

Interaction between Doxorubicin and the Resistance Modifier Stilbene on Multidrug Resistant Mouse Lymphoma and Human Breast Cancer Cells

MARIA-JOSÉ U. FERREIRA¹, NOÉLIA DUARTE¹, NORA GYÉMÁNT², RITA RADICS², GEORGY CHEREPNEV³, ANDRAS VARGA⁴ and JOSEPH MOLNÁR²

¹CECF, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal;

²Department of Medical Microbiology, University of Szeged, Szeged, Hungary;

Departments of ³Medical Immunology (Charité) and ⁴Molecular Parasitology, Humboldt University, Berlin, Germany

Abstract. The hydroxystilbene *trans*-3,5,3',4'-tetrahydroxystilbene (piceatannol) (**1**), isolated from the methanol extract of *Euphorbia lagascae* defatted seeds, was methylated to yield the derivatives *trans*-3,5,3',4'-tetramethoxystilbene (**2**), (*trans*-3,5-dihydroxy-3',4'-dimethoxystilbene) (**3**) and *trans*-3,5,3'-trihydroxy-4'-methoxystilbene (**4**). The structures of the compounds were assigned by spectroscopic methods (IR, ¹H-NMR, ¹³C-NMR and MS). The ability of piceatannol (**1**) and the three methylated derivatives to modulate the transport activity of P-glycoprotein (P-gp) and apoptosis induction on the L5178 mouse lymphoma cell line containing the human MDR1 gene was studied by flow cytometry. The reversal of multidrug-resistance (MDR) was investigated by measuring the accumulation of rhodamine-123, a fluorescent substrate analog of doxorubicin, in cancer cells. Verapamil was applied as a positive control. For the evaluation of the compounds as apoptosis inducers, tumor cells were stained with FITC-labelled annexin-V and propidium iodide. The tetramethylated derivative (**2**) was found to be a powerful inhibitor of P-gp activity. Compounds **1** and **2** showed an increased apoptotic effect in the MDR subline, the most active being piceatannol (**1**). Furthermore, in the combination chemotherapy model, the interaction between doxorubicin and the resistance modifier **2** was studied *in vitro*. The results of checkerboard experiments indicated that the type of interaction was additive between doxorubicin and compound **2** on the human MDR1 gene-transfected mouse lymphoma cells. However, in the MCF7/dox human breast cancer cells, the interaction was

non-additive. The degree of additive and non-additive interactions were close to the borderline of the *FIX* values corresponding to the two types of interactions.

Stilbenes are naturally occurring phytoalexins, biosynthesized via the phenylpropanoid pathway and found in a wide range of plant sources existing mainly as *trans* stereoisomers (1). The most well-known stilbene is resveratrol (*trans*-3,4',5-trihydroxystilbene), which is produced by a number of unrelated plants, such as grapevines (*Vitis vinifera*), peanuts (*Arachis hypogaea*), mulberries (*Morus* species) and pines (*Pinus* species) in response to stress, injury, ultraviolet irradiation and fungal infection. It was reported to have potent anti-inflammatory, anticancer and anti-oxidant properties (1, 2). Its anti-oxidant properties seem to contribute to the role of red wine in the prevention of cardiovascular diseases when moderately consumed. Piceatannol (*trans*-3,5,3',4'-tetrahydroxystilbene), an anti-cancer compound that differs from resveratrol by having an additional aromatic hydroxyl group, was first isolated and identified as an antileukemic agent, from the seeds of *Euphorbia lagascae* (3). It is mainly incorporated in the diet through the intake of grapes and red wine. Most of the reported studies regarding piceatannol have been focused on its potent protein-tyrosine kinase inhibitory properties (4). It has been suggested that resveratrol may act as a pro-drug of piceatannol that would be the compound responsible for the biological effect on tumor cells. In fact, it was reported that resveratrol undergoes metabolism by cytochrome P 450 enzyme CYP1B1, overexpressed in a wide variety of human tumors, to piceatannol. This enzyme catalyzes aromatic hydroxylation reactions (5).

