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New isopimarane diterpenes and nortriterpene with cytotoxic activity from *Ephorbia alatavica* Boiss

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ABSTRACT

Three new isopimarane diterpenes and one new nor-triterpenes, along with five known diterpenes were isolated from the whole areal part of *Ephorbia alatavica* Boiss. The structures of the new compounds (**1–4**) were determined based on extensive spectroscopic analysis, including HR-ESIMS, 1D and 2D NMR data. A plausible biosynthetic pathway for new compounds (**1–4**) were hypothesized. All isolated compounds were screen for cytotoxicity activity against MCF-8, HeLa and A549 cell lines in vitro by MTT assay. New compound **1** and known **9** showed potential cytotoxic activities with IC_{50} values of 15.327 μg/mL, 23.066 μg/mL against MCF-8 cell lines, compound**1** showed noteworthy cytotoxic activity with IC_{50} 13.033 μg/mL against A549 cancer cell line. New compounds **2, 4** and **4** showed moderate cytotoxic activities three human cancer lines with IC_{50} value around 50 μg/mL, which compared with positive control doxorubicin (DOX).

1. Introduction

The plants of genus *Euphorbia* (e.g., Euphorbia sororia) are commonly used in traditional Uighur medicines for cure abdominal pain, abdominal distention, skin disease, and paralysis [1]. Common species of this genus, such as *Euphorbia macrorrhiza*, *Euphorbia sororia* and *Euphorbia soongarica* have been studied by our group, and reported different types of compounds including diterpenes [2–5], triterpenoids [6], lignins and flavonoids. *Euphorbia alatavica* Boiss, belonging to the Euphorbia genus, is a perennial herb, widely distributed in the Xinjiang Uighur Autonomous Region of China and Central Asia country [7]. In previous study of *E. alatavica* have been isolated two new triterpenoids, flavonoids, lignins and phenolic compounds [8]. Continuous efforts on this plant to find bioactive components, have resulted in the isolation of terpenes, including isopimarane type diterpenes, nor-tritepenes and some tetracyclic diterpenes. Terpenes, as a structurally diverse and functionally noteworthy group of natural products, are well known for their broad range of bioactivities, such as cytotoxicity [9–11], antimicrobial and bacterial [12,13], anti-malarial [14], anti-infammatory [15], TRAIL resistance [16] and multi drug resistance activities [17].

In this investigation describes the structural elucidation of nine terpene compounds isolated by chromatographic methods from the acetone extraction of the *E. alatavica*. Three of them were new diterpenoids (**1, 2, 4**) and one was new nortriterpene (**4**), along with five known diterpenes (**6–10**). All of the above isolated compounds were screened of their cytotoxicity in vitro against three human cancer cell lines MCF-8, HeLa and A549.

2. Experimental section

2.1. General experimental procedures

Optical rotations were recorded on a Rudolph RS Autopol VI automatic polarimeter (Rudolph Research Analytical, Flanders, NJ, USA) in MeOH at 26 °C. UV data were obtained on a Shimadzu UV-2550 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). NMR spectra were recorded on a Varian VNMRS-600 spectrometer (Agilent Technologies, USA) (600 MHz for ^{1}H and 150 MHz for ^{13}C NMR) and VNMRS-400 spectrometer (Agilent Technologies, USA) (400 MHz for ^{1}H and 100 MHz for ^{13}CNMR) in CD_3OD or $CDCl_3$ with TMS as an internal reference. The HR–ESI–MS data were collected with a QStar Elite mass spectrometer (AB SCIEX, Framingham, MA, USA). Analytical HPLC was carried on a Dionex UltiMate 3000 instrument (Thermo, Waltham, MA, USA), with UV detection, using Waters XSELECTTM CSHTM C18 (4.7 × 250 mm, 6 μm) column. Semipreparative HPLC was conducted on a Shimadzu LC-20A instrument (Shimadzu Corporation,

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Table 1
^{1}H NMR Data of new compounds (**1–4**).
^{1}H NMR spectroscopic data for **1–4** (Data were recorded in CD_3OD at 400 MHz(**1–3**); Compound **4** data was recorded in $CDCl_3$ at 600 MHz).

