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Two new jatrophane diterpenes from the roots of *Euphorbia nicaeensis*

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Abstract: In the previous study fifteen jatrophane diterpenes were isolated from the *Euphorbia nicaeensis* latex. Fourteen of them have been shown to be potent P-glycoprotein (P-gp) inhibitor in two MDR cancer cells (NCI-H460/R and DLD1-TxR). The aim of this study was to determine whether and which jatrophane diterpenes can be isolated from the root of the plant, and then to examine their inhibition power on P-glycoprotein of selected cancer cell lines (NCI--H460, DLD1, U87, NCI-H460/R, DLD1-TxR and U87-TxR). Two previously undescribed jatrophane diterpenes were isolated from the root of *E. nicaeensis* collected in Deliblato Sand (Serbia). The structures of the isolated compounds were determined using 1D and 2D NMR, as well as HRESIMS data. The results obtained by MTT assay showed different antitumor potential of these two jatrophanes. Compound **1** inhibited cell growth of non-small cell lung carcinoma cell lines NCI-H460 and NCI-H460/R, as well as glioblastoma cell lines U87 and U87-TxR, while jatrophane **2** was almost completely inactive in the suppression of cancer cell growth in a given range of concentrations. The obtained results also showed that the isolated compounds have an inhibitory effect on P-glycoprotein, as well as that their inhibitory potential is similar.

Keywords: terpenoids; *Euphorbiaceae*; P-glycoprotein; MDR.

INTRODUCTION

Jatrophane diterpenes are secondary metabolites characteristic only for the family Euphorbiaceae, primarily for the plants of genus *Euphorbia*.[1] More than 350 jatrophane derivatives have been isolated up to now, of which only four have

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been isolated from the genus *Jatropha*, while the rest have been isolated from the genus *Euphorbia*.[1,2] These macrocyclic diterpenes are mostly constructed of a bicyclic [10.3.0] pentadecane system that can be highly functionalized. The great diversity in the structure of these compounds is enabled by the different oxygenation of the jatrophane skeleton, the presence of different ester groups, but also by the possibility of these compounds to appear in different conformations of the twelve-membered ring. The jatrophane diterpenes have been investigated for decades in search for new drugs that prevent multidrug resistance (MDR).[3,4] The MDR is a major medical problem because more and more tumours are becoming resistant to applicable drugs. For this reason, it is necessary to find a way to overcome MDR so that drugs can perform their function again. By the examination of the development of cell resistance to the drugs used, it was found that one of the mechanisms is the overexpression of P-glycoprotein (P-gp).[3–5] Overexpression of P-gp prevents the accumulation of the drug in a cancer cell and thus reduces the therapeutic dose of the drug and increases the resistance of tumour cells. The previous research showed that many naturally occurring jatrophane derivatives have shown higher potencies than ordinary used P-gp inhibitors cyclosporin A or verapamil; hence, they are promising candidates for further drug research.[6–8] Our previous study showed that jatrophane diterpenes isolated from *E. nicaeensis* latex have significant MDR activity, and the major mechanism of their action was inhibition of P-gp expression.[9] The aim of this study was to isolate the jatrophane diterpenes from the root of *E. nicaeensis*, and to examine their inhibitory on P-gp.

EXPERIMENTAL

Plant material

The roots of *E. nicaeensis* was collected from wild stock at Deliblato Sands (Serbia), collection site at latitude: 44°56'57" N and longitude: 21°11'13" E, in May 2018. The plant was identified by Professor Petar Marin, University of Belgrade – Faculty of Biology, Institute of Botany. Voucher specimen (No. 16,855) has been deposited at the Herbarium of Botanical Garden "Jevremovac" University of Belgrade, Belgrade (Serbia).

