TRITERPENOIDS AS ANTIPROLIFERATIVE AGENTS IN CLASSICAL AND ATYPICAL MULTIDRUG RESISTANT CANCER CELLS

Lage H[1], Ramalhete C[2], Mulhovo S[3] and Ferreira MJU[2]

[1]Charité Campus Mitte, Institute of Pathology, Schumannstr. 20/21, D-10117 Berlin, Germany; [2]iMed.UL, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal; [3]Escola Superior Técnica, Departamento de Ciências Agro-Pecuárias, Universidade Pedagógica, Campus de Lhanguene, Av. de Moçambique, Mozambique.

Introduction

Resistance of cancer cells to multiple classes of structurally and mechanistically unrelated antitumor drugs can be defined as multidrug resistance (MDR), and it is one of the major causes of chemotherapy failure. The most significant mechanism of MDR, referred as typical or classical, results from altered cell membrane transport due to over-expression of transporter proteins that act as efflux pumps, such as P-glycoprotein (Pgp/MDR1). Conversely, MDR cells without overexpression of transporter proteins are referred as atypical MDR cells and their resistance has been associated with altered DNA topoisomerase II. Topoisomerases are nuclear enzymes crucial to DNA replication, transcription, and recombination. According to some authors, atypical MDR may result from altered expression of some metabolizing enzymes [1]. Therefore, a promising approach to overcome MDR is the development of compounds that are selectively cytotoxic to resistant cancer cells.

Figure 1. *Momordica balsamina.*

Aim of the study

The aim of this work was to evaluate the antiproliferative activity of several cucurbitane-type triterpenoids (Fig. 2) isolated from *Momordica balsamina* (Fig. 1), a medicinal African plant also used as food, in three human drug-sensitive cancer cell lines: gastric, pancreatic and colon carcinomas, and in classical and atypical multidrug resistant sublines .

Table 1. Cytotoxicity of triterpenic compounds **1**-**10** in parental and in different multidrug-resistant EPG85-257 gastric carcinoma cells.

Compound	EPG85-257P [1]	EPG85-257RDB [2]		EPG85-257RNOV [3]	
	IC_{50}	IC_{50}	RR[4]	IC_{50}	RR[4]
1	7.9 ± 0.4	2.5 ± 0.3	0.32	6.59 ± 0.36	0.83
2	19.8 ± 0.3	13.1 ± 1.5	0.66	16.8 ± 2.86	0.85
3	21.4 ± 4.0	19.9 ± 1.6	0.92	53.8 ± 9.11	2.51
4	19.3 ± 0.6	21.2 ± 1.6	1.1	10.4 ± 2.32	0.54
5	21.8 ± 0.2	210 ± 77.5	9.7	17.0 ± 1.15	0.78
6	8.03 ± 1.0	63.5 ± 2.3	7.9	7.04 ± 0.27	0.88
7	>100	>100		>100	
8	20.4 ± 0.4	14.5 ± 3.5	0.71	14.3 ± 2.19	0.70
9	49.0 ± 0.6	24.4 ± 1.4	0.49	23.2 ± 3.71	0.47
10	20.4 ± 0.1	17.8 ± 0.4	0.87	18.9 ± 0.78	0.93
Etoposide	0.105 ± 0.008	6.2 ± 0.3	59.0	1.55 ± 0.09	14.8

[1] EPG85-257P: parental, drug-sensitive gastric carcinoma cell line; [2] EPG85-257RDB: gastric carcinoma cell line with classical MDR phenotype; [3] EPG85-257RDB: gastric carcinoma cell line with atypical MDR phenotype. [4] RR: Relative resistance.

Table 2. Cytotoxicity of triterpenic compounds **1**-**10** in parental and in different multidrug-resistant EPP85-181 pancreatic carcinoma cells.

Compound	EPP85-181P [1]	EPP85-181RDB [2]		EPP85-181RNOV [3]	
	IC_{50} (μM)	IC_{50} (μM)	RR[4]	IC_{50} (μM)	RR[4]
1	6.7 ± 0.1	6.8 ± 0.9	1.0	8.0 ± 0.3	1.2
2	19.9 ± 0.2	8.6 ± 0.5	0.43	17.1 ± 2.0	0.86
3	55.1 ± 5.9	85.6 ± 3.5	1.56	33.6 ± 6.8	0.61
4	19.7 ± 0.3	23.9 ±0.8	1.21	13.7 ± 4.6	0.69
5	29.0 ± 6.1	100 ± 0	3.44	20.8 ± 0.02	0.72
6	19.4 ± 0.65	77.1 ± 3.1	3.97	14.9 ± 1.99	0.77
7	>100	>100		>100	
8	21.5 ± 0.73	22.1 ± 1.37	1.02	22.2 ± 1.8	1.03
9	69.2 ± 0.18	66.7 ± 0.65	0.96	61.0 ± 4.74	0.88
10	20.3 ± 0.02	18.9 ± 1.84	0.93	20.7 ± 1.79	1.02
Etoposide	0.58 ± 0.03	62.0 ± 4.2	106.9	4.5 ± 0.7	7.8

[1] EPP85-181P: parental, drug-sensitive pancreatic carcinoma cell line; [2] EPP85-181RDB: pancreatic carcinoma cell line with classical MDR phenotype; [3] EPP85-181RDB: pancreatic carcinoma cell line with atypical MDR phenotype. [4] RR: Relative resistance

Table 3. Cytotoxicity of triterpenic compounds **1**-**10** in parental and in different multidrug-resistant HT-29 colon carcinoma cells.

