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Cytotoxic macrocyclic diterpenoids from *Jatropha multifida*

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ABSTRACT

Nine new macrocyclic diterpenoids (**1**–**9**), jatromultones A-I, along with eight known analogues (**10**–**17**) were isolated from the trunks of *Jatropha multifida*. The structures of the new compounds, including their absolute configurations, were elucidated by combination of spectroscopic analysis, single crystal X-ray diffraction, $Rh_2(OCOCF_3)_4$-induced CD method, and chemical correlations. All compounds were screened for the cytotoxicity against five cancer cell lines, including one drug-resistant cell line, and seven compounds exhibited significant activity with IC_{50} values less than 10 μM. Compound **4** with IC_{50} values ranging from 2.69 to 6.44 μM toward all cell lines was selected for further mechanistic study, which showed that **4** could arrest cell cycle at G2/M phase and induce apoptosis. The brief structure-activity relationships (SARs) of these macrocyclic diterpenoids were also discussed.

1. Introduction

Macrocyclic diterpenoids refer to a group of diterpenoids with at least a seven-membered carbon ring or even bigger one in the structure. They are usually highly oxygenated and functionalized and are mainly reported from the Euphorbiaceae sp. [1,2]. Euphorbia diterpenoids, such as ingenanes, lathyranes, jatrophanes, tiglianes, daphnanes, etc., all belong to this compound class. These diterpenoids have attracted considerable interests for the last decades due to their diverse scaffolds and wide range of biological activities [3–5]. For instance, picato, an ingenane diterpenoid (5/7/7/3-ring system) isolated from *Euphorbia peplus*, was approved by FDA in 2012 for the treatment of actinic keratosis [6]. Prostratin, a tigliane diterpenoid (5/7/6/3-ring system) isolated from *Homalanthus nutans*, was one of the most promising molecule in HIV therapy [7].

Jatropha multifida L. (Euphorbiaceae) is an ornamental shrub mainly distributed in South America and South China. Previous investigation on this plant revealed that it was a rich source of structurally diverse macrocyclic diterpenoids mainly represented by lathyranes (5/11/3-ring system). Some of them possessed the novel architectures, such as multidione featuring a phenolic moiety and a long side chain [8], and multifidone possessing an expanded carbon ring A [9]. These diterpenoids commonly showed intriguing cytotoxic and antimicrobial activities [8–10].

In our continuing efforts toward discovering structurally intriguing anti-cancer diterpenoids from the Euphorbiaceae plants [11–13], nine new macrocyclic diterpenoids and eight known analogues were isolated from the trunks of *J. multifida*. The structures of new compounds, including their absolute configurations, were elucidated by combination of spectroscopic analysis, single crystal X-ray diffraction, and chemical evidence. All compounds were screened for the antiproliferative activity against a panel of cancer cell lines, including one drug-resistant cell line, and seven compounds exhibited significant activity with IC_{50} values less than 10 μM. Herein, details of the isolation, structural elucidation, cytotoxicity, and possible mechanism of these compounds are described.

2. Materials and methods

2.1. General experimental procedures

X-ray data were collected using an Agilent Xcalibur Nova X-ray diffractometer. Melting points were measured on an X-4 melting instrument and were uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer. IR spectra were determined on a Bruker Tensor 37 infrared spectrophotometer with KBr disks. NMR spectra were measured on a Bruker AM-400 spectrometer at 25 °C. ESIMS and HRESIMS were carried out on a Finnigan LCQ Deca instrument. The absorbance was detected at 490 nm using a multifunction microplate reader (Molecular Devices, Flex Station 3) and analyzed using GraphPad Prism 5 (GraphPad Inc., La Jolla, CA, USA). A Shimadzu LC-20AT equipped with a SPD-M20A PDA detector was used for HPLC, and a YMC-pack ODS-A column (250 × 10 mm, S-5 μm,

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12 nm) together with a Phenomenex chiral column (250 × 10 mm, 5 μm) were used for semipreparative HPLC separation. Silica gel (300–400 mesh, Qingdao Haiyang Chemical Co. Ltd.), reversed-phase C_{18} (RP-C_{18}) silica gel (12 nm, S-50 μm, YMC Co. Ltd.), and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) were used for column chromatography (CC). All solvents were of analytical grade (Guangzhou Chemical Reagents Company, Ltd.). Annexin-V/FITC and Cell cycle were purchased from Keygen Biotech, China. MTT was purchased from Sigma, USA.

