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Synthesis of heterocycle-based analogs of resveratrol and their antitumor and vasorelaxing properties

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ABSTRACT

New resveratrol (RES) analogs were developed by replacing the aromatic 'core' of our initial naphthalene-based RES analogs with *pseudo*-heterocyclic (salicylaldoxime) or heterocyclic (benzofuran, quinoline, and benzothiazole) scaffolds. The resulting analogs were tested for their antiproliferative and vasorelaxing effect, two typical properties shown by RES. Some of the new compounds confirmed strong antiproliferative activities, comparable to that previously found with the most active naphthalene-based analog. In particular, 3-(3,5-dihydroxyphenyl)-7-hydroxyquinoline exhibited the most potent antiproliferative effect (IC_{50} = 17.4 μM). In vascular assays, the highest levels of potency (pIC_{50} = 4.92) and efficacy (E_{max} = 88.2%) were obtained with 2-(3,5-dihydroxyphenyl)-6-hydroxybenzothiazole. A conformational analysis of these compounds indicated that the antiproliferative activity on MDA-MB-231 cancer cells can be correlated to a common sterical profile of the most active compounds and, in particular, to the spatial arrangement of the three phenolic groups. Furthermore, the vasorelaxing properties showed a good correlation with the electronic properties measured through the electrostatic molecular potential (ESP).

1. Introduction

Resveratrol (*trans*-3,5,4′-trihydroxystilbene, RES), a dietary polyphenol isolated from edible materials such as red wine, grape skins, and peanuts, is one of the most widely studied natural stilbenoids since it shows remarkable cancer chemopreventive and cardioprotective activities.[1] We have previously shown that the effect of RES on cancer cell growth is associated to its ability to interfere with the sphingolipid pathways. In particular, it was found to induce apoptosis by triggering the de novo biosynthesis of endogenous ceramide, a bioactive sphingolipid.[2,3] Some of the biological effects of RES, such as inhibition of cancer growth, reduction of cholesterol levels, and prevention of osteoporosis, resemble those found with many other phytoestrogens and, therefore, have been associated to its ability to bind the estrogen receptors α and β.[4] Moreover, this molecule proved to efficiently reduce the risk of cardiovascular diseases.[5] This phenomenon, which may be attributed to the antioxidant activity of resveratrol, has been proven in several studies;[6,7] it is able to counteract the damages caused by reactive oxygen species (ROS) and to inhibit low density lipoprotein (LDL) peroxidation leading to atherogenesis.[8] More recently, it was shown that resveratrol can have anti-hypertensive properties due to both endothelium-independent and endothelium-mediated vasorelaxing effects. These vasoactive responses are mainly linked to the activation of large-conductance calcium-activated potassium channels (BK) expressed on vascular smooth muscle and endothelial cells, respectively.[9,10] Furthermore, additional cardioprotective effects of resveratrol may be related to the NO release caused by the activation of the BK channels.[9] All these beneficial effects on human health have prompted the use of RES as a therapeutic agent. Unfortunately, RES is characterized by a very low bioavailability,[11] mainly due to extensive phase II metabolism consisting of in vivo transformation into sulfate and glucuronic conjugates, mostly on 3-OH.[12] Besides, a molecular point of environmental, chemical, and metabolic instability in RES is constituted by the stilbene double bond, which may undergo *E*/*Z*-isomerization[13] or other types of oxidative transformations.[14]

So far there have been several reports on synthetic analogs of RES, in an attempt to determine the structural requirements for resveratrol-like biological activities and, at the same time, to extend the chemical diversity of this type of polyphenols, in view of possible improvements of their biopharmacological profiles.

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Antitumor assays with MDA MB 468 cells of some 3-hydroxy-(*E*)-stilbenes revealed an apoptosis-inducing activity in the low micromolar range.[15] Contemporarily, our group developed some naphthalene-based RES analogs endowed of proapoptotic properties against MDA-MB-231 breast cancer cells.[16] Later, other scientists have extended the series of naphthalene analogs of RES, displaying strong antitumor effects.[17]

In an attempt to broaden the chemical diversity of resveratrol analogs, we now report further transformations of our initial naphthalene-based analogs of RES, which leads to the development of polyphenols containing *pseudo*-heterocyclic or heterocyclic scaffolds. In particular, our design started from our previously found most active RES analog **1** (Fig. 1),[16] whose naphthalene core was replaced by the following portions: salicylaldoxime (**2**), benzofuran (**3**), quinoline (**4** and **5**), and benzothiazole (**6**–**9**). It is important to notice that the tri-hydroxylated pattern of RES was substantially maintained in all cases. The only compound bearing only two phenol groups was **2**, but in this case the presence of the oxime OH group in the six-membered *pseudo*-cycle formed by an intramolecular H-bond, can isosterically replace one phenol moiety, as we had previously verified in the field of ER ligands.[18]

These compounds were submitted to two different biological evaluations in order to assess both their growth-inhibitory properties in cancer cells, and their vasorelaxing effects, since these are some of the most important activities that have been reported to be associated to RES itself.

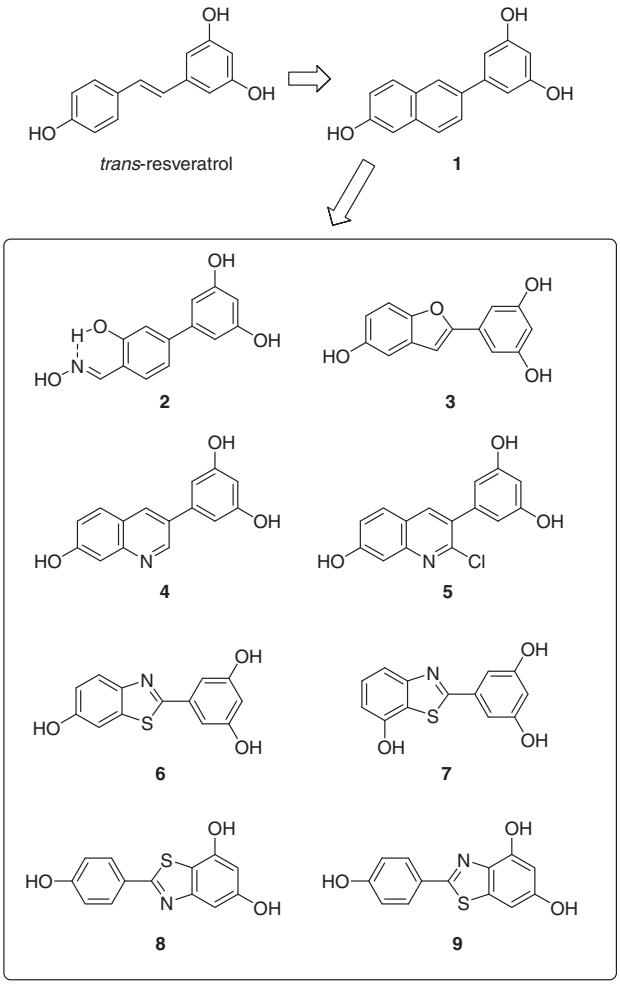


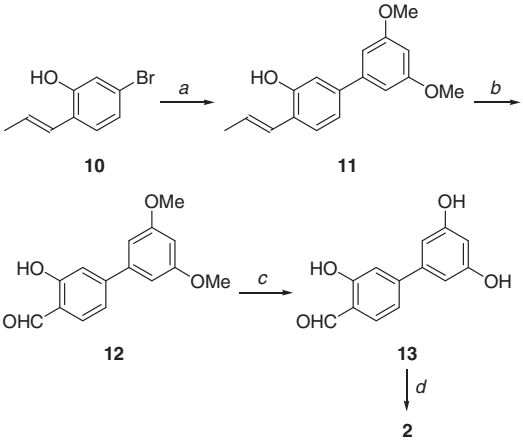
Figure 1. Chemical structures of resveratrol analogs containing naphthalene (**1**), *pseudo*-heterocycle (**2**), or heterocycle-portions (**3**–**9**).

