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Jatrophane diterpenoids with multidrug resistance-modulating activity from *Euphorbia mongolica* Prokh.

Dóra Rédei [a], Peter Forgo [a], Joseph Molnár [b], Pál Szabó [c], Tumur Zorig [d], Judit Hohmann [a,*]

[a] *Department of Pharmacognosy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary*
[b] *Department of Medical Microbiology and Immunology, University of Szeged, Dóm tér 10, H-6720 Szeged, Hungary*
[c] *Institute of Structural Chemistry Chemical Research Centre of the Hungarian Academy of Sciences, Pusztaszeri út 59-67, H-1025 Budapest, Hungary*
[d] *Reference Laboratory for Medicines, Public Health Institute, Central PO Box 1193, 211613 Ulaanbaatar, Mongolia*

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ABSTRACT

Four novel (**1**–**4**) and one known (**5**) diterpene polyesters with the jatrophane skeleton were isolated from a methanol extract of the aerial parts of the East Asian weed *Euphorbia mongolica* Prokh. The isolated compounds were characterized structurally and evaluated for multidrug resistance (MDR) reversing activity on human MDR gene-transfected L5178 mouse lymphoma cells; all these compounds were found to modulate the intracellular drug accumulation. The results highlighted some aspects of the structural requirements of jatrophane diterpenes as MDR modulators.

1. Introduction

Multidrug resistance (MDR), i.e., cellular resistance to structurally and functionally unrelated chemotherapeutic drugs, has become the major obstacle to successful chemotherapy in recent years. The most significant mechanism of MDR in tumor cells is associated with the overexpression of P-glycoprotein (P-gp), which acts as an energy-dependent drug efflux pump. The overexpression of P-gp results in reduced drug accumulation in tumor cells and subsequently increases the possibility of chemotherapeutic failure. Although some chemical compounds (verapamil, quinidine, cyclosporine A, progesterone, VX-710, PSC-833, etc.) have been reported to reverse MDR both in vitro and in vivo, these MDR-reversing agents still proved unsuitable for further clinical trials as a consequence of their insufficient potency, unacceptable toxicities or solubility limitations.[1,2] In the past few decades, extensive studies have been performed with the aim of developing effective resistance modulators from natural sources.[3–5] A promising group of anti-MDR natural products are the macrocyclic diterpenes from Euphorbiaceae species. The majority of the tested diterpenes based on the jatrophane and lathyrane skeletons exhibited a very strong modulation of P-gp.[6–11] Some in vitro experiments have revealed the synergistic interaction of Euphorbia diterpenes with antitumor drugs (doxorubicin and epirubicin) in their antiproliferative effects.[12,13]

We earlier reported that jatrophane diterpenes isolated from *Euphorbia mongolica* Porkh. demonstrate a concentration-dependent effect in inhibiting the efflux-pump activity of mouse lymphoma cells.[14] The present study was conducted to search for further potent MDR-reversing compounds in this plant. We report here the isolation and structure elucidation of four new diterpenes (**1**–**4**), in addition to the known compound **5**, from a methanol extract of *E. mongolica*. The MDR-reversing activities of all these isolated compounds were tested.

2. Results and discussion

The methanol extract of the dried aerial parts of *E. mongolica* collected from Govi Gurvan-Sajhan, Mongolia, was partitioned between water and dichloromethane. The organic layer was fractionated by column chromatography (CC) on polyamide and by vacuum liquid chromatography (VLC) on silica gel. Selected fractions from these separations were further purified by rotation planar chromatography (RPC), preparative TLC, and HPLC, to yield five compounds (**1**–**5**) (Fig. 1).

* Corresponding author. Tel.: +36 62 546453; fax: +36 62 545704; e-mail address: hohmann@pharm.u-szeged.hu (J. Hohmann).

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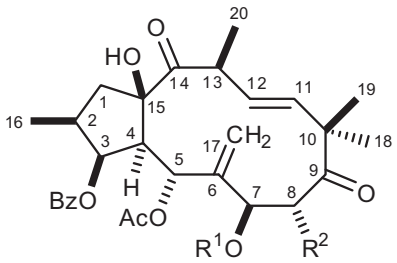

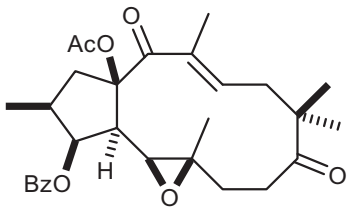

5

	R^1	R^2
1	Ac	H
2	Prop	OAc
3	Bu	OAc
4	*i*Bu	OAc

Ac = acetyl, Bu = *n*-butanoyl, *i*Bu = isobutanoyl, Bz = benzoyl, Prop = propanoyl

Fig. 1. Structures of compounds **1**–**5**.

2.1. Characterization of the compounds

Compound **1**, an amorphous solid, has the molecular formula $C_{31}H_{38}O_9$, determined via the quasimolecular ion peak at *m*/*z* 494.2312 $[M-CH_3COOH]^+$ in the HREIMS and supported by the hydrogen and carbon atom counts in the NMR spectra (Table1).

