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Antiangiogenic Resveratrol Analogues by Mild *m*-CPBA Aromatic Hydroxylation of 3,5-Dimethoxystilbenes

Carmela Spatafora[a], Giuseppina Basini[b], Laura Baioni[b], Francesca Grasselli[b], Alfio Sofia[a] and Corrado Tringali[a]*

[a]*Dipartimento di Scienze Chimiche, Università di Catania, Viale A. Doria 6, I-95125 Catania, Italy*

[b]*Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti, Università di Parma, Via del Taglio 8, I-43100 Parma, Italy*

ctringali@unict.it

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A mild treatment of the resveratrol analogue 3,5,4'-trimethoxystilbene **2** with *m*-CPBA afforded two hydroxylated methoxystilbenes **5** and **6** by direct aromatic hydroxylation. A similar protocol was applied to other stilbenes bearing a 3,5-dimethoxy moiety, namely tetramethoxystilbenes **7** and **10** to obtain respectively the hydroxylated analogues **8**, **9** and **11**, **12**. The substrate **2** and the new compounds **5**, **8** and **11** were evaluated as anti-angiogenic agents and proved significantly active in the range 1 – 100 μM.

Keywords: antiangiogenic activity, resveratrol analogues, methoxystilbenes, hydroxylation, *m*-CPBA.

E-resveratrol (*E*-3,5,4′-trihydroxystilbene, **1**, Figure 1), originally isolated from *Veratrum grandiflorum* and later obtained from *Polygonum cuspidatum* [1], is one of the main polyphenols present in red wine. It has became popular mainly due to the so-called 'French paradox'[2]. Starting from the first studies focused on its possible role in preventing cardiovascular heart diseases (CHD) [3a], as well as on its cancer chemopreventive properties [3b], this stilbenoid has recently attracted the attention of an increasing number of researchers. In particular, *E*-resveratrol exhibits promising antiproliferative, pro-apoptotic and antiangiogenic properties [4a-4e]. Nevertheless, the available *in vivo* studies indicate that **1**, although absorbed in high extent by the organism, has a poor bioavailability [4f], and may be converted *in vivo* into compounds lacking of its anti-proliferative activity [4d]. Thus, a number of resveratrol analogues have been recently evaluated as anti-tumor agents, in the hope to obtain compounds with higher activity and / or bioavailability. Among these, polymethoxystilbenes are an interesting sub-group including compounds with significant antitumor properties [5a]. In particular, the presence of a 3,5-dimethoxy moiety is frequently associated with noticeable biological activity. In addition, some *in vivo* studies indicate that methoxystilbenes

1 R = H
2 R = CH_3
3 $R_1 = R_2 = H$
4 $R_1 = OCH_3$, $R_2 = OH$

Figure 1: Structures of compounds **1 – 4**.

undergo different metabolic conversion and have a higher bioavailability with respect to resveratrol [5b]. In recent studies the simple *E*-resveratrol derivative 3,5,4'-trimethoxystilbene **2** (Figure 1), and its *Z*-isomer (**3**, Figure 1), structurally related to the potent tubulin inhibitor and vascular disrupting agent combretastatin A4 (**4**, Figure 1) [5c], proved far more active than **1** both as antiproliferative [5b] and antiangiogenic agents [5d].

In particular, **2** proved 30 to 100 times more potent than **1** in inhibiting endothelial cell proliferation, sprouting, collagen gel invasion, and morphogenesis. *In vivo*, **2** caused the rapid stasis of blood flow and regression of intersegmental vessels in the trunk of zebrafish embryos. In addition, it inhibited blood vessel growth and caused the disappearance of pre-existing blood vessels in the chick embryo.

A rising interest towards new vessel growth reducing agents has been observed in the last decade, due to the possible applications of these substances in cancer therapy, as well as in other degenerative diseases involving new vessel growth. Unfortunately, while the major stimula triggering the neovascularization process are overall well characterized, the mechanisms underlying the negative control of angiogenesis are still not completely known. In a previous study, several of us attempted to unravel physiological mechanisms of neovascularisation [6a-6d] focusing, more recently, on its negative control [7a-7c]. Various angiogenesis inhibitors have been developed so far; their efficacy has been evaluated in different *in vitro* and *in vivo* assays, and their clinical evaluation is in progress [7d]. Recently, the anti-VEGF antibody bevacizumab has been shown to exert antiangiogenic effects in patients with cancer, leading to U.S. Food and Drug Administration approval for colorectal cancer treatment [7e].

