Antiproliferative Activity of Methylated Analogues of *E*- and *Z*-Resveratrol

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The stilbenoids *E*-resveratrol (*E*-3,5,4′-trihydroxystilbene, **1**), *E*-3,5,4′-trimethoxystilbene (**2**), *E*-3,4,4′-trimethoxystilbene (**3**) and *E*-3,4′-dimethoxy-5-hydroxystilbene (**4**) were converted by photoisomerization to their corresponding *Z*-isomers **5**–**8**. Compounds **1**–**8** were subjected to antiproliferative activity bioassays towards a set of four different human cancer cell lines, namely DU-145 (androgen not responsive human prostate tumor), LNCaP (androgen responsive human prostate tumor), M-14 (human melanoma) and KB (human mouth epidermoid carcinoma). The methylated analogues of **1** are more active than the natural lead in the majority of bioassays. The most active compound was *Z*-3,5,4′-trimethoxystilbene (**6**), which showed against DU-145 and LNCaP cells GI_{50} values close to those of the anticancer drug vinorelbine; **6** resulted more active than its *E*-isomer **2** towards DU-145, LNCaP and especially KB cell lines. A number of methylated *Z*-isomers displayed a higher activity than their *E*-isomers, but *E*-resveratrol (**1**) was more active than *Z*-resveratrol (**5**) towards all the tested cell lines.

Key words: Resveratrol Analogues, Antiproliferative Activity

Introduction

E-Resveratrol (*E*-3,5,4′-trihydroxystilbene, **1**), a natural phytoalexin found in grapes as well as in other plants, has been the subject of intensive studies focusing on its possible role in preventing cardiovascular heart diseases (CHDs) (Bradamante *et al.*, 2004). This was evidenced by the so-called 'French paradox', namely the inverse correlation between a high-fat diet and low mortality risk of heart disease, observed in some French southern regions (Renaud and de Lorgeril, 1992) and attributed to red wine consumption. Although this hypothesis achieved wide popularity for resveratrol, more recently this stilbenoid has been reported in the literature for a variety of promising biological activities (Aggarwal and Shishodia, 2006), among them are antioxidant activity (Belguendouz *et al.*, 1997), inhibition of cyclooxygenase (Maccarrone *et al.*, 1999), inhibition of platelet aggregation (Pace-Asciak *et al.*, 1996), antioestrogenic activity (Gehm *et al.*, 1997). In particular, resveratrol showed cancer chemopreventive activity in assays representing antiinitiation, antipromotion and antiprogression activity (Jang *et al.*, 1997) and was found to be a DNA polymerase inhibitor (Sun *et al.*, 1998). Further evidences showed that **1** is able to inhibit cell growth and to induce apoptosis (programmed cell death) in various human cancer cell lines (Aggarwal and Shishodia, 2006; Schneider *et al.*, 2000; Joe *et al.*, 2002; Kuo *et al.*, 2002). Very recently **1** has been shown to induce apoptosis and inhibit angiogenesis in human breast cancer xenografts *in vivo* (Garvin *et al.*, 2006). The antiproliferative properties of resveratrol appear promising in view of the optimization of this natural 'lead compound' and the possible use of resveratrol-derived stilbenoids as cancer chemopreventive agents or adjuvants of the current anticancer drugs. Nevertheless, notwithstanding that a number of chemical modifications accompanied by biological evaluation has been carried out on resveratrol, the key structural requirements to enhance its antiproliferative activity remained, at least in part, undisclosed. In this frame, we have recently carried out a study on lipophilic resveratrol derivatives: a series of acylated, methylated and hydrogenated resveratrol analogues were prepared and subjected to a MTT

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bioassay towards DU-145 cell cultures (Cardile *et al.*, 2005). Our results showed that *E*-3,5,4′-trimethoxystilbene (**2**) is considerably more active than **1** towards DU-145 cells. This is in agreement with other studies pointing out the interesting antiproliferative and pro-apoptotic properties of resveratrol ethers bearing two or three methyl groups (Roberti *et al.*, 2003; Pettit *et al.*, 2002) indicating, in particular, compound **2** and its *Z*-stereoisomer **6** as highly antiproliferative agents, the latter being noticeably more active (Schneider *et al.*, 2003). From the results reported in these studies it emerges that in the majority of the bioassays against tumor cells, carried out on *E*-, *Z*-couples of resveratrol analogues, the *Z*-isomers resulted more active than those with *E*-configuration. In contrast with this trend, *E*-resveratrol (**1**) proved more active than its *Z*-isomer in all the reported bioassays.

