SYNERGISTIC EFFECT BETWEEN MACROCYCLIC DITERPENES AND EPIRUBICINE ON RESISTANT CANCER CELLS

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INTRODUCTION

The aim of this study was to search for new multidrug resistance reversal agents from *Euphorbia* species. In this way, three new jatophane diterpenes (1 – 3) isolated from *E.tuckeyana* (Fig. 1) and three new lathyrane esters (4 - 6) obtained through acylation reations of latilagascene B (Fig. 4), have been screened for their potential P-gp modulating properties on mouse resistance cancer cell lines. Furthermore, in the combination chemotherapy model, the interaction between the cytostatic epirubicine and the six diterpenes were also evaluated.

Figure 1. *Euphorbia tuckeyana*

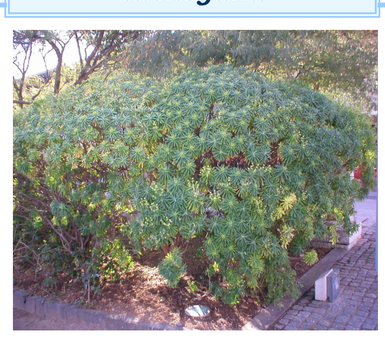
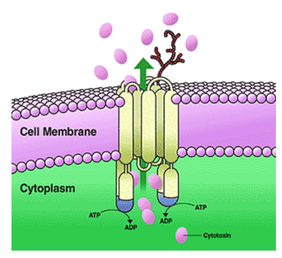


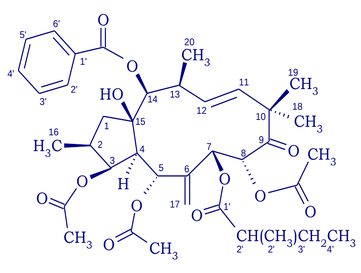
Figure 2. P-gp – a drug efflux pump[3]



ABOUT MULTIDRUG RESISTANCE

One of the most effective strategies in biological systems to demonstrate resistance to cytotoxic drugs is the efflux of these compounds out of the cell, via membrane transporter proteins (Eg. P-glycoprotein, Fig. 2). This phenomenon is called **multidrug resistance** (**MDR**) and is considered to be one of the major difficulty in cancer treatment.

Figure 3. New jatrophane diterpenes isolated from *Euphorbia tuckeyana*



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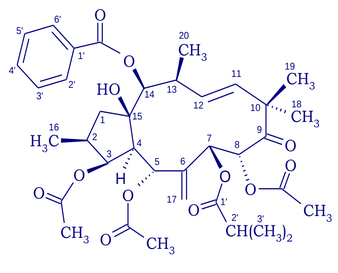

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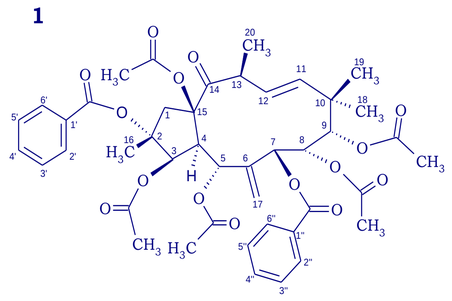

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Table 1. MDR reversal effects of compounds 1 -6 on L5178 resistant cell line.

Compounds	Concentration (μg/mL)	FSC[a]	SSC[a]	FL-1[a]	FAR
PAR+R123[b]	–	623.59	158.89	711.39	
MDR+R123[c]	–	698.49	185.04	11.02	
Verapamil	10	612.50	193.64	47.50	3.41
1	4	668.84	240.39	554.00	39.82
	40	664.00	226.76	1375.55	98.88
2	4	669.50	245.69	347.56	24.98
	40	670.47	230.79	1353.24	97.28
3	4	670.95	230.19	1127.36	81.04
	40	665.70	242.43	1476.67	106.15
4	4	634.27	459.88	478.33	68.92
	40	584.82	458.03	496.62	71.56
5	4	604.68	463.33	427.35	61.58
	40	563.66	465.42	541.26	77.99
6	4	619.97	452.84	433.40	62.45
	40	563.92	465.78	376.52	54.25
DMSO	20 μL	655.80	268.69	15.12	1.08

[a]FSC: Forward scatter count of cells in the samples; SSC: Side scatter count of cells in the samples; FL-1: Mean fluorescence intensity of the cells. Fluorescence activity ratio: values were calculated by using the equation given in the experimental section; [b]PAR control: a parental cell without MDR gene; [c]MDR: a parental cell line transfected with human MDR1 gene.

Latilagascene B: 4 μg/mL → FAR = 28.1;