2. Results and discussion

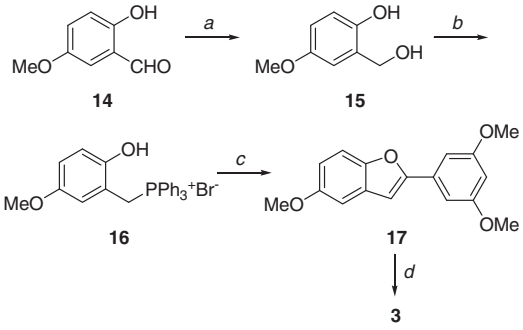
2.1. Synthetic chemistry

The synthesis of salicylaldoxime **2** (Scheme 1) started from *trans*-β-methylstyrene derivative **10**,[19] which was submitted to a palladium-catalyzed cross-coupling reaction with 3,5-dimethoxyphenylboronic acid, prepared as previously reported.[20] Oxidative cleavage of the double bond of the resulting biphenyl derivative **11** with sodium periodate in the presence of catalytic amounts of osmium tetroxide, yielded salicylaldehydes **12**. Overnight treatment of **12** with boron tribromide caused the removal of the two *O*-methyl groups and subsequent condensation of the resulting aldehyde (**13**) with hydroxylamine hydrochloride produced final oxime **2**, obtained as a single *E*-diastereoisomeric form; this is probably due to the fact that only this isomer can form intramolecular hydrogen bonds that contribute to the energetic stabilization of the product. This selectivity had already been verified for other oxime analogs we had developed as estrogen receptor ligands.[21] Anyway, the *E*-configuration of **2** was confirmed by the chemical shift value of the oxime proton, which was found downfield from the 8 ppm value (δ = 8.39 ppm, see Section 4).[22]

The synthesis of benzofuran **3** is shown in Scheme 2 and started from 2-hydroxy-3-methoxybenzaldehyde **14**, which was initially reduced with sodium borohydride to the corresponding benzyl alcohol **15**. Subsequent reaction with triphenylphosphine



Scheme 1. Reagents and conditions: (a) 3,5-dimethoxyphenylboronic acid, $Pd(OAc)_2$, PPh_3, aqueous 2 M K_3PO_4, dioxane, 80 °C, 16 h [77%]; (b) OsO_4, $NaIO_4$, dioxane–H_2O, 2 h [68%]; (c) BBr_3, CH_2Cl_2, −78 °C to rt, overnight [44%]; (d) $NH_2OH{\cdot}HCl$, MeOH-H_2O, rt, 18 h [39%].



Scheme 2. Reagents and conditions: (a) $NaBH_4$, EtOH, rt, 1 h [85%]; (b) $PPh_3{\cdot}HBr$ CH_3CN, reflux, 1 h [84%]; (c) (1) 3,5-dimethoxybenzoic acid, DCC, DMAP, CH_2Cl_2, overnight; (2) Et_3N, dioxane, reflux, overnight [47%]; (d) BBr_3, CH_2Cl_2, −78 °C to rt, overnight [47%].

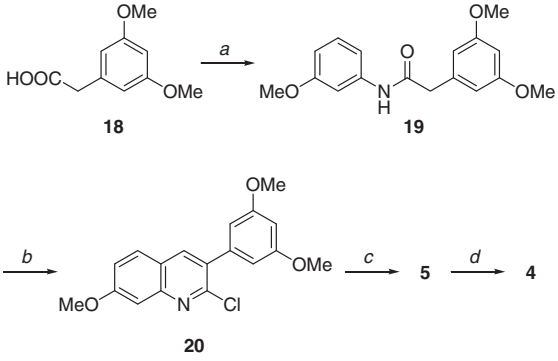
hydrobromide formed phosphonium salt **16**, which was submitted to an initial condensation with 3,5-dimethoxybenzoic acid, followed by a base-promoted thermal cyclization. These sequence afforded trimethoxy-substituted benzofuran **17**, which was then deprotected with BBr_3, leading to final compound **3**.

Quinoline derivatives **4** and **5** were synthesized as described in Scheme 3. An initial transformation of (3,5-dimethoxyphenyl)acetic acid **18** into its corresponding acid chloride, followed by its condensation with *m*-anisidine in the presence of a polymer-supported base (PS-DIEA), produced amide **19**. Subsequent cyclization of **19** in the presence of $POCl_3$ formed the quinoline scaffold of compound **20**. Removal of the three *O*-methyl groups with BBr_3 afforded 2-chloquinoline **5**. The other quinoline analog **4** was obtained by a catalytic hydrogenation, which caused a proto-dechlorination reaction of **5**.

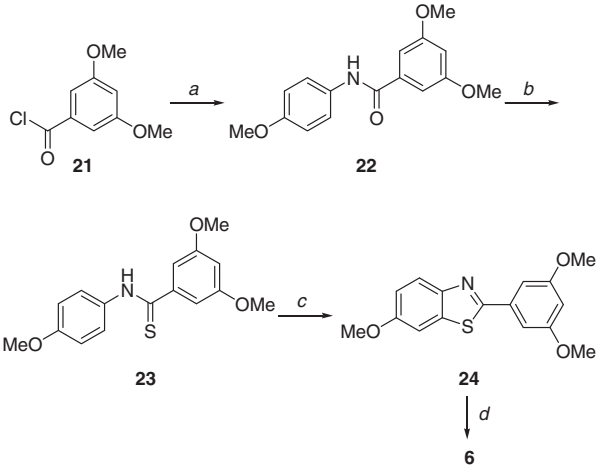
The synthesis of benzothiazole **6** (Scheme 4) starts with a condensation of 3,5-dimethoxybenzoyl chloride **21** with *p*-anisidine, to form amide **22**. Treatment of this amide with the Lawesson's reagent produced thioamide **23**, which was then submitted to an oxidative cyclization promoted by potassium ferricyanide. The resulting trimethoxy-substituted benzothiazole **24** was treated with BBr_3 to yield final product **6**.

Benzothiazole **7** was obtained following the same synthetic sequence, as shown in Scheme 5. This time, acid chloride **21** was condensed with *m*-anisidine, thus producing amide **34** and, after treatment with Lawesson's reagent, thioamide **35**. In this case, the oxidative cyclization step produced, as predicted, a 1:1 mixture of two isomeric benzothiazoles **36** and **37**. Much to our surprise, while BBr_3-promoted O-demethylation of **36** led to the desired product **7**, the same treatment of regioisomer **37** caused its decomposition to a complex mixture, so we were not able to obtain the trihydroxy-substituted regioisomer of **7**.

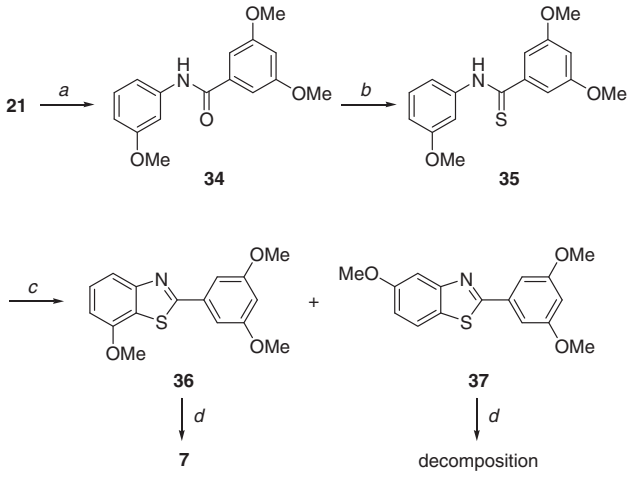
This reaction sequence was also employed in the synthesis of benzothiazoles **8** and **9**, as shown in Scheme 6. Condensation of 4-methoxybenzoyl chloride **38** with 3,5-dimethoxyaniline afforded amide **39**. Lawesson's reagent produced the oxygen/sulfur atom exchange in the amide group and the resulting thioamide **41** was submitted to ferricyanide-promoted cyclization. Benzothiazole **43** was then deprotected with BBr_3, yielding final product **8**. The other benzothiazole analog **9** was obtained following an identical synthetic pathway, with the only modification consisting in the condensation of **38** with 2,4-dimethoxyaniline.



