$$FAR = \frac{MDR\ treated / MDR\ control}{parental treated / parental control}$$

Inhibition of P-glycoprotein Activity by Curcubitane-type Triterpenes and their Interaction with Doxorubicin on Resistant Cancer Cells

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INTRODUCTION

The overexpression of P- glycoprotein (P-gp) is one of the mechanisms of multidrug resistance (MDR), responsible for the failure of cancer treatment. One strategy to restore the effectiveness of the anti-cancer drugs is to co-administer compounds that are not toxic themselves, but inhibit these efflux pumps. These compounds have been called MDR inhibitors, MDR modulators, MDR reversal agents or chemosensitizers (Fig.1). In recent years, several compounds have been reported as MDR modulators, obtained either from natural origin or by synthesis. However, in spite of the great number of MDR inhibitors known, no effective modulator without side effects is still available for the clinical practice.[1]

The aim of this study was to search for new multidrug reversal agents from *Momordica balsamina* L. (Fig.2). In this way, three new cucurbitane-type triterpenoids (**1**-**3**), a known compound (**4**) and five new acylated derivatives (**5**-**9**) prepared through acylation reactions of compound **4**, have been evaluated for their potential ability as MDR modulators (Fig.3). Furthermore, the antiproliferative effects of the anticancer drug doxorubicin and the most effective modulators, in combination, was also studied.

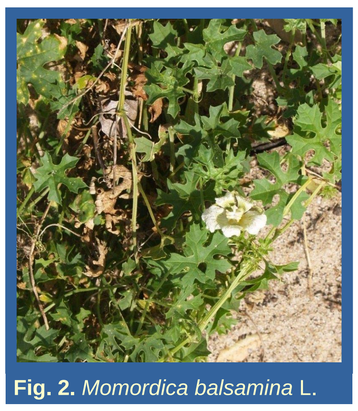

Fig. 2. *Momordica balsamina* L.

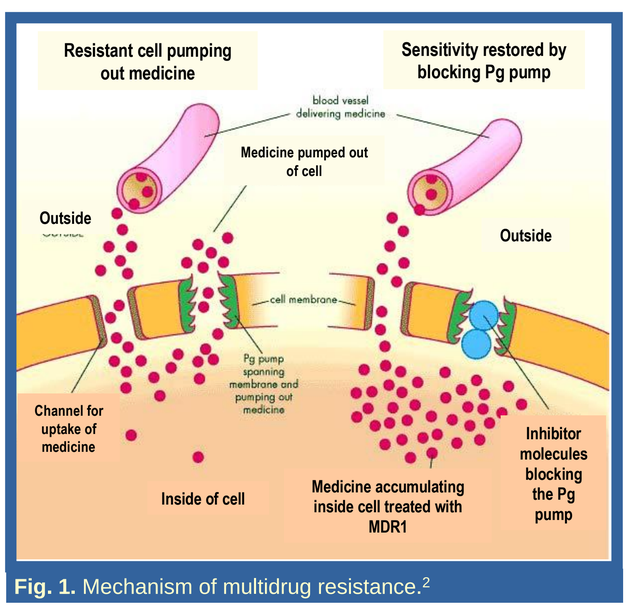

Fig. 1. Mechanism of multidrug resistance.[2]

RESULTS

Cucurbitacins **1** - **9** were evaluated for their MDR-reversing activities on L15178 mouse T-lymphoma cell line transfected with the human *MDR1* by using the rhodamine-123 exclusion test. The results are summarized in Table 1.

Compounds with FAR values higher than 1 were considered active as P-gp inhibitors and those with FAR values higher than 10 strong MDR modulators.[2]

At the highest concentration (20 µM) all compounds, excepting compound **8**, were found to be strong P-gp inhibitors (FAR = 20.0 – 104.2), exhibiting compound **2** (FAR = 5.98 and 104.2 at 2 and 20 µM, respectively) and compound **3** (FAR = 1.45 and 89.4 at 2 and 20 µM, respectively) the highest effects, and a manifold activity when compared to that of the positive control verapamil (FAR = 7.4 – 8.6 at 22 µM concentration).

Compounds **1**, **8** and **9** were found to be inactive at 2 µM concentration. At this concentration, compounds **4** (FAR = 42.1 and 46.0 at 2 and 20 µM, respectively) and **5** (FAR = 35.2 and 34.2 at 2 and 20 µM, respectively) exhibited the highest effect in reversing MDR.

In further experiments, some of the most effective resistance modulators (**2**-**5**) were studied in combination with doxorubicin, on the same cell line, using the checkerboard microplate method. All these compounds exhibited a synergistic effect with doxorubicin (Table 2 and Fig. 4). The most effective compound was compound **2**, which expressed a significant synergy having a fractional inhibitory index of 0.18.

