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Triterpenoids from *Momordica balsamina* with a Collateral Sensitivity Effect for Tackling Multidrug Resistance in Cancer Cells

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ABSTRACT

The collateral sensitivity effect is among the most promising strategies for overcoming multidrug resistance in cancer. In this work, 28 cucurbitane-type triterpenoids (**1**–**28**), previously isolated from the African medicinal plant *Momordica balsamina* and its derivatives, were evaluated for their collateral sensitivity effect on three different human cancer entities, gastric (EPG85-257), pancreatic (EPP85-181), and colon (HT-29), each with two different multidrug-resistant variants. One was selected for its resistance to daunorubicin (EPG85-257RDB, EPP85-181RDB, HT-29RDB) and the other was selected for its resistance to mitoxantrone (EPG85-257RNOV, EPP85-181RNOV, HT-29RNOV). On gastric cell lines, the best results were obtained for compounds **3** and **10**, which exhibited a collateral sensitivity effect together with high antiproliferative activity. In turn, on colon cancer cell lines, the best multidrug resistance-selective antiproliferative effects were observed for derivatives **11, 13**, and **15**, which showed collateral sensitivity effects against both resistant variants. Compounds **11** and **3** were also the most selective against the multidrug resistance pancreatic cells lines. Some compounds, such **6, 10, 11** and **15**, were previously found to be strong P-glycoprotein modulators, thus highlighting their potential as promising leads for overcoming multidrug resistance in cancer cells.

Introduction

In spite of all the valuable chemotherapy regimens to treat cancer, it represents a major health problem worldwide, especially due to the high incidence of MDR phenotypes. MDR is characterized by cross-resistance of tumors to multiple structurally and functionally unrelated drugs. One of the most relevant and studied MDR mechanisms of tumor cells is correlated with the overexpression of P-gp (P-gp/ABCB1/MDR1), encoded by the *ABCB1* gene, which belongs to the superfamily of ABC transporters [1]. The overexpression of this ABC transporter, resulting from an association of intrinsic and acquired drug resistance factors, is evident in tumor tissues from patients, reducing the intracellular accumulation of the anticancer drug and thus compromising the efficacy of treatment [2]. As a consequence of the efflux function of P-gp, clinical chemotherapeutic agents, such as paclitaxel and Adriamycin, or the selective kinase inhibitors erlotinib and sorafenib suffered a reduction of efficacy [3–5].

Numerous strategies to overcome MDR have been explored, including the development of P-gp modulators to restore drug accumulation, the design of novel drugs that avoid recognition and efflux, and the use of small molecules that selectively kill MDR

ABBREVIATIONS

ABC	ATP-binding cassette
ABCB1	ATP-binding cassette, subfamily B
CI	confidence interval
EPG85-257P	parental gastric cancer cells
EPG85-257RDB	gastric cancer cells selected against daunorubicin
EPG85-257RNOV	gastric cancer cells selected against mitoxantrone
EPP85-181RDB	pancreatic cancer cells selected against daunorubicin
EPP85-181P	parental pancreatic cancer cells
EPP85-181RNOV	pancreatic cancer cells selected against mitoxantrone
MDR	multidrug resistance
MDR1	multidrug resistance gene 1
P-gp	P-glycoprotein
RDB	daunorubicin
RNOV	mitoxantrone
RR	relative resistance
SRB	sulforhodamine B

cells but not the nonresistant parental cells [6]. The latter, named collateral sensitivity effect, represents a new promising therapeutic approach for eradicating resistant cells. It has been considered as resulting from genetic alterations accumulated during the development of resistance towards one agent that are associated with the development of hypersensitivity to a second one [1]. Thus, it is thought that the development of novel treatment strategies exploiting collateral sensitivity could improve cancer treatment from refractory tumors by being resensitized to drugs through the selective killing of MDR cells, or by preventing the development of the MDR phenotype through coadministration during chemotherapy. This is a widely observed phenomenon, found not only in P-gp-expressing cancer cells, but also in tumors overexpressing other ABC transporters such as the multidrug resistance protein 1 (MRP1/ABCC1) and the breast cancer resistance protein (BCRP/ABCG2) [7].

In spite of being an old concept, observed firstly in resistant bacteria, the complex mechanisms by which compounds exert a collateral sensitivity effect are not yet clearly understood and are still under investigation [8].