The multidrug resistance (MDR) of tumor cells is one of the major problems in cancer chemotherapy. One of the most important forms of resistance is related to an increased drug efflux, mediated by P-glycoprotein (P-gp), a

Correspondence to: Prof. Joseph Molnár, Department of Medical Microbiology and Immunobiology, University of Szeged, H-6720, Szeged, Hungary. Fax: +36 62 545-113, e-mail: molnarj@comser.szote.u-szeged.hu

Key Words: Stilbenes, *Euphorbia lagascae*, multidrug resistance, apoptosis.

member of a superfamily of ATP-dependent membrane transport proteins, which functions as a drug efflux pump and removes substrates out of tumor cells in a unidirectional manner. In tumor cells expressing P-gp, this results in reduced intracellular drug concentrations, leading to loss of therapeutic efficacy. The main anticancer compounds that are subject to P-gp-mediated MDR are the anthracyclins (doxorubicin, daunorubicin), vinca alkaloids (vinblastine, vincristine), colchicine, epipodophyllotoxins (etoposide, teniposide) and paclitaxel. Other ABC transporters, such as the multidrug resistance associated protein (MRP1), lung cancer resistance-related protein (LRP) and breast cancer-resistance protein (BCRP) are also related to the increased drug efflux of cancer cells. Most antitumor agents that can be substrates of P-gp are also MRP1 substrates, although these may involve a previous metabolism to glutathione conjugates. Whereas P-gp transports mainly lipophilic compounds, MRP1 transports organic anions, including glutathione and glucuronide conjugates (6, 7).

In this study, piceatannol (**1**), isolated from *Euphorbia lagascae* and three methylated derivatives (**2-4**) were evaluated as MDR modulators and apoptosis-inducers on multidrug resistant mouse lymphoma cells. Furthermore, the antiproliferative effects of the anticancer drug doxorubicin and the resistance modifier **2**, in combination, were studied on human MDR1 gene-transfected mouse lymphoma and doxorubicin-resistant human breast cancer cell lines.

Materials and Methods

Plant material. *Euphorbia lagascae* Spreng. was collected in Cova da Beira, Coimbra, Portugal, and identified by Dr. Teresa Vasconcelos of Instituto Superior de Agronomia, University of Lisbon, Portugal. A voucher specimen (n° 323) was deposited at the herbarium of Instituto Superior de Agronomia.

Compounds. Four stilbenes, whose structures are presented in Figure 1 were employed: *trans*-3,5,3',4'-tetrahydroxystilbene (piceatannol) (**1**), *trans*-3,5,3',4'-tetramethoxystilbene (**2**), (*trans*-3,5-dihydroxy-3',4'-dimethoxystilbene) (**3**) and *trans*-3,5,3'-trihydroxy-4'-methoxystilbene (rhapontigenin or 3-methoxyresveratrol) (**4**). All compounds were dissolved in DMSO.

Extraction and isolation of piceatannol. The air-dried powdered seeds of *Euphorbia lagascae* (1.4 kg) were extracted twice with n-hexane (7 L) at room temperature, to give after concentration under vacuum, 484 g of an apolar extract that was not further investigated. The defatted seeds were again extracted two times with methanol (7 L) at room temperature, yielding, after concentration, 34.4 g of a brown residue that was re-suspended in a mixture of methanol-water (6:1). This suspension was successively extracted with n-hexane (3x500 ml), ether (3x500 ml) and n-butanol (2x250 ml), to give the corresponding hexane (11.4 g), ether (12 g) and n-butanol (2.56 g) extracts. The ether extract was subjected to column chromatography over silica gel, eluted with mixtures of