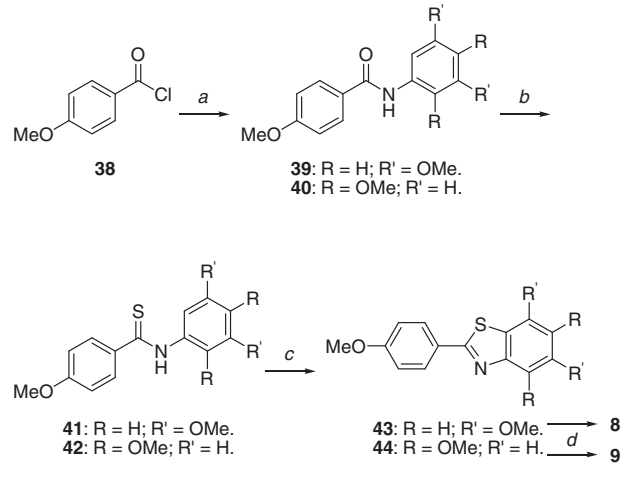
Scheme 3. Reagents and conditions: (a) (1) $SOCl_2$, CH_2Cl_2, 60 °C, 4 h; (2) *m*-anisidine, CH_2Cl_2, DMAP, PS-DIEA, rt, overnight [74%]; (b) $POCl_3$, DMF, 75 °C, 1.5 h [55%]; (c) BBr_3, CH_2Cl_2, −78 °C to rt, overnight [84%]; (d) H_2 (1 atm), Pd–C, Et_3N, EtOH, 24 h [13%].



Scheme 4. Reagents and conditions: (a) *p*-anisidine, CH_2Cl_2, DMAP, PS-DIEA, rt, overnight [61%]; (b) Lawesson's reagent, 130 °C, 3 h [67%]; (c) $K_3Fe(CN)_6$, aq NaOH 30%, EtOH, 85 °C, 30 min, then rt overnight [74%]; (d) BBr_3, CH_2Cl_2, −78 °C to rt, overnight [49%].



Scheme 5. Reagents and conditions: (a) *m*-anisidine, CH_2Cl_2, DMAP, PS-DIEA, rt, overnight [31%]; (b) Lawesson's reagent, 130 °C, 3 h [52%]; (c) (1) $K_3Fe(CN)_6$, aq NaOH 30%, EtOH, 85 °C, 30 min; (2) rt, overnight [70%]; (d) BBr_3, CH_2Cl_2, −78 °C to rt, overnight [49%].



Scheme 6. Reagents and conditions: (a) 3,5-dimethoxyaniline or 2,4-dimethoxyaniline, CH_2Cl_2, DMAP, PS-DIEA, rt, overnight [**39**: 89%; **40**: 74%]; (b) Lawesson's reagent, 130 °C, 3 h [**41**: 70%; **42**: 72%]; (c) (1) $K_3Fe(CN)_6$, aq NaOH 30%, EtOH, 85 °C, 30 min; (2) rt, overnight [**43**: 70%; **44**: 49%]; (d) BBr_3, CH_2Cl_2, −78 °C to rt, overnight [**8**: 41%; **9**: 66%].

2.2. Antiproliferative properties

Resveratrol analogs were compared for their growth-inhibitory effect on MDA-MB-231 breast cancer cells. Cells were treated with compounds **1–9** at different concentrations, ranging from 0.25 μM to 64 μM for 6 days. The sulphorhodamine B (SRB) cell proliferation assay was used. The proliferation index at the beginning of the experiment (time 0) was set at 1. The IC_{50} for the new resveratrol analogs **2–9** are reported in Table 1, together with the IC_{50} of resveratrol (**1**) and the previously reported naphthalene derivative **1**. Some of the new compounds (**2**, **4**, **6**, and **7**) confirmed strong antiproliferative activities which are comparable to that previously found with naphthalene-based analog **1**. We were pleased to find that the replacement of the naphthalene scaffold of **1** with a salicylaldoxime-based pseudocycle, as in compound **2**, substantially maintained the IC_{50} value in the low micromolar range, thus confirming in this case the suitability of this isosteric replacement for phenols.[18] Quinoline **4** exhibited the most potent antiproliferative effect (IC_{50} = 17.4 μM) among these new compounds, whereas its 2-chloro-substituted analog **5** suffered from a nearly threefold reduction of the activity. Similarly, benzofuran **3** showed a moderate potency, with an IC_{50} of 46.1 μM. The two benzothiazoles bearing a single hydroxy group in their bicyclic portion **6** and **7** displayed noticeable antiproliferative effects, whereas their fellow compound, based on the 4,6-dihydroxybenzothiazole scaffold, was either less active (**8**). Finally, 5,7-dihydroxybenzothiazole **9** displayed no significant activity in the micromolar range.

2.3. Vasorelaxing properties

The vasorelaxing properties of RES and its analogs **1–9** were studied on in vitro vascular smooth muscle preparation of isolated rat aortic rings. Data reported in Table 1 concern vasorelaxing efficacy of the synthesised compounds and RES, which were evaluated as the maximal vasorelaxing response at a concentration of 30 μM, expressed as a percentage of the contractile tone induced by 30 mM KCl (E_{max}, %). Potency is given as the pIC_{50}, calculated as the negative logarithm of the molar concentration of the test compounds evoking a 50% reduction in the contractile tone induced by 30 mM KCl. The pIC_{50} values could not be calculated for compounds with efficacy below 50% at 30 μM. Six out of the nine compounds tested exhibited a vasorelaxing effect generally comparable, albeit slightly less potent, to that of RES, which in our hands showed a pIC_{50} of 5.16 and a E_{max} of 83%. Napthalene analog **1**[16] was tested here for the first time as a vasorelaxing agent and it shows a pIC_{50} of 4.89 with an efficacy of 78.5%. Among the heterocyclic-based compounds, the highest levels of potency (pIC_{50} = 4.92) and efficacy (E_{max} = 88.2%) were obtained with benzothiazole **6**. Its close analog **7** displayed similar levels of potency (pIC_{50} = 4.81) and efficacy (E_{max} = 76.8%). Dihydroxylated benzothiazole **8** proved to be a poor vasorelaxing agent in this assays (E_{max} <40% at 40 μM), whereas the other dihydroxy-substituted benzothiazole **9** showed again significant vasorelaxing effects (pIC_{50} = 4.90 and E_{max} = 80.6%). Oxime **2** displayed a rather weak activity on the vascular smooth muscle, with an efficacy lower than 50%, thus proving that in this specific pharmacodynamic feature at the vascular level, differently from the case of the antiproliferative mechanisms, the phenol/salicylaldoxime replacement was not tolerated. Benzofuran **3** showed good vasorelaxing properties, with potency (pIC_{50} = 4.81) and efficiency (E_{max} = 77.2%) comparable to those obtained with naphthalene derivative **1** and benzothiazole **7**. A remarkable efficacy (E_{max} = 84.6%) was reached by 2-chloroquinoline **5**, which also displayed a noticeable pIC_{50} value (4.80), whereas the formal remotion of the chlorine atom from **5**, leading to compound **4**, produced a dramatic reduction of the vasorelaxing effectiveness (E_{max} <20%). In order to correlate the vascular effects with the activation of potassium currents, the synthesised compounds endowed of satisfactory levels of vasorelaxing efficacy (E_{max} >50%) were also tested in the presence of tetraethylammonium chloride (TEA, 10 mM), which acts as a non-selective blocker of BK channels, as well as other different types of voltage-operated potassium channels.