These observations prompted us, in continuation of our studies on resveratrol analogues [8a,8b], to synthesize further methoxystilbenes as new antiangiogenic agents.

As detailed in the following sections, we employed a mild treatment of substrate **2** with *m*-CPBA at RT to obtain two hydroxylated methoxystilbenes **5** and **6**.

Analogously, a similar protocol was applied to the tetramethoxystilbenes **7** and **10** to obtain respectively the hydroxylated analogues **8**, **9** and **11**, **12**. Among these resveratrol analogues, we selected the substrate **2** and novel compounds **5**, **8** and **11** for an evaluation of their angiogenic properties employing a previously developed method.

In contrast to the variety of completely methoxylated stilbenoids prepared by standard synthetic methods [5b], methoxystilbenes bearing one free phenol group have been rarely reported, and when obtained it has been by cumbersome synthetic methodologies, normally affording a mixture of *cis* and *trans* isomers [9,10]. This may account for the scarcity of literature data about the biological properties of hydroxylated polimethoxystilbenes. In searching for simple methods to obtain this kind of resveratrol analogues, we were attracted by the unexpected result of a standard treatment of substrate **2** with *m*-CPBA, carried out as a side project in our laboratory to obtain resveratrol analogues with an epoxide function. An unexpected major product of this reaction was found to be 2-hydroxy-3,5,4'-trimethoxystilbene **5** which indicated direct aromatic hydroxylation of ring A in the stilbene nucleus, without epoxidation of the central double bond (Scheme 1). Hydroxylation at positions 2 and 4 suggested that this unexpected reaction could be *via* regioselective aromatic electrophilic substitution, of the activated *ortho* and *para* positions.

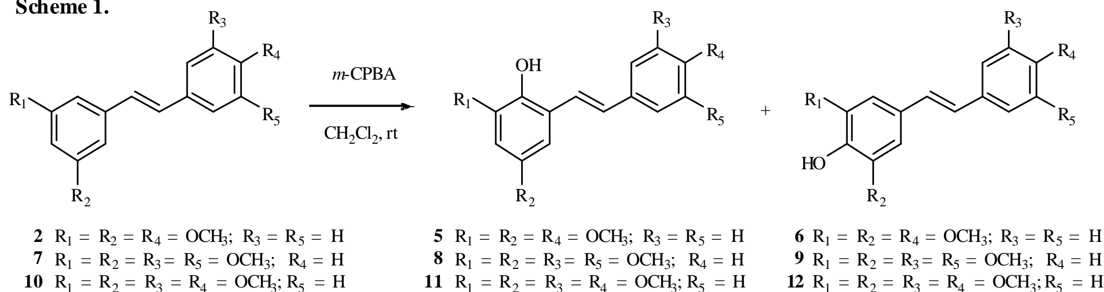
This was readily confirmed by a calculation of atomic charges obtained *via* PM3 semi-empirical optimisation (Figure 2). In fact model compound **2**, displays higher values at C-2, C-4 and C-6 (3,5-dimethoxy substituted ring) than at positions C-2', C-3', C-5', C-6' (4'-methoxy substituted ring).

A recent article on the synthesis of methoxy-substituted phenols by peracid oxidation presented convincing evidence for a two-step electrophilic substitution mechanism [11].

To test the applicability of this novel hydroxylation method of methoxystilbenes we synthesized further two substrates bearing 3,5-dimethoxy groups on rings A and 3,5 or 3,4-dimethoxy groups on ring B.

According to the above cited methodology (Wittig olefination) the reaction of 3,5-dimethoxy-benzaldehyde or 3,4-dimethoxybenzaldehyde and diethyl (3,5-dimethoxybenzyl) phosphonate was carried out to obtain respectively the 3,5,3',5'-tetramethoxystilbene **7** and 3,5,3',4'-tetramethoxy-stilbene **10**.

Scheme 1.



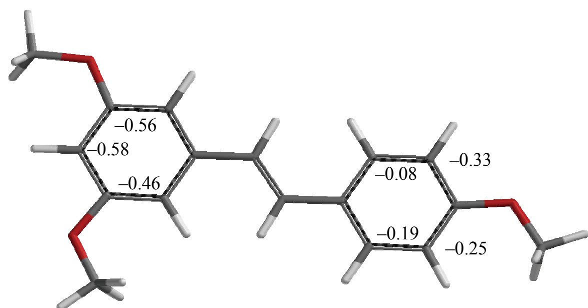


Figure 2: PM3 atomic charges for **2**.