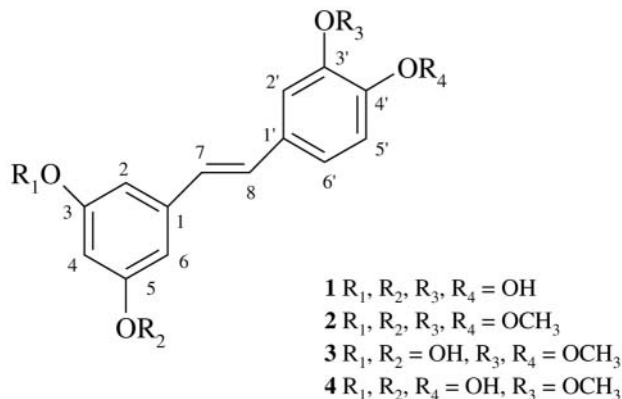


Figure 1. Chemical structures of stilbenes **1-4**.

dichloromethane/ethyl acetate and ethyl acetate/methanol of increasing polarity, to obtain nine crude fractions (A to I). The crude fraction D (1.2 g), eluted with dichloromethane/ethyl acetate (7:3 and 13:1), was recrystallized twice with the same pair of solvents to yield 584 mg of pure compound **1**.

Methylation of piceatannol (1). Five mL of an ethereal solution of diazomethane was added dropwise to a solution of compound **1** (80 mg on absolute methanol). The reaction was followed by TLC. The purification of the compounds was performed by preparative chromatography (chloroform/methanol 9:1, twice) to yield the tetramethylated (*trans*-3,5,3',4'-tetramethoxystilbene, **2**, 50 mg), as well as the dimethylated (**3**, *trans*-3,5-dihydroxy-3',4'-dimethoxy-stilbene, 8 mg) and the monomethylated (**4**, *trans*-3,5,3'-trihydroxy-4'-methoxystilbene, 11 mg) derivatives which were identified by spectroscopic methods (IR, ¹H-NMR, ¹³C-NMR and MS) (8-13).

Octanol/water partition coefficient (Log₁₀ P) calculation. The theoretical Log₁₀ P was determined by using the JME molecular editor (version March 2006, <http://www.molinspiration.com/>).

Cell cultures. The L5178 mouse T-cell lymphoma cells were transfected with pHa MDR1/A retrovirus, as previously described (14). The MDR1-expressing cell lines were selected by culturing the infected cells with 60 ng/mL colchicine to maintain the expression of the MDR phenotype. The L5178 (parent) mouse T-cell lymphoma cells and the human MDR1-transfected subline were cultured in McCoy's 5A medium supplemented with 10% heat-inactivated horse serum L-glutamine and antibiotics.

The drug-resistant subline of the human breast cancer MCF7 (ECACC 86012803; KCR) cells were grown in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum supplemented with antibiotics. In every second week, 1 μM doxorubicin was added to the medium, so as to maintain the P-gp expression. The breast cancer MDA-MB-231 (ATCC: HTB-26) cells were grown in Leibovitz's L-15 medium with 2 mM L-glutamine and 10% fetal bovine serum supplemented with antibiotics. The MDA-MB-231 cell line does not require CO₂. Cell viability was determined by the trypan blue extrusion test.

Assay for rhodamine 123 accumulation test. The harvested cells were resuspended in serum-free McCoy's 5A medium and were distributed into Eppendorf tubes at a density of 2×10^6 cell/mL. Then, 2 to 20 μ L of the stock solution (1 mg/mL in DMSO) of the tested compounds were added and the samples were incubated for 10 min at room temperature. Following the addition of 10 μ L of rhodamine 123 to the samples (5.5 mM final concentration), the cells were further incubated for 20 min at 37°C, were washed twice and were resuspended in 0.5 mL phosphate-buffered saline (PBS) for analysis. The fluorescence uptake of the cells was measured by flow cytometry using a Beckton Dickinson FACScan instrument equipped with an argon laser. The fluorescence excitation and emission wavelengths were 488 nm and 520 nm, respectively. Verapamil was used as a positive control, and the influence of DMSO on the cells was monitored. The mean fluorescence intensity was calculated as a percentage of the control for the parental (PAR) 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 (15, 16).

$$R = \frac{\text{MDR treated/MDR control}}{\text{parental treated/parental control}}$$