Isolation and purification

The roots (152 g) were dried, grounded and extracted with 96 % ethanol with heating (2 h) and then left overnight at room temperature. The obtained extract (25 g) was then subjected to the column chromatography (dry flash (SiO_2, eluent petroleum ether/acetone, gradient 10/0 → 1/9), Table S-I). Progress of separation was followed by TLC (precoated Merck silica gel 60 F_{254} plates) and ^{1}H-NMR spectra. The fraction that contained jatrophanes was eluted with 20 % acetone. That fraction was further separated using the column chromatography (dry flash (SiO_2, isocratic, petroleum ether/acetone 97/3)) to afford subfraction F1. The subfraction F1 was further purified by the preparative normal phase liquid chromatography (NP-LC), using an Agilent Technologies 1260 series liquid chromatograph equipped with diode-array detector (λ = 210 nm), autosampler and thermostated column compartment, under the following conditions: injection volume 500 µL (c ~10 mg mL^{-1}, acetone), Zorbax RX-Sil column (250 mm×9.4 mm; 5 µm), column temp. 24 °C, mobile phase 4.00 mL min^{-1},

isocratic, acetone/petroleum ether 12.5/87.5. The obtained fractions F1a and F1b were finally purified by the preparative reversed phase liquid chromatography (RP-LC) using Agilent Technologies 1100 series liquid chromatograph equipped with diode-array detector (λ 210 and 264 nm), autosampler and thermostated column compartment. For the separation of the compounds, the following LC parameters have been applied: the injection volume 1000 μL ($c \approx 10$ mg mL^{-1}, MeOH), Zorbax XDB-C18 column (250 mm×9.4 mm; 5 μm), column temp. 20 °C, mobile phase 4.00 mL min^{-1}. The mobile phase consisted of two solvents: MilliQ water (solvent A) and CH_3CN (solvent B). The following gradient was set: 40–80 % B, 0–10 min, 80–90 % B, 10–15 min. ESI-MS spectra were recorded on Agilent Technologies 6550 Funnel Q-TOF MS instrument in positive ion mode with MeOH/H_2O 1/1 with 0.2 % HCOOH as the carrying solvent solution. The samples were dissolved in MeOH (MS hypergrade purity). The selected values were as follows: capillary voltage = 3,500 V, fragmentor voltage = 175 V, nozzle voltage = 1,000 V, skimmer 1 = 65 V, octopole RF peak = 750 V, desolvatation gas (nitrogen) temperature 200 °C, desolvatation gas (nitrogen) flow 14 L min^{-1}, sheat gas (nitrogen) flow 11 L min^{-1}. From subfraction F1a (23.4 mg) the compound **1** (1.2 mg) was obtained, while from fraction F1b (2.3 mg) the compound **2** (0.8 mg) was obtained. IR spectra were recorded on a Thermo Scientific Nicolet 6700 FT-IR. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 500 Avance III spectrometer (500.26 and 125.80 MHz, respectively) using $CDCl_3$ as a solvent and TMS as the internal standard. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hz.

Drugs

Tariquidar (TQ) was kindly provided by Dr. Sven Rottenberg from The Netherlands Cancer Institute, Amsterdam. TQ was diluted in dimethyl sulfoxide and 10 μM aliquots were kept at –20 °C. Jatrophane diterpenes (**1** and **2**) were kept as 20 mM stocks in 100 % ethanol at –20 °C. Working solutions of 200 μM were prepared in 10 % ethanol.

Cells and cell culture

The NCI-H460, DLD1, and U87 cell lines were purchased from the American Type Culture Collection, Rockville, MD, USA. NCI-H460/R cells were selected from NCI-H460 cells by their continuous culturing in a medium containing stepwise increasing concentrations of doxorubicin for three months.[10] Similarly, DLD1-TxR and U87-TxR cells were selected from DLD1 and U87 cells, respectively, by continuous exposure to the stepwise increasing concentrations of paclitaxel during six to nine months.[11] NCI-H460, NCI-H460/R, DLD1 and DLD1--TxR were grown in RPMI 1640 medium supplemented with 10 % fetal bovine serum, 1 % L-glutamine, and 1 % antibiotic–antimycotic mixture, while U87 and U87-TxR were cultivated in MEM supplemented with 10 % fetal bovine serum, 1 % L-glutamine, 1 % antibiotics, and 1 % non-essential amino acids. All cell lines were sub-cultured two-times per week using 0.25 % trypsin/EDTA and seeded into a fresh medium at the following densities: 8,000 cells cm^{-2} for NCI-H460, NCI-H460/R, DLD1 and DLD1-TxR, and 16,000 cells cm^{-2} for U87 and U87-TxR.