Table 1. MDR reversal effects of compounds **1 – 9** on L 5178 resistant cell line

Compound	Conc. (µM)	FL-1	FAR	Compound	Conc. (µM)	FL-1	FAR
PAR+R123	—	974.2					
MDR+R123	—	15.0					
Verapamil	22.0	154.5	8.6				
1	2.0	13.95	1.07	**6**	2.0	102.1	5.7
	20.0	579.8	44.3		20.0	671.2	37.5
2	2.0	78.4	5.98	**7**	2.0	30.3	1.7
	20.0	1365.1	104.2		20.0	726.8	40.6
3	2.0	19.0	1.45	**8**	2.0	16.2	0.9
	20.0	1171.1	89.4		20.0	42.3	2.4
4	2.0	365.7	42.1	**9**	2.0	18.0	1.0
	20.0	399.5	46.0		20.0	388.7	21.7
5	2.0	671.2	35.2	**DMSO**	10 µl	13.75	0.8
	20.0	612.7	34.2				

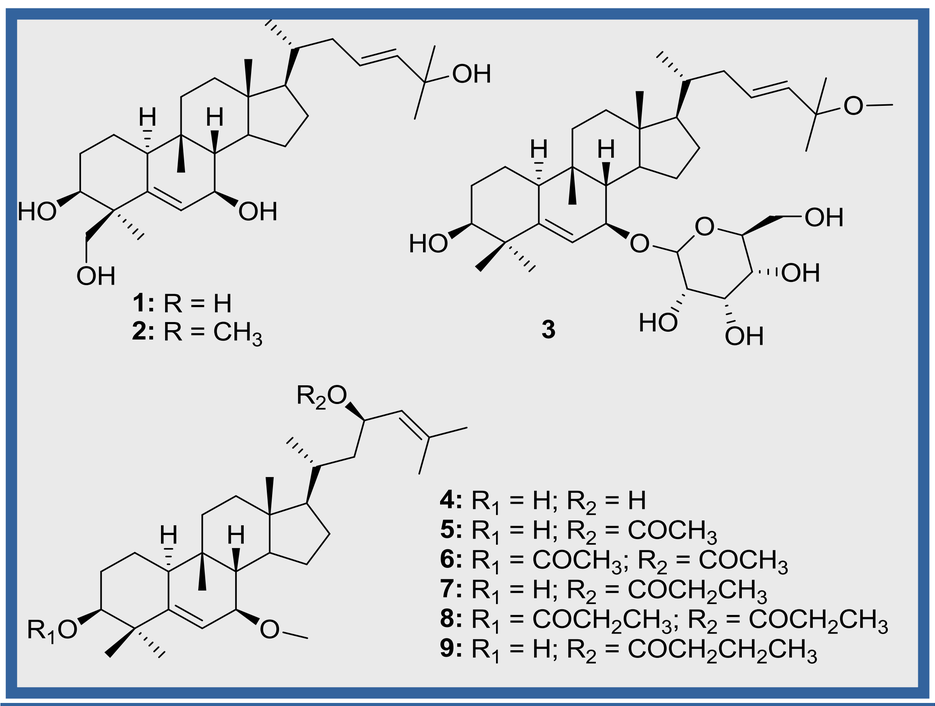

Fig. 3. New cucurbitacins (1-3) isolated from *Momordica balsamina* and derivatives obtained through acylation of compound 4.

Table 2. *In vitro* effects of compounds **2-5** in combination with doxorubicin

Compound	FIX value	Interaction
2	0.18	Synergism
3	0.37	Synergism
4	0.28	Synergism
5	0.44	Synergism

CONCLUSIONS

Some of the tested triterpenes have shown to enhance drug retention by strongly modulating the efflux pump activity mediated by P-glycoprotein. The most active compounds were compounds **2**, **3** and **4**. Furthermore, compounds **2 – 5** synergistically enhance the effect of the anticancer drug doxorubicin, when used in combination.

In conclusion, these results indicate that cucurbitane-type triterpenes can be considered as very promising lead compounds for the reversal of multidrug resistance.

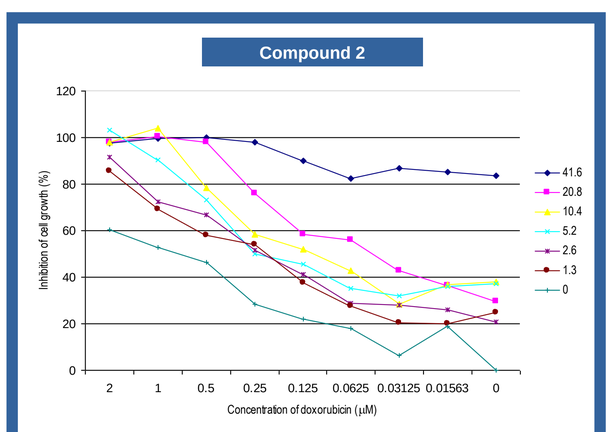

Fig. 4. Effect of compound 2, [41.5 - 0 µM] in combination with doxorubicin on L5178 MDR cell line

MATERIALS AND METHODS

Isolation of Compounds: The powdered aerial parts of *Momordica balsamina* were extracted at room temperature with methanol. The methanol extract was further fractionated, by liquid-liquid extraction. The resulting methanol:H_2O extract was then studied by chromatographic methods, until the isolation of pure compounds **1**-**4**. Compound **4** was derivatized using several acylating reagents to afford compounds **5**-**9**. All the structures were deduced from their physical and spectroscopic data, including 2D NMR experiments (COSY, HMQC, HMBC and NOESY).

MDR Reversal Assay and Checkerboard Microplate Method: These assays were done in *MDR1* gene-transfected mouse lymphoma cells as previously described.[4] An activity ratio (FAR) was calculated on the basis of the measured fluorescence values (FL-1) measured via the following equation: FAR = (FL-1MDR treated/FL-1MDR control)/(FL-1parental treated/ FL-1parental control).

Acknowledgement: FCT, Portugal (PhD grant SFRH/BD/22321/2005).

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