In this scenario, we planned to acquire further data on the role of the configuration of the double bond concerning the antiproliferative activity of resveratrol and its methylated analogues. In particular, we evaluated the *E*- and *Z*-stereoisomers of resveratrol and three further stilbenoids bearing two or three methoxy groups towards a set of four different human cancer cell lines. As detailed below, *E*-resveratrol (**1**), *E*-3,5,4′-trimethoxystilbene (**2**), *E*-3,4,4′-trimethoxystilbene (**3**) and *E*-3,4′-dimethoxy-5-hydroxystilbene (**4**) were converted by photoisomerization to their corresponding *Z*-isomers **5**–**8**. These eight compounds were subjected to antiproliferative activity bioassays and the results of this study are reported here.

Material and Methods

General

Resveratrol (**1**) was purchased from Sigma; all reagents were of commercial quality and were used as received (Merck and Sigma-Aldrich); solvents were distilled and dried using standard techniques.

The ^{1}H NMR spectra (in CD_3OD or $CDCl_3$) were recorded on a Varian Unity Inova spectrometer at 500 MHz and performed at constant temperature (27 °C). The chemical shifts are reported as δ (ppm) referenced to TMS as internal standard.

Electron impact mass spectra (EI-MS) were recorded on a ZAB 2-SE instrument.

The products were purified by preparative liquid chromatography (PLC) on Polyamide CC6 (50–160 μm; Macherey-Nagel), Polyamide 11 or silica gel DIOL (40–63 μm; Merck); the elution system is reported below for each compound. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck), Polyamide 6 UV_{254} precoated plastic sheets (0.1 mm; Macherey-Nagel) or Polyamide 11. Polyamide 11 powder, used for coating home-made TLC plates or packing PLC columns, was prepared in our laboratory starting from commercially available Polyamide 11 pellets (Aldrich). To this end, the pellets were suspended in conc. formic acid and stirred until a solution was obtained. After drop-wise addition of methanol a precipitate was obtained which was separated by filtration and repeatedly washed with water/methanol (80:20) until neutralization.

*Methylation of E-resveratrol (**1**)*

500 mg (2.19 mmol) of compound **1** were placed into a boiling flask and dispersed with 200 mL of acetone and 300 mg of anhydrous potassium carbonate. To this suspension 300 μL of dimethyl sulphate were added. The resulting mixture was heated for 24 h under reflux. Acetone was removed from the mixture by a rotary evaporator. The resulting mixture was purified by PLC (silica gel, CH_2Cl_2 in *n*-hexane from 20 to 100%) to obtain 440 mg (74.4% yield) of **2** and 41.3 mg (7.3% yield) of **4**.

E-3,5,4′-Trimethoxystilbene (**2**). PLC was monitored by TLC on silica gel plates, eluted with MeOH/CH_2Cl_2 (7:93, v/v), R_f = 0.67. – EI-MS: *m/z* = 270 $[M]^+$. – ^{1}H NMR (CD_3OD): δ = 7.47 (2H, d, *J* = 8.0 Hz, H-2′, H-6′), 7.08 (1H, d, *J* = 16.0 Hz, H-β), 6.94 (1H, d, *J* = 16.0 Hz, H-α), 6.90 (2H, d, *J* = 8.0 Hz, H-3′, H-5′), 6.68 (2H, d, *J* = 2.0 Hz, H-2, H-6), 6.37 (1H, br t, H-4), 3.81 (9H, s, 3-OCH_3, 5-OCH_3, 4′-OCH_3). The ^{1}H NMR spectrum recorded in $CDCl_3$ was in perfect agreement with that previously reported in the literature (Mannila *et al.*, 1993).