Cell viability assay

Cell viability was assessed by MTT assay (Sigma, St. Louis, MO, USA). MTT assay is based on the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide into formazan dye by active mitochondria of living cells. Briefly, cells were seeded in 96-well tissue culture plates (2,000 cells/well for NCI-H460, NCI-H460/R, DLD1 and DLD1-TxR; 4,000 cells/well for U87 and U87-TxR) and incubated overnight in 100 μL of appropriate medium. Afterwards, cells were treated with the increasing concentrations of jatrophane diter-

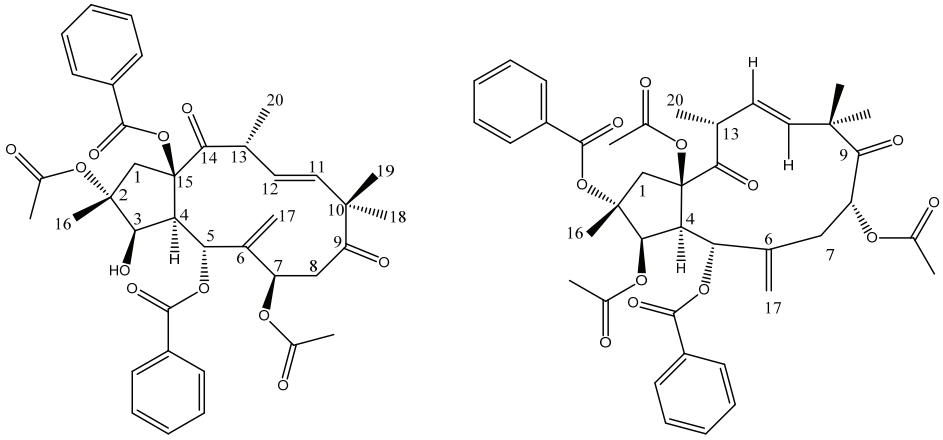
penes **1** and **2** (1, 5, 10, 20 and 50 μM). All treatments lasted 72 h. At the end of treatment period, 100 μL of MTT solution (1 mg mL^{-1}) was added to each well and plates were incubated at 37 °C for 4 h. The formazan product was dissolved in 100 μL DMSO. The absorbance of obtained dye was measured at 570 nm with reference wavelength at 690 nm using an automatic microplate reader (Multiskan Sky, Thermo Scientific, Waltham, MA, USA). IC_{50} value was defined as concentration of each drug that inhibited cell growth by 50 %. IC_{50} was calculated by non-linear regression analysis using log (inhibitor) *vs*. normalized response in GraphPad Prism 8.0.2 software.

Rhodamine 123 accumulation assay

The function of P-glycoprotein was analysed by flow cytometry exploiting the ability of its substrate rhodamine 123 to emit fluorescence. The increased level of rhodamine 123 accumulation positively correlated with the inhibited P-glycoprotein function. TQ was used as a positive control. The MDR cancer cells were suspended in 3.5 mL centrifuge tubes in a 5 μM rhodamine 123-containing medium. Then, the cells were immediately treated with jatrophanes **1** and **2** (10 μM) and TQ (50 nM) and incubated at 37 °C in 5 % CO_2 for 30 min. The samples were washed twice, suspended in 1 mL of cold phosphate-buffered saline, and analysed using a flow cytometer (Partec, Münster, Germany) and the data were analysed by Summit 4.3 (DAKO, Carpinteria, CA, USA). The fluorescence of rhodamine123 was assessed on green fluorescence channel 1 (FL1). At least 20000 events were assayed for each sample.

RESULTS AND DISCUSSION

Chemical analyses on the roots of *E. nicaeensis* afforded two unreported jatrophane diterpenes (Fig. 1). The structures of the isolated compounds and their relative configurations were established on the basis of spectroscopic analysis includeing 1D and 2D NMR (COSY, NOESY, HSQC, HMBC) and HRESIMS data.



1 **2**

Fig. 1. The structures of the isolated jatrophane diterpenes.

