Phenolic Compounds as Selective Antineoplasic Agents against Multidrug-resistant Human Cancer Cells

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- antiproliferatives
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- stilbenes
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Abstract

Twelve phenolic compounds, including three stilbenes, two flavonoids, two coumarins, one neolignan, and four lignans, isolated from *Euphorbia* and *Pycnanthus* species or obtained by derivatization, were assayed for their potential antineoplastic efficacy in three human cancer cell lines: gastric (EPG85-257), pancreatic (EPP85-181), and colon (HT-29) carcinomas as well as derived multidrug-resistant sublines. In each case, two different multidrug-resistant variants, i.e., cell lines with classical and atypical MDR phenotype, were used. The majority of the MDR cancer sublines showed increased sensitivities to the studied compounds when compared to the parental sublines. The most active compound was the flavonoid naringenin, found to be 15-fold more effective against the atypical MDR subline of gastric carcinoma than in parental drug-sensitive cells. Furthermore, the stilbene *trans*-3,5,3′,4′-tetramethoxypiceatannol and the lignans 4′-hydroxy-3,3′,4-trimethoxylignan and heliobuphthalmin also exhibited high antineoplasic activities against the classical MDR subline derived from gastric carcinoma. The results of this study suggest that some phenolic compounds might be valuable for the treatment of multidrug-resistant cancer cells.

Introduction

The major clinical obstacle to the successful chemotherapy of neoplastic diseases is the development of drug resistance. Combination chemotherapy was supposed to overcome this phenomenon by treating with multiple agents that exert their effects by different mechanisms. However, cancer cells respond by development of multidrug resistance (MDR), i.e., simultaneous resistance to a panel of mechanistically and structurally diverse drugs [1]. When multidrug-resistant phenotype is depending on the enhanced activity of the drug extrusion pump MDR1/P-gp, the MDR is referred as classical MDR; in the case of independence from MDR1/P-gp, the drug-resistant phenotype is designated as atypical MDR [2]. One possible approach to overcome MDR is to find out new anticancer drugs without cross-resistance in cancer cells exhibiting a multidrug-resistant phenotype. Consequently, there is an urgent need to identify new cancer chemotherapeutic leads. Natural products have long been regarded as excellent sources for drug discovery and development. In fact, the wide structural diversity of secondary metabolites, due to the presence of chirality and functionality represents an extremely rich biogenetic resource, providing also pointers for rational drug design [3,4]. Natural product derived drugs represent 60% of the chemotherapeutic agents currently in use for all types of cancers [3]. In recent years, there is a growing interest in the study of lignans because some of them exhibit a remarkable antineoplastic activity. In particular, the lignan podophyllotoxin, isolated from *Podophyllum sp*, was used as a lead structure for its semisynthetic derivatives etoposide and teniposide, widely employed in the treatment of a variety of malignant diseases [5]. Stilbenes have as well been considered attractive candidates in the therapeutic development of anticancer drugs [6]. An important example is the stilbene piceatannol that was first isolated as an antileukemic compound from *Euphorbia lagascae* seeds and, later on, identified as a potent tyrosine kinase inhibitor [7,8]. There are also various reports on the antiproliferative and apoptosis induction activities of piceatannol and other stilbenes against several cell lines and animal models [6,9]. On the other hand, flavonoids have been described as exerting