2.2. Plant material

Trunks of *J. multifida* were collected in May 2016 from Xishuangbanna Tropical botanical Garden (XTBG), Chinese Academy of Sciences, Mengla County, Yunnan Province, China. The plant was identified by Prof. You-Kai Xu of XTBG, where a voucher specimen (accession number: SHH201607) was deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

2.3. Extraction and isolation

The air-dried powder of the trunks of *J. multifida* (12.5 kg) was extracted with 95% EtOH (3 × 5 L) at room temperature (rt) to give 980 g of crude extract. The extract was suspended in H_2O (3 L) and partitioned with EtOAc (3 × 3 L). The EtOAc extract (280 g) was subjected to MCI gel CC eluted with a MeOH/H_2O gradient (6:4 → 10:0) to afford six fractions (I-VI). Fr. II (27 g) was subjected to silica gel CC (PE/CH_2Cl_2, 10:1 → 0:1) to give four fractions (IIa-IId). Fr. IIc (1.1 g) was separated by silica gel CC (PE/acetone, 50:1) to give **17** (17 mg) and IIc1 (17 mg). IIc1 was followed by semi-preparative HPLC (MeCN/H_2O, 90/10, 3 mL/min) to give **3** (5 mg, t_R 12.0 min) and **4** (4 mg, t_R 13 min). Fr. IId (128 mg) was purified on silica gel CC (CH_2Cl_2) to give **10** (67 mg). Fr. III (12 g) was subjected to silica gel CC (CH_2Cl_2/MeOH, 400:1 → 10:1) to give six fractions (IIIa-IIIf). Fr IIIb (550 mg) was separated by RP-C_{18} silica gel CC (MeOH/H_2O, 7:3 → 10:0) to yield **12** (34.2 mg), **9** (21 mg) and **14** (108 mg). Fr. III c (6.5 g) was subjected to silica gel CC (CH_2Cl_2/MeOH, 100:1) to give three fractions (IIIc1-IIIc3). Fr. IIIc2 (1.4 g) was separated by RP-C_{18} silica gel CC (MeOH/H_2O, 7:3 → 10:0), followed by semi-preparative HPLC (MeCN/H_2O, 70/30, 3 mL/min) to give **13** (53 mg, t_R 14.5 min), **15** (24 mg, t_R 17.5 min), and **16** (18 mg, t_R 18 min). Fr. IIIc3 (600 mg) was subjected to silica gel CC (CH_2Cl_2/MeOH, 100:1 → 10:1) to give three fractions (IIIc3a-IIIc3c). Fr. IIIc3a (120 mg) was subjected to RP-C_{18} silica gel CC (MeOH/H_2O, 7:3 → 10:0), followed by semi-preparative chiral HPLC (MeOH/H_2O, 80/20, 3 mL/min) to give **1** (3.1 mg, t_R 10.6 min) and **2** (3.1 mg, t_R 11.6 min). Fr. IIIc3c (340 mg) was applied to silica gel CC (PE/acetone, 80:1 → 0:1), followed by semi-preparative chiral HPLC (MeOH/H_2O, 85/15, 3 mL/min) to give **5** (3.8 mg, t_R 15.6 min) and **6** (4.1 mg, t_R 17.6 min). Fr. IV (520 mg) was further purified by a Sephadex LH-20 column using MeOH as eluent to give **11** (48 mg) and IVa-IVd. IVb (35 mg) was further purified by semi-preparative HPLC (MeOH/H_2O, 90/10, 3 mL/min) to give **8** (6 mg, t_R 14.6 min) and **7** (4 mg, t_R 17.0 min).