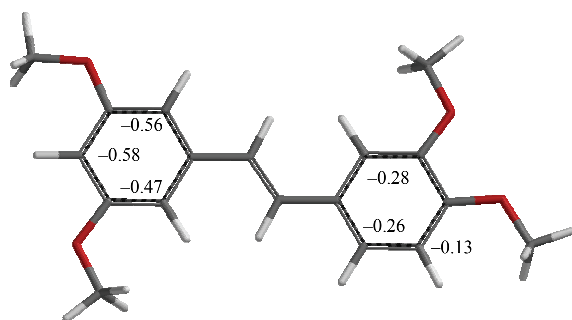


Figure 3: PM3 atomic charges for **10**.

Analogous to **2**, substrate **7** readily reacted at RT with *m*-CPBA to give a reaction mixture which remained unmodified after prolonged time, with a 68% conversion. Two main products were characterized by spectroscopy as 2-hydroxy-3,5,3',5'-tetramethoxystilbene **8** and 4-hydroxy-3,5,3',5'-tetramethoxystilbene **9** (see Scheme 1). Finally, when **10** was treated with *m*-CPBA under the same conditions employed for **7**, a reaction mixture was rapidly obtained, which remained unmodified after prolonged time, with a 66 % conversion. The main products, after spectral characterization, were established as 2-hydroxy-3,5,3',4'-tetramethoxy-stilbene **11** and 4-hydroxy-3,5,3',5'-tetramethoxy-stilbene **12** (see Scheme 1). This in agreement with the atomic charge density calculations, showing higher values at C-2, C-4 and C-6 and lower values at C-2', C-5' and C-6' (Figure 3).

Compounds **5**, **8** and **11** are to our knowledge previously unreported. Compound **9** has been reported obtained by Wittig olefination at –78°C under nitrogen, using 3,5-dimethoxybenzyltriphenyl-phosphonium bromide and with a protected aldehyde; final deprotection gave a mixture of the *cis/trans*-stilbenes (3:7) [9]; compound **12** has been previously obtained by a Knoevenagel condensation [10]. It is also worthy of note here that compound **6** has been recently reported as a putative metabolite of 3,4,5,4'-tetramethoxy-stilbene (DMU-212), this latter being under preclinical evaluation as a potential anti-tumor prodrug that undergoes metabolic activation by cytochrome P450 enzymes affording hydroxylated metabolic products [5b,12]. This suggests that hydroxylated polymethoxystilbenes are good candidates as potential antitumor agents.

The potential of compounds **5**, **8** and **11** to interfere with vessel growth has been investigated by means of a previously reported angiogenesis bioassay [7c]. This method consists of a three dimensional fibrin gel support in which we include dextran microcarriers beads with adhering porcine aortic endothelial cells (AOC). The AOC growth was assessed after 48 and 96 h of incubation. Our data indicated that all these compounds with the exception of **11**, exerted an inhibitory effect ($p<0.001$) on the angiogenic process at all the concentrations tested. With regard to compound **11**, our data indicated that the lowest concentration, which is ineffective after 48 h, became effective ($p<0.001$) after 96 h even if showing a weaker effect ($p<0.001$) in comparison to the other compounds. Moreover, 1 and 10 µM of compound **11** exerted a significant inhibitory effect ($p<0.001$) after 48 h of incubation although displaying a lesser potency; after the 96 h incubation time, the effectiveness of **11** resulted in enhancement, thus suggesting that this compound could require a longer time to exert its effect. Overall, after the 48 h incubation, compounds **2**, at the concentration 1 and 10 µM, and **5**, at 100 µM, resulted the most potent ($p<0.001$). More interestingly, after 96 h, compound **5** appeared the most effective ($p<0.001$), since it displayed the most relevant suppressive effect at the concentrations of 1, 10 and 100 µM (Figure 4 and 5; Photos 1-4).

To the best of our knowledge, this is the first application of a direct aromatic hydroxylation by *m*-CPBA to polymethoxystilbenes. The method here reported, although limited in % conversion and product yield, could be of valuable help for the rapid and mild preparation of limitate amounts of these hydroxylated derivatives, allowing a first-step biological evaluation. In addition, this methodology is of potential usefulness in direct ring hydroxylation of many substrates bearing an aromatic ring with positions highly activated to the aromatic electrophilic substitution. The easy availability of compounds **5**, **8** and **11** allowed us to test these compounds as potential antiangiogenic agents, in comparison with **2**.