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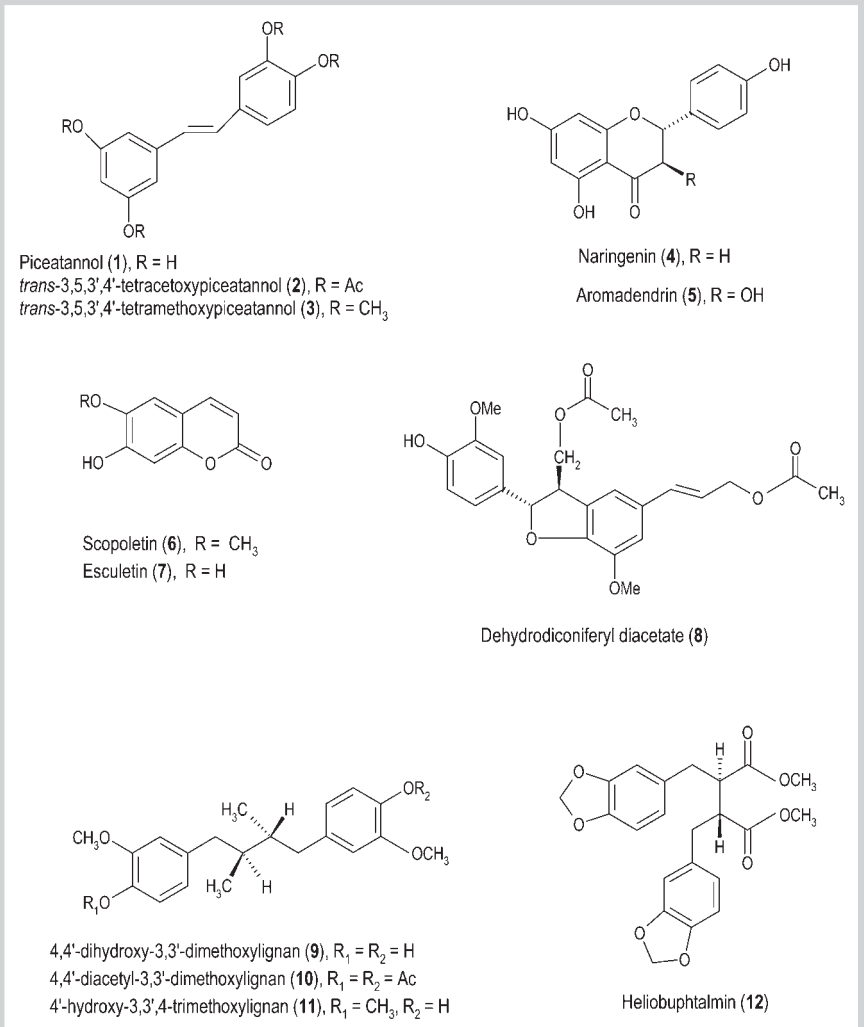

Fig. 1 Chemical sructures of the twelve tested compounds.

a wide range of biochemical and pharmacological effects. Their antitumor and cancer-preventing properties have been some of the most investigated and have been attributed to a wide variety of mechanisms, including antiproliferative activity [10, 11].

Continuing our search for anticancer agents from plant sources, in this work we studied the potential antineoplasic activity of several phenolic compounds, previously isolated from *Euphorbia* and *Pycnanthus* species (**1**, **4**–**9**, and **12**), or obtained by derivatization (**2**, **3**), together with the new acetylated (**10**) and methylated (**11**) derivatives of compound **9**. These compounds were evaluated in sensitive gastric, pancreatic, and colon human cancer cells and in their classical and atypical multidrug-resistant variants.

Materials and Methods

General experimental procedures

The NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer (^{1}H 400 MHz; ^{13}C 100.61 MHz) using $CDCl_3$ as solvent. MS was taken on a Kratos MS25RF mass spectrometer (70 eV). TLC was performed on SiO_2 F_{254} plates (Merck 5554 and 5744) and visualized under UV radiation by spraying with sulphuric acid-acetic acid-water (1 : 20 : 4) followed by heating.