Table 1
^{1}H NMR data of compounds **1–9** in $CDCl_3$ (Recorded at 400 MHz, δ in ppm, J in Hz).

No.	1	2	3	4	5	6	7	8	9
1	α 2.90, dd (18.5, 2.3) β 2.67, dd (18.5, 4.1)	α 2.84, dd (18.5, 3.1) β 2.69, dd (18.5, 3.1)			4.85, dd (2.0, 2.0)	5.27, dd (6.3, 1.6)	α 3.09, dd (19.4, 1.6) β 2.21, dd (19.4, 6.5)	7.16, d (1.0)	7.66, s
2			2.71, m	2.31, m	2.44, m	2.61, m	2.37, m		
3			6.42, dd (6.4, 1.1)	5.76, brs					
4								3.08, s	
5	4.68, dd (4.1, 2.3)	4.70, dd (3.1, 3.1)			3.22, brd (7.7)	3.25, brd (8.9)	3.56, brs	4.21, s	6.42, s
7	α 1.75, m	α 1.99, dd (14.2, 6.6)	5.79, m	5.76, brs	α 2.24, m	α 2.24, m	a 1.23, m	a 1.60, m	a 1.87, m
	β 2.00, dd (14.1, 6.5)	β 1.73, m			β 2.53, m	β 2.53, dd (11.4, 5.9)	b 2.38, m	b 2.15, m	b 2.75, m
8	α 1.79, m	α 1.79, m	2.59, td (11.8, 3.9)	2.57, dd (12.0, 5.5)	2.17, m	2.19, m	a 0.93, m	α 1.71, m	α 1.30, m
	β 1.10, m	β 1.09, m					b 1.81, m	β 0.55, m	β 0.58, m
9	0.68, ddd (10.5, 9.0, 7.0)	0.68, m	2.35, m	2.33, m	0.89, m	0.87, m	0.27, m	0.29, m	0.70, m
10									
11	0.47, ddd (11.2, 9.0, 6.5)	0.46, ddd (11.1, 8.9, 6.5)	α 1.91, m	α 1.89, m	0.55, m	0.55, m	0.72, m	0.29, m	0.28, m
			β 1.47, m	β 1.46, m					
12	α 2.37, dd (15.5, 6.4)	α 2.38, dd (15.6, 6.4)	a 2.38, m	a 2.27, m	1.41, m	1.42, m	a 1.01, m	a 1.54, m	a 0.95, m
	β 1.27, m	β 1.27, m	b 2.43, m	b 2.42, m			b 1.74, m	b 1.71, m	b 1.23, m
13					2.42, m	2.40, m	1.79, m	3.77, m	2.95, m
14			3.19, d (11.8)	3.15, d (12.0)			5.22, brs		
16	1.39, s	1.40, s	1.07, d (7.3)	1.36, d (7.7)	1.27, d (7.7)	1.20, d (7.5)	1.15, d (7.5)	1.84, d (1.0)	1.94, s
17	0.83, s	0.85, s	1.81, s	1.83, s	4.03, s 4.70, s	4.07, s 4.68, s	1.09, s	1.27, s	1.61, s
18	1.03, s	1.03, s	4.82, s 4.84, s	4.81, s 4.83, s	0.91, s	0.91, s	0.90, s	0.73, s	0.56, s
19	1.05, s	1.05, s	1.58, s	1.58, s	1.10, s	1.10, s	1.05, s	0.96, s	0.96, s
20	1.25, s	1.24, s	4.09, s	4.13, s	1.14, d (6.6)	1.15, d (6.6)	0.95, d (6.8)	1.13, d (6.8)	1.12, d (6.7)
			4.75, s	4.75, s					
3-OAc			2.02, s	2.01 , s					