Aiming at giving some insights into the collateral sensitivity phenomenon, several hypotheses have been considered, such as the ability of collateral sensitivity agents to generate reactive oxygen species via stimulation of P-gp ATPase activity, take advantage of P-gp-expressing cells sensitivity to changes in energy levels, stimulate the extrusion of endogenous subtracts, which is essential for cell survival, or induce perturbation of the biophysical properties of membranes. Nevertheless, the number of experimental studies providing evidence for these explanations is scarce, and several mechanisms might be involved depending on the compound [8]. While collateral sensitivity agents in P-gp-overexpressing cancer cells appear to act through different biochemical mechanisms, in relation to MRP1-overexpressing cancer cells, there is experimental evidence that they mainly act as stimulators of MRP1-mediated glutathione efflux, thus modifying redox balance, which selectively triggers apoptosis of resistant cells overexpressing this ABC protein [7].

Momordica balsmamina L. (Cucurbitaceae), commonly called African pumpkin, is an herb commonly found in tropical and subtropical regions of Africa and Asia. It presents high nutritional and medicinal value, being extensively used as food and traditional medicine [9]. A wide variety of cucurbitane-type triterpenoids with different biological activities has been isolated from the *Momordica* genus, namely from *Momordica charantia* [10, 11].

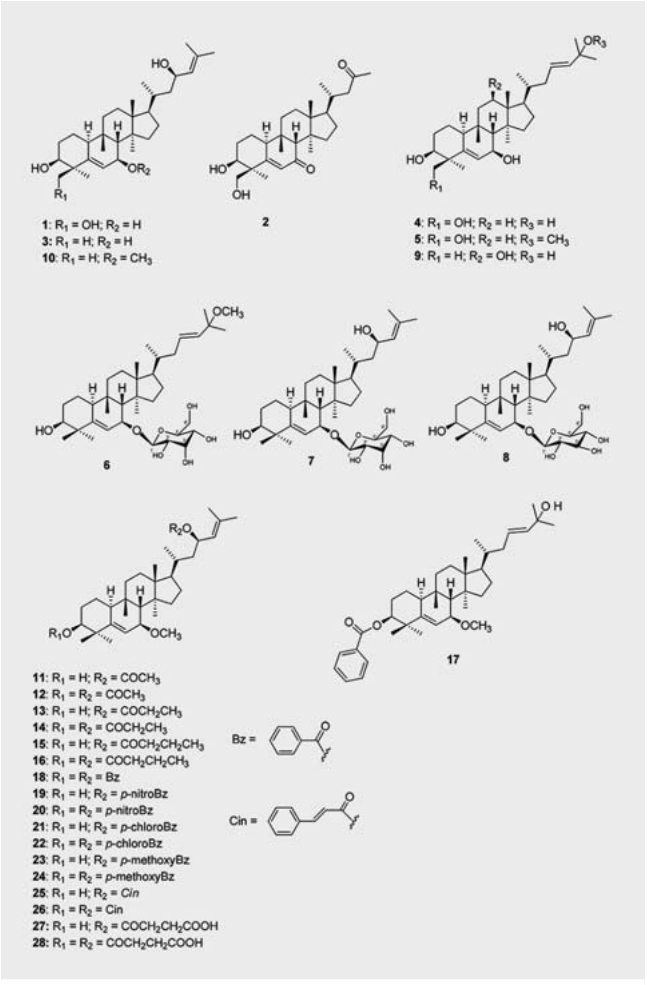
In our previous investigation of the methanol extract of the aerial parts of *M. balsamina,* several cucurbitanes isolated or obtained by derivatization showed they were potent inhibitors of P-gp efflux pump activity [12, 13]. Moreover, they were also able to inhibit the efflux pump systems of resistant strains of gram-positive bacteria [14].

In the present work and continuing our search for plant-derived compounds that can circumvent MDR [15–20], triterpenoids **1**–**28** were evaluated for their potential collateral sensitivity effect on colon, gastric, and cancer cell models (drug sensitive and drug resistant sublines) well characterized for MDR [17].

Results and Discussion

The phytochemical study of the methanol extract of the aerial plant parts of *M. balsamina* lead to the isolation of several triterpenes (**1**–**10**) (▶ **Fig. 1**) with the cucurbitane skeleton, as previously described [12, 21–23]. Karavilagenin C [**10**, 7β-methoxycucurbita-5,24-diene-3β,23(*R*)-diol], the major compound, allowed for the generation of a small library of mono- and di-acyl derivatives at C-23 and/or C-3, bearing alkanoyl, aroyl, and cinnamoyl moieties (**11**–**28**) (▶ **Fig. 1**) [12, 24]. These compounds were previously evaluated at non-cytotoxic doses for their ability as P-glycoprotein modulators on MDR1 mouse lymphoma cells [12, 13]. It was concluded that different substitution patterns, at both the tetracyclic nucleus and the side chain, led to distinct inhibition of this efflux pump activity [12, 13].