Assay for apoptosis induction. The assay was carried out according to the protocol of Alexis Bichemicals (17) with little modification. The cells were incubated in the presence of the compounds for 40 min at 37°C, then the samples were washed in PBS. The harvested cells were resuspended in culture medium and distributed to 24-well tissue culture plates in 1 mL aliquots, followed by the incubation of the plate for 24 h at 37°C, 5% CO₂. The treated cells were then transferred into small centrifuge tubes, centrifuged, washed in 0.5 mL PBS and resuspended in 195 μ L binding buffer. Annexin V-FITC (4.5 μ L) was added to the samples, which were incubated at room temperature for 10 min in the dark. Finally, the cells were washed in PBS, resuspended in 190 μ L binding buffer and 10 μ L of a 20 μ g/mL propidium iodide stock solution were added to the samples (final conc. 1 μ g/mL). The fluorescence activity (FL-1, FL-2) of the cells was measured and analyzed on a Becton Dickinson FACScan instrument.

Assay for antiproliferative effect. The effects of increasing concentrations of the drugs alone on cell growth were tested in 96-well flat-bottomed microtiter plates. The compounds were diluted in a volume of 50 μ L medium. Then, 1×10^4 cells in 0.1 mL of medium were added to each well, with the exception of the medium control wells. The culture plates were further incubated at 37°C for 72 h. At the end of the incubation period, 20 μ L of MTT (thiazolyl blue, Sigma, St. Louis, MO, USA) solution (from a 5 mg/mL stock) was added to each well. After incubation at 37°C for 4 h, 100 μ L of sodium dodecyl sulfate (SDS) (Sigma) solution (10%) was measured into each well and the plates were further incubated at 37°C overnight. The cell growth was determined by measuring the optical density (OD) at 550 nm (ref. 630 nm) with a Dynatech MRX vertical beam ELISA reader. Inhibition of cell growth (as a percentage) was determined according to the formula:

$$100 - \frac{\text{OD sample} - \text{OD medium control}}{\text{OD cell control} - \text{OD medium control}} \times 100$$

Table I. Effect of compounds 1-4 on reversal of multidrug resistance (MDR) on human MDR1 gene-transfected mouse lymphoma cells.

Samples	Concentration (μ g/mL)	FSC ^a	SSC ^a	FL-1 ^a	Fluorescence activity ratio
PAR + R123 ^b	-	517.43	145.32	924.46	-
PAR - R123	-	541.46	160.25	1003.20	-
MDR + R123 ^c	-	565.24	181.35	7.35	-
Verapamil	10	576.40	192.00	137.30	18.68
1	4	549.87	175.71	6.59	0.89
	40	538.44	178.86	6.09	0.82
2	4	555.01	172.82	411.67	56.01
	40	590.01	179.17	551.72	75.06
3 ^d	2.7	397.00	153.99	9.86	1.43
4	4	546.13	172.47	8.34	1.13
	40	542.71	167.21	10.08	1.37
DMSO	20 μ L	532.18	172.43	5.93	0.80

^aFSC: 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.

^bPAR control: a parental cell without MDR gene.

^cMDR: a parental cell line transfected with human MDR1 gene.

^dThe results for compound 3 were obtained from another assay.

Checkerboard microplate method as a model for combination therapy. This method was applied to study the effects of drug interactions between resistance modifiers and cytotoxic compounds on cancer cells.

The effects of the anticancer drug doxorubicin and the resistance modifiers in combination were studied on various cancer cell lines. The dilutions of doxorubicin (A) were made in a horizontal direction, while the dilutions of resistance modifiers (B) vertically in the 100 μ L microtiter plate. The cell suspension in the tissue culture medium was distributed into each well in 100 μ L containing 5×10^4 cells. The plates were incubated for 72 h at 37°C in a CO₂ incubator. The cell growth rate was determined after MTT staining and the intensity of the blue color was measured on a Dynatech MRX vertical beam ELISA reader. Drug interactions were evaluated according to the following system:

$FIC_A = ID_{50A \text{ in combination}} / ID_{50A \text{ alone}}$
 $FIC_B = ID_{50B \text{ in combination}} / ID_{50B \text{ alone}}$
 ID=inhibitory dose
 FIC=fractional inhibitory concentration
 FIX=fractional inhibitory index
 $FIX = FIC_A + FIC_B$
 FIX=0.51-1 Additive effect
 FIX <0.5 Synergism
 FIX=1-2 Indifferent effect
 FIX >2 Antagonism

Results and Discussion

The seeds of *Euphorbia lagascae* were sequentially extracted with hexane and methanol. The methanol extract was submitted to successive chromatographic fractionation and purification, as described in the Materials and

Table II. Effect of compounds **1-4** on apoptosis induction on a resistant mouse lymphoma cell line (MDR1) at 40 µg/mL.

Samples	Concentration (µg/mL)	Early apoptosis %	Total apoptosis %	Necrosis %
Cell control without staining		0.02	0.04	0.10
Cell control annexin-V+ PI ^a +		2.98	18.11	2.26
Cell control annexin-V- PI- 1.0% DMSO		0.08	18.31	0.01
M-627 ^b control+	40	0.02	98.00	1.81
<i>trans</i> -3,5,3',4'-tetrahydroxystilbene (1)	40	1.52	62.82	2.90
<i>trans</i> -3,5,3',4'-tetramethoxystilbene (2)	40	1.60	24.30	2.48
<i>trans</i> -3,5- dihydroxy-3',4'-dimethoxystilbene (3)	40	1.63	23.56	2.18
<i>trans</i> -3,5,3'-trihydroxy-4'-methoxystilbene (4)	40	1.35	13.52	1.45

^aPI: propidium iodide;

^b12H-benzo(α)-phenothiazine.

Table III. Effect of compounds **1-4** on apoptosis induction on a resistant mouse lymphoma cell (MDR1) at 10 µg/mL.

Samples	Concentration (µg/mL)	Early apoptosis %	Total apoptosis %	Necrosis %
Cell control without staining		0.02	0.04	0.10
Cell control annexin-V+PI+		2.09	8.89	6.41
Cell control annexin-V- PI- 1.0% DMSO		0.98	8.40	7.29
M-627 ^a control +	50	0.00	78.02	21.91
<i>trans</i> -3,5,3',4'-tetrahydroxystilbene (1) ^b	4	6.75	36.52	0.83
<i>trans</i> -3,5,3',4'-tetramethoxystilbene (2)	10	5.00	18.98	3.77
<i>trans</i> -3,5- dihydroxy-3',4'-dimethoxystilbene (3)	10	2.19	8.47	4.70
<i>trans</i> -3,5,3'-trihydroxy-4'-methoxystilbene (4)	10	2.05	10.17	5.24

^a12H-benzo(α)-phenothiazine.

^bThe results for compound **1** were obtained from other an assay. M-627 control + (4 µg/mL): early apoptosis 1.72 %, total apoptosis 65.01 %, necrosis 16.30%.

Methods, to afford *trans*-3,5,3',4'-tetrahydroxystilbene (piceatannol) (**1**) that was methylated with diazomethane to yield the derivatives *trans*-3,5,3',4'-tetramethoxystilbene (**2**), (*trans*-3,5-dihydroxy-3',4'-dimethoxystilbene) (**3**) and *trans*-3,5,3'-trihydroxy-4'-methoxystilbene (**4**).

The effects of stilbenes **1-4** were evaluated on the reversion of multidrug resistance, by using the rhodamine 123 exclusion test and apoptosis induction in mouse lymphoma cells. Verapamil was applied as a control. Two concentrations (4 and 40 µg/mL) were used in the experiments. The results for MDR are displayed in Table I. It is interesting to note that the results indicate that the four structurally related stilbenes differ significantly in their interaction with P-gp. As shown in Table I, *trans*-3,5,3',4'-tetramethoxystilbene (**2**) strongly inhibit P-gp activity dose-dependently (fluorescence activity ratios R=56.01 at 4 µg/mL; R=75.06 at 40 µg/mL) and with a higher activity when compared to that of the positive control verapamil (R=18.68 at 10 µg/ml concentration). Conversely, piceatannol (**1**), and its derivatives **3** and **4** were found to be ineffective in the MDR reversal assay.