Benzothiazole derivative **6** showed the highest levels of vasorelaxing potency and efficacy, nevertheless these effects were completely unaffected by TEA. This experimental observation indicates that the activity of **6** on vascular smooth muscle is not linked to the activation of hyperpolarizing potassium currents. Hence, an additional experimental protocol was designed to further support this hypothesis. In particular, it is widely known that high levels of membrane depolarization due to high extracellular concentrations of potassium ions can dramatically and non-specifically reduce the vasorelaxing effects of vasorelaxing agents acting through the activation of every type of membrane potassium channels.[23] Therefore, the vasorelaxing effect of **6** was also evaluated in rat aortic rings pre-contracted with KCl 60 mM and, again, they were completely unaffected by this experimental condition, confirming that activation of potassium channels does not play a role in the vascular activity of **6**. The effects of compound **2** were only poorly, and not significantly, affected by TEA, suggesting that the activation of potassium channels might play only a modest role (if any) in the vascular effects of this salicylaldoxime derivative.

On the other hand, the vasodilator effects of resveratrol, **1**, **3**, **5**, **7**, and **9** were significantly antagonised by TEA, indicating a clear involvement of potassium channels in their pharmacodynamic mechanism of action. Compound **5**, showing the highest level of efficacy among these subset of molecules exhibiting TEA-sensitive effects, was selected for a more accurate mechanistic investigation and was tested in the presence of iberiotoxin (IbTX, 100 nM), a highly selective blocker of BK channels. This scorpion toxin significantly antagonised the effects of **5**, thus demonstrating that the vasodilator effect of this compound is due to the activation of vascular BK potassium channels.

Table 1
Antiproliferative activities and vasorelaxing properties of resveratrol and its analogs **1–9**

Compound	MDA-MB-231 IC_{50}[a] (μM)	Rat aortic rings pIC_{50}[b]	E_{max}[c] at 30 μM	TEA[d] 10 mM
Resveratrol	20.5	5.16 ± 0.13	83.0 ± 5.0	+
1	12.7[e]	4.89 ± 0.03	78.5 ± 8.0	+
2	19.2	n.c.	47.3 ± 8.6	—[g]
3	46.1	4.81 ± 0.08	77.2 ± 19.8	+
4	17.4	n.c.	17.2 ± 3.0	n.t.
5	47.4	4.80 ± 0.02	84.6 ± 6.6	+[h]
6	19.4	4.92 ± 0.01	88.2 ± 2.7	—[f]
7	18.1	4.81 ± 0.02	76.8 ± 2.6	+
8	67.6	n.c.	39.6 ± 13.7	+
9	>100	4.90 ± 0.04	80.6 ± 5.6	+

[a] IC_{50} (μM) was calculated as the mean of the proliferation values obtained at 6 days of treatment with each compound used at different concentrations in three independent experiments. Standard errors are not shown for the sake of clarity and were never higher than 15% of the means.
[b] Negative logarithm of the molar concentration evoking an half-reduction of the contractile tone induced by KCl 20 mM (n.c. = not calculable).
[c] Maximal vasorelaxing effects induced by the concentration 30 μM, expressed as a % of the contractile tone induced by KCl 20 mM.
[d] The vasorelaxing effects were significantly (+) or were not significantly (−) antagonised by TEA 10 mM (n.t. = not tested).
[e] See Ref. 16.
[f] Additional test: the effects were not affected also by high KCl concentrations (60 mM).
[g] TEA induced modest and not significant influences.
[h] Additional test: IbTX 100 nM antagonised the vasorelaxing effects.

2.4. Computational chemistry

The structures of resveratrol and compounds **1**–**9** were minimized at 'ab initio' SCF 6-31G(d) level through GAUSSIAN03 program.[24] Compounds **3** and **6**–**9** showed to prefer a completely planar conformation, while the other derivatives displayed an angle of about 45° between the two planar aromatic systems, with the exception of compound **5** where this angle was much larger (70°) due to the presence of the chlorine in alpha position of the quinoline scaffold. The conformational analysis of compound **2** confirmed the presence of the intramolecular H-bond between the phenolic OH group and the oxime nitrogen atom, as already found in estrogen receptor ligands.[18,19]

With the aim of explaining the activity differences of compounds **1**–**9** in the two separate pharmacological assays, namely, the measurements of antiproliferative activity in MDA-MB-231 cancer cells and the vasorelaxing properties in vascular smooth muscle, their structural superimpositions with resveratrol was taken into consideration. These superimpositions were obtained through the ROCS 2.2 software,[25] which is a shape-similarity method based on the Tanimoto-like overlap of volumes and superimposes the molecules on this basis. Figure 2 evidences the good superimpositions found for compounds **1**, **2**, **4**, and **6** with resveratrol (Fig. 2A), whereas significant differences are evident for compounds **3**, **5**, **8**, and **9** (Fig. 2B). Therefore, compounds **1**, **2**, **4**, and **6** seem to be able to interact with biological targets in a manner very similar to resveratrol, since all three phenolic groups are sterically placed in comparable arrangements. These computational results find a good correlation with the results deriving from the antiproliferative experiments. In fact, compounds **1**, **2**, **4**, and **6** display good antiproliferative activities on MDA-MB-231 cells, that are similar to the activity level associated to resveratrol, whereas compounds **3**, **5**, **8**, and **9** generally showed lower antiproliferative activities. In particular, the worst superimposition is observed for compound **9**, which is also the least active derivative. This analysis does not seem to work for compound **7**, which shows a good activity although the superimposition of one of its three hydroxyls to the *p*-OH of resveratrol is rather poor. However, there is still the possibility that a H-bond acceptor group of an hypothetical biological target (still not identified for resveratrol anticancer activity), indicated as G in Figure 2C, is placed in a position such as it can interact with both the phenolic group of the resveratrol and the corresponding OH of the benzothiazole portion of **7**.

On the other hand, the results about cardiovascular activity of resveratrol and compounds **1**–**9** indicate that they generally possess similar appreciable activities, with the exception of compounds **2**, **4**, and **8**. Therefore, in this case, the same theoretical analysis based on structural superimpositions, reported above for the interpretation of the antiproliferative data, is not able to represent reliable structure–activity correlations. Hence, we analyzed electronic properties of resveratrol and compounds **1**–**9**, such as the electrostatic molecular potential (ESP), which was calculated through the GAUSSIAN03 program. The ESP was calculated at SCF 6-31G(d) level for the preferred conformation and it is visualized through the GAUSSVIEW program on the isosurface corresponding to a value of the total electron density of 0.0004 atomic unit. All compounds were aligned according to the above mentioned results of the shape similarity analysis. In Figure 3 the ESP of resveratrol and compounds **1**–**9** are reported. Red regions correspond to negative ESP while blue regions correspond to positive ESP. The results of this analysis show a common feature present in the three inactive compounds **2**, **4**, and **8**: there is negative ESP in spatially corresponding regions, which is otherwise not present in the active compounds. This negative-potential area is generated in compound **2** by the phenolic oxygen of the salicylaldoxime portion, in compound **4** by the nitrogen of the quinoline moiety and in compound **8** by the nitrogen of the benzothiazole system.