In this work, aiming at finding effective compounds for overcoming MDR, compounds **1**–**28** were assessed for their potential collateral sensitivity effect on three different cancer cell models: gastric (EPG85-257), pancreatic (EPP85-181), and colon (HT-29) cancer cells. For each cancer cell model, one sensitive cell line and two resistant sublines, selected for resistance to RDB and to RNOV, were tested. The characteristics of these MDR cell lines are well known and the same cancer cell models were used with a similar purpose in other studies [15–17, 25–27] The collateral sensitivity effect was assessed by determining the RR (calculated as the ratio of the IC_{50} of a compound against a resistant line divided by the IC_{50} against the corresponding parental line). Compounds with an RR < 1 kill MDR cells more effectively than parental cells, and when they exhibit an RR ≤ 0.50 they have a collateral sensitivity effect. An RR ≥ 2.0 expresses a compound that has resistance to a drug and is simultaneously cross-resistance to others [28]. The RR ratio only evaluates selectivity towards resistant cells. Thus, when selecting a compound with a collateral sen-



▶ **Fig. 1** Structures of compounds **1**–**28**.

sitivity effect for further studies, the antiproliferative values should also be considered. The anticancer drugs etoposide and cisplatin were used as positive controls.

The antiproliferative activity and collateral sensitivity effect (RR values) of compounds **1**-**28** are summarized in ▶ **Tables 1**–**3**. As can be observed, a significant antiproliferative effect (IC_{50} < 10 µM) in parental drug sensitive cell lines was observed for compounds **6** [EPG85-257, EPP85-181, and HT-29, IC_{50} = 9.5 µM (CI 7.2–11.8 µM), 7.1 µM (CI 6.9–7.3 µM), and 6.7 µM (CI 6.6–6.8 µM), respectively], **7** [EPG85-257, IC_{50} = 9.2 µM (CI 8.0–10.4 µM)], **10** [EPG85-257, EPP85-181, IC_{50} = 7.9 µM (CI 7.4–8.4 µM) and 6.7 µM (CI 6.6–6.8 µM), respectively], **11** [HT-29, IC_{50} = 7.9 µM (CI 4.1–11.4 µM)], and **15** [EPG85-257, IC_{50} = 8.0 µM (CI 6.9–9.1 µM)]. The remaining compounds showed a moderate/weak antiproliferative effect in parental drug-sensitive cell lines or were inactive (▶ **Tables 1**–**3**). Regarding MDR sublines, when the IC_{50} values were compared with those found for their specific drug-sensitive counterpart cell line, an increased sensitivity (RR < 1) was observed for most of the compounds (▶ **Fig. 2**), mainly for resistant cancer gastric and colon cancer cell lines. Moreover, a collateral sensitivity effect (RR ≤ 0.50) was observed for the natural compounds balsaminol F [**3**, IC_{50} = 6.2 µM (CI 5.7–6.7 µM); RR = 0.43] and karavilagenin C [**10**, IC_{50} = 2.5 µM (CI 2.2–2.8 µM); RR = 0.32] against the gastric EPG85-257 RDB subline (▶ **Table 1** and **Fig. 2**), with a high concomitant antiproliferative activity, which was comparable to that found for the positive controls [cisplatin, IC_{50} = 4.0 µM (CI 3.7–4.3 µM); RR = 1; etoposide, IC_{50} = 6.2 µM (CI 5.9–6.5 µM); RR = 59]. A collateral sensitivity effect was also found for compounds **4** and **27** on the same cells, although with a lower antiproliferative effect. Similarly, on the gastric EPG85-257 RNOV variant, a collateral sensitivity effect was observed for compounds **3, 4, 6**, and **9**, and was associated with strong antiproliferative activity for compounds **3** [IC_{50} = 7.2 µM (CI 6.4–8.0 µM); RR = 0.50] and **6** [IC_{50} = 4.5 µM (CI 2.5–6.5 µM); RR = 0.47]. By using the nonparametric Kruskal-Wallis rank test, a statistical difference with p = 0.053 in the IC_{50} values was detected between the three gastric cell lines, and was more significant (p = 0.010, one tail) between EPG85-257 RDB and EPG85-257 RNOV cells.