Position	Compound **1** (δ_H)	Compound **2** (δ_H)	Compound **3** (δ_H)	Compound **4** (δ_H)
1a	1.61, m	2.86, m	2.84, m	1.70, m
1b	1.51, m	1.63, m	1.66, m	1.05, m
2a	1.63, overlapped	2.82, m	2.80, m	1.64, m
2b	1.63, overlapped	2.17, m	2.14, m	1.63, m
3	3.41, *β*, m			4.48, *β*, dd (5.4,5.4 Hz)
4				
5	1.91, *α*, overlapped	1.62, *α*, m	1.57, *α*, m	0.84, *α*, m
6a	1.93, m	2.18, overlapped	2.15, overlapped	1.52, overlapped
6b	1.61, m	1.93, overlapped	1.94, overlapped	1.45, overlapped
7a		5.56, d (4.8 Hz)	5.55, d (4.4 Hz)	1.59, overlapped
7b	4.28, *β*, m			1.29, overlapped
8				
9	2.29, *α*, overlapped	2.19, *α*, overlapped	1.94, *α*, overlapped	1.33, *α*, overlapped
10				
11	1.76[a], overlapped, 1.76[b], overlapped	3.82, *β*, dd (10.4, 2.4 Hz)	3.56, *β*, dd (9.6, 9.6 Hz)	1.52[a], overlapped,1.52[b], overlapped
12	3.69, *β*, m	3.42, *β*, d (2.4 Hz)	3.24, *α*, d (9.6 Hz)	1.51[a], overlapped
				1.09[b], m
13				1.71, *β*, overlapped
14	5.52, s	2.54[a], br d (14.0 Hz) 1.73[b], br d (14.0 Hz)	2.12[a], overlapped 1.96[b], overlapped	
15	5.90, dd(10.8,6.8 Hz)	5.98, dd (17.6, 10.8 Hz)	5.89, dd (17.6,10.8 Hz)	1.63[a],1.18[b], overlapped
16a	5.11, dd(10.4, 2.4 Hz)	5.00, d (17.6 Hz)	5.02, d (17.6 Hz)	1.91, m
16b		5.01, d(10.8 Hz)	5.00, d(10.8 Hz)	1.53, overlapped
17	1.15, *β*, s	0.90, *β*, s	0.88, *β*, s	2.32, *β*, m
18	0.89, *β*, s	1.24, *β*, s	1.25, *β*, s	0.98, *β*, s
19				0.87, *β*, s
20				
21				
22				5.69, s
23				2.11, s
24				0.88, *α*, s
4-CH_3 (*α*)	0.97, s	1.15, s	1.15, s	0.86 s
4- CH_3 (*β*)	0.90, s	1.06, s	1.06, s	0.85 s
3-OAc				1.25, s
21-OMe				3.49, s

Table 2
^{13}C NMR Data of compound (**1–4**).
^{13}C NMR spectroscopic data for **1–4** (Data were recorded in CD_3OD at 100 MHz; Compound **4** data was recorded in $CDCl_3$ at 150 MHz).

Position	Compound **1** (δ_C)	Compound **2** (δ_C)	Compound **3** (δ_C)	Compound 4 (δ_C)
1	32.6	41.22	42.4	38.9
2	26.5	35.7	35.7	23.8
3	76.6	219.5	219.4	81.0
4	38.24	48.9	48.8	38.1
5	42.4	54.02	53.8	56.1
6	27.06	24.8	24.8	18.3
7	86.6	124.4	125.0	35.5
8	135.9	135.6	134.8	40.8
9	45.5	53.1	57.3	50.9
10	39.3	37.1	37.6	37.3
11	27.4	70.6	72.9	21.5
12	74.3	79.1	80.9	24.9
13	44.1	42.1	42.4	45.7
14	135.3	40.5	46.5	49.9
15	144.04	147.4	147.9	31.9
16	115.1	112.2	112.3	27.9
17	26.7	22.2	16.4	51.9
18	15.3	14.6	14.7	15.7, *β*
19				16.5, *β*
20				166.4
21				169.1
22				113.9
23				16.8
24				16.1
4-CH_3 (*α*)	29.01	26.1	26.1	28.1
4- CH_3 (*β*)	22.7	23.2	23.2	16.6
3-OAc				171.2
3-OCOMe				21.5
21-OMe				50.9

Kyoto, Japan), with UV detection, using a Waters XSELECTTMCSHTM C18 (11 × 250 mm, 6 μm, Waters Co., Milford, MA, USA) column. The detection wavelengths were set to 210 and 230 nm. Silica gel (100–200 mesh, 200–300 mesh, jiangyou silica gel Development Co. Ltd., Yantai, China) was used for open column chromatography (CC). Solvents were analytical grade (Baishi Chemical Co. Ltd., Tianjin, China) for open CC and HPLC grade (Merck, Germany) for HPLC analysis.

2.2. Plant material

The whole dried plant of *E. alatavica* was collected in July 2015, from Ili alatao mountain area in Xinjiang, People's Republic of China, plant was identified by Prof. Y. Feng of Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences. A voucher specimen (No. XJIPC150826) was deposited at Xinjiang Technical Institute of Physics and Chemistry Chinese Academy of Sciences.

2.3. Extraction and isolation

The dried and ground stems of *E. alatavica* (14.0 kg) were extracted with acetone at room temperature for 9 times. Evaporation of the solvent under reduced pressure give a dark brown crude extract (831.4 g). Crude extract was suspended in acetonitrile, and then partitioned with n-hexane, produce a hexane soluble fraction (498.4 g) and an acetonitrile (ACN) fraction (328 g). The acetonitrile -soluble extract (328 g) was subjected to column chromatography over silica gel (100–200 mesh, 11 × 100 cm, 7 kg) eluting with petroleum ether (PE)–acetone (100:0–0:100, v/v) to yield thirteen fractions (Fr.1–Fr.14). The fraction Fr.9 (7.1 g) was separated by a silica gel column using a gradient of n-hexane EtOAc (100:0–0:100, v/v) to