The apoptosis-inducing activity of the four compounds is summarized in Table II and Table III. According to these data, the stilbenes **1** and **2** can be considered as apoptosis inducers being the most active piceatannol (**1**).

The four compounds tested have very similar structures differing only in the substitution pattern of the benzene rings; compound **1** has four free hydroxyl groups and in compounds **3** and **4**, two and one of the hydroxyl groups are replaced by a methoxyl group, respectively. Compound **2** contains four methoxyl groups. In terms of molecular properties, the effect of these substituents in compound **2** are very significant because of their influence over several molecular properties, namely the lipophilicity (octanol/water partition coefficients, Log₁₀ P=4.18, see Table IV), which appears as a general requirement for the MDR modifiers. P-gp is an H-bond donor and, according to several authors (18, 19), the modifiers should be H-bond acceptors. Our results corroborate with this statement. The substitution of the hydroxyls of piceatannol, which showed no significant activity as an MDR modifier, by methoxyl groups drastically enhanced

Table IV. Correlation between multidrug resistance and the theoretical octanol/water partition coefficient ($\text{Log}_{10} P$).

Sample	Fluorescence activity ratio (4 $\mu\text{g/mL}$) ^a	$\text{Log}_{10} P$
1	0.89	2.49
4	1.13	2.80
3	1.43	3.11
2	56.01	4.18

^aConcentration of compound 3: 3 $\mu\text{g/mL}$.

Table V. Antiproliferative effect of *trans*-3,5,3',4'-tetramethoxystilbene (2) on mouse T lymphoma cell line and drug resistant subline of MCF7 (KCR).

Samples	L5178 ID ₅₀ values ($\mu\text{g/mL}$)	KCR ID ₅₀ values ($\mu\text{g/mL}$)
<i>trans</i> -3,5,3',4'-tetramethoxystilbene (2) ^a	12.9	20.63

^aCompound 2 was dissolved in DMSO, but the DMSO content remained under 2.14%.

Table VI. *In vitro* effects of *trans*-3,5,3',4'-tetramethoxystilbene (2) in combination with doxorubicin on human MDR1 gene-transfected mouse lymphoma and doxorubicin-resistant human breast cancer cell lines.

Samples	MDR		KCR	
	FIX	Interaction	FIX	Interaction
<i>trans</i> -3,5,3',4'-tetramethoxystilbene (2)	0.855	additive	1.10	Indifferent effect

the activity in the tetramethylated derivative (2). The role of the methoxyl groups in the biological action on P-gp involves electron charge transfer type of interaction between the tetramethyl piceatannol and its binding site on the membrane inserted P-gp molecule.

In further experiments, the *in vitro* effects of the MDR modifier 2 (*trans*-3,5,3',4'-tetramethoxystilbene), in combination with doxorubicin on human MDR1 gene-transfected mouse lymphoma and the MCF7/dox human breast cancer cells, were examined (Table V and Table VI; Figure 2 and Figure 3). It was observed that compound 2 showed an additive effect on the human MDR1 gene-transfected mouse lymphoma cells. On the MCF7/dox human breast cancer cell line, no interaction was observed. The ineffectiveness of compound 2 on MCF7/dox can be