E-3,4′-Dimethoxy-5-hydroxystilbene (**4**). PLC was monitored by TLC on silica gel plates, eluted with MeOH/CH_2Cl_2 (7:93, v/v), R_f = 0.41. – EI-MS: *m/z* = 256 $[M]^+$. – ^{1}H NMR (CD_3OD): δ = 7.46 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 7.03 (1H, d, *J* = 16.3 Hz, H-β), 6.91 (2H, d, *J* = 8.6 Hz, H-3′, H-5′), 6.89 (1H, d, *J* = 16.3 Hz, H-α), 6.58 (1H, br t, H-2), 6.56 (1H, br t, H-6), 6.27 (1H, t, *J* = 2.2 Hz, H-4), 3.81 (3H, s, 3-OCH_3), 3.78 (3H, s, 4′-OCH_3).

The ^{1}H NMR spectrum recorded in $CDCl_3$ was in perfect agreement with that previously reported in the literature (Gonzales *et al.*, 1993).

*Synthesis of E-3,4,4′-trimethoxystilbene (**3**)*

4-Methoxybenzylchloride (1.1 mL, 8.13 mmol) was heated with excess of triethyl phosphite (1.85 mL, 10.6 mmol) to 130 °C to give diethyl (4-methoxybenzyl)phosphonate (1.82 g, 7.1 mmol, yield 87%). This latter was cooled to 0 °C, and dry DMF (10 mL) and 0.41g (7.6 mmol) of sodium methoxide were added. To this solution, 1.18 g (7.1 mmol) of 3,4-dimethoxybenzaldehyde was added and the mixture was allowed to stand at room temperature for 1 h. Then it was heated to 100 °C and allowed to stand at this temperature for 1 h. After cooling, the reaction mixture was stirred overnight and subsequently quenched with water and extracted with diethyl ether. The combined organic layers were washed with water and dried over Na_2SO_4, affording 1.57 g of product **3** (82% yield).

E-3,4,4′-Trimethoxystilbene (**3**). PLC was monitored by TLC on silica gel plates, eluted with MeOH/CH_2Cl_2 (7:93, v/v), R_f = 0.65. – EI-MS: *m/z* = 270 $[M]^+$. – ^{1}H NMR (CD_3OD): δ = 7.45 (2H, d, *J* = 9.0 Hz, H-2′, H-6′), 7.15 (1H, d, *J* = 2.0 Hz, H-2), 7.05 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 7.00 (1H, d, *J* = 16.5 Hz, H-β), 6.97 (1H, d, *J* = 8.0 Hz, H-5), 6.94 (1H, d, *J* = 16.5 Hz, H-α), 6.90 (2H, d, *J* = 9.0 Hz, H-3′, H-5′), 3.88 (3H, s, 3-OCH_3), 3.84 (3H, s, 4-OCH_3), 3.80 (3H, s, 4′-OCH_3). The ^{1}H NMR spectrum recorded in $CDCl_3$ was in perfect agreement with that previously reported in the literature (Cardile *et al.*, 2005).

Photoisomerization

Irradiation experiments were performed in a 200 mL quartz vessel using a Rayonet photochemical reactor equipped with a variable number of "black light" phosphor lamps with emission in the 310–390 nm range and a maximum at 350 nm. The fluence rate at the irradiation position was measured to be 5 mW/cm^2. A 2×10^{-4} M solution (200 mL) of each compound (**1**–**4**) in ethanol was irradiated in the reactor for 10 min under nitrogen bubbling. The irradiated solution was then taken to a small volume under vacuum and charged onto the appropriate Polyamide PLC column to separate the *Z*-product from the residual *E*-isomer. All photoisomerizations were obtained with 80–82% conversion, based on ^{1}H NMR measurements.