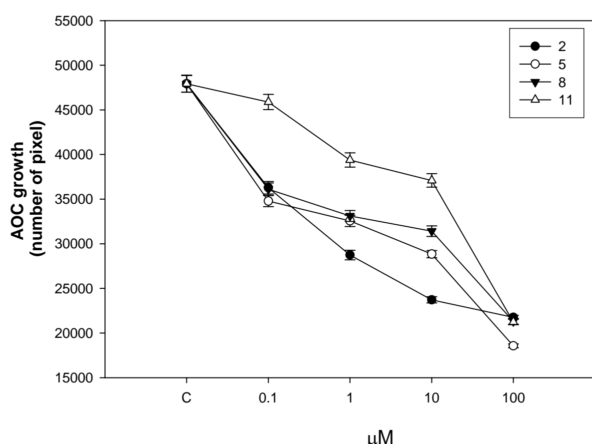


Figure 4: Effect of the treatment with compounds **2**, **5**, **8** and **11** (0.1, 1, 10 and 100 µM) for 48 h on AOC growth. Data represent mean ± SEM of 4 replicates/treatment repeated in 4 different experiments

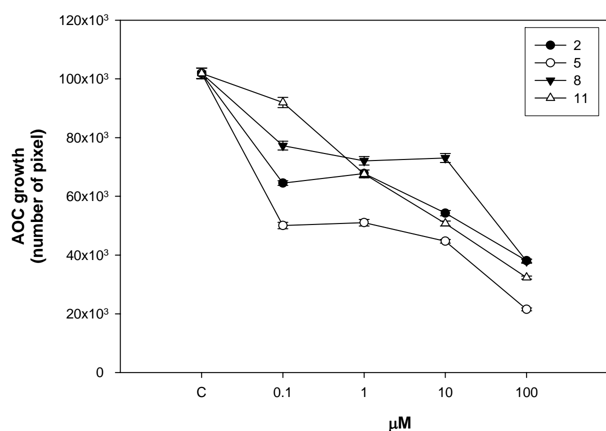


Figure 5: Effect of the treatment with compounds **2**, **5**, **8** and **11** (0.1, 1, 10 and 100 µM) for 96 h on AOC growth. Data represent mean ± SEM of 4 replicates/treatment repeated in 4 different experiments.

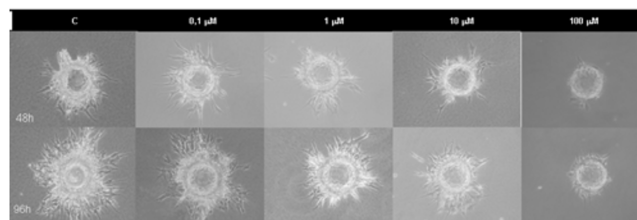


Photo 1: Phase contrast micrographs showing AOC growth at 48 h and 96 h in fibrin gel matrix. Cells were cultured in CM or treated with compound **2** at the concentrations of 0.1, 1, 10 and 100 µM.

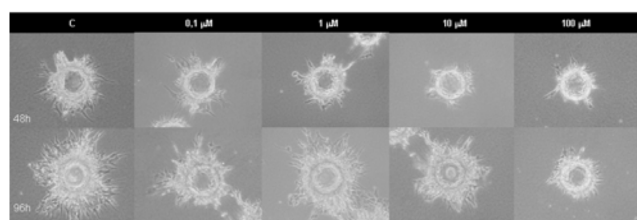


Photo 2: Phase contrast micrographs showing AOC growth at 48 h and 96 h in fibrin gel matrix. Cells were cultured in CM or treated with compound **5** at the concentrations of 0.1, 1, 10 and 100 µM.

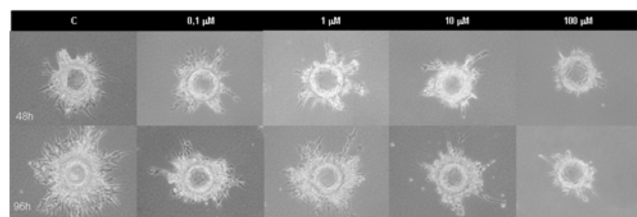


Photo 3: Phase contrast micrographs showing AOC growth at 48 h and 96 h in fibrin gel matrix. Cells were cultured in CM or treated with compound **8** at the concentrations of 0.1, 1, 10 and 100 µM.

