 64.9, 70.6, 79.0, 80.5, 137.6, 140.9), and five quaternary carbons (δ_C 74.7, 80.5, 88.8, 138.9, 171.7). These data suggested that compound **5** might be a sesquiterpene lactone of guaianolide type. The HMBC correlations (Fig. 5) from H-6 (δ_H 5.46, dd, J = 10.2, 9.8 Hz) to C-4 and C-8, H_2-13 to C-7 and C-12, H_2-9 to C-1, C-7 and C-14, H-5 to C-10 and C-15, H-3 to C-1, and H-1 to C-2, together with the succeeding ^{1}H-^{1}H COSY correlations of H-2/H-3/H-5/H-6/ H-7/H-8/H_2-9, further specified the structure of **5**, which is closely related to the known compound Artemdubolide G isolated from *A. dubia* [8]. Comparison of these two compounds revealed that a hydroxy rather than an acetyl group of Artemdubolide G was attached to C-8 (δ_H 4.56, δ_C 70.6 *vs* δ_H 5.17, δ_C 73.8) in compound **5**. The relative configuration of **5** was established by the ROESY experiment (Fig. 5). The ROESY correlations of H-6/H-8, H-5/OH-1, H-5/H-7, and H-5/H-15 defined H-5, H-7, H_3-15, and OH-1 were α-oriented while H-6 and H-8 were β-oriented. In addition, the strong ROESY correlations of H-6/OH-10 and H-8/OH-10 indicated a β-orientation for OH-10. Accordingly, the structure of **5** was determined and named artemdubolide I.

Table 2 ^{1}H and ^{13}C NMR (125 MHz) spectroscopic data for compounds 5 (in pyridine-d_5) and 13 (in $CDCl_3$)

No.	5		13	
	δ_H (multi, J in Hz)[a]	δ_C	δ_H (multi, J in Hz)[b]	δ_C
1	7.24 (br s, OH)	88.8 (C)	3.96 (t, J = 8.6 Hz)	79.0 (C)
2	6.17 (d, J = 5.8 Hz)	137.6 (CH)	–	–
3	6.21 (d, J = 5.8 Hz)	140.9 (CH)	2.82 (ddq, J =17.1, 8.8, 2.1 Hz) 2.16 (ddq, J = 17.1, 8.3, 2.1 Hz)	38.0 (CH_2)
4	5.12 (br s, OH)	80.5 (C)	–	145.3 (C)
5	2.78 (d, J = 10.2 Hz)	64.9 (CH)	2.32 (br d)	55.3 (C)
6	5.46 (dd, J = 10.2, 9.8 Hz)	79.0 (CH)	4.00 (t, J = 10.6 Hz)	81.2 (C)
7	4.70 (m)	48.3 (CH)	2.39 (m)	50.8 (C)
8	4.56 (m)	70.6 (CH)	1.67-1.59 (m) 2.07 (dtd, J = 13.6, 4.0, 2.3 Hz)	21.7 (CH_2)
9	2.51 (dd, J = 14.0, 5.1 Hz) 2.04 (dd, J = 14.0, 10.6 Hz)	48.7 (CH_2)	1.95 (ddd, J = 13.0, 4.0, 2.4 Hz) 1.33 (td, J = 13.0, 4.4 Hz)	34.6 (CH_2)
10	7.59 (br s, OH)	74.7 (C)	–	47.3 (C)
11	–	139.8 (C)	–	138.7 (C)
12	–	171.7 (C)	–	171.1 (C)
13	6.85 (br d, J = 2.8 Hz) 6.56 (dd, J = 2.8, 2.7 Hz)	124.5 (CH_2)	6. 08 (d, J = 3.3 Hz) 5.41 (d, J = 3.3 Hz)	117.1 (CH_2)
14	1.66 (s, 3H)	29.3 (CH_3)	0.82 (s, 3H)	11.8 (CH_3)
15	1.78 (s, 3H)	27.2 (CH_3)	4.90 (q, J = 2.1 Hz) 5.20 (q, J = 2.6 Hz)	108.1 (CH_2)