Compound **1**, $[\alpha]^{20}$; D = –266.3 (c 0.08, acetone), was isolated as a colourless amorphous substance with the molecular formula $C_{38}H_{42}O_{11}$, as determined by the

^{1}H- and ^{13}C-NMR data (Table S-II of the Supplementary material to this paper), as well as the (+)ESI-HRMS *m*/*z*: calculated for [$C_{38}H_{42}O_{11}$ + Na^{+}] 697.2800, observed 697.2612, and the IR (ATR): 2974*s*, 1736*s*, 1230*s*, 1121*m*, 1023*m*, cm^{-1}. Its ^{13}C-NMR spectra showed 38 carbon signals, including two keto carbonyls at 208.29 (C-9) and 204.69 (C-14), four ester carbons at 169.55 (C-1'), 163.90 (C-1''), 170.28 (C-1'''), and 165.96 (C-1^{vi}), two double bond pairs (one geminally substituted (140.78 and 116.48, C-6(17)), and one *trans* substituted (135.48 and 133.22, C-11(12))), eight aromatic carbons (129.50 (C-2''), 129.45 (C-3'') 129.24 (C-4''), 129.34 (C-5''), 130.04 (C-2^{vi}), 130.69 (C-3^{vi}) 133.85 (C-4^{vi}), and 133.94 (C-5^{vi})), five oxygenate carbons (91.30 (C-2), 77.92 (C-3), 73.94 (C-5), 76.78 (C-7) and 91.83 (C-15)), and six methyl groups (18.58 (C-16), 26.91 (C-18), 23.29 (C-19), 18.33 (C-20), 20.92 (C-2'), and 20.86 (C-2^{iv})). The ^{1}H-NMR and COSY spectra revealed the presence of four separate *J*-coupling networks (**A**–**D**): **A**) H-1α/H-1β (δ_H 3.95 *d* and 2.56 *d*); **B**) H-3/H-4/H-5 (δ_H 4.71 *brs*, 2.85 *d* and 5.55 *brs*); **C**) H-7/H-8α/H-8β (δ_H 4.97 *d*, 2.43 *dd* and 2.02 *d*); **D**) H-11/H-12/H-13/H-20 (δ_H, 5.22 *d*, 5.54 *dd*, 3.60 *dq* and 1.22 *d*), Figs. S-1 and S-3, Supplementary material). The large vicinal coupling constants of signals H-11 (δ_H 5.22, J = 16.0 Hz) and H-12 (δ_H 5.54, J = 16.0; 10 Hz) indicated *E* geometry of the double bond at C-11 (Fig. S-1 of the Supplementary material). The COSY fragments were connected using the long-range C–H correlations in the HMBC spectrum. The long-range heteronuclear couplings ($^{2\text{-}4}J_{C,H}$) of the carbons of C-15 with H-1α, H-1β, H-3, H5β and H-16 confirmed the presence of five-membered ring (ring A). The HMBC correlations between H-5 and C-6, as well as C-6 with H-7 and H-17a/17b made it possible to link the COSY fragments **A** and **B**. The long-range correlation between C-9 with H-8, H-11, H-18 and H-19 connected COSY fragments **B** and **C**. The linkage of fragment **B** with five-membered ring was enabled by the HMBC correlations between C-14 and H-1α, H-1β, H-4, H-12, H-13 and H-20 (Fig. S-4). At the end, it was concluded that the isolated compound **1** is a jatrophane diterpene with two keto groups (C-9 and C-14), and esters at positions C-2, C-5, C-7 and C-15, respectively, and with a free hydroxyl group at C-3. Benzoyl ester at C-5 was proved from HMBC correlation of the carbonyl signal (163.90) with the proton H-5, while acetate at C-7 was identified from the HMBC correlation of the carbonyl signal (170.28) with the proton H-7 (Fig. S-4). The other two ester groups were connected from NOESY correlations, because those ester groups were attached to the quaternary carbons. The proton of the second benzoate (8.10 ppm) showed coupling with the protons H-5β, H-13β and H-16β, as well as with the protons of acetate at the C-7 position (Fig. S-5).

According to the chemical shift of C-15 (91.83) and HMBC correlations of C-15 mentioned above, C-15 position of the benzoate was deduced. The protons of the methyl group (1.75 ppm) of the second acetate exhibited NOESY correl-

ation with the protons H1α, H3α, and the proton from the benzoate bound at the C-5 position, indicating that this acetate is bound at the C-2 position (Fig. S-5).