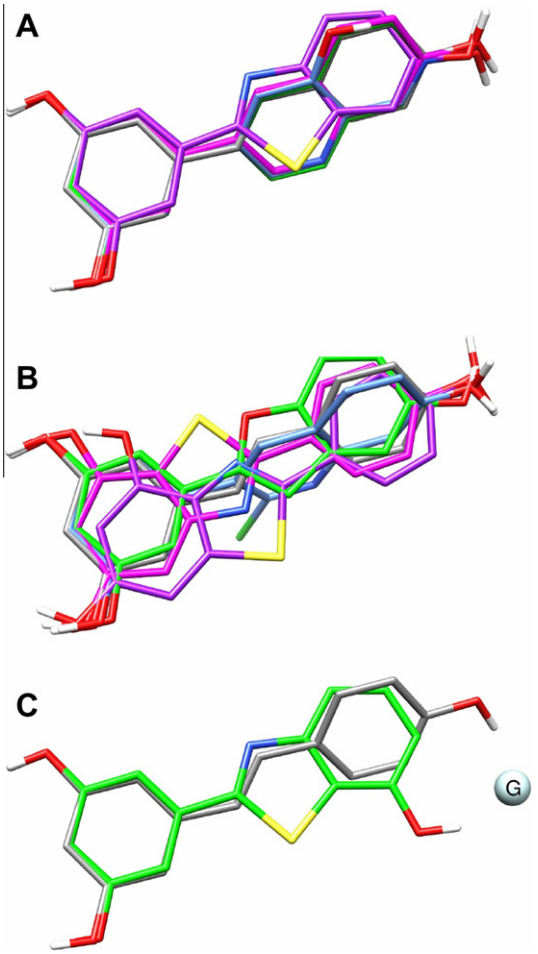


Figure 2. (A) Superimposition of resveratrol (gray) with active compounds **1** (green), **2** (light blue), **4** (magenta), and **6** (purple). (B) Superimposition of resveratrol (gray) with inactive compounds 3 (green), **5** (light blue), **8** (magenta), and **9** (purple). (C) Superimposition of resveratrol (gray) with compound **7**. The position of an hypothetical H-bond acceptor group G is indicated.

As shown by Figure 3, active compounds **1**, **3**, **5**–**7**, and **9** do not possess a similar negative ESP in this region. In the case of the active chloro-quinoline **5** which is very similar to its inactive non-chlorinated analog **4**, the lack of the negative ESP in the concerned region of **5** is due to a different conformation that it assumes when compared to **4**, due to the presence of the α-Cl atom in the quinoline moiety, which forces the dihedral angle between the two planar aromatic systems to a much larger value (70°), as seen before. Therefore, these data would suggest that the presence of a negative ESP in this region could be responsible for the unfavorable vasorelaxing properties associated to resveratrol analogs **2**, **4**, and **8**.

3. Conclusion

This study was carried out with the aim of finding new derivatives of resveratrol and of its naphthalene-based analog **1**, by introducing heterocyclic systems into their structures, in order to increase the polarity of these systems which, eventually, may be useful in the future in improving the poor pharmacokinetic properties associated to resveratrol itself. The resulting analogs were tested for two typical properties shown by resveratrol: tumor antiproliferative action and vasorelaxing effect. A conformational

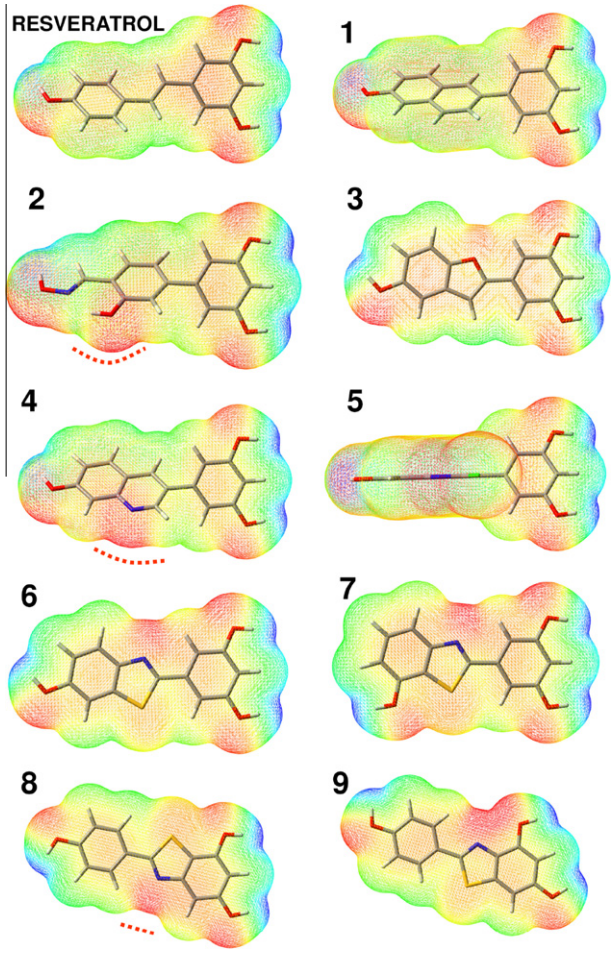


Figure 3. ESP of resveratrol and compounds **1**–**9**. Red regions correspond to negative ESP, blue region to positive ESP, and green region to neutral ESP. The red regions of compounds **2**, **4**, and **8** correlable with a low affinity are evidenced.

analysis of these compounds indicated that the antiproliferative activity on MDA-MB-231 cancer cells can be correlated to a common sterical profile of the most active compounds and, in particular, to the spatial arrangement of the three phenolic groups. Furthermore, the vasorelaxing properties of these analogs showed a good correlation with the electronic properties measured through the ESP. These theoretical observations point out that the structural requirements for mimicking resveratrol anticancer effects are likely to be different from those needed to obtain a resveratrol-like vasorelaxing action, and may indicate the future directions to be followed in the design of new analogs of this powerful, but still poorly drug-like, natural polyphenol.

4. Experimental section

4.1. Chemistry

NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Electron impact (EI, 70 eV) mass spectra were obtained on a ThermoQuest Finnigan GCQ Plus mass spectrometer. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040–0.06 mm; Merck) or gravity column (Kieselgel 60, 0.063–0.200 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F254) sheets that were visualized under a UV lamp. Evaporation was performed in vacuo (rotating evaporator).

4.1.1. 5-(3,5-Dimethoxy)phenyl-2-(prop-1-enyl)phenol (11)

To a solution of **10** (300 mg, 1.41 mmol) in dioxane (7 mL) was added, under nitrogen flux, 3,5-dimetoxyphenylboronic acid (615 mg, 3.38 mmol), $Pd(PPh_3)_4$ (104 mg, 0.09 mmol) and 3.7 mL of a 2 M aqueous solution of K_3PO_4. The resulting mixture was stirred at 80 °C overnight in a sealed vial. The crude mixture was then diluted with water and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 95:5), affording **11** (295 mg, 1.09 mmol, 77% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.94 (dd, 3H, J = 6.5, 1.6 Hz), 3.84 (s, 6H), 6.25 (m, 1H), 6.45 (t, 1H, J = 2.2 Hz), 6.60 (dd, 1H, J = 14.5, 1.4 Hz), 6.70 (d, 2H, J = 2.2 Hz), 7.01 (d, 1H, J = 1.5 Hz), 7.11 (dd, 1H, J = 8.1, 1.6 Hz), 7.35 (d, 1H, J = 7.9 Hz).

4.1.2. 4-(3,5-Dimethoxy)phenylsalicylaldehyde (12)

A solution of **11** (120 mg, 0.44 mmol) in dioxane (4.7 mL) was treated with H_2O (2.0 mL), $NaIO_4$ (219 mg, 1.02 mmol) and a solution of OsO_4 in *t*-BuOH (0.014 mL). The resulting mixture was stirred at 50 °C for 2 h, then cooled to rt, diluted with H_2O and extracted with $CHCl_3$. The organic phase was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 95:5), affording **12** (77 mg, 0.30 mmol, 68% yield) as a white powder. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.85 (s, 6H), 6.53 (t, 1H, J = 2.2 Hz), 6.75 (d, 2H, J = 2.2 Hz), 7.20–7.26 (m, 2H), 7.61 (d, 1H, J = 7.9 Hz), 9.92 (br s, 1H), 11.12 (br s, 1H).