Tested compounds

Twelve compounds, whose structures are presented in **Fig. 1**, were tested for their potential antiproliferative activity in sensitive and multidrug-resistant cancer cell lines: the stilbenes piceatannol (**1**), *trans*-3,5,3′,4′-tetraacetoxypiceatannol (**2**), and *trans*-3,5,3′,4′-tetramethoxypiceatannol (**3**), the flavonoids naringenin (**4**) and aromadendrin (**5**), the coumarins scopoletin (**6**) and esculetin (**7**), the neolignan dehydrodiconiferyl diacetate (**8**), and the lignans (−)-dihydroguaiaretic acid (4,4′-dihydroxy-3,3′-dimethoxylignan, **9**), 4,4′-diacetyl-3,3′-dimethoxylignan (**10**), 4′-hydroxy-3,3′,4-trimethoxylignan (**11**), and heliobuphthalmin (**12**). The purity of the compounds was more than 95% (HPLC). All the compounds were dissolved in DMSO. Compounds **1, 7**, and **6, 8** were isolated from the methanol extracts of *E. lagascae* defatted seeds and aerial parts, respectively [12–14]. Compounds **4** and **5** were isolated from the methanol extract of *E. tuckeyana* aerial parts [15]. Compounds **2** and **3** were obtained by acetylation and methylation reactions of piceatannol [12, 16]. Compounds **9** and **12** were isolated from the CH_2Cl_2 extract of *P. angolensis* stem bark [17]. Compounds **10** and **11** were obtained by acetylation and methylation reactions of (−)-dihydroguaiaretic acid (**9**), as described below.

Acetylation of (−)-dihydroguaiaretic acid

50 mg of (−)-dihydroguaiaretic acid (**9**) were suspended in Ac_2O (1.0 mL) and pyridine (1.0 mL). After stirring at room temperature for 24 hours, the excess of reagents were eliminated with N_2. Purification was made by preparative chromatography ($CHCl_3$-MeOH, 39 : 1) yielding 52 mg of compound **10** (4,4′-diacetyl-3,3′-dimethoxylignan).

4,4′-diacetyl-3,3′-dimethoxylignan (**10**): EIMS, *m/z* (rel. int.): 414 $[M]^+$ (2), 372 (28), 330 (51), 137 (100). ^{1}H RMN (400 MHz, $CDCl_3$): δ 6.92 (*d, J* = 8.0 Hz, H-5 and H-5′), 6.67 (*dd, J* = 1.6 and 8.0 Hz, H-6 and H-6′), 6.66 (*br s*, H-2 and H-2′), 3.78 (*s*, OMe-3 and OMe-3′), 2.61 (*dd, J* = 6.8 and 13.6 Hz, H-7a and H-7′a), 2.46 (*dd, J* = 8.0 and 13.6 Hz, H-7b and H-7′b), 2.33 (*s*, OCOMe-4 and OCOMe- 4′), 1.81 (*m*, H-8 and H-8′), and 0.87 (*d, J* = 6.4 Hz, H-9 and H-9′).

Methylation of (−)-dihydroguaiaretic acid

5 mL of an ethereal solution of diazomethane was added drop-by-drop to a solution of compound **9** (50 mg in 2 mL of anhydrous ether). The reaction occurred overnight and then was followed by TLC. The purification of the compounds was performed by preparative chromatography (CH_2Cl_2-acetone, 19 : 1) to yield 14.5 mg of compound **11** (4′-hydroxy-3,3′,4-trimethoxylignan).

4′-hydroxy-3,3′,4-trimethoxylignan (**11**): EIMS, *m/z* (rel. int.): 344 $[M]^+$ (35), 152 (30), 151 (100), 137 (75), 28 (17). ^{1}H RMN (400 MHz, $CDCl_3$): δ 6.82 (*d, J* = 8.0 Hz, H-5′), 6.78 (*d, J* = 8.0 Hz, H-5), 6.65 (*dd, J* = 2.0 and 8.0 Hz, H-6), 6.61 (*dd, J* = 2.0 and 8.0 Hz, H-6′), 6.59 (*d, J* = 2.0 Hz, H-2), 6.56 (*d, J* = 2.0 Hz, H-2′), 5.49 (*s*, OH-4′), 3.88 (*s*, OMe-4), 3.83 (*s*, -OMe-3 and -OMe-3′), 2.56 (*dd, J* = 6.8 and 13.6 Hz, H-7a and H-7′a), 2.41 (*dd, J* = 6.8 and 13.6 Hz, H-7b and H-7′b), 1.77 (*m*, H-8 and H-8′), and 0.85 (*d, J* = 7.2 Hz, H-9 and H-9′).