The relative configuration of **1** was deduced by the interpretation of the NOESY spectrum and coupling constants (Fig. S-5). The H-5/H-4 and H-5/H-8β NOE correlations, the absence of correlation between H-5 and H-17a/b, and a small value of $^3J_{4,5}$ (3 Hz) suggested that **1** belong to the *exo*-type conformation with exomethylene 6,17-double bond.[12] The configuration of proton H-4 was determined biogenetically as H-4α,[13] and the NOE interactions of H-4α with H-3α implied 3β-OH orientation. The correlation of H-1β with methyl group at C-16 suggested β orientation of H-16, and α-orientation of 2α-OAc. The NOE correlation H-5β/H-13β confirmed the orientation of the methyl group at C-13 (H-20α), as well as 5α-OBz and H-18α. The NOE interactions between H-4α and H-7α indicated 7β-OAc. The above evidences confirmed the structure of **1** as 2α,7β-diacetyloxy-3β-hydroxy-5α,15β-dibenzoyloxyjatropha-6(17),11*E*-diene--9,14-dione.

Compound **2**, $[\alpha]^{20}$; D = –68.8 (c = 0.08 g mL^{-1}, methanol), IR (ATR): 2976*s*, 1734*s*, 1235*s*, 1122*m*, 1021*s* cm^{-1}, was isolated as a colourless amorphous substance with the molecular formula $C_{40}H_{44}O_{12}$, as deduced by the ^{1}H- and ^{13}C-NMR data (Table S-II), as well as the (+)ESI-HRMS *m*/*z*: calculated for [$C_{40}H_{44}O_{12}$ + H^+] 717.2906, observed 717.2904. The structure of compound **2** was similar to the structure of **1**. Significant structural differences between these two compounds were in the number of ester groups, as well as in the conformation of the jatrophane skeleton. In contrast to the compound **1**, in which there were four ester groups, in compound **2** there were five (two benzoates and three acetates). The positions of ester groups in this molecule were determined on the basis of $^3J_{C,H}$ HMBC correlations of the ester carbonyls and neighbouring protons from the jatrophane skeleton (Fig. S-8). The attachment of the acetates was proved from HMBC correlations of the carbonyl signal at 169.50 with the proton H-3 (δ_H 6.01), as well as the proton H-8 (δ_H 5.13) and carbonyl 170.57. The correlation of proton H-5 (δ_H 5.77) and carbonyl signal at 165.64 enabled the binding of one benzoate to the C-5 position. The binding sites of the remaining two esters were determined on the basis of the NOE correlations and chemical shifts of carbons' positions of attachment (C-2 and C-15), as in compound **1**. The relative configuration of **2** was determined by the interpretation of the NOESY spectrum (Fig. S-9). The H-5/H-17a and H-8/H-17b NOE correlations, as well as large value of $^3J_{4,5}$ (6 Hz) suggested that **2** belonged to the *endo*-type conformation with exomethylene 6,17-double bond perpendicular to the main plane,[12] that is usually adopted in compounds lacking substituent at C-7.[14,15]

All NMR spectra and spectroscopic data for compounds **1** and **2** are given in the Supplementary material.

Jatrophane diterpenoids are well-known for their multidrug resistance (MDR) modulating potential due to the direct interaction and the inhibition of P-glycoprotein.[9,16,17] The effect of **1** and **2** on the cell growth of six human cancer cell lines (three pairs of sensitive and corresponding MDR cell lines) were examined. The results obtained by MTT assay showed different antitumor potential of these two jatrophanes. Specifically, **1** inhibited cell growth of non-small cell lung carcinoma cell lines NCI-H460 and NCI-H460/R, as well as glioblastoma cell lines U87 and U87-TxR with IC_{50} values between 10 μM and 20 μM (Table I). Both colorectal carcinoma cell lines, DLD1 and DLD1-TxR, were resistant to **1** with IC_{50} values over 50 μM (Table I, Fig. 2). However, MDR cell lines (NCI-H460/R and U87-TxR) were not resistant to **1**, meaning that the IC_{50} values of MDR cell lines were not significantly increased in comparison with the IC_{50} values of the corresponding sensitive cell lines (NCI-H460 and U87, respectively). On the contrary, jatrophane **2** was almost completely inefficient in the suppression of cancer cell growth in a given range of concentrations (1–50 μM). Only glioblastoma cell line U87 responded to **2** treatment with IC_{50} value around 20 μM (Table I, Fig. 2).