4.1.3. 4-(3,5-Dihydroxy)phenylsalicylaldehyde (13)

A solution of **12** (77 mg, 0.30 mmol) in 5.0 mL of anhydrous CH_2Cl_2 was cooled to −78 °C under nitrogen flux and treated with a 1 M solution of BBr_3 in Et_2O (1.8 mL, 1.8 mmol). The mixture was stirred at rt overnight, then quenched with water and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 6:4), affording pure **13** (30 mg, 0.13 mmol, 44% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 6.45 (t, 1H, J = 2.2 Hz), 6.68 (d, 2H, J = 2.2 Hz), 7.14 (d, 1H, J = 1.6 Hz), 7.29 (dd, 1H, J = 8.1, 1.6 Hz), 7.82 (d, 1H, J = 8.1 Hz), 10.04 (br s, 1H).

4.1.4. 4-(3,5-Dihydroxy)phenylsalicylaldoxime (2)

A solution of **13** (25 mg, 0.11 mmol) in methanol (0.2 mL) was added to a solution of hydroxylamine hydrochloride (15 mg, 0.22 mmol) in water (0.02 mL). The mixture was stirred at rt for 18 h. Partial removal of the solvent, followed by dilution with water and extraction with AcOEt, afforded a crude product which was purified by column chromatography (*n*-hexane/ethyl acetate 1:1), affording pure product **2** (8.9 mg, 0.04 mmol, 39% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 6.39 (t, 1H, J = 2.1 Hz), 6.64 (d, 2H, J = 2.2 Hz), 7.10 (m, 1H), 7.14 (dd, 1H, J = 8.0, 1.7 Hz), 7.41 (d, 1H, J = 7.9 Hz), 8.39 (s, 2H), 10.12 (br s, 1H), 10.55 (br s, 1H). MS *m/z* 245 (M^+, 100).

4.1.5. 2-Hydroxy-5-methoxybenzyl alcohol (15)

Sodium borohydride (62 mg, 1.65 mmol) was added to a cooled (ice bath) solution of 2-hydroxy-5-methoxybenzaldehyde **14** (500 mg, 3.29 mmol) in EtOH (5 mL). The reaction mixture was stirred at rt for 1 h. After the solvent was removed, the residue was treated with 1 N aqueous solution of HCl and extracted with Et_2O. The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrate to give compound **15** (432 mg, 2.80 mmol,

85% yield) as an oil. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.72 (s, 3H), 4.72 (s, 2H), 6.59 (s, 1H), 6.73 (m, 2H).

4.1.6. (2-Hydroxy-5-methoxybenzyl)-triphenylphosphonium bromide (16)

A solution of **15** (351 mg, 2.28 mmol) and triphenylphosphine hydrobromide (783 mg, 2.28 mmol) in CH_3CN (7 mL) was refluxed for 1 h. The solid was filtered and washed with CH_3CN to give **16** (918 mg, 1.92 mmol, 84% yield) that was used for the next step without further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.38 (s, 3H), 4.87 (d, 2H, J = 14.7 Hz), 6.33 (s, 1H), 6.65–6.71 (m, 2H), 7.67–7.89 (m, 15H), 9.34 (s, 1H).

4.1.7. 2-(3,5-Dimethoxy)phenyl-5-methoxybenzofuran (17)

A solution of dicyclohexylcarbodiimide (485 mg, 2.35 mmol) in dry CH_2Cl_2 (1 mL) was added to a solution of **16** (343 mg, 1.88 mmol), DMAP (34.4 mg, 0.28 mmol), and 3,5-dimethoxybenzoic acid (900 mg, 1.88 mmol) in dry CH_2Cl_2 (18 mL), under nitrogen flux. The mixture was stirred overnight at rt, then concentrated under vacuum. The residue, dissolved in dry dioxane (9 mL), was treated with triethylamine (1.5 mL, 10.64 mmol) and the resulting mixture was refluxed overnight. After cooling to rt, the solution was filtered and the solvent removed under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 9:1), affording product **17** (250 mg, 0.88 mmol, 47% yield) as a solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.86 (s, 3H), 3.87 (s, 6H), 6.47 (t, 1H, J = 2.3 Hz), 6.89 (dd, 1H, J = 9.0, 2.6 Hz), 6.95 (s, 1H), 7.00 (d, 2H, J = 2.4 Hz), 7.04 (d, 1H, J = 2.6 Hz), 7.41 (d, 1H, J = 8.8 Hz).

4.1.8. 2-(3,5-Dihydroxy)phenyl-5-hydroxybenzofuran (3)

A solution of 17 (120 mg, 0.43 mmol) in 5 mL of anhydrous CH_2Cl_2 was cooled to −78 °C under nitrogen flux and treated with a 1 M solution of BBr_3 in Et_2O (4,5 mL, 4,5 mmol). The mixture was stirred at rt overnight, then quenched with water and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 1:1), affording product **3** (49 mg, 0.20 mmol, 47% yield) as a white solid. ^{1}H NMR (acetone-d_6) δ (ppm): 6.39 (t, 1H, J = 2.2 Hz), 6.82 (dd, 1H, J = 8.8, 2.4 Hz), 6.89 (d, 2H, J = 2.2 Hz), 7.01 (d, 1H, J = 2.6 Hz), 7.03 (d, 1H, J = 0.7 Hz), 7.35 (d, 1H, J = 8.8 Hz). MS *m/z* 242 (M^+, 100).

4.1.9. *N*-(3-Methoxyphenyl)-3,5-dimethoxyphenylacetamide (19)

To a solution of 3,5-dimethoxyphenylacetic acid **18** (627 mg, 3.20 mmol) in dry CH_2Cl_2 (32 mL) was added $SOCl_2$ (0.93 mL, 12.80 mmol). The reaction mixture was stirred at 60 °C for 4 h. The solution was then concentrated under nitrogen flux and the residue containing crude acid chloride was treated with a solution of *m*-anisidine (328 mg, 2.66 mmol) in a minimum amount of dry CH_2Cl_2, PS-DIEA (973 mg, 3.19 mmol) and DMAP (catalytic amount). The mixture was stirred at room temperature overnight, then neutralized with aq satd K_2CO_3 and extracted with CH_2Cl_2. The organic phase was dried and evaporated to afford a crude residue that was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2), yielding pure **19** (540 mg, 1.79 mmol, 74% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.66 (s, 2H), 3.78 (s, 3H), 3.81 (s, 6H), 6.43 (t, 1H, J = 2.2 Hz), 6.47 (d, 2H, J = 2.2 Hz), 6.64 (ddd, 1H, J = 8.3, 2.4, 1.0 Hz), 6.85 (ddd, 1H, J = 8.3, 2.4, 1.0 Hz), 7.17 (t, 1H, J = 8.24 Hz), 7.23 (m, 1H).

4.1.10. 2-Chloro-3-(3,5-dimethoxyphenyl)-7-methoxyquinoline (20)

Phosphorus oxychloride (0.21 mL, 2.31 mmol) was added, dropwise, to well stirred and cooled (0 °C, ice bath) *N*,*N*-dimethylformamide (0.04 mL). After 30 min, amide **19** (100 mg, 0.33 mmol) was added at the same temperature. The ice bath was then removed and the mixture was heated at 75 °C for 1.5 h. The mixture was quenched with water and extracted with EtOAc. The organic phase was dried and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 9:1), affording pure **20** (198 mg, 0.60 mmol, 55% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.85 (s, 6H), 3.96 (s, 3H), 6.54 (t, 1H, J = 2.2 Hz), 6.65 (d, 2H, J = 2.2), 7.20 (dd, 1H, J = 9.0, 2.7 Hz), 7.39 (d, 1H, 2.7 Hz), 7.71 (d, 1H, J = 9.0 Hz), 8.03 (s, 1H).