Cell lines, cell culture, and cell proliferation assay

Human cancer cell lines and drug-resistant sublines were grown in modified Leibovitz L-15 medium (Biowhittaker) as described previously [15, 18, 19]. Drug-resistant cell lines were established from parental cell lines by continuous exposure of the cells to stepwise increasing concentrations of antineoplastic agents [20]. For maintenance of drug-resistant phenotypes, the medium of drug-resistant sublines was supplemented with the selection agent. Resistance to etoposide, cisplatin (both from Gry-Pharma), and tested compounds was assessed using a proliferation assay based on sulforhodamine B (SRB) staining. 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. 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 (Sigma) 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. Mean IC_{50} values and standard deviations were calculated from at least two independent experiments in triplicate for each cell line by using the Prism software (GraphPad Software, Inc.). Relative resistance (RR) values were also determined as:

RR = (IC_{50} resistant cell line)/(IC_{50} parental-drug sensitive cell line)

Results and Discussion

In our search for bioactive compounds from plant sources, several phenolic compounds were isolated from *Euphorbia lagascae* [12–14], *Euphorbia tuckeyana* [15], and *Pycnanthus angolensis* [17], or obtained by derivatization reactions [12, 16]. These compounds include the stilbenes piceatannol (**1**), *trans*-3,5,3′,4′-tetraacetoxypiceatannol (**2**), and *trans*-3,5,3′,4′-tetramethoxypiceatannol (**3**), the flavonoids naringenin (**4**) and aromadendrin (**5**), the coumarins scopoletin (**6**) and esculetin (**7**), the neolignan dehydrodiconiferyl diacetate (**8**), as well as the lignans (−)-dihydroguaiaretic acid (4,4′-dihydroxy-3,3′-dimethoxylignan, **9**) and heliobuphthalmin (**12**, ◉ **Fig. 1**).

Continuing in this study our search for antineoplasic agents, the lignan (−)-dihydroguaiaretic acid (**9**) was derivatized to afford the new compounds 4,4′-diacetyl-3,3′-dimethoxylignan (**10**) and 4′-hydroxy-3,3′,4-trimethoxylignan (**11**). The structure elucidation of these compounds was based on a comparison of their MS and NMR spectra with those of (−)-dihydroguaiaretic acid (**9**). The twelve phenolic compounds listed above were investigated for their potential antiproliferative activity in several human cancer cell lines which were derived from three different tumor entities: gastric (EPG85-257), pancreatic (EPP85-181), and colon cancer cells (HT-29). Furthermore, in each case two different multidrug-resistant variants of these cells were also investigated: cell lines with a classical MDR phenotype (associated with the overexpression of MDR1/P-gp, EPG85-257RDB, EPP85-181RDB, and HT-29RDB) and cell lines with an atypical MDR phenotype (no enhanced expression of MDR1/P-gp, EPG85-257RNOV, EPP85-181RNOV, and HT-29RNOV). For assessment of cytotoxicity of the twelve tested compounds, the IC_{50} values of each agent were determined by proliferation assays in each of the different cell variants after continuous exposure for 5 days. The etoposide and cisplatin-specific IC_{50} values were considered as positive controls for maintenance of the drug-resistant phenotype. Relative resistance (RR) values were also determined as the relation between the IC_{50} of the resistant cell line and the IC_{50} of the parental drug-sensitive cell line. When the sensitivity against a given compound was less than 10% of the corresponding parental cell line, the compound was assessed to be highly efficient in this drug-resistant cell line [18, 19].

The IC_{50} and RR values of drug-resistant cell variants in comparison to the drug-sensitive parental cell lines are summarized in ◉ **Tables 1**, **2** and **3** for the three EPG85-257 gastric, EPP85-181 pancreatic, and HT-29 colon carcinomas, respectively. As it can be observed, in parental drug-sensitive cell lines, all the tested compounds (**1**–**12**) showed a moderate/weak antiproliferative effect or were inactive. In contrast, when comparing with the parental sublines, some of the multidrug-resistant variants showed increased sensitivities to the studied compounds, particularly the EPG85-257RDB subline.