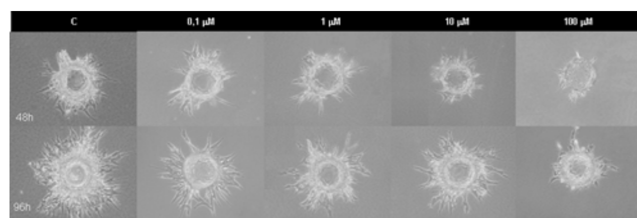


Photo 4: Phase contrast micrographs showing AOC growth at 48 h and 96 h in fibrin gel matrix. Cells were cultured in CM or treated with compound **11** at the concentrations of 0.1, 1, 10 and 100 µM

In our assay, the 3,5,4'-trimethoxystilbene **2** was confirmed as a potent antiangiogenic agent determining until to a 65% reduction in new vessel growth. More interesting, its 2-hydroxy analogue **5** resulted in even more activity since after 96 h of incubation, at the highest concentration tested, it produced a 80% inhibition in neovascularization. Moreover, the other stilbenoids **8** and **11** tested in the angiogenesis bioassay have been demonstrated to negatively control new vessel growth even if to a lesser extent then compounds **2** and **5**. In particular, compound **11** showed a marked increase of the activity during the 48h to 96 h incubation. These data suggest that the resveratrol-like substitution (3,5,4'-trimethoxy) is the more effective in inhibition of new vessel growth, and the presence of a further hydroxy group may have a role in improving the effectiveness with time, possible due to increased hydrophilicity. In the presence of four methoxy groups and one hydroxy group, as in compounds **8** and **11**, the 3,5-dimethoxy substitution pattern appears more effective than the 3,4-dimethoxy pattern; of course, further data are required for a more complete understanding of the structure-activity relationships for these compounds. In conclusion, these data, although preliminary, may be of interest in view of possible optimization of these stilbenoids as antiangiogenic agents for therapeutic use.

Experimental

General method: All reagents were of commercial quality and were used as received (Merck and Sigma-Aldrich); all reagents for angiogenesis bioassay were obtained from Sigma unless otherwise specified.

Solvents were distilled using standard techniques. *m*-Chloroperbenzoic acid (*m*-CPBA) of 99% assay was obtained by washing the commercial 77% material (Aldrich) whit a phosphate buffer of pH 7.5, and drying the residue under reduced pressure. Melting points were determined on a Kofler apparatus. Mass spectra were recorded in ESI positive mode on a Micromass ZQ2000 spectrometer (Waters). ^1H and ^{13}C NMR spectra were run on a Varian Unity Inova spectrometer at 500 and 125 MHz, respectively, in $CDCl_3$ or C_6D_6 solutions with TMS as internal standard. *J* values are given in Hertz. IR spectra were taken with an Perkin-Elmer Spectrum BX FT-IR System spectrophotometer using CCl_4 as solvent. UV spectra were recorded on a double-beam Lambda 25 spectrophotometer (Perkin-Elmer) using CH_3CN as solvent. All reactions were monitored by TLC on commercially available precoated plates (silica gel 60 F 254) and the products were visualized with cerium sulphate solution. Silica gel 60 was employed for column chromatography. Elemental analyses were performed on a Perkin-Elmer 240B microanalyzer.

Compounds **2**, **7** and **10** were synthesized through an Arbuzov rearrangement followed by a Horner-Emmons-Wadsworth reaction, as previously reported [8b]. According to a previously reported method, this procedure affords the *E*-stilbenoid with a minimal percentage of its *Z*-isomer [13].

Synthesis of 3,5,4'-tri-methoxystilbene (2): 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. The resultant solution was cooled to 0°C, and dry DMF (10 mL) and 0.41g (7.6 mmol) of sodium methoxide added. To this solution was added 1.18 g (7.1 mmol) of 3,5-dimethoxybenzaldehyde and the mixture was allowed to stand at room temperature for 1 h. Then it was heated to 100°C 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 (Na_2SO_4), affording 1.57 g of the product **2** (82.3 % yield after crystallization); white solid mp 55 - 56°C (MeOH); ESIMS: 271 $[M+H]^+$; ^1H and ^{13}C NMR data are in agreement with literature data [14].