Table 1
NMR data on compound **1** [$CDCl_3$, 500 MHz (^{1}H), 125 MHz (^{13}C), δ (ppm)]

Atom	^{1}H	^{13}C	HMBC (H No.)	NOESY (H No.)
1α	2.47 dd (14.4, 8.5)	46.8	16, 15-OH	13
1β	1.96 dd (14.4, 12.2)			16,15-OH
2	2.35–2.39 m	38.8	16	3, 4, 13
3	5.86 t (3.5)	77.3	1α, 5, 16	2, 4
4	2.89 dd (3.5, 3.0)	51.1	15-OH	2, 3, 11, 13
5	5.68 s	71.3	17a, 17b	
6	—	139.6	17b	
7	5.66–5.69 m	65.4	17a, 17b	
8α	2.25 dd (12.0, 10.7)	n.o.[a]		
8β	2.92 d (12.0)			19
9	—	208.4	8α, 18, 19	
10	—	50.8	11, 12, 18, 19	
11	5.96 d (16.1)	137.0	18, 19	4, 13, 18
12	5.68 dd (16.1, 9.6)	131.4	20	19, 20
13	3.60 dq (9.6, 6.6)	43.6	11, 12, 20	1α, 2, 4, 11
14	—	212.7	20, 15-OH	
15	—	85.2	1β, 3, 15-OH	
16	1.06 d (6.6)	14.1		1β
17a	5.51 s	120.5		15-OH
17b	5.30 s			
18	1.23 s	23.3	11, 19	11
19	1.18 s	24.0	11, 18	8α, 12
20	1.41 d (6.6)	21.2		12
5-*O*-Acetyl	—	169.2	5-COMe	
	1.73 s	20.4		
7-*O*-Acetyl	—	170.0	7-COMe	
	1.98 s	21.2		
3-*O*-Benzoyl	—	165.7	3, 2′, 6′	
1′	—	130.0	3′, 5′	
2′, 6′	8.11 d (7.1)	129.7	3′, 5′, 4′	
3′, 5′	7.45 t (7.7)	128.4	2′, 6′, 4′	
4′	7.56 t (7.4)	133.0	2′, 3′, 5′, 6′	
15-OH	4.22 s	—		1β, 17a

[a] ^{13}C NMR signal of C-8 could not be observed.

The ^{1}H NMR spectrum suggested the presence of one benzoyl and two acetyl groups in the molecule. The skeletal carbons and directly bonded hydrogen atoms were assigned by means of JMOD and HSQC experiments. Excluding the resonances of ester moieties, the signals of two tertiary (δ_H 1.18 s and 1.23 s, δ_C 24.0 and 23.3) and two secondary (δ_H 1.06 d and 1.41 d, δ_C 14.1 and 21.2) methyl groups, three methylenes (δ_H 1.96 dd, 2.47 dd, 2.25 dd, and 2.92 d, δ_C 46.8), including one *exo*-methylene (δ_H 5.30 s and 5.51 s, δ_C 120.5,), six methines (δ_H 2.35–2.39 m, 2.89 dd, 3.60 dq, 5.66–5.69 m, 5.68 s, and 5.86 t, δ_C 38.8, 51.1, 43.6, 65.4, 71.3, and 77.3), one disubstituted carbon–carbon double bond (δ_C 131.4 and 137.0, δ_H 5.68 dd and 5.96 d) and five quaternary carbons (δ_C 50.8, 85.2, and 139.6), including two keto groups (δ_C 208.4 and 212.7) were observed. One proton signal (δ_H 4.22 s), which did not exhibit any correlation in the HSQC spectrum, suggested one hydroxy group in the molecule. The ^{1}H–^{1}H COSY correlations determined three structural fragments (A) (δ_H 2.47 dd, 1.96 dd, 2.35–2.39 m, 5.86 t, 2.89 dd, and 5.68 s), (B) (δ_H 5.66–5.69 m, 2.92 d, and 2.25 dd), and (C) (5.96 d, 5.68 dd, 3.60 dq, and 1.41 d). The sequences A, B, and C, tertiary methyls and quaternary carbons were connected by means of an HMBC experiment. The long-range correlation of the quaternary carbon at δ_C 85.2 (C-15) with the signals at δ_H 1.96 (H-1β), 5.86 (H-3), and 4.22 (15-OH), and the HMBC cross-peaks between δ_C 51.1 (C-4) and 4.22 (15-OH) revealed that structural element A, together with one hydroxy-substituted quaternary carbon, forms a methyl-substituted five-membered ring. The *exo*-methylene protons (H-17a,b) at δ_H 5.51 and 5.30 showed an HMBC correlation to the two oxymethine carbons at δ_C 71.3 (C-5) and 65.4 (C-7) and to the carbon at δ_C 139.6 (C-6), respectively, indicating the connection of sequences A and B through a quaternary carbon bearing the *exo*-methylene group. Similarly, the $^2J_{C,H}$ and $^3J_{C,H}$ couplings of the keto group at δ_C 208.4 (C-9) and the quaternary carbon at δ_C 50.8 (C-10) (Table 1) demonstrated that units B and C are connected. The position of the second keto group at C-14 was established on the basis of the HMBC correlation of the carbon at δ_C 212.7 with 15-OH (δ_H 4.22) and H-20 (δ_H 1.41). The position of the benzoyl group at C-3 was evident from the cross-peak between the carbonyl carbon signal at δ_C 165.7 and the proton signal at δ_H 5.86 (H-3). Two acetate groups were placed at C-5 and C-7 in consequence of the bond correlations between the acetate CO (δ_C 169.2 and 170.0) and the oxymethine protons (δ_H 5.68 and 5.67). Therefore, compound **1** has