When considering the results obtained for the stilbenes (**1**–**3**), it can be observed that in the drug-resistant subline EPG85-257RDB (associated with the classical MDR phenotype) derived from gastric carcinoma, piceatannol (**1**) and its tetramethylated derivative (**3**) were found to be as effective as the positive control etoposide (IC_{50} = 6.2 µM) and slightly less effective than cisplatin (IC_{50} = 4.0 µM), showing IC_{50} values of 5.6 and 6.3 µM, respectively (◉ **Table 1**). Furthermore, *trans*-3,5,3′,4′-tetramethoxypiceatannol (**3**) exhibited a RR value of 0.18 and thus can be considered very effective against this cell line. On the other hand, when compared to piceatannol, its acetoxy derivative (**2**) showed just a

Table 1 Cytotoxicity of phenolic compounds **1**–**12** in parental, drug-sensitive, and in different multidrug-resistant EPG85-257 gastric carcinoma cells.

Compound	EPG85-257P[a]	EPG85-257RDB[b]		EPG85-257RNOV[c]	
	IC_{50} (μM)	IC_{50} (μM)	RR[d]	IC_{50} (μM)	RR[d]
Stilbenes					
▶ **1**	10.5 ± 1.8	5.6 ± 1.1	0.53	26.0 ± 3.2	2.48
▶ **2**	61.7 ± 6.4	14.9 ± 2.6	0.24	16.7 ± 3.3	0.27
▶ **3**	34.7 ± 3.3	6.3 ± 0.6	0.18	48.8 ± 4.2	1.40
Flavonoids					
▶ **4**	95.0 ± 8.6	27.0 ± 2.4	0.28	6.2 ± 0.8	0.06
▶ **5**	> 100	64.0 ± 6.6	< 0.64	29.0 ± 2.5	< 0.29
Coumarins					
▶ **6**	> 100	> 100	n.c	62.0 ± 8.1	< 0.62
▶ **7**	17.0 ± 2.3	12.5 ± 2.0	0.74	16.0 ± 2.4	0.94
Lignans					
▶ **8**	> 100	77.2 ± 7.8	< 0.77	> 100	n.c
▶ **9**	20.0 ± 2.3	7.7 ± 1.7	0.38	8.8 ± 1.6	0.44
▶ **10**	48.3 ± 4.9	15.5 ± 2.1	0.32	34.2 ± 2.8	0.71
▶ **11**	24.6 ± 2.1	4.7 ± 0.6	0.19	6.3 ± 0.8	0.25
▶ **12**	27.9 ± 2.6	3.5 ± 0.4	0.12	23.3 ± 2.5	0.84
Etoposide	0.105 ± 0.008	6.2 ± 0.3	59	1.55 ± 0.09	14.8
Cisplatin	4.4 ± 0.4	4.0 ± 0.3	1	2.6 ± 0.2	0.6

[a] EPG85-257P: parental, drug-sensitive gastric carcinoma cell line; [b] EPG85-257RDB: gastric carcinoma cell line with classical MDR phenotype; [c] EPG85-257RNOV: gastric carcinoma cell line with atypical MDR phenotype; [d] RR: relative resistance in relation to the parental, drug sensitive cell line EPG85-257P; n.c: not calculated

Table 2 Cytotoxicity of phenolic compounds **1**–**12** in parental, drug-sensitive, and in different multidrug-resistant EPP85-181 pancreatic carcinoma cells.