[a] at 500 MHz; [b] at 600 MHz

Compound **13** was obtained as a colorless oil. Its molecular formula was designated as $C_{14}H_{18}O_3$ by HR-ESI-MS. The IR spectrum showed the absorption bands for hydroxyl (3441 cm^{-1}), γ-lactone (1793 cm^{-1}), and terminal olefinic (1662 and 1249 cm^{-1}) groups. The ^{13}C and DEPT NMR spectrum showed 14 carbon signals assigned to one methyl (δ_C 11.8), five methylenes (δ_C 21.7, 34.6, 38.0, 108.1, 117.1), four methines (δ_C 50.8, 55.3, 79.0, 81.2), and four quaternary

carbons (δ_C 47.3, 138.7, 145.3, 171.1) (Table 2). ^{1}H NMR data (Table 2) displayed characteristic signals of an eudesmanolide including a singlet methyl (δ_H 0.82, s), an *α*-methyl-*γ*-lactone moiety (δ_H 6.08 and 5.41, each d, J = 3.3 Hz, δ_H 4.00, t, J = 10.6 Hz), one terminal double bond (δ_H 5.20, d, J = 2.6 Hz; δ_H 4.90, d, J = 2.1 Hz). All these data suggested that compound **13** is a 2-nor eudesmanolide [9]. The ^{1}H–^{1}H COSY correlations of H-1/H-3, H-5/-6, H-7/H-8, and H-8/H-9, and the key HMBC correlations from H_2-13 to C-11 and C-12, H_3-14 to C-1, C-5, C-9 and C-10, and H_2-15 to C-3, C-4 and C-5 (Fig. 6) further supported that **13** possesses a structure of a noreudesmane sesquiterpene.

Table 3 The IC_{50} values of the inhibition activities of compounds 4, 6–7, 10–13 against the LPS-induced NF-κB activation on a THP1-Dual cell model

Compounds	IC_{50} (μmol·L^{-1})	Compounds	IC_{50} (μmol·L^{-1})
4	12.42	**10**	9.31
6	2.93	**11**	2.27
7	24.65	**12**	5.94
8	2.06	**13**	21.93
SC75741	> 100	JSH23	> 100

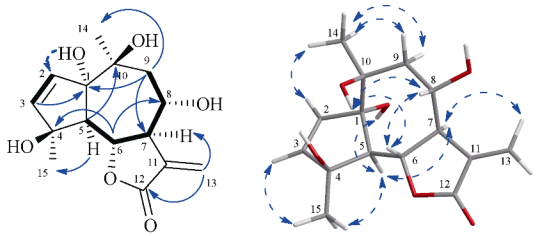

Fig. 5 ^{1}H-^{1}H COSY (bold), key HMBC (H→C), and ROESY (↔) correlations of compound 5

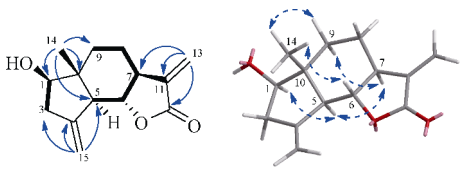

Fig. 6 ^{1}H-^{1}H COSY (bold), key HMBC (H→C), and NOESY (↔) correlations of compound 13

The relative configuration of **13** was determined by the coupling constants and the NOESY spectrum (Fig. 6). The large coupling constants for H-6 (δ_H 4.00, t, J = 10.6 Hz) suggested that H-6 should be in a *β* orientation, and H-5 (δ_H 2.32, br d) and H-7 (δ_H 2.39, m) being *α*-oriented, respectively. Therefore, the orientation of H_3-14 suggested to be *β*-oriented according to NOESY correlations of H_3-14/H-6. Furthermore, the orientation of H-1 was defined as *α*-oriented based on the H-1/H-5 correlation (Fig. 6). Since the first similar compound was identified from plant *Ajania przewalskii* and named ajaniaolide A [9], compound **13** was accordingly named ajaniaolide B.

Along with the new compounds **1**, **5**, and **13**, 10 known compounds (Fig. 1) were identified also from this plant as artemisiane A (**2**) [6], millifolide A (**3**) [10], achillinin C (**4**) [11], 3*β*-chloro-4*α*, 10*α*-dihydroxy-1*α*, 2*α*-epoxy-5*α*, 7*αH*-guaia-11(13)-en-12, 6*α*-olide (**6**) [12], 3*α*-chloro-4*β*, 10*α*-dihydroxy-1*β*, 2*β*-epoxy-5*α*, 7*αH*-guai-11(13)-en-12, 6*α*-olide(**7**) [13], rupicolin B (**8**) [14], hydroxyachillin (**9**) [14], iso-*seco*-tanapartholide (**10**)[10], *seco*-tanapartholide A (**11**) [10], and 5-*epi*-*seco*-tanapartholide A (**12**) [10], respectively, by comparing their spectroscopic data with those in literature.