40 μg/mL → FAR = 102..1

Figure 4. Lathyrane diterpenes obtained through acylation of latilagascene B

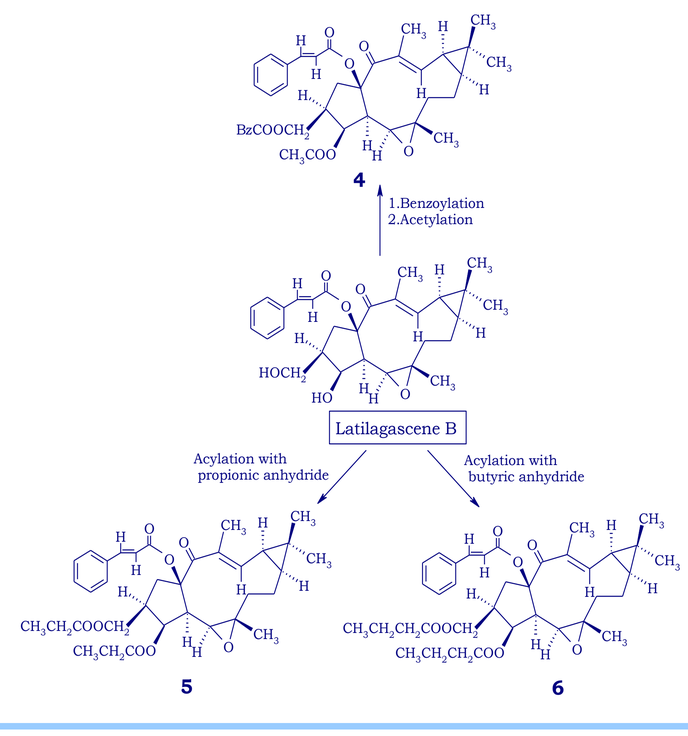


Figure 5. ^{1}H NMR spectrum of compound 3.

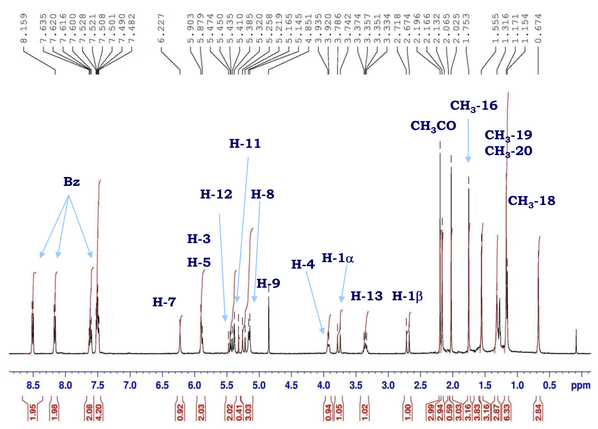
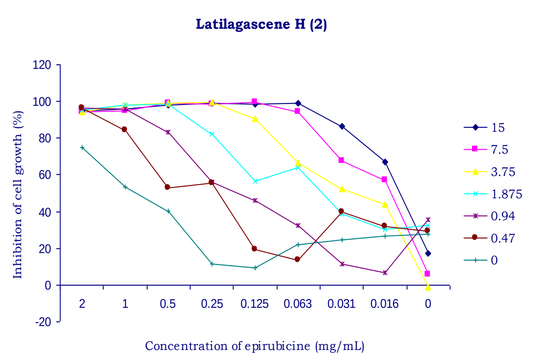


Table 2. In vitro effects of compounds 1 – 6 in combination with epirubicine.

Compounds	FIX value	Interaction
1	0.180	Synergism
2	0.250	Synergism
3	0.085	Synergism
4	0.144	Synergism
5	0.070	Synergism
6	0.137	Synergism

Figure 6. Effect of latilagascene H (5), [15 - 0 μg/mL] in combination with epirubicine on L5178 MDR cell line



Materials and Methods

Compounds: Three new jatrophane diterpenes named tuckeyanol A (**1**), tuckeyanol B (**2**) and euphotuckeyanol (**3**) were isolated from the methanol extract of *Euphorbia tuckeyana*, by chromatographic methods. Latilagascenes G (**4**), H (**5**) and I (**6**) were obtained by acylation reactions of latilagascene B, previously isolated from *Euphorbia lagascae*. All structures were established by the combination of physical and spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR and MS), (Fig. 5). All compounds were dissolved in DMSO.

MDR modulation assay: Cells: L5178 Y mouse T-lymphoma parental cell line transfected with the pHa MDR1/A retrovirus. The Rhodamine 123 (R123) uptake assay was done as described elsewhere.[1,2] Verapamil (5 μl of a 2.0 mM solution) was used as a positive control. The mean fluorescence intensity was calculated as a percentage of the control for the parental and MDR cell lines as compared to untreated cells. An activity ratio (R) was calculated on the basis of the measured fluorescence values (FL-1) measured via the following equation: R = (FL-1 MDR treated/FL-1 MDR control)/(FL-1 parental treated/FL-1 parental control). The **Checkerboard microplate method** as a model for combination therapy was done as described in the literature.[1,2] The effects of the anticancer drug epirubicine and the resistance modifiers in combination were studied. Drug interactions were evaluated according to the following system:

FICA = ID50A in combination / ID50A alone; FICB = ID50B in combination / ID50B alone; ID = inhibitory dose; FIC = fractional inhibitory concentration; **FIX** = fractional inhibitory index; **FIX** = FICA+ FICB; **FIX** = 0.51-1: Additive effect; **FIX** < 0.5: Synergism; **FIX** = 1 – 2: Indifferent effect; **FIX** > 2: Antagonism

RESULTS AND CONCLUSION

- All the studied diterpenes were found to strongly increase drug retention in the cells, by inhibiting the efflux pump activity mediated by P-gp (Table 1).
- All the compounds exhibited a slightly dose-dependent activity, which was stronger than the positive control, verapamil.
- The acylation of the free hydroxyl groups of latilagascene B enhance drastically the anti-MDR activity (at 4 μg/mL) in the ester derivatives **4** – **6**.
- As can be observed in Table 2, all compounds showed a synergistic effect with epirubicine, in the combination chemotherapy. The more effective compound was latilagascene H (FIX = 0.07, Fig. 6).

References: [1]Duarte *et al*, Planta Medica 2006, 72: 162; [2]Duarte *et al*, Bioorg. Med. Chem. 2007, 15: 546; [3]http://www.altcorp.com/AffinityLabeling/pglycoprotein.htmwww.xenova.co.uk/dc_xr9576.html