Compound	EPP85-181P[a]	EPP85-181RDB[b]		EPP85-181RNOV[c]	
	IC_{50} (μM)	IC_{50} (μM)	RR[d]	IC_{50} (μM)	RR[d]
Stilbenes					
▶ **1**	27.0 ± 2.4	36.0 ± 2.9	1.33	20.0 ± 2.1	0.74
▶ **2**	76.6 ± 8.2	65.1 ± 7.3	0.85	58.6 ± 6.9	0.76
▶ **3**	67.2 ± 8.2	60.8 ± 7.8	0.90	64.6 ± 8.5	0.96
Flavonoids					
▶ **4**	65.0 ± 4.5	73.0 ± 4.6	1.12	88.0 ± 4.9	1.35
Coumarins					
▶ **7**	14.5 ± 2.3	12.5 ± 1.6	0.86	8.0 ± 0.7	0.55
Lignans					
▶ **9**	44.0 ± 5.2	28.0 ± 4.3	0.63	37.0 ± 4.6	0.84
▶ **10**	66.4 ± 7.6	49.3 ± 5.5	0.74	63.2 ± 6.9	0.95
▶ **11**	15.7 ± 1.8	14.8 ± 1.7	0.94	27.4 ± 3.2	1.74
▶ **12**	35.3 ± 3.8	19.4 ± 2.5	0.55	28.9 ± 3.1	0.81
Etoposide	0.58 ± 0.03	62.0 ± 4.2	106.9	4.5 ± 0.7	7.8
Cisplatin	0.08 ± 0.01	0.09 ± 0.01	1.2	2.6 ± 0.2	34

Compounds **5, 6,** and **8** were ineffective in the sensitive and resistant variants of pancreatic carcinoma cells (IC_{50} > 100 μM); [a] EPP85-181P: parental, drug-sensitive pancreatic carcinoma cell line; [b] EPP85-181RDB: pancreatic carcinoma cell line with classical MDR phenotype; [c] EPP85-181RNOV: pancreatic carcinoma cell line with atypical MDR phenotype; [d] RR: relative resistance in relation to the parental, drug sensitive cell line EPP85-181P; n.c: not calculated

moderate effect against this multidrug-resistant subline (IC_{50} = 14.9 μM, ◉ **Table 1**).

Regarding the results obtained for flavonoids (**4, 5**) and coumarins (**6, 7**), it should be observed that except for compound **4**, all the compounds showed a moderate activity or were inactive against EPG85-257P gastric cancer cells and its drug-resistant cell variants. Nevertheless, the best result was obtained in the multidrug-resistant EPG85-257RNOV subline associated with no MDR1/P-gp expression, for the flavanone naringenin (**4**), which exhibited a significant IC_{50} (6.2 μM, ◉ **Table 1**) and a RR value of 0.06, being 15-fold more effective in this resistant cell line than in parental drug-sensitive cells. Compounds **4** and **5** only differ in the substitution pattern of the six-membered heterocyclic ring, suggesting that the presence of an extra hydroxyl group at C-3 in aromadendrin (**5**) may be responsible for its smaller activity.

Concerning the set of lignans (**8**–**12**), 4′-hydroxy-3,3′,4-trimethoxylignan (**11**) and heliobuphthalmin (**12**) showed significant antiproliferative activities against the subline EPG85-257RDB that overexpresses MDR1/P-gp (IC_{50} = 4.7 and 3.5 μM and RR = 0.19 and 0.12, respectively, ◉ **Table 1**). In fact, this multidrug resistance subline is 5-fold and 8-fold more sensitive than the parental drug-sensitive cells and therefore, lignans **11** and **12** could be considered very effective against EPG85-257RDB cells.

Although the structures of dibenzylbutane-type lignans (**9**–**11**) are very similar, it could be observed that they considerably differ in their activities. As shown by the results, acetylation of compound **9** resulted in a decrease of antiproliferative activity for

Table 3 Cytotoxicity of phenolic compounds **1**–**12** in parental, drug-sensitive, and in different multidrug-resistant HT-29 colon carcinoma cells.