All isolates were evaluated their inhibitory activities against activation of NF-κB induced by lipopolysaccharides (LPS) on a model based on the THP1-Dual cells. THP1-Dual™ cells were derived from the human THP-1 monocyte cell line by stable integration of two inducible reporter constructs, and they express a secreted embryonic alkaline phosphatase (SEAP) reporter gene driven by an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site.[15] The activation of NF-κB was measured by monitoring SEAP levels in cell culture supernatants. The results showed that the inhibition rates of compounds **4**, **6**–**8**, **10**–**13** fell into the range of 60-100% at the concentration of 20 μmol·L^{-1}. The IC_{50} values of these seven compounds were further assessed, ranging from 2 to 25 μmol·L^{-1} (Table 3). It is noticed that most monomeric sesquiterpenes showed stronger inhibitory activities than the dimerized sesquiterpenoids such as compounds **1**–**3**, but no further evidence was provided for the structure-activity relationship.

In summary, this is the first phytochemical investigation of *A. heptapotamica* endemic in Kazakhstan. A total of 13 sesquiterpene lactones were afforded, including four dimers and nine monomers (five guaianolide and three *seco*-guaianolide). The co-occurring dimeric and monomeric compounds suggested that dimers could be biosynthetically constructed from the same or different monomers through different types of linkages. Compounds **1** and **2** could be postulated as esterified products from a guaianolide and a *seco*-guaianolide type of sesquiterpene lactone. Compound **3** could be an ether product derived from two *seco*-guanolide monomers. Compound **4** could be a product presumably resulted from a Diels-Alder reaction between a guaianolide and a *seco*-guaianolide sesquiterpenes. Most of the isolated monomeric sesquiterpenes showed strong inhibition against activation of NF-κB induced by LPS, which might be correlated to the anti-inflammatory effects reported for some of the *Artemisia* plants rich in different types of sesquiterpenoids [16-17].

Experimental

General experimental procedure

Optical rotations were obtained on a Rudolph Research Analytical Autopol VI 90079 polarimeter (Hackettstown, NJ). UV spectra were measured on a Shumadzu UV-2550 UV–vis spectrophotometer. IR spectra were recorded with a Thermo

Nicolet FTIR IS5 spectrometer. Analytical HPLC and ESI-MS spectra were performed on a Waters 2695 instrument with a 2998 PAD coupled with a Waters Acquity ELSD and a Waters 3100 SQDMS detector. Preparative HPLC was performed on a Varian PrepStar instrument with an Alltech 3300 ELSD detector (Columbia, MD, USA) using a Waters Sunfire RP C18 column (5 μm, 30 mm × 150 mm). Semi-preparative HPLC was performed on a Waters 2690 instrument with a Waters 996 UV detector using a YMC HPLC column (S-5 μm, 250 mm × 10 mm). HR-ESI-MS spectra were recorded on a Waters Synapt G2-Si Q-Tof mass detector. 1D and 2D NMR spectra were recorded using a Bruker AVANCE III 500 or 600 MHz instrument. Chemical shifts were reported in ppm (δ) with coupling constants (*J*) in hertz. MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Industries, Japan), Econosep C18 60A (50 μm, DIKMA, China), silica gel (100−200 and 300−400 mesh, Qingdao Haiyang Chemical Co., Ltd., China), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography (CC). TLC was carried out on precoated silica gel 60 F_{254} aluminum sheets (Merck, Germany), and the TLC spots were viewed at 254 nm and visualized by 5% sulfuric acid in alcohol containing 10 mg·mL^{-1} of vanillin. *OD* values were recorded on a SpectraMax Plus Microplate Reader (Molecular Devices). THP1-Dual™ cells (Catalog # thpd-nfis), lipopolysaccharide (LPS)-B5 (Catalog # tlrl-b5lps), and QUANTI-Blue™ were purchased from InvivoGen. FBS was obtained from Gibco Australia. NF-κB inhibitors SC75741 and JSH23 with > 98% purity were purchased from Selleck Chemicals.

Plant materials

The flowering whole plant of *A. heptapotamica* were collected in August, 2018, from Almaty region, Kazakhstan, and identified by Dr. Alibek Ydyrys of Al-Farabi Kazakh National University. Specimens (9358-H) were deposited in the Herbarium of Laboratory Plant Biomorphology, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan.