Z-3,5,4′-Trihydroxystilbene (**5**). From 25 mg of **1**, irradiated as above, 17.8 mg of the *Z*-isomer were obtained after purification by Polyamide 11 PLC, eluted with EtOH/H_2O (80:20, v/v). The separation was monitored by TLC on Polyamide 11 plates eluted with EtOH/H_2O (80:20, v/v), R_f (**5**) = 0.36, R_f (**1**) = 0.10. – EI-MS: *m/z* = 228 $[M]^+$. – ^{1}H NMR (CD_3OD): δ = 7.09 (2H, d, *J* = 8.4 Hz, H-2′, H-6′), 6.62 (2H, d, *J* = 8.4 Hz, H-3′, H-5′), 6.41 (1H, d, *J* = 12.2 Hz, H-β), 6.32 (1H, d, *J* = 12.2 Hz, H-α), 6.21 (2H, d, *J* = 2.2 Hz, H-2, H-6), 6.11 (1H, t, *J* = 2.2 Hz, H-4).

Z-3,5,4′-Trimethoxystilbene (**6**). This compound (18.6 mg) was obtained starting from 33.6 mg of **2** following irradiation and purification on a Polyamide CC6 column (EtOH/H_2O 62:38, v/v). Analytical controls were performed on Polyamide CC6 TLC plates eluted with EtOH/H_2O (62:38, v/v), R_f (**6**) = 0.62, R_f (**2**) = 0.37. – EI-MS: *m/z* = 270 $[M]^+$. – ^{1}H NMR (CD_3OD): δ = 7.16 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.77 (2H, d, *J* = 8.8 Hz, H-3′, H-5′), 6.51 (1H, d, *J* = 12.2 Hz, H-β), 6.42 (1H, d, *J* = 12.2 Hz, H-α), 6.39 (2H, d, *J* = 2.3 Hz, H-2, H-6), 6.30 (1H, t, *J* = 2.3 Hz, H-4), 3.74 (3H, s, 4′-OCH_3), 3.62 (6H, s, 3-OCH_3, 5-OCH_3). The ^{1}H NMR spectrum recorded in $CDCl_3$ was in perfect agreement with that previously reported in the literature (Koh *et al.*, 2001).

Z-3,4,4′-Trimethoxystilbene (**7**). From 42.5 mg of **3** 13.6 mg of the *E*-isomer **7** were obtained after irradiation and PLC on Polyamide CC6 (EtOH/H_2O 48:52, v/v). The separation was monitored by TLC on Polyamide CC6 plates eluted with EtOH/H_2O (48:52, v/v), R_f (**7**) = 0.33, R_f (**3**) = 0.12. – EI-MS: *m/z* = 270 $[M]^+$. – ^{1}H NMR (CD_3OD): δ = 7.18 (2H, d, *J* = 8.1 Hz, H-2′, H-6′), 6.84–6.79 (overlapped multiplets, 5H, H-2, H-5, H-6, H-3′, H-5′), 6.47 (1H, d, *J* = 12.1 Hz, H-β), 6.44 (1H, d, *J* = 12.1 Hz, H-α), 3.80 (3H, s, 4-OCH_3), 3.76 (3H, s, 4′-OCH_3), 3.58 (3H, s, 3-OCH_3).

Z-3,4′-Dimethoxy-5-hydroxystilbene (**8**). 18.2 mg of the title compound were obtained after photoisomerization of 26 mg of **4** and subsequent PLC purification on Polyamide 11 (EtOH/H_2O 65:35, v/v). The separation was monitored by TLC on Polyamide 11 plates eluted with EtOH/H_2O (65:35, v/v), R_f (**8**) = 0.23, R_f (**4**) = 0.05. – EI-MS: *m/z* = 256 $[M]^+$. – ^{1}H NMR (CD_3OD): δ = 7.17 (2H, d, *J* = 8.7 Hz, H-2′, H-6′), 6.77 (2H, d, *J* = 8.7 Hz, H-3′, H-5′), 6.48 (1H, d, *J* = 12.1 Hz, H-β), 6.40 (1H, d, *J* = 12.1 Hz, H-α), 6.30 (2H, d, *J* = 2.2 Hz, H-2, H-6), 6.21 (1H, t, *J* = 2.2 Hz, H-4),

3.75 (3H, s, 4′-OCH_3), 3.61 (3H, s, 3-OCH_3). The ^{1}H NMR spectrum recorded in $CDCl_3$ was in perfect agreement with that previously reported in the literature (Pettit *et al.*, 2002).