TABLE I. Cancer cell growth inhibition (IC_5 / μM, average ± standard deviation) induced by jatrophanes **1** and **2**

Cell line	Compound	
	1	**2**
NCI-H460	17.63±2.08	> 50
NCI-H460/R	20.98±2.79	> 50
DLD1	> 50	> 50
DLD1-TxR	> 50	> 50
U87	10.97±1.41	20.12±1.96
U87-TxR	15.49±3.57	> 50

Our previous results demonstrated that jatrophane diterpenoids are able to selectively inhibit cancer cell growth without harming normal cells.[18] Besides, jatrophanes showed potential to reverse resistance to paclitaxel and doxorubicin in non-small cell lung carcinoma cells.[9,18] This chemosensitization effect of jatrophanes is related to their ability to inhibit P-glycoprotein function. Therefore, the P-glycoprotein interaction with **1** and **2** in three human MDR cancer cell lines (NCI-H460/R, DLD1-TxR, and U87-TxR), using functional rhodamine 123 accumulation assay, was tested. The obtained results showed that jatrophanes **1** and **2** have similar potency in the inhibition of P-glycoprotein function (Table II). The potential of the tested compounds to inhibit P-glycoprotein activity in MDR cancer cell lines is expressed as fluorescence activity ratio (*FAR*), while sensitization index (*SI*) was used to compare their effects in MDR cancer cell lines with the rhodamine 123 accumulation in untreated sensitive cancer cell lines (Table II).

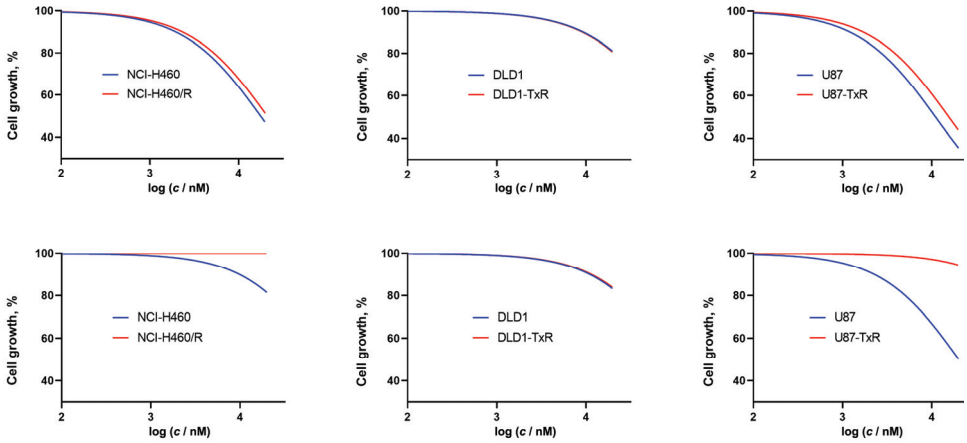


Fig. 2. Non-linear regression analysis of cell growth inhibition induced by jatrophanes **1** (above) and **2** (below). MTT results obtained with **1** and **2** treatments in NCI-H460, NCI-H460/R, DLD1, DLD1-TxR, U87, and U87-TxR cells (absorbance at 570 nm corrected with 690 nm) were transformed into percentages of cell growth normalized to untreated cells and presented in $\log_{10}$ scale of concentrations using GraphPad Prism 8.0.2 software.

TABLE II. P-glycoprotein inhibition induced by **1** and **2** in MDR cancer cell lines

Medium	NSCLC			Colorectal carcinoma			Glioblastoma		
	MFI[a]	*FAR*[b]	*SI*[c]	*MFI*	*FAR*	*SI*	*MFI*	*FAR*	*SI*
Sensitive cells[d]	100.30±2.93[e]		100.0	101.16±2.81		100.0	182.70±1.57		100.0
MDR cells	16.32±3.51		16.3	25.27±10.20		25.0	24.44±3.99		13.4
1[f]	114.62±10.92	7.0	114.3	202.93±1.34	8.0	200.6	149.80±0.98	6.1	82.0
2	92.41±2.92	5.7	92.1	212.55±1.55	8.4	210.1	144.94±1.00	5.9	79.3
TQ[g]	92.95±2.88	5.7	92.7	130.34±1.42	5.2	128.8	120.92±1.32	4.9	66.2