Compound	HT-29P[a]	HT-29RDB[b]		HT-29RNOV[c]	
	IC_{50} (μM)	IC_{50} (μM)	RR[d]	IC_{50} (μM)	RR[d]
Stilbenes					
▶ **1**	56.0 ± 4.8	55.0 ± 4.8	0.98	38.0 ± 4.3	0.68
▶ **2**	77.8 ± 12.3	96.0 ± 14.8	1.23	70.0 ± 10.9	0.90
▶ **3**	84.5 ± 9.2	89.4 ± 10.6	1.06	78.0 ± 8.7	0.92
Flavonoids					
▶ **4**	> 100	> 100	n.c	73.0 ± 10.5	< 0.73
Coumarins					
▶ **7**	24.0 ± 3.4	19.0 ± 2.2	0.79	17.0 ± 2.0	0.70
Lignans					
▶ **9**	54.0 ± 3.8	54.0 ± 4.1	1.0	55.0 ± 3.6	1.0
▶ **10**	51.9 ± 4.4	69.7 ± 5.7	1.34	71.0 ± 5.9	1.37
▶ **11**	45.6 ± 3.7	67.9 ± 5.2	1.49	66.4 ± 6.1	1.46
▶ **12**	70.0 ± 8.2	> 100	< 1.43	> 100	< 1.43
Etoposide	2.3 ± 0.3	26.0 ± 1.7	11.3	35.0 ± 2.6	15.2
Cisplatin	3.8 ± 0.1	2. 7 ± 0.1	0.7	3.8 ± 0.1	1

Compounds **5, 6,** and **8** were ineffective in the sensitive and resistant variants of colon carcinoma cells (IC_{50} > 100 μM). [a] HT-29P: parental, drug-sensitive colon carcinoma cell line; [b] HT-29RDB: colon carcinoma cell line with classical MDR phenotype; [c] HT-29RNOV: colon carcinoma cell line with atypical MDR phenotype; [d] RR: relative resistance in relation to the parental, drug sensitive cell line HT-29P; n.c: not calculated

compound **10**, against all the three sublines of EPG85-257 gastric carcinoma cells (**Table 1**). On the other hand, compound **11**, which differs from compound **9** only by the presence of a methoxyl group at C-4 instead of a hydroxyl group, showed an increasing activity particularly against the EPG85-257RDB subline. Besides, both compounds **9** and **11** showed a moderate activity (IC_{50} = 8.8 μM, RR = 0.44 and IC_{50} = 6.3 μM, RR = 0.25, respectively) in multidrug-resistant EPG85-257RNOV cells, suggesting that the antiproliferative activity is dependent from the individual drug resistance phenotype.

With regard to the activity against the three sublines of pancreatic carcinoma cells (EPP85-181), most of the tested compounds exhibited just a moderate antiproliferative activity (**Table 2**). In this cell line, the lowest IC_{50} values were obtained for the coumarin-type compound esculetin (**7**) against the two multidrug-resistant sublines EPP85-181RDB and EPP85-181RNOV (IC_{50} = 12.5 and 8.0 μM). Similar results were obtained for the colon carcinoma cells (HT-29, **Table 3**). Actually, these cell lines (EPP85-181 and HT-29) appeared markedly more resistant than the gastric carcinoma cells, which may indicate a tissue-dependence.

In previous studies we have reported that *trans*-3,5,3′,4′-tetramethoxystilbene (**3**) strongly modulates the efflux pump P-glycoprotein on the human *MDR1* gene-transfected mouse lymphoma cells in a dose-dependent manner. Additionally, when tested in combination with doxorubicine, compound **3** showed an additive antiproliferative effect [12], while in other assays it was found to be an effective inhibitor of the activity of voltage-gated potassium Kv 1.3 channels, which are important to inhibit the proliferation of prostate, breast, and colon cancer cells [21]. Furthermore, the stilbenes piceatannol (**1**) and *trans*-3,5,3′,4′-tetraacetoxypiceatannol (**2**), as well as the flavonoids naringenin (**4**) and aromadendrin (**5**) were found to be effective inhibitors of the multidrug-resistance associated protein 1 (MRP1) [16].

In summary, together with our previous data, the results obtained lead us to conclude that some phenolic compounds, particularly the stilbene *trans*-3,5,3′,4′-tetramethoxypiceatannol (**3**), the flavonoid naringenin (**4**), and the lignans 4′-hydroxy-3,3′,4-trimethoxylignan (**11**) and heliobuphthalmin (**12**), may be interesting as potential scaffolds for the development of new drugs against resistant human cancer cells since they appear not to be extruded by ABC-transporters.

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