Cell cultures

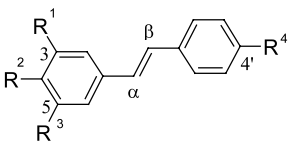
Androgen not responsive human prostate tumor (DU-145), androgen responsive human prostate tumor (LNCaP), human melanoma (M-14) and human mouth epidermoid carcinoma (KB) cells were purchased from the American Type Culture Collection (Rockville, 146 MD, USA). The LNCaP, M-14 and KB cell lines were grown in RPMI-1640 medium (Sigma) supplemented with 10% fetal calf serum, 1 mM L-glutamine and 10 μL/mL penicillin-streptomycin. DU-145 human prostate cancer cells were maintained in Earle Minimal Essential Medium (EMEM), containing 10% fetal calf serum, 1 mM L-glutamine, antibiotics (50 IU/mL penicillin and 50 μg/mL streptomycin) and 1% non-essential amino acids. All the cells were incubated at 37 °C in 5% CO_2/95% air atmosphere, routinely split 1:2 each week, used between the 4th and 5th passage and treated one day before they reached the confluence.

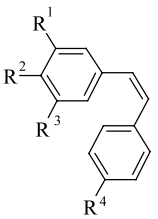
Antiproliferative assay (MTT test)

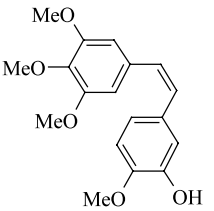
The MTT assay, used to evaluate the cell viability, measures the cellular capacity of various mitochondrial dehydrogenase enzymes to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a blue formazan product. The method described by Mosmann (1983) was employed. Briefly, experimental cancer cultures (1×10^4) were set up in flat-bottomed 200 μL microplates, incubated at 37 °C in a humidified 5% CO_2/95% air mixture and, 24 h later, treated with the compound under test for 72 h (before cell harvesting) at the concentrations of 50, 25, 12.5 and 6.25 μM, except for compounds **1**, **3**, **4**, **5**, and **7**, tested also at the concentrations 200 and 100 μM, and compound **6**, tested at the concentrations 0.5, 0.1, 0.05 and 0.025 μM. Vinorelbine was used as reference and tested at 5, 0.5, 0.1, 0.05, 0.025 and 0.0125 μM. 4 h before the end of the culture, 20 μL of 0.5% MTT in phosphate buffer saline were added to each microwell. After incubation with the reagent, the supernatant was removed and replaced by 100 μL of dimethyl sulfoxide. All the bioassays were carried out in the dark to avoid possible undesired photoisomerization of the compounds during the test. The optical density of each sample was measured with a microplate spectrophotometer reader (Titertek Multiskan, DAS, Milan, Italy) at λ 550 nm.

Results and Discussion

E-Resveratrol (**1**) was subjected to photoisomerization by irradiation at 350 nm and afforded *Z*-resveratrol (**5**). *E*-3,5,4′-Trimethoxystilbene (**2**) was obtained by methylation of **1** and, by photoisomerization under the same conditions used for **1**, gave *Z*-3,5,4′-trimethoxystilbene (**6**). *E*-3,4,4′-Trimethoxystilbene (**3**) was synthesized from 3,4-dimethoxybenzaldehyde and diethyl (4-methoxybenzyl)phosphonate in two steps through an Arbuzov rearrangement followed by a Wittig-Horner reaction, as previously reported by us