Compounds **11** [IC_{50} = 9.8 µM (CI 7.4–12.2 µM); RR = 0.51] and **3** [IC_{50} = 8.5 µM (CI 7.4–9.6 µM); RR = 0.55] were the most selective against the pancreatic EPP85-181RDB cells. On the pancreatic EPP85-181RNOV subline, an RR < 1 was also found for several compounds (▶ **Table 2** and **Fig. 2**), indicating that they exerted a higher antiproliferative effect against the MDR-derived line than the parental one. Among them, compounds **3, 11**, and **13** exhibited the lowest IC_{50} values (11.7–13.7 µM). For the pancreatic cancer cell lines, a significant statistical difference was found between the IC_{50} values (p = 0.03), reflecting a different antiproliferative effect of the compounds on both parental and resistant cancer cell lines. This effect was corroborated by the p values obtained when IC_{50} values of the parental cell line and EPP85-181RDB subline (p = 0.016, one tail) and IC_{50} values of EPP85-181RDB and EPP85-181RNOV (p = 0.009, one tail) were compared.

Regarding colon cancer cell lines (▶ **Table 3**), the best MDR-selective antiproliferative effects were found for karavilagenin C [**10**, IC_{50} = 6.8 µM (CI 5.2–8.4 µM); RR = 0.49, EPG85-257 RDB; IC_{50} = 6.7 µM (CI 5.8–7.6 µM); RR = 0.49, EPG85-257 RNOV] and some of its derivatives, with a collateral sensitivity effect being observed against both resistant variants. When comparing both the antiproliferative and the relative resistance ratio (▶ **Table 3** and **Fig. 2**), the best results were obtained for the acyl derivatives karavoate A [**11**, IC_{50} = 3.1 µM (CI 1.8–4.4 µM); RR = 0.39, EPG85-257 RDB; IC_{50} = 2.3 µM (CI 2.250–3.35 µM); RR = 0.29, EPG85-257 RNOV], karavoate C [**13**, IC_{50} = 7.1 µM (CI 7.09–7.10 µM); RR = 0.51, EPG85-257 RDB; IC_{50} = 4.9 µM (CI 4.3–5.5 µM); RR = 0.36, EPG85-257 RNOV], and karavoate E [**15**, IC_{50} = 6.9 µM (CI 6.7–7.1 µM); RR = 0.45, EPG85-257 RDB; IC_{50} = 4.0 µM (CI 2.8–5.2 µM); RR = 0.26, EPG85-257 RNOV].

When analyzing the results of compounds **1**–**28**, lipophilicity seems to be detrimental for antiproliferative activity, although no statistical correlation with antiproliferative activity was found. In fact, higher *log* p values (≥ 8.5) (**Table S1**, Supporting Information), observed for the acyl derivatives, were always associated with a lack of antiproliferative effect (IC_{50} > 100 µM).

As mentioned before, these compounds (**1**–**28**) were previously assessed for their ability to modulate the transport activity of P-gp in a functional assay [12, 13]. Interestingly, some compounds

▶ **Table 1** Antiproliferative activity of compounds **1–28** in gastric carcinoma cells: EPG85-257P (parental), EPG85-257RDB (MDR phenotype), and EPG85-257RNOV (MDR phenotype).