Synthesis of 3,5,3',5'-tetramethoxystilbene (7): 3,5-dimethoxybenzylbromide (1.72 g, 11 mmol) was heated with excess of triethyl phosphite (4 mL, 10.8 mmol) to 130°C to give diethyl (4-methoxybenzyl)phosphonate. The resultant solution was cooled to 0° C, and dry DMF (20 mL) followed by 0.81 g (15 mmol) of sodium methoxide were added. To this solution was added 1.92 g (11.5 mmol) of 3,5-dimethoxybenzaldehyde and the mixture was allowed to stand at room temperature for 1 h. Then it was heated to 100°C for 1 h. After cooling, the reaction mixture was stirred overnight and subsequently quenched with water-methanol (2:1) affording 2.93 g of the product **7** (88.8 % yield crystallization) as white solid, mp 131-132°C (MeOH); ESIMS: 301 $[M+H]^+$; NMR and IR data are in agreement with literature data [15,16].

Synthesis of 3,5,3',4'-tetramethoxystilbene (10): 3,5-dimethoxybenzylbromide (1.70 g, 7.3 mmol) was heated with excess of triethyl phosphite (1.26 mL, 7.3 mmol) to 130°C to give diethyl (4-methoxybenzyl)phosphonate. The resultant solution was cooled to 0° C, and dry DMF (10 mL) followed by 0.432 g (8 mmol) of sodium methoxide were added. To this solution was added 1.39 g (8.3 mmol) of 3,4-dimethoxybenzaldehyde and the mixture was allowed to stand at room temperature for 1 h. Then it was heated to 100°C for 1 h. After cooling, the reaction mixture was stirred overnight and subsequently quenched with water and extracted with CH_2Cl_2. The combined organic layers were washed with water and dried over Na_2SO_4, affording 1.92 g of the product **10** (87.6 % yield crystallization) as white solid, mp 63 – 64°C (MeOH); ESIMS: *m/z* 301 $[M+H]^+$; NMR data are in agreement with literature data [17].

General Procedure for hydroxylation of compounds 2, 7 and 10 with *m*-CPBA: To a stirred solution of the substrate in CH_2Cl_2 (0.105 mmol/mL) a solution of *m*-CPBA in CH_2Cl_2 (0.150 mmol/mL) was added at room temperature. Then, the reaction mixture was washed with a $NaHSO_3$ solution and subsequently with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*; the residues were submitted to flash-chromatography on 3 x 25 cm silica gel column, eluted with EtOAc in *n*-hexane (from 0% to 30%), to afford the purified products **5**, **8** and **11**.

2-Hydroxy-3,5,4'-trimethoxystilbene (5)

White solid; mp 100-102°C (MeOH), Yield, 21%.

R_f (*n*-hexane/EtOAc 70:30) 0.58.

IRν_{max}: 3558, 3003, 2954, 2938, 2868, 1605, 1511, 1489, 1465, 1431, 1281, 1252, 1237, 1200, 1174, 1151, 1082, 1057, 1041, 966, 926, 826 cm^{-1}.

UV λ_{max} (ε): 217 (3.04 E+04), 303 (2.99 E+04).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.82 (s, 3H, 4'-OCH_3), 3.83 (s, 3H, 5-OCH_3), 3.88 (s, 3H, 3-OCH_3), 5.54 (bs, 1H, OH), 6.40 (d, J = 2.5, 1H, H-4), 6.66 (d, J = 2.5, 1H, H-6), 6.90 (d, J = 8.5, 2H, H-2' and H-6'), 7.07 (d, J = 16.5, 1H, H-α), 7.28 (d, J = 16.5, 1H, H-β), 7.47 (d, J = 8.5, 2H, H-3' and H-5').
^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.1 (3-OCH_3), 55.6 (4'-OCH_3), 55.9 (5-OCH_3), 98.3 (C-4), 100.7 (C-6), 113.9 (C-3' and C-5'), 120.8 (C-α),123.5 (C-1'), 127.6 (C-2' and C-6'), 128.7 (C-β), 130.5 (C-1), 137.6 (C-5), 147.2 (C-3), 152.9 (C-2), 159.1 (C-4').
ESIMS: 309 $[M+Na]^+$, 595 $[2M+Na]^+$.
Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.45; H, 6.32.