1	R^1 = OH	R^2 = H	R^3 = OH	R^4 = OH	**5**	R^1 = OH	R^2 = H	R^3 = OH	R^4 = OH	**9**
2	R^1 = OMe	R^2 = H	R^3 = OMe	R^4 = OMe	**6**	R^1 = OMe	R^2 = H	R^3 = OMe	R^4 = OMe	
3	R^1 = OMe	R^2 = OMe	R^3 = H	R^4 = OMe	**7**	R^1 = OMe	R^2 = OMe	R^3 = H	R^4 = OMe	
4	R^1 = OMe	R^2 = H	R^3 = OH	R^4 = OMe	**8**	R^1 = OMe	R^2 = H	R^3 = OH	R^4 = OMe	

Fig. 1. Structures of compounds **1**–**9**: *E*-3,5,4′-trihydroxystilbene (**1**), *E*-3,5,4′-trimethoxystilbene (**2**), *E*-3,4,4′-trimethoxystilbene (**3**), *E*-3,4′-dimethoxy-5-hydroxystilbene (**4**), *Z*-3,5,4′-trihydroxystilbene (**5**), *Z*-3,5,4′-trimethoxystilbene (**6**), *Z*-3,4,4′-trimethoxystilbene (**7**), *Z*-3,4′-dimethoxy-5-hydroxystilbene (**8**) and combretastatin A-4 (**9**).

Table I. Cell growth inhibition[a] of *E*-resveratrol (**1**) and its analogues **2**–**8**.

Compound	DU-145[b]	LNCaP[c]	M-14[d]	KB[e]
1	22.7 ± 1.3	35.1 ± 2.4	31.0 ± 3.1	72.8 ± 2.6
2	4.7 ± 1.2	20.0 ± 1.9	12.1 ± 1.7	36.0 ± 2.8
3	4.8 ± 0.7	2.0 ± 0.2	65.9 ± 5.1	5.3 ± 0.9
4	8.4 ± 0.8	49.2 ± 1.5	80.5 ± 4.5	50.0 ± 3.2
5	56.0 ± 2.4	99.8 ± 4.2	98.2 ± 6.3	73.0 ± 4.8
6	2.9 ± 0.4	1.5 ± 0.3	40.0 ± 3.8	0.10 ± 0.05
7	29.1 ± 1.9	24.5 ± 2.7	100.0 ± 7.6	30.1 ± 2.3
8	5.7 ± 1.1	38.2 ± 3.3	46.6 ± 3.2	13.0 ± 1.5
Vinorelbine	2.5 ± 0.5	3.1 ± 0.6	3.5 ± 0.8	0.014 ± 0.008

[a] Data are reported as GI_{50} (μM) ± SD, calculated after 72 h of continuous exposure relative to untreated controls. Values are the mean (± SD) of three experiments performed in quadruplicate.
[b] DU-145, androgen not responsive human prostate tumor.
[c] LNCaP, androgen responsive human prostate tumor.
[d] M-14, human melanoma.
[e] KB, human mouth epidermoid carcinoma.

(Cardile *et al.*, 2005). Compound **3** was irradiated to obtain *Z*-3,4,4′-trimethoxystilbene (**7**). Finally, *E*-3,4′-dimethoxy-5-hydroxystilbene (**4**) was obtained by partial methylation of **1** and photoisomerization of **4** gave *Z*-3,4′-dimethoxy-5-hydroxystilbene (**8**).

Compounds **1**–**8** (Fig. 1) were subjected to antiproliferative activity bioassays towards the following cell lines: DU-145 (androgen not responsive human prostate tumor), LNCaP (androgen responsive human prostate tumor), M-14 (human melanoma) and KB (human mouth epidermoid carcinoma). The anticancer drug vinorelbine was used as positive control. The GI_{50} values are reported in Table I.

Looking at the cell line viability, DU-145 and LNCaP are more sensitive to the tested stilbenoids, whereas KB and especially M-14 are more resistant, with the exception of the potent activity of compound **6** against KB cells (GI_{50} = 0.10 μM). On the whole, these data are only in partial agreement with previous literature reports. The methylated analogues of **1** are more active than the natural lead in the majority of bioassays. *E*-Resveratrol (**1**) is more active than *Z*-resveratrol (**5**) towards all the tested cell lines: nevertheless, there is a considerable difference between **1** and **5** in the activity towards DU-145, LNCaP and M-14 cells, whereas the GI_{50} values towards KB cells are substantially identical for both isomers.