4.1.11. 2-Chloro-3-(3,5-dihydroxyphenyl)-7-hydroxyquinoline (5)

A solution of **20** (80 mg, 0.24 mmol) in 2.6 mL of anhydrous CH_2Cl_2 was cooled at −78 °C, under flux of nitrogen, and treated with a 1 M solution of BBr_3 in Et_2O (2.9 mL, 2.90 mmol). The mixture was then stirred at rt overnight, then quenched with water and extracted with ethyl acetate. The organic phase were dried and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 1:1), affording **5** (60 mg, 0.21 mmol, 84% yield) as a white solid. ^{1}H NMR (acetone-d_6) δ (ppm): 6.44 (t, 1H, J = 2.2 Hz), 6.49 (d, 2H, J = 2.2), 7.27 (dd, 1H, J = 7.8 Hz, 2.5 Hz), 7.31 (d, 1H, 2.5 Hz), 7.90 (d, 1H, J = 7.8 Hz), 8.16 (s, 1H), 8.46 (br, 3H). MS *m/z* 287 (M^+, 100).

4.1.12. 3-(3,5-Dihydroxyphenyl)-7-hydroxyquinoline (4)

Compound **5** (50 mg, 0.17 mmol) was dissolved in EtOH (5 mL), and Et_3N (0.07 mL), then Pd/C 10% (22 mg) was added and the mixture was allowed to react under H_2 for 24 h. The mixture was filtered on a Celite pad and the solution was evaporated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 9:1), affording product **4** (12 mg, 0.05 mmol, 13% yield) as a white solid. ^{1}H NMR (acetone-d_6) δ (ppm): 6.43 (t, 1H, J = 2.2 Hz), 6.75 (d, 2H, J = 2.2 Hz), 7.25 (dd, 1H, J = 8.8, 2.3 Hz), 7.40 (d, 1H, J = 2.2 Hz), 7.89 (d, 1H, J = 8.8 Hz), 8.32 (d, 1H, J = 2.3 Hz), 8.49 (br, 2H), 9.01 (d, 1H, J = 2.3 Hz), 9.10 (br, 1H). MS *m/z* 253 (M^+, 7).

4.1.13. *N*-(*p*-Methoxyphenyl)-3,5-dimethoxybenzamide (22)

3,5-Dimethoxybenzoyl chloride **21** (736 mg, 3.67 mmol), PS-DIEA (1.040 g, 3.67 mmol) and DMAP (catalytic amount) were added to a solution of *p*-anisidine (377 mg, 3.06 mmol) in dry CH_2Cl_2. The mixture was stirred at rt overnight, then filtered and evaporated under vacuum. The crude residue was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1), affording product **22** (593 mg, 2.06 mmol, 61% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.81 (s, 3H), 3.84 (s, 6H), 6.61 (t, 1H, J = 2.2 Hz), 6.91 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.7 Hz), 6.97 (d, 2H, J = 2.2 Hz), 7.52 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.7 Hz), 7.70 (br, 1H).

4.1.14. *N*-(*p*-Methoxyphenyl)-3,5-dimethoxythiobenzamide (23)

A solution of **22** (593 mg, 2.06 mmol) and Lawesson's reagent (500 mg, 1.24 mmol) in chlorobenzene (3.0 mL) was boiled at 130 °C for 3 h. The mixture was evaporated and the crude residue was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2), affording **23** (417 mg, 1.37 mmol, 67% yield) as a solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.85 (s, 9H), 6.58 (t, 1H, J = 2.2 Hz), 6.97 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.98 (d, 2H, J = 2.2 Hz), 7.64 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.4 Hz).

4.1.15. 2-(3,5-Dimethoxyphenyl)-6-methoxybenzothiazole (24)

Compound **23** (300 mg, 0.99 mmol) was wetted with a little amount of ethanol, and 30% aqueous NaOH solution (1.3 mL) was added. The mixture was diluted with water to provide a final suspension of 10% aqueous NaOH. This mixture was added

portionwise (1 mL, 1 min intervals) to a stirred solution of potassium ferricyanide (1.302 g, 3.96 mmol) in water, at 85 °C. The reaction mixture was heated for a further 30 min and then allowed to cool to rt and stirred overnight. The mixture was filtered and the residue dissolved in $CHCl_3$, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 9:1), affording **24** (220 mg, 0.73 mmol, 74% yield) as a solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.89 (s, 6H), 3.90 (s, 3H), 6.57 (t, 1H, J = 2.3 Hz), 7.09 (dd, 1H, J = 8.8, 2.6 Hz), 7.20 (d, 2H, J = 2.3 Hz), 7.35 (d, 1H, J = 2.6 Hz), 7.95 (d, 1H, J = 8.8 Hz).

4.1.16. 2-(3,5-Dihydroxyphenyl)-6-hydroxybenzothiazole (6)

A solution of **24** (210 mg, 0.69 mmol) in 7.6 mL of anhydrous CH_2Cl_2 was cooled to −78 °C under nitrogen flux and treated with a 1 M solution of BBr_3 in Et_2O (7.3 mL, 8.33 mmol). The mixture was stirred at rt overnight, then quenched with water and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 1:1), affording **6** (89 mg, 0.34 mmol, 49% yield) as a solid. ^{1}H NMR (CD_3COCD_3): 6.50 (t, 1H, J = 2.1 Hz), 7.05 (dd, 1H, J = 8.8, 2.3 Hz), 7.08 (d, 2H, J = 2.1 Hz), 7.43 (d, 1H, J = 2.3 Hz), 7.83 (d, 1H, J = 8.8 Hz), 8.56 (br, 2H), 8.75 (br, 1H). MS *m/z* 259 (M^+, 40).

4.1.17. *N*-(*m*-Methoxyphenyl)-3,5-dimethoxybenzamide (34)

To a solution of *m*-anisidine (0.34 mL, 3.06 mmol) in dry dichloromethane, 3,5-dimethoxybenzoyl chloride **21**, PS-DIEA (1.04 g, 3.67 mmol) and DMAP (catalytic amount) were added. The mixture was stirred overnight at rt, then filtered and evaporated under vacuum. The crude residue was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1), affording **34** (300 mg, 1.04 mmol, 31% yield) as a solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.84 (s, 3H), 3.85 (s, 6H), 6.62 (t, 1H, J = 2.2 Hz), 6.72 (ddd, 1H, J = 8.0, 2.3, 0.8 Hz), 6.97 (d, 2H, J = 2.2 Hz), 7.06 (ddd, 1H, J = 8.0, 2.3, 0.8 Hz), 7.25 (t, 1H, J = 8.0 Hz), 7.44 (t, 1H, J = 2.3 Hz), 7.75 (br, 1H).

4.1.18. *N*-(*m*-Methoxyphenyl)-3,5-dimethoxythiobenzamide (35)

A solution of **34** (300 mg, 1.04 mmol) and Lawesson's reagent (253 mg, 0.63 mmol) in chlorobenzene (1.5 mL) was boiled at 130 °C for 3 h. The mixture was evaporated and the crude residue was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2), affording **35** (163 mg, 0.54 mmol, 52% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.84 (s, 9H), 6.57 (m, 1H), 6.84 (m, 1H), 6.96 (m, 2H), 7.29 (m, 2H), 7.64 (m, 1H), 8.98 (br, 1H).