Compound	HT-29P [1]	HT-29RDB [2]		HT-29RNOV [3]	
	IC_{50} (μM)	IC_{50} (μM)	RR[4]	IC_{50} (μM)	RR[4]
1	14.4 ± 0.60	6.8 ± 1.37	0.49	6.7 ± 0.76	0.48
2	7.1 ± 2.16	2.6 ± 0.22	0.36	2.3 ± 0.04	0.31
3	80.0 ± 1.28	22.2 ± 1.49	0.28	24.3 ± 5.5	0.30
4	13.8 ± 2.75	7.13 ± 0.03	0.52	4.9 ± 0.55	0.35
5	151.7 ± 14.78	88.8 ± 6.0	0.59	115.5 ± 8.0	0.76
6	15.4 ± 0.49	4.0 ± 1.1	0.26	6.9 ± 0.17	0.45
7	>100	>100		>100	
8	21.2 ± 0.18	20.0 ± 0.42	0.94	17.5 ± 0.56	0.82
9	60.4 ± 0.43	31.3 ± 1.26	0.51	60.9 ± 3.66	1.00
10	20.1 ± 0.07	19.9 ± 0.43	0.99	19.1 ± 0.68	0.95
Etoposide	2.3 ± 0.3	26.0 ± 1.7	11.3	35.0 ± 2.6	15.2

[1] HT-29P: parental, drug-sensitive colon carcinoma cell line; [2] HT-29RDB: colon carcinoma cell line with classical MDR phenotype; [3] HT-29RNOV: colon carcinoma cell line with atypical MDR phenotype. [4] RR: Relative resistance

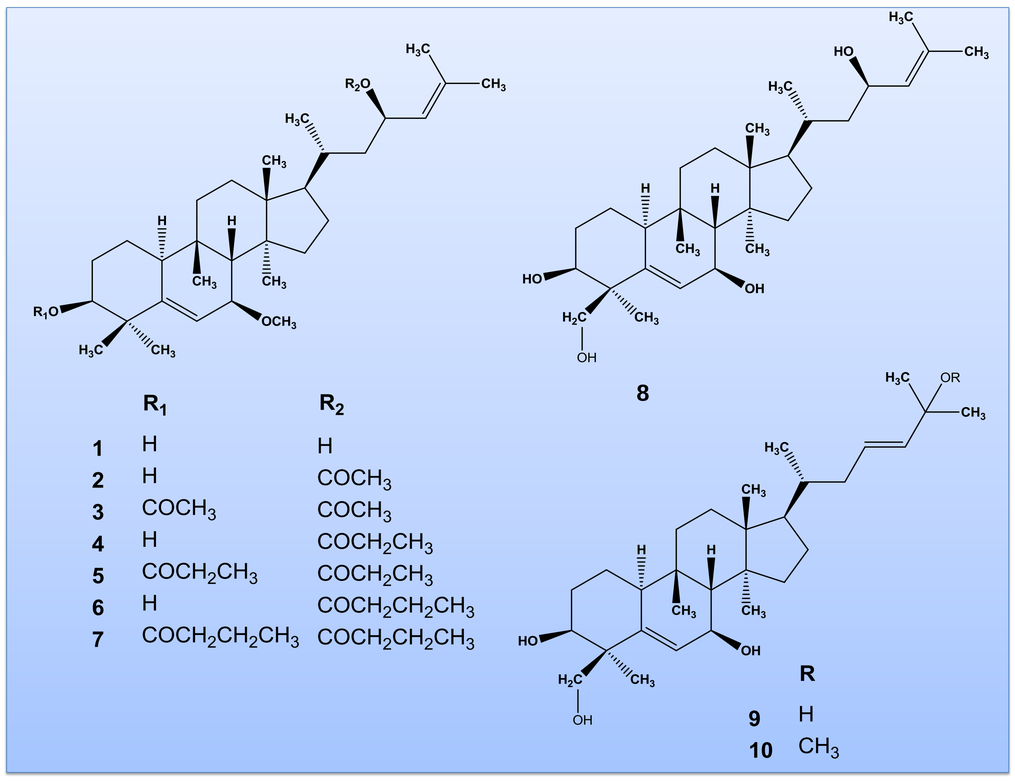

Figure 2. Structure of compounds isolated from *M. balsamina* **1**-**10**.

Results and Conclusions

Four cucurbitane-type triterpenoids isolated from the aerial parts of *Momordica balsamina* (**1**, **8**-**10**) and six alkanoyl derivatives (**2**-**7**) of compound **1** were investigated for their antiproliferative activity in three human drug sensitive cancer cell lines: gastric (EPG85-257P), pancreatic (EPP85-181RDB) and colon carcinomas (HT-29P).

Moreover, two different multidrug resistant variants of these cells were investigated: cell lines with classical MDR phenotype (associated with the expression of P-gp / MDR1 transporters: EPG85-257RDB, EPP85-181RDB and HT-29RDB) and cell lines with atypical MDR phenotype (associated with altered topoisomerase II expression: EPG85-257RNOV, EPP85-181RNOV and HT-29RNOV). As control, the etoposide-specific IC_{50}-values were measured, (Tables 1-3). Relative resistance (RR) values in relation to the parental drug-sensitive cell line were also determined.

In parental drug sensitive cell lines, none of the studied compounds were more efficient than the classical topoisomerase II inhibitor – etoposide. In some of the cases, drug resistance cancer sublines showed increased sensitivities to the tested compounds.

The best results were obtained with the alkanoyl derivatives compounds **2** - **4** in drug-resistant subline HT-29RNOV (colon carcinoma), showing a markedly antineoplasic activity (RR values between 0.30 and 0.35).

In conclusion, alkanoyl derivatives of compound 1 may be interesting as leads for the development of new anticancer agents.

References: [1] Teodori, E. et al (2006) Curr Drug Targets 7: 893-909.