Methylated *Z*-isomers display a higher activity than their relevant *E*-isomers in the majority of the bioassays, but this includes some cases where the activity of *E*- and *Z*- isomers are comparable. *Z*-3,4′-dimethoxy-5-hydroxystilbene (**8**) resulted more active than its *E*-isomer **4** towards all the cell lines; conversely, *E*-3,4,4′-trimethoxystilbene (**3**) was always more active than its *Z*-isomer **7**. The most active compound was *Z*-3,5,4′-trimethoxystilbene (**6**), which resulted more active than its *E*-isomer **2** towards DU-145, LNCaP and especially KB cell lines and showed towards DU-145 and LNCaP cells GI_{50} values close to those of the anticancer drug vinorelbine.

Summing up, on the basis of the data reported here and literature references, a general rule, stating that stilbenoids with *Z*-configuration at the double bond display a considerably higher antiproliferative activity than their *E*-isomers, cannot be established. It is also difficult to trace general conclusions on structure-activity relationships for the *E*-, *Z*-stilbenoids on the basis of the data reported here. In our opinion, it is clear that the *Z*-configuration of the stilbene nucleus does not always provide, alone, a higher antiproliferative activity than the *E*-geometry. One important factor is undoubtedly the presence of methoxy groups: the trimethoxystilbenes **2** and **6** proved to be the most active compounds, followed by the other stilbenoids with three or two methoxy groups. The antiproliferative activity of *Z*-3,5,4′-trimethoxystilbene (**6**) has been related to its structural analogy with the well-known antimitotic combretastatins, isolated from *Combretum caffrum*, and in particular with combretastatin A-4 (**9**) (Fig. 1) (Pettit *et al.*, 1995). The X-ray study of **9** established that the two aromatic rings are not coplanar (the normals to the phenyl ring planes are inclined

66° to each other) (Cushman *et al.*, 1992). In addition, a SAR study on combretastatin A-4 and other related stilbenoids and phenanthrenes showed that the active *Z*-stilbenoids take on a preferred conformation with the aromatic rings inclined to each other, and this may play a role in preferential binding to a receptor site. On this basis, the higher activity of some methylated *Z*-analogues of resveratrol (in particular those bearing OMe or OH groups in 3 and 5 positions) may be related to a preferred conformation where the two aromatic rings are not coplanar. Nevertheless, some still unclarified factors have to be claimed to justify the higher antiproliferative activity of some *E*-stilbenoids, and in particular of *E*-resveratrol (**1**). Of course, further structural modifications of the resveratrol nucleus accompanied by extensive biological evaluation are required for a better understanding of the structure-activity relationships of this important stilbenoid and its analogues.

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Aggarwal B. B. and Shishodia S. (eds.) (2006), Oxidative Stress and Disease, Vol 20, Resveratrol in Health and Disease. CRC Press LLC, Boca Raton, FL, pp. 1–712.

Belguendouz L., Fremont L., and Linard A. (1997), Resveratrol inhibits metal ion-dependent and independent peroxidation of porcine low-density lipoproteins. Biochem. Pharmacol. **53**, 1347–1355.

Bradamante S., Barenghi L., and Villa A. (2004), Cardiovascular protective effects of resveratrol. Cardiovasc. Drug Rev. **22**, 169–188.

Cardile V., Lombardo L., Spatafora C., and Tringali C. (2005), Chemo-enzymatic synthesis and cell-growth inhibition activity of resveratrol analogues. Bioorg. Chem. **33**, 22–33.

Cushman M., Nagarathnam D., Gopal D., He H. M., Lin C. M., and Hamel E. (1992), Synthesis and evaluation of analogues of (*Z*)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as potential cytotoxic and antimitotic agents. J. Med. Chem. **35**, 2293–2306.

Garvin S., Oellinger K., and Dabrosin C. (2006), Resveratrol induces apoptosis and inhibits angiogenesis in human breast cancer xenografts *in vivo*. Cancer Lett. **231**, 113–122.

Gehm B. D., McAndrews J. M., Chien P.-Y., and Jameson J. L. (1997), Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. Proc. Natl. Acad. Sci. USA **94**, 14138–14143.

Gonzales M. J. T. G., Pinto M. M. M., Kijjoa A., Anantachoke C., and Herz W. (1993), Stilbenes and other constituents of *Knema austrosiamensis*. Phytochemistry **32**, 433–438.

Jang M., Cai L., Udeani G. O., Slowing K. V., Thomas C. F., Beecher C. W. W., Fong H. H. S., Farnsworth N. R., Kinghorn A. D., Mehta R. G., Moon R. C., and Pezzuto J. M. (1997), Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science **275**, 218–220.

Joe A. K., Liu H., Suzui M., Vural M. E., Xiao D., and Weinstein I. B. (2002), Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines. Clin. Cancer Res. **8**, 893–903.

Koh D., Park K. H., Jung J., Yang H., Mok K. H., and Lim Y. (2001), Complete assignment of the ^{1}H and ^{13}C NMR spectra of resveratrol derivatives. Magn. Res. Chem. **39**, 768–770.

Kuo P.-L., Chiang L.-C., and Lin C.-C. (2002), Resveratrol-induced apoptosis is mediated by p53-dependent pathway in Hep G2 cells. Life Sci. **72**, 23–34.

Maccarrone M., Lorenzon T., Guerrieri P., and Finazzi Agrò A. (1999), Resveratrol prevents apoptosis in K562 cells by inhibiting lipoxygenase and cyclooxygenase activity. Eur. J. Biochem. **265**, 27–34.

Mannila E., Talvitie A., and Kolehmainen E. (1993), Anti-leukaemic compounds derived from stilbenes in *Picea abies* bark. Phytochemistry **33**, 813–816.

Mosmann T. (1983), Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Meth. **65**, 55–63.

Pace-Asciak C. R., Rounova O., Hahn S. E., Diamandis E. P., and Goldberg D. M. (1996), Wines and grape juices as modulators of platelet aggregation in healthy human subjects. Clin. Chim. Acta **246**, 163–182.

Pettit G. R., Singh S. B., Boyd M. R., Hamel E., Pettit R. K., Schmidt J. M., and Hogan F. (1995), Antineoplastic agents. 291. Isolation and synthesis of combretastatins A-4, A-5, and A-6(1a). J. Med. Chem. **38**, 1666–1672.

Pettit G. R., Grealish M. P., Jung M. K., Hamel E., Pettit R. K., Chapuis J.-C., and Schmidt J. M. (2002), Antineoplastic agents. 465. Structural modification of resveratrol: sodium resverastatin phosphate. J. Med. Chem. **45**, 2534–2542.

Renaud S. and de Lorgeril M. (1992), Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet **339**, 1523–1526.

Roberti M., Pizzirani D., Simoni D., Rondanin R., Baruchello R., Bonora C., Buscemi F., Grimaudo S., and Tolomeo M. (2003), Synthesis and biological evaluation of resveratrol and analogues as apoptosis-inducing agents. J. Med. Chem. **46**, 3546–3554.

Schneider Y., Vincent F., Duranton B., Badolo L., Gosse F., Bergmann C., Seiler N., and Raul F. (2000), Antiproliferative effect of resveratrol, a natural component of grapes and wine, on human colonic cancer cells. Cancer Lett. **158**, 85–91.

Schneider Y., Chabert P., Stutzmann J., Coelho D., Fougerousse A., Gosse F., Launay J.-F., Brouillard R., and Raul F. (2003), Resveratrol analog (*Z*)-3,5,4′-trimethoxystilbene is a potent anti-mitotic drug inhibiting tubulin polymerization. Int. J. Cancer **107**, 189–196.

Sun N. J., Woo S. H., Cassady J. M., and Snapka R. M. (1998), DNA polymerase and topoisomerase II inhibitors from *Psoralea corylifolia*. J. Nat. Prod. **61**, 362–366.