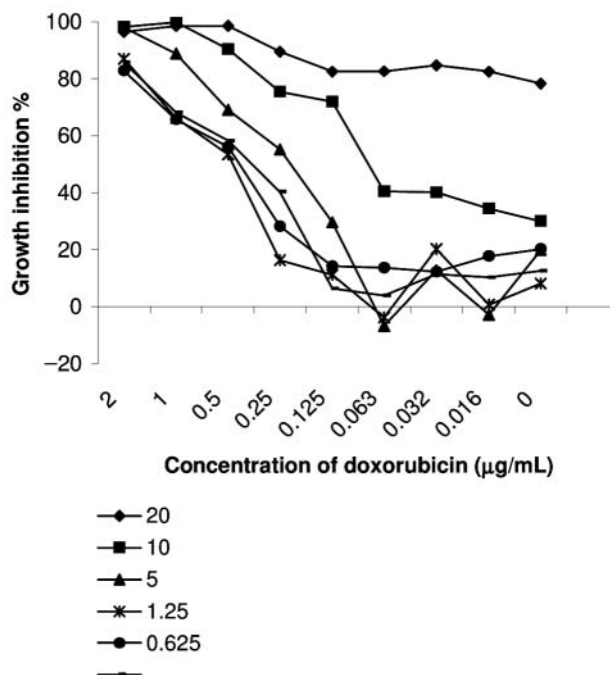


Figure 2. Effect of *trans*-3,5,3',4'-tetramethoxystilbene (2) in combination with doxorubicin on the human MDR1 gene-transfected mouse lymphoma cell line (treatments with 20, 10, 5, 1.25 0.625 and 0 $\mu\text{g/mL}$ *trans*-3,5,3',4'-tetramethoxystilbene).

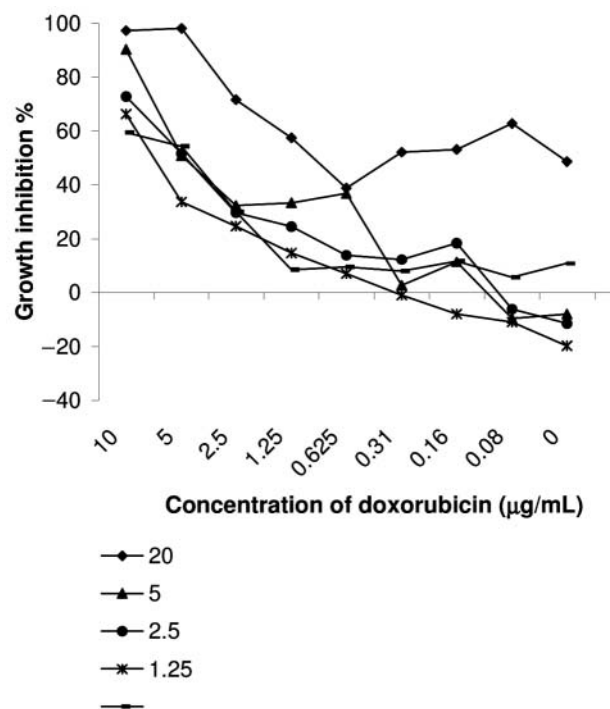


Figure 3. Effect of *trans*-3,5,3',4'-tetramethoxystilbene (2) in combination with doxorubicin on doxorubicin-resistant KCR human breast cancer cell (treatments with 20, 5, 2.5, 1.25 and 0 $\mu\text{g/mL}$ *trans*-3,5,3',4'-tetramethoxystilbene).

explained by the presence of both MRP and MDR on the cell membrane. The second possibility is that additive and non-additive effects measured on the mouse lymphoma and human breast cancer cells are close to the borderline of the FIX values, meaning that electron charge transfer complexes play no significant role in the combined interaction between doxorubicin and compound **2** in the human breast cancer cells, since the expression of MDR P-gp is relatively weak in these cells. A third hypothesis is that doxorubicin has targets other than P-gp, which are not directly related to the moderate efflux pump activity in the MCF7/dox cells.

Acknowledgements

This work was supported by FCT, Portugal (POCTI, Quadro Comunitário de Apoio III) and by Szeged Foundation of Cancer Research, Hungary. The authors thank Dr. Teresa Vasconcelos (ISA, University of Lisbon, Portugal) for identification of the plant and Dr. Zoltán Kiss (CanCure, Michigan, USA) for doxorubicin-resistant human breast cancer cell line (KCR).