Extraction and isolation

The air-dried whole plant of *A. heptapotamica* (12 kg) were ground and extracted with 95% EtOH three times, 7 days each. Combined percolates were evaporated under reduced pressure to obtain a crude residue (1.52 kg). The residue suspended in water and partitioned with petroleum ether (PE), CH_2Cl_2 (DCM), and EtOAc (EA), successively. The DCM fraction (155 g) was applied to a MCI gel column chromatography (CC) using 30, 40, 50, 60, 70, and 95% aqueous EtOH (*V*/*V*) as eluent. The 30% EtOH fraction (20 g) was further separated by CC over Sephadex LH-20 eluted with $CHCl_3$–MeOH (1 : 1) to give five fractions (A_1–A_5). Subsequently, fraction A_2 was separated by repeated CC over Sephadex LH-20 (MeOH) to give three subfractions (A_2A–A_2A_2). A_2A (450 mg) was subjected to ODS CC (MeOH–H_2O, from 50 : 50 to 100 : 0) to give seven subfractions A_2A_1–A_2A_7. Subfractions A_2A_3 (37 mg), A_2A_5 (21 mg), A_2A_6 (51 mg) was further purified by semi-preparative HPLC (CH_3CN in H_2O from 5% to 95%, 30 min, 3 mL·min^{-1}) to yield compounds **1** (4 mg), **2** (5 mg), **3** (3 mg), and **9** (4 mg). The similar procedures were applied for the 40% and 50% EtOH fractions, affording compounds **4** (3 mg), **6** (86 mg), **7** (2 mg), **8** (369 mg), and **9** (15 mg).

The EA fraction (90 g) was applied to a polyamide gel column eluted with aqueous EtOH (0, 20, 40, and 95% in a step manner). The water part (21 g) was subjected to CC over Sephadex LH-20 ($CHCl_3$–MeOH, 1 : 1) to give seven fractions (Fr1–Fr7). Fraction Fr4 (3.58 g) was further applied to silica gel CC eluting with a step solvent system of PE–EA (10 : 1, 1 : 1, 0 : 1) to give 10 subfractions (Fr4-1–Fr4-10). Subfraction Fr4-10 (592 mg) passed through a column of Sephadex LH-20 (MeOH), giving subfraction Fr4-10A. Subsequently, Fr4-10A (525 mg) was purified over ODS (MeOH–H_2O from 98 : 2 to 25 : 75) and silica gel (PA–EA, 7 : 1, 1 : 1, 0 : 1) to yield compounds **11** (19 mg), **12** (54 mg), and **13** (15 mg). Fraction Fr6 was applied to CC of silica gel (PE–EA, 8 : 1 to 1 : 1, then acetone) to give eight subfractions (Fr6-1–Fr6-8). Subfraction Fr6-8 was further purified by Sephadex LH-20 ($CHCl_3$–MeOH, 1 : 1) and then repeated CC over silica gel to afford **5** (13 mg).

Artemisiane E (**1**): Colorless oil, $[\alpha]_D^{18}$ + 38 (*c* 0.1, MeOH); IR(KBr) ν_{max}: 3439, 2959, 2957, 2868, 2853, 1768, 1709, 1657, 1462, 1378, 1063 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HR-ESI-MS *m/z* 617.2242 $[M + COOH]^-$ (Calcd. for $C_{31}H_{37}O_{13}$, 617.2240).

Artemdubolide I (**5**): Colorless gum, $[\alpha]_D^{18}$ + 194 (*c* 0.1, MeOH); IR(KBr) ν_{max}: 3444, 2957, 2928, 2847, 1742, 1468, 1381, 1084, 1036, 966 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HR-ESI-MS *m/z* 341.1242 $[M + COOH]^-$ (Calcd. for $C_{16}H_{21}O_8$, 341.1242).

Ajaniaolide B (**13**): Colorless oil, $[\alpha]_D^{20}$ + 6 (*c* 0.1, MeOH); IR(KBr) ν_{max}: 3441, 2930, 2848, 1793, 1662, 1249 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HR-ESI-MS *m/z* 233.1183 $[M - H]^-$ (Calcd. for $C_{14}H_{17}O_3$, 233.1183).

Anti-inflammation assay

THP1-Dual™ cells were plated into 96-well plate at a density of 1 × 10^5/well, tested compounds were added into the cells at indicated concentration for 1 hour, and then stimulated with 1 μg·mL^{-1} of lipopolysaccharide (LPS)-B5. After 24 h, 20 μL of each cell supernatant were obtained and added into 180 μL of resuspended QUANTI-Blue™ per well and incubated for 1 h at 37 °C. The SEAP levels were measured using a spectrophotometer at 650 nm. The inhibition rate was calculated as following: Inhibition Rate = $[1 - (OD_{cpd} - OD_{untreated})/(OD_{LPS} - OD_{untreated})] \times 100\%$. The IC_{50} values were calculated by concentration−response curve fitting using the four-parameter method. NF-κB inhibitors SC75741 and JSH23 was used as positive controls.

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