[a]The measured mean fluorescence intensity (*MFI*) was used for the calculation of the fluorescence activity ratio (*FAR*); [b]*via* the following equation: $FAR = MFI_{MDRtreated}/MFI_{MDRcontrol}$; [c]the sensitivity index (*SI*) was calculated on the basis of the measured mean fluorescence intensity (*MFI*) expressed *via* the following equation: $SI = (MFI_{MDRtreated} \times 100)/MFI_{sensitive\ control}$; [d]sensitive cancer cell lines and their MDR counterparts used in the study: non-small cell lung carcinoma-NSCLC (NCI-H460 and NCI-H460/R), colorectal carcinoma (DLD1 and DLD1-TxR) and glioblastoma (U87 and U87-TxR); [e]*MFI*±*SEM* (standard error of mean); [f]jatrophanes were applied at the same concentration of 10 μM; [g]*TQ* was applied at 50 nM

SI values reflect the capacity of **1** and **2** to restore the rhodamine 123 accumulation in MDR cancer cell lines close to the level of accumulation observed in sensitive cancer cell lines. On SI values scale, the strong sensitization exists when $50 << SI < 100$ and there is a complete blockade of P-glycoprotein function when $SI >> 100$ (the level of accumulated rhodamine 123 in MDR cancer cells after treatment with compounds that are P-glycoprotein inhibitors exceeds the level obtained in sensitive cancer cells). Importantly, TQ, a non-competitive inhibitor of P-glycoprotein,[19] was equally or less potent than **1** and **2** in all tested MDR cancer cell lines (Table II). The highest increases in the rhodamine 123 accumulation and

complete blockade of P-glycoprotein (*SI* > 100) were observed in non-small lung carcinoma cells (non-small cell lung cancer – NSCLC) NCI-H460/R treated with **1** and in colorectal carcinoma cells DLD1-TxR treated with both jatrophanes and TQ (Table II). Similar strong sensitization in DLD1-TxR was achieved with jatrophanes **2**, **4**–**6** and **15** that were isolated from the latex of *Euphorbia dendroides*.[17]

CONCLUSION

In conclusion, the jatrophane diterpenoides **1** and **2** completely blocked P-glycoprotein in MDR NSCLC and colorectal carcinoma cells showing even higher potential than TQ in MDR colorectal carcinoma and glioblastoma cells. Therefore, both jatrophanes could be valuable as sensitizing agents capable to decrease the effective concentrations of drugs which are P-glycoprotein substrates. Importantly, jatrophane **1** exerted cell growth inhibitory effect in NSCLC and glioblastoma cells, indicating that this compound could also have considerable anticancer properties. Generally, these jatrophane diterpenoides can be used as lead compounds for drug development and the improvement of chemotherapy.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11038, or from the corresponding author on request.

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ИЗВОД

ДВА НОВА ЈАТРОФАНСКА ДИТЕРПЕНА ИЗ КОРЕНА *Euphorbia nicaeensis*

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У претходном истраживању, петнаест дитерпена јатрофанског типа изоловано је из латекса *Euphorbia nicaeensis*. Њих четрнаест показала су се као снажни инхибитори P-гликопротеина (P-gp) у две MDR ћелијске линије рака (NCI-H460/R и DLD1-TxR). Циљ ове студије био је да се утврди да ли је и које јатрофанске дитерпене могуће изоловати из корена биљке, а затим испитивање њихове инхибиторне моћи на P-гликопротеину одабраних ћелијских линија рака (NCI-H460, DLD1, U87, NCI-H460/R, DLD1-TxR и U87-TxR). Два претходно непозната јатрофана изолована су из корена *E. nicaeensis* прикупљеног у Делиблатској пешчари. Структуре изолованих једињења одређене су применом 1D и 2D NMR метода, као и HRESIMS експеримента. Резултати добијени MTT тестом показали су различит антиканцерогени потенцијал ова два јатрофана. Једињење **1** је инхибирало раст ћелија ћелијских линија неситноћелијског карцинома

плућа NCI-H460 и NCI-H460/R, као и ћелијских линија глиобластома U87 и U87-TxR, док је јатрофан **2** био готово потпуно неефикасан у сузбијању раста ћелија карцинома у датом концентрационом опсегу. Добијени резултати су такође показали да **1** и **2** имају инхибиторно дејство на P-гликопротеин, као и да је њихов инхибиторни потенцијал сличан.

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