References

- Roupe KA, Remsberg CM, Yáñez JA and Davies NM: Pharmacometrics of stilbenes: Sequestering towards the clinic. *Curr Clin Pharm* 1: 81-101, 2006.
- Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S and Takada Y: Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res* 24: 2783-2840, 2004.
- Ferrigni NR, McLaughlin JL, Powell RG and Smith CR Jr: Use of potato disc and brine shrimp bioassays to detect activity and isolate piceatannol as the antileukemic principle from the seeds of *Euphorbia lagascae*. *J Nat Prod* 47: 347-352, 1984.
- Geahlen RL and McLaughlin J: Piceatannol (3,4,3',5'-tetrahydroxy-*trans*-stilbene) is a naturally occurring protein-tyrosine kinase inhibitor. *Biochem Biophys Res Commun* 165: 241-245, 1989.
- Potter GA, Patterson LH, Wanogho E, Perry PJ, Butler PC, Ijaz T, Ruparelia KC, Lamb JH, Farmer PB, Stanley LA and Burke MD: The cancer preventative agent resveratrol is converted to the anticancer agent piceatannol by the cytochrome P450 enzyme CYP1B1. *Br J Cancer* 86: 774-778, 2002.
- Krishna R and Mayer LD: Modulation of P-glycoprotein (P-GP) mediated multidrug resistance (MDR) using chemosensitizers: Recent advances in the design of selective MDR modulators. *Curr Med Chem – Anti-Cancer Agents* 1: 163-174, 2001.
- Avendaño C and Menéndez JC: Inhibitors of multidrug resistance to antitumor agents (MDR). *Curr Med Chem* 9: 159-193, 2002.
- Cardona ML, Fernandez MI, Garcia M and Pedro J: Synthesis of natural polyhydroxystilbenes. *Tetrahedron* 42: 2725-2730, 1986.
- Gill MT, Bajaj R, Chang C, Nichols D and McLaughlin J: 3,3',5'-tri-O-methylpiceatannol and 4,3',5'-tri-O-methylpiceatannol: improvements over piceatannol in bioactivity. *J Nat Prod* 50: 36-40, 1987.
- Talvitie A, Mannila E and Kolehmainen E: Synthesis of some biologically active compounds from stilbenes isolated from the bark of *Picea abies*. *Liebigs Ann Chem* 399-401, 1992.
- Kim S, Ko H, Park J, Jung S, Lee S and Chun Y: Design, synthesis and discovery of novel *trans*-stilbene analogues as potent and selective human cytochrome P450 1B1 inhibitors. *J Med Chem* 45: 160-164, 2002.
- Thakkar K, Geahlen R and Cushman M: Synthesis and protein-tyrosine kinase inhibitory activity of polyhydroxylated stilbene analogues of piceatannol. *J Med Chem* 36: 2950-2955, 1993.
- Teguo P, Decendit A, Krisa S, Deffieux G, Vercauteren J and Méridon J: The accumulation of stilbene glycosides in *Vitis vinifera* cell suspension cultures. *J Nat Prod* 59: 1189-1191, 1996.
- Cornwell MM, Pastan I and Gottesmann MM: Certain calcium channel blockers bind specifically to multidrug-resistant human KB carcinoma membrane vesicles and inhibit drug binding to P-glycoprotein. *J Biol Chem* 262: 2166-2170, 1987.
- Weaver JL, Szabo G, Pine PS, Gottesman MM, Goldenberg S and Aszalos A: The effect of ion channel blockers, immunosuppressive agents, and other drugs on the activity of the multidrug transporter. *Int J Cancer* 54: 456-461, 1993.
- Kessel D: Exploring multidrug resistance using Rhodamine 123. *Cancer Commun* 1: 145-149, 1989.
- Koopmann G, Rentelinger CP, Kuijten GA, Koehnen RM, Pals ST and Van OMH: Annexin V flow cytometric detection of phosphatidyl-serine expression on B cells undergoing apoptosis. *Blood* 84: 1115-1120, 1994.
- Seelig A: A general pattern for substrate recognition by P-glycoprotein. *Eur J Biochem* 251: 252-2261, 1998.
- Wiese M and Pajeva IK: Structure-activity relationships of multidrug resistance reversers. *Curr Med Chem* 8: 685-713, 2001.

Received March 15, 2006

Accepted July 7, 2006