4-Hydroxy-3,5,4'-trimethoxystilbene (6)
Pale-yellow amorphous powder; yield 8.5%.
R_f (*n*-Hexane/EtOAc 70:30) 0.32.
IR ν_{max}: 3557, 2955, 2940, 2838, 1601, 1594, 1490, 1465, 1430, 1237, 1205, 1153, 1057, 965, 929, 828 cm^{-1}.
UV λ_{max} (ε): 303 (2.03 E+04).
^{1}H NMR (C_6D_6, 500 MHz): δ 3.31 (s, 3H, 4'-OCH_3), 3.37 (s, 6H, 3-OCH_3 and 5-OCH_3), 5.37 (bs, 1H, OH), 6.66 (s, 2H, H-2 and H-6), 6.83 (d, J = 8.0, 2H, H-2' and H-6'), 6.95 (d, J = 16.0, 1H, H-α), 7.01 (d, J = 16.0, 1H, H-β), 7.39 (d, J = 8.0, 2H, H-3' and H-5').
^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.4 (4'-OCH_3), 56.3 (3-OCH_3 and 5-OCH_3), 103.1 (C-2 and C-6), 114.2 (C-3' and C-5'), 126.2 and 126.7 (C-α and C-β), 127.7 (C-2' and C-6'), 130.2 (C-1), 131.1 (C-1'), 134.5 (C-4), 147.4 (C-3 and C-5), 159.1 (C-4').

2-Hydroxy- 3,5,3',5'-tetramethoxystilbene (8)
White solid; mp 119-121°C (MeOH); yield 23% .
R_f (*n*-Hexane/EtOAc 70:30) 0.46.
IR ν_{max}: 3557, 2955, 2940, 2838, 1601, 1594, 1490, 1465, 1430, 1237, 1205, 1153, 1057, 965, 929, 828 cm^{-1}.
UV λ_{max} (ε): 2.27 (2.30 E+04), 303 (2.03 E+04).
^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.82 (s, 3H, 5-OCH_3), 3.83 (s, 6H, 3'-OCH_3 and 5'-OCH_3), 3.88 (s, 3H, 3-OCH_3), 5.57 (bs, 1H, OH), 6.39 (d, J = 2.5, 1H, H-4), 6.66 (d, J = 2.5, 1H, H-6), 6.70 (d, J = 2.0, 2H, H-2' and H-6'), 7.09 (d, J = 16.5, 1H, H-α), 7.39 (d, J = 16.5, 1H, H-β).
^{13}C NMR ($CDCl_3$, 125 MHz) δ 55.4 (3'-OCH_3 and 5'-OCH_3), 55.7 (5-OCH_3), 56.1 (3-OCH_3), 98.9 (C-4'), 100.0 (C-4), 101.0 (C-6), 104.6 (C-2' and C-6'), 123.0 (C-α), 123.5 (C-1'), 129.3 (C-β), 138.08 (C-1), 139.8 (C-5), 147.3 (C-3), 153.2 (C-2), 160.9 (C-3').
ESIMS: 317 $[M+H]^+$, 339 $[M+Na]^+$.
Anal. Calcd for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.27; H, 6.49.

4-Hydroxy- 3,5,3',5'-tetramethoxystilbene (9)
White amorphous powder; yield 10.5%.
R_f (*n*-hexane/EtOAc 70:30) 0.25.
ESIMS: 317 $[M+H]^+$, 355 $[M+K]^+$.
NMR and IR data are in agreement with literature data [9].

2-Hydroxy-3,5,3',4'-tetramethoxystilbene (11)
White solid; mp 124-125°C (MeOH); yield 19 %.
R_f (*n*-Hexane/EtOAc 70:30) 0.35.
IR ν_{max}: 3554, 2998, 2940, 2833, 1601, 1544, 1492, 1246, 1205, 1154, 1058, 976, 929, 830 cm^{-1}.
UV λ_{max} (ε): 220 (2.40 E+04), 326 (2.2 E+04).
^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.82 (s, 3H, 5-OCH_3), 3.88 (s, 3H, 3-OCH_3), 3.90 (s, 3H, 4'-OCH_3), 3.94 (s, 3H, 3'-OCH_3), 5.56 (bs, 1H, OH), 6.42 (d, J = 2.5, 1H, H-4), 6.67 (d, J = 2.5, 1H, H-6), 6.86 (d, J = 8.0, 1H, H-5'), 7.08 (d, J = 8.0, 1H, H-6'), 7.10 (bs, 1H, H-2'), 7.35 (d, J = 16.0, 1H, H-β), 7.81 (d, J = 16.0, 1H, H-α);
^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.7 (5-OCH_3), 55.8 (4'-OCH_3), 55.9 (3'-OCH_3), 56.0 (3-OCH_3), 98.5 (C-4), 100.8 (C-6), 108.8 (C-2'), 111.2 (C-5'), 119.9 (C-6'), 121.6 (C-α), 123.4 (C-1), 129.1 (C-β), 130.9 (C-1'), 137.7 (C-2), 147.3 (C-3), 148.8 (C-4'); 149.0 (C-3'); 153.0 (C-5).
ESIMS: *m/z* 317 $[M+H]^+$, 339 $[M+Na]^+$.
Anal. Calcd for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.31; H, 6.52.