4.1.19. 2-(3,5-Dimethoxyphenyl)-5-methoxybenzothiazole (37) and 2-(3,5-dimethoxyphenyl)-7-methoxybenzothiazole (36)

Compound **35** (163 mg, 0.54 mmol) was wetted with a little amount of ethanol, and 30% aqueous NaOH solution (0.6 mL) was added. The mixture was diluted with water to provide a final suspension of 10% aqueous NaOH. This mixture was added portionwise (1 mL, 1 min intervals) to a stirred solution of potassium ferricyanide (709 mg, 2.15 mmol) in water at 85 °C. The reaction mixture was heated for a further 30 min and then allowed to cool to rt and stirred overnight. The mixture was filtered and the residue dissolved in $CHCl_3$, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 9:1), affording a mixture of **37** and **36** (70% yield). ^{1}H NMR ($CDCl_3$) δ (ppm), (asterisk denotes peaks of compound **36**): 3.89 (s, 6H+6H), 3.91 (s, 3H), 4.01* (s,3H), 6.59 (t, 1H+1H, J = 2.2 Hz), 6.84* (d, 1H, J = 8.3 Hz), 7.04* (dd, 1H, J = 8.3, 2.6 Hz), 7.24 (d, 2H, J = 2.2 Hz), 7.27* (d, 2H, J = 2.2 Hz), 7.44 (t, 1H, J = 8.2 Hz), 7.56* (d, 1H, J = 2.6 Hz), 7.10 (d, 1H, J = 8.2 Hz), 7.74 (d, 1H, J = 8.2 Hz).

4.1.20. 2-(3,5-Dihydroxyphenyl)-7-hydroxybenzothiazole (7)

A solution of the mixture of **37** and **36** (90 mg, 0.30 mmol) in 3.3 mL of anhydrous CH_2Cl_2 was cooled to −78 °C under nitrogen flux and treated with a 1 M solution of BBr_3 in Et_2O (3.2 mL, 3.56 mmol). The mixture was stirred at rt overnight, then quenched with water and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 1:1), affording **7** (19 mg, 0.07 mmol, 49% yield) as a solid, while the trihydroxy-substituted regioisomer of **7** was not isolated. ^{1}H NMR (CD_3COCD_3) δ (ppm): 6.54 (t, 1H, J = 2.1 Hz), 6.91 (dd, 1H, J = 7.9, 0.7 Hz), 7.16 (d, 2H, J = 2.1 Hz), 7.35 (t, 1H, J = 7.9 Hz), 7.54 (dd, 1H, J = 7.9, 0.7 Hz), 8.59 (br, 2H), 9.37 (br, 1H). MS *m/z* 259 (M^+, 100).

4.1.21. *N*-(3,5-Dimethoxy)phenyl-4-methoxybenzamide (39)

To a solution of 3,5-dimethoxyaniline (250 mg, 1.63 mmol) in dry CH_2Cl_2 (3 mL), *p*-anisoylchoride 38 (401 mg, 1.35 mmol), PS-DIEA (425 mg, 1.96 mmol), and DMAP (catalytic amount) were added. The mixture was stirred at rt overnight, then filtered and evaporated under vacuum. The crude residue was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1), affording **39** (463 mg, 1.61 mmol, 89% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.81 (s, 6H), 3.88 (s, 3H), 6.27 (t, 1H, J = 2.2 Hz), 6.89 (d, 2H, J = 2.2 Hz), 6.98 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 7.82 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.6 Hz).

4.1.22. *N*-(3,5-Dimethoxy)phenyl-4-methoxythiobenzamide (41)

A solution of **39** (200 mg, 0.70 mmol) and Lawesson's reagent (170 mg, 0.42 mmol) in chlorobenzene (1.0 mL) was boiled at 130 °C for 3 h. The mixture was evaporated and the crude residue was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2), affording **41** (148 mg, 0.49 mmol, 70% yield) as a solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.79 (s, 6H), 3.86 (s, 3H), 6.38 (t, 1H, J = 2.1 Hz), 6.91 (m, 4H), 7.82 (m, 2H).

4.1.23. 2-(*p*-Methoxyphenyl)-5,7-dimethoxybenzothiazole (43)

Compound **41** (100 mg, 0.33 mmol) was wetted with a little amount of ethanol, and 30% aqueous NaOH solution (0.4 mL) was added. The mixture was diluted with water to provide a final suspension of 10% aqueous NaOH. This mixture was added portionwise (1 mL, 1 min intervals) to a stirred solution of potassium ferricyanide (435 mg, 1.32 mmol) in water at 85 °C. The reaction mixture was heated for a further 30 min and then allowed to cool to rt and stirred overnight. The mixture was filtered and the residue dissolved in $CHCl_3$, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 9:1), affording **43** (60 mg, 0.20 mmol, 70% yield) as a solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.88 (s, 3H), 3.90 (s, 3H), 3.96 (s, 3H), 6.48 (d, 1H, J = 2.0 Hz), 6.99 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 7.16 (d, 1H, J = 2.0 Hz), 8.01 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.6 Hz).

4.1.24. 2-(*p*-Hydroxyphenyl)-5,7-hydroxybenzothiazole (8)

A solution of **43** (60 mg, 0.20 mmol) in 2.2 mL of anhydrous CH_2Cl_2 was cooled to −78 °C under nitrogen flux and treated with a 1 M solution of BBr_3 in Et_2O (1.98 mL, 1.98 mmol). The mixture was stirred at rt overnight, then quenched with water and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 4:6), affording **8** (21 mg, 0.08 mmol, 41% yield) as a solid. ^{1}H NMR (acetone-d_6) δ (ppm): 6.50 (d, 1H, J = 2.1 Hz), 6.98 (d, 1H, J = 2.1 Hz), 6.99 (AA′XX′, 2H, J_{AX} = 6.8 Hz, $J_{AA'/XX'}$ = 2.0 Hz), 7.97 (AA′XX′, 2H, J_{AX} = 6.8 Hz, $J_{AA'/XX'}$ = 2.0 Hz). MS *m/z* 259 (M^+, 49).

4.1.25. *N*-(2,4-Dimethoxy)phenyl-4-methoxybenzamide (40)

To a solution of 2,4-dimethoxyaniline (328 mg, 2.66 mmol) in dry CH_2Cl_2 (3 mL), *p*-anisoylchoride **38** (401 mg, 1.35 mmol), PS-DIEA (510 mg, 2.35 mmol) and DMAP (catalytic amount) were added. The mixture was stirred at rt overnight, then filtered and evaporated under vacuum. The crude residue was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1), affording **40** (418 mg, 1.45 mmol, 74% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.82 (s, 3H), 3.87 (s, 3H), 3.90 (s, 3H), 6.52 (d, 1H, J = 2.6 Hz), 6.53 (dd, 1H, J = 9.4, 2.6 Hz), 6.98 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 7.85 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 8.26 (br, 1H), 8.38 (d, 1H, J = 9.4 Hz).

4.1.26. *N*-(2,4-Dimethoxy)phenyl-4-methoxythiobenzamide (42)

A solution of **40** (250 mg, 0.87 mmol) and Lawesson's reagent (213 mg, 0.52 mmol) in chlorobenzene (1.3 mL), was boiled at 130 °C for 3 h. The mixture was evaporated and the crude residue was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2), affording **42** (190 mg, 0.63 mmol, 72% yield) as a solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.84 (s, 3H), 3.86 (s, 3H), 3.89 (s, 3H), 6.54 (d, 1H, J = 2.1 Hz), 6.55 (dd, 1H, J = 9.5, 2.1 Hz), 6.93 (AA′XX′, 2H, J_{AX} = 8.8 Hz), 7.87 (AA′XX′, 2H, J_{AX} = 8.8 Hz), 8.91 (d, 1H, J = 9.5 Hz), 9.34 (br, 1H).

4.1.27. 2-(*p*-Methoxyphenyl)-4,6-dimethoxybenzothiazole (44)

Compound **42** (190 mg, 0.62 mmol) was wetted with a little amount of ethanol, and 30% aqueous NaOH solution (0.6 mL) was added. The mixture was diluted with water to provide a final suspension of 10% aqueous NaOH. This mixture was added portionwise (1 mL, 1-min intervals) to a stirred solution of potassium ferricyanide (822 mg, 2.50 mmol) in water at 85 °C. The reaction mixture was heated for a further 30 min and then