Compound	EPG85-257P	EPG85-257RDB		EPG85-257RNOV	
	IC_{50} (μM)[1] (CI 95%) (μM)	IC_{50} (μM) [1] (CI 95%) (μM)	RR[2]	IC_{50} (μM) [1] (CI 95%) (μM)	RR[2]
Balsaminol A (**1**)	20.4 (20.0–20.8)	14.5 (10.5–18.5)	0.71	12.1 (7.1–17.1)	0.59
Balsaminol D (**2**)	> 100	56.0 (43.8–68.2)	< 0.56	74.7 (67.9–81.5)	0.75
Balsaminol F (**3**)	14.4 (10.1–18.7)	6.2 (5.7–6.7)	0.43	7.2 (6.4–8.0)	0.50
Balsaminagenin A (**4**)	49.0 (48.4–49.6)	24.4 (22.9–25.9)	0.50	23.2 (19.0–27.4)	0.47
Balsaminagenin B (**5**)	20.4 (20.4–20.4)	17.5 (15.2–19.8)	0.86	18.2 (15.1–21.3)	0.89
Balsaminoside A (**6**)	9.5 (7.2–11.8)	> 100	> 10.52	4.5 (2.5–6.5)	0.47
Balsaminoside B (**7**)	9.2 (8.0–10.4)	64.2 (61.6–66.8)	6.98	5.0 (2.2–7.8)	0.54
Balsaminoside C (**8**)	19.8 (19.1–20.5)	58.8 (49.8–67.8)	2.97	11.5 (4.8–18.2)	0.58
Cucurbalsaminol A (**9**)	67.0 (64.1–69.9)	54.7 (46.4–63.0)	0.82	31.9 (24.0–39.8)	0.48
Karavilagenin C (**10**)	7.9 (7.4–8.4)	2.5 (2.2–2.8)	0.32	6.6 (6.2–7.0)	0.84
Karavoate A (**11**)	19.8 (19.4–20.2)	13.1 (10.7–15.5)	0.66	16.8 (12.2–21.4)	0.85
Karavoate B (**12**)	21.4 (16.9–25.9)	19.9 (18.1–21.7)	0.93	53.8 (43.5–64.1)	2.51
Karavoate C (**13**)	19.3 (18.6–20.0)	21.2 (19.4–23.0)	1.10	10.4 (7.8–13.0)	0.54
Karavoate D (**14**)	21.8 (21.5–22.1)	> 100	> 4.59	17.0 (15.7–18.3)	0.78
Karavoate E (**15**)	8.0 (6.9–9.1)	63.5 (60.9–66.1)	7.94	7.0 (6.7–7.3)	0.88
Karavoate G (**17**)	21.9 (21.2–22.6)	> 100	> 4.57	16.4 (13.2–19.6)	0.75
Karavoate I (**19**)	19.6 (19.0–20.2)	74.1 (66.0–82.2)	3.78	14.3 (13.5–15.1)	0.73
Karavoate K (**21**)	73.8 (65.2–82.4)	> 100	> 1.36	63.3 (61.6–65.0)	0.86
Karavoate M (**23**)	20.0 (19.2–20.8)	> 100	> 5.00	17.9 (15.2–20.6)	0.90
Karavoate O (**25**)	22.9 (21.9–23.9)	> 100	> 5.42	18.8 (17.1–20.5)	0.82
Karavoate Q (**27**)	40.3 (27.3–53.3)	18.7 (18.0–19.4)	0.46	27.9 (25.7–30.1)	0.69
Karavoate R (**28**)	66.6 (66.0–67.2)	55.7 (54.9–56.5)	0.84	62.9 (59.0–66.8)	0.94
Etoposide	0.105 (0.1–0.1)	6.2 (5.9–6.5)	59	1.55 (1.4–1.7)	14.8
Cisplatin	4.4 (3.9–4.9)	4.0 (3.7–4.3)	1	2.6 (2.4–2.8)	0.6

Compounds **16**, **18**, **20**, **22**, **24**, and **26** were ineffective in the sensitive and resistant variants of carcinoma cells (IC_{50} > 100 μM). [1] The IC_{50} values with 95% confidence intervals (CI 95%) given in parentheses indicate the mean of n = 3 to 4 independent experiments (each concentration was performed in triplicate per experiment). [2] RR is the relative resistance ratio determined by dividing the mean IC_{50} against a resistant line by the mean IC_{50} against a parental line

▶ **Table 2** Antiproliferative activity of compounds **1–28** in pancreatic carcinoma cells: EPP85-181P (parental), EPP85-181RDB (MDR phenotype), and EPP85-181RNOV (MDR phenotype).

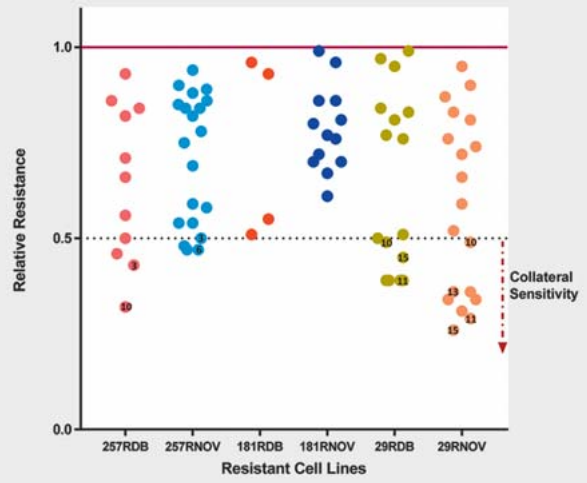
Compounds	EPP85-181P	EPP85-181RDB		EPP85-181RNOV	
	IC_{50} (μM) [1] (CI 95 %) (μM)	IC_{50} (μM) [1] (CI 95 %) (μM)	RR[2]	IC_{50} (μM) [1] (CI 95 %) (μM)	RR[2]
Balsaminol A (**1**)	21.5 (20.7–22.3)	22.1 (20.6–23.6)	1.03	22.2 (20.2–24.2)	1.03
Balsaminol D (**2**)	91.0 (90.2–91.8)	> 100	> 1.10	> 100	> 1.10
Balsaminol F (**3**)	15.4 (11.8–19.0)	8.5 (7.4–9.6)	0.55	11.7 (9.6–13.8)	0.76
Balsaminagenin A (**4**)	69.2 (69.0–69.4)	66.7 (66.0–67.4)	0.96	56.3 (45.6–67.0)	0.81
Balsaminagenin B (**5**)	20.3 (20.2–20.3)	18.9 (16.8–21.0)	0.93	20.7 (18.7–22.7)	1.02
Balsaminoside A (**6**)	7.1 (6.9–7.3)	> 100	> 14.08	9.6 (9.4–9.8)	1.35
Balsaminoside B (**7**)	10.2 (6.4–14.0)	67.0 (65.4–68.6)	6.57	17.5 (14.5–20.5)	1.72
Balsaminoside C (**8**)	21.0 (20.4–21.6)	66.0 (64.9–67.1)	3.14	20.1 (19.6–20.6)	0.96
Cucurbalsaminol A (**9**)	69.0 (66.8–71.2)	70.0 (68.8–71.2)	1.01	68.5 (67.6–69.4)	0.99
Karavilagenin C (**10**)	6.7 (6.6–6.8)	6.8 (5.8–7.8)	1.00	6.7 (4.1–9.3)	1.00
Karavoate A (**11**)	19.1 (17.4–20.8)	9.8 (7.4–12.2)	0.51	13.4 (10.4–16.4)	0.70
Karavoate B (**12**)	55.1 (48.4–61.8)	85.6 (81.6–89.6)	1.55	33.6 (25.9–41.3)	0.61
Karavoate C (**13**)	19.7 (19.4–20.0)	23.9 (23.0–24.8)	1.21	13.7 (8.4–19.0)	0.70
Karavoate D (**14**)	29.0 (22.1–35.9)	> 100	> 3.45	20.8 (20.78–20.82)	0.72
Karavoate E (**15**)	19.4 (18.7–20.1)	77.1 (73.6–80.6)	3.97	14.9 (12.6–17.2)	0.77
Karavoate G (**17**)	62.1 (47.6–76.6)	> 100	> 1.61	63.7 (44.3–83.1)	1.03
Karavoate I (**19**)	23.9 (23.6–24.2)	> 100	> 4.18	20.6 (20.4–20.8)	0.86
Karavoate K (**21**)	> 100	> 100	n. d.	66.8 (65.6–68.0)	< 0.67
Karavoate M (**23**)	23.7 (23.1–24.3)	> 100	> 4.22	20.4 (20.2–20.6)	0.86
Karavoate O (**25**)	28.2 (27.7–28.7)	> 100	> 3.55	22.5 (20.8–24.2)	0.80
Karavoate Q (**27**)	49.1 (41.1–57.1)	58.6 (55.2–62.0)	1.19	55.5 (54.9–56.1)	1.13
Karavoate R (**28**)	68.6 (68.1–69.1)	70.3 (68.4–72.2)	1.02	71.4 (70.4–72.4)	1.04
Etoposide	0.58 (0.57–0.59)	62.0 (57.2–66.8)	106.9	4.5 (3.7–5.3)	7.8
Cisplatin	0.08 (0.07–0.09)	0.09 (0.07–0.1)	1.2	2.6 (2.4–2.8)	34

Compounds **16, 18, 20, 22, 24**, and **26** were ineffective in the sensitive and resistant variants of carcinoma cells (IC_{50} > 100 μM). [1] The IC_{50} values with 95 % confidence intervals (CI 95 %) given in parentheses indicate the mean of n = 3 to 4 independent experiments (each concentration was performed in triplicate per experiment). [2] RR is the relative resistance ratio determined by dividing the mean IC_{50} against a resistant line by the mean IC_{50} against a parental line

▶ **Table 3** Antiproliferative activity of compounds **1**–**28** in colon carcinoma cells: HT-29P (parental), HT-29RDB (MDR phenotype), and HT-29RNOV (MDR phenotype).

Compounds	HT-29P	HT-29RDB		HT-29RNOV	
	IC_{50} (μM) [1] (CI 95%) (μM)	IC_{50} (μM) [1] (CI 95%) (μM)	RR[2]	IC_{50} (μM) [1] (CI 95%) (μM)	RR[2]
Balsaminol A (**1**)	21.2 (21.0–21.4)	21.0 (20.4–21.6)	0.99	17.5 (16.9–18.1)	0.83
Balsaminol D (**2**)	> 100	79.0 (73.0–85.0)	< 0.79	79.0 (72.1–85.9)	< 0.79
Balsaminol F (**3**)	11.9 (11.1–12.6)	9.2 (7.5–10.9)	0.77	7.0 (6.7–7.3)	0.59
Balsaminagenin A (**4**)	60.4 (59.9–60.9)	57.3 (49.0–65.6)	0.95	31.3 (29.9–32.7)	0.52
Balsaminagenin B (**5**)	20.1 (20.0–20.2)	20.0 (19.5–20.5)	1.00	19.1 (18.3–19.9)	0.95
Balsaminoside A (**6**)	6.7 (6.6–6.8)	7.1 (6.5–7.7)	1.06	4.8 (2.6–7.0)	0.72
Balsaminoside B (**7**)	18.2 (16.0–20.4)	61.7 (57.5–65.9)	3.39	15.9 (14.9–16.9)	0.87
Balsaminoside C (**8**)	27.8 (27.0–28.6)	66.4 (66.2–66.6)	2.39	31.4 (26.3–36.5)	1.13
Cucurbalsaminol A (**9**)	66.4 (64.8–68.0)	55.9 (49.0–62.8)	0.84	48.9 (39.5–58.3)	0.74
Karavilagenin C (**10**)	13.8 (13.1–14.5)	6.8 (5.2–8.4)	0.49	6.7 (5.8–7.6)	0.49
Karavoate A (**11**)	7.9 (4.1–11.4)	3.1 (1.8–4.4)	0.39	2.3 (2.25–3.35)	0.29
Karavoate B (**12**)	61.5 (49.4–73.6)	30.7 (8.5–52.9)	0.50	19.3 (12.3–26.3)	0.31
Karavoate C (**13**)	13.8 (10.7–16.9)	7.1 (7.09–7.1)	0.51	4.9 (4.3–5.5)	0.36
Karavoate E (**15**)	15.4 (14.9–15.9)	6.9 (6.7–7.1)	0.45	4.0 (2.8–5.2)	0.26
Karavoate G (**17**)	80.1 (77.2–83.0)	67.1 (52.6–81.6)	0.83	27.5 (25.3–29.7)	0.34
Karavoate I (**19**)	27.7 (26.5–28.9)	22.5 (21.6–23.4)	0.81	22.3 (20.5–24.1)	0.81
Karavoate M (**23**)	28.7 (27.8–29.6)	14.7 (9.4–20.0)	0.39	18.9 (17.9–19.9)	0.66
Karavoate O (**25**)	71.3 (68.0–74.6)	27.9 (19.9–35.9)	0.39	25.9 (22.5–29.3)	0.36
Karavoate Q (**27**)	61.3 (59.1–63.5)	24.2 23.8–24.6)	0.39	21.4 (21.2–21.6)	0.34
Karavoate R (**28**)	69.4 (68.2–70.6)	67.4 (66.7–68.1)	0.97	62.2 (57.4–67.0)	0.90
Etoposide	2.3 (2.0–2.6)	26.0 (24.1–27.9)	11.3	35.0 (32.1–37.9)	15.2
Cisplatin	3.8 (3.7–3.9)	2.7 (2.6–2.8)	0.7	3.8 (3.7–3.9)	1

Compounds **14**, **16**, **18**, **20**–**22**, **24**, and **26** were ineffective in the sensitive and resistant variants of carcinoma cells (IC_{50} > 100 μM). [1] The IC_{50} values with 95% confidence intervals (CI 95%) given in parentheses indicate the mean of n = 3 to 4 independent experiments (each concentration was performed in triplicate per experiment). [2] RR is the (elative resistance ratio determined by dividing the mean IC_{50} against a resistant line by the mean IC_{50} against a parental line.



▶ **Fig. 2** Compounds that showed greater sensitivity to the MDR sublines than to the corresponding parental cell line: relative resistance, the ratio between the mean IC_{50} against a resistant line by the mean IC_{50} against a parental line (RR), lower than 1.0. Compounds **4, 6, 9–13, 15, 17, 23, 25**, and **27** exhibited a collateral effect (RR ≤ 0.50). The tagged relative resistance points correspond to compounds that presented the best collateral sensitivity effect values concomitant with significant antiproliferative activity.

classified as strong P-gp modulators (**1, 4–7, 10–13, 15**) also showed a significant collateral sensitivity effect on some of the resistant cell sublines [12, 13]. For instance, karavilagenin C (**10**), which presented a very strong P-gp-mediated MDR reversal activity at a very low concentration [12], was able to kill the resistant gastric cell line EPG85 (RR = 0.32) more efficiently. Although to a lesser extent, a selective antiproliferative effect was also observed against both resistant colon HT-29 cell sublines (RR < 0.50). On the other hand, the monoacetylated derivative of **10**, karavoate A (**11**), which was also able to modulate P-gp [12], exhibited a collateral sensitivity [16]effect against both resistant HT-29 colon carcinoma cell variants (HT-29RDB, RR = 0.39; HT-29RNOV, RR= 0.29). As expected, similar results were also found for the efflux pump modulator karavoate E (**15**), which differs from compound **11** in the number of carbons of the ester moiety. Similarly, a collateral sensitivity effect (RR = 0.47) was also observed for the strong P-gp modulator **6** [12] against EPG85-257RNOV gastric cancer cells.

The exact molecular mechanisms mediating a sensitization of different multidrug-resistant cancer cell variants to alternative triterpenoids have still not been evaluated and are beyond the scope of this investigation.

In conclusion, MDR is a complex phenomenon, involving several biochemical mechanisms. Thus, some of these triterpenes, such as compounds **6, 10, 11**, and **15**, by acting as both P-gp modulators and collateral sensitivity agents, might be promising leads for overcoming MDR cancer cells and are worthy of further studies.

Materials and Methods

Tested compounds

Compounds **1**–**10**, namely, balsaminol A (**1**), balsaminol D (**2**), balsaminol F (**3**), balsaminagenin A (**4**), balsaminagenin B (**5**), balsaminosides A–C (**6**–**8**), cucurbalsaminol A (**9**), and karavilagenin C (**10**), were previously isolated from the methanol extract of *M. balsamina* as reported [12, 21–23]. Compound **10**, isolated in a large amount, gave rise to 18 compounds, namely, karavoates A–R (**11**–**28**), by using several alkanoyl and aroyl acylating reagents, as described [12, 24]. The purity of the compounds was more than 95% by HPLC. All of the compounds were dissolved in DMSO.

Cell lines and cell culture

The human cancer cell lines (EPG85-257P, EPP85-181P, and HT-29P) and their drug-resistant sublines (EPG85-257RNOV, EPG85-257RDB, EPP85-181RNOV, EPP85-181RDB, HT-29RNOV, and HT-29RDB) were grown in Leibovitz L-15 medium (Biowhittaker) supplemented with 10% fetal calf serum (GIBCO/BRL), 1 mM L-glutamine, 6.25 mg/L fetuin, 80 IE/L insulin, 2.5 mg/mL transferrin, 0.5 g/L glucose, 1.1 g/L $NaHCO_3$, 1% minimal essential vitamins, and 20 000 kIE/L trasylolina in a humidified atmosphere of 5% CO_2 at 37 °C. The drug-resistant cell lines were established from parental cell lines by continuous exposure of the cells to stepwise increasing concentrations of antineoplastic agents as described previously [29]. For maintenance of drug-resistant phenotypes, the medium of the drug-resistant sublines was supplemented with the selective agent as described previously [30]. The used cytotoxic drugs daunorubicin (Farmitalia Carlo Erba), mitoxantrone (Lederle), etoposide (Bristol-Myers), and cisplatin (GRY-Pharm) showed purities for application in clinical settings.

Cell proliferation assay

The antiproliferative activity of the compounds was assessed using a proliferation assay based on SRB staining as described previously [17]. Briefly, 800 cells per well were seeded in 96-well plates in triplicate. After 24 h attachment, the particular agent was added in a dilution series for 5 days incubation (5% CO_2 at 37 °C). Cells were fixed by chilled 10% trichloroacetic acid for 1 h at 4 °C, and washed five times with tap water before staining was performed with 0.4% SRB in 1% acetic acid for 10 min at room temperature. After washing with 1% acetic acid, drying, and resolubilization in 20 mM Tris-HCl (pH 10), absorbance was measured at 562 nm against the reference wavelength of 690 nm. Etoposide and cisplatin were used as positive controls. Mean IC_{50} values with a 95% confidence interval were calculated from four independent experiments in triplicate for each cell line by using Prism software (GraphPad Software, Inc.). RR values were determined as $IC_{50(\text{resistant cells})}/IC_{50(\text{parental cells})}$.

Statistical analysis

Analysis using the nonparametric Kruskal-Wallis rank test (a probability value of $p < 0.05$ was considered statistically significant) was carried out to identify differences between the three cells lines of each group. The Mann-Whitney test was used to examine

the statistical significance (a probability value of $p < 0.05$ was considered statistically significant) of differences in the mean IC_{50} values between two independent groups. The Real Stats package of Excel software was used.

Supporting information

Physicochemical properties of compounds **1–28** are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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