4-Hydroxy- 3,5,3',4'-tetramethoxystilbene (12)
White amorphous powder; yield 5.2 %.
R_f (*n*-hexane/EtOAc 70:30) 0.25;
ESIMS: 317 $[M+H]^+$, 355 $[M+K]^+$.
NMR and IR data are in agreement with literature data [10].

Endothelial cell culture: An immortalized porcine aortic endothelial cell line (AOC) [18] was generously provided by José Yelamos (Department of Immunology, IMIM-Hospital del Mar, Barcelona Biomedical Research Park, Barcelona, Spain). In all experiments, AOC at 19th passage were used and seeded in culture medium (CM) composed by M199 supplemented with sodium bicarbonate (2.2 mg/mL), bovine serum albumin (BSA 0.1%), penicillin (100 IU/mL), streptomycin (100 μg/mL), amphotericin B

(2.5 μg/mL), selenium (5 ng/mL) and transferrin (5 μg/mL).

Three-dimensional endothelial cell culture on a fibrin gel support: The microcarrier-based fibrin gel angiogenesis assay was performed as described by Basini *et al*. [7c] Briefly, 12.5 mg gelatin-coated cytodex-3 microcarriers in 1 mL PBS were incubated for 3 h to hydrate. After two washings in PBS and one in CM, the microcarriers were put in flasks containg 5 mL CM; AOC (5 x 10^5) were added and cultured for 24 h in order to let the endothelial cells coat the microcarriers. For the fibrin gel preparation, 40 μl microcarriers covered by AOC were pipetted into 6 well plates containing a solution of fibrinogen (1 mg/mL PBS, pH 7.6), added with 1250 IU thrombine (250 μl). Fibrin gels were allowed to polymerize for 30 min at 37°C, then they were equilibrated for 60 min with 2 mL M199. After a change of the medium, AOC were treated with VEGF (100 ng/mL; PeproTech EC Ltd, London, UK) in the presence or absence of 0.1 1, 10 or 100 μM of **2**, **5**, **8** or **11**. Plates were incubated at 37°C under humidified atmosphere (5% CO_2). AOC were cultured for 96 h, renewing totally the treatment after 48h.

Quantification of AOC growth on fibrin gel matrix: Endothelial cell proliferation in the fibrin gel matrix was evaluated by means of the public domain NIH Program Scion Image Beta 4.02 (Scion Corporation, MA, USA (http://rsb.info.nih.gov/nih-image/). Ten pictures were taken for each gel at 48 and 96 h; images were converted into greyscale, resized to 50% (Paintbrush Software, MS Office) and saved as Bitmap 24bit format compatible with Scion. The modified images were then imported into the program and measurements were made drawing the perimeter of the area occupied by AOC expressed as number of pixel. In order to validate the measurement of the area covered by AOC in fibrin gels as a reliable method to evaluate cell proliferation, fibrin gels were stained by the nuclear dye bis-benzimide (Hoechst 33258, 20 μg/mL in PBS for 60 min) and examined by the fluorescence microscope [7a,7b].

This procedure was performed 20 times; for each experiment the number of nuclei was counted under fluorescence and pictures of the aera covered by AOC were taken in order to measure the surface covered in the fibrin gel. A strong correlation was observed between the area covered by AOC and the number of nuclei found in the same area (r = 0.96).

Statistical analysis: Bioassays were repeated at least 4 times (4 replicates/treatment). Experimental data are presented as mean ± SEM; statistical differences between treatments were calculated with Multifactorial ANOVA using Statgraphics package (STSC Inc., Rockville, MD, USA). When significant differences were found, means were compared by Scheffè's F test; p values < 0.05 were considered to be statistically significant.

The calculations were performed utilizing the PM3 semiempirical hamiltonian as implemented in MOPAC 2007 [19] package using Winmostar as Gui interface [20]; full geometry optimization was carried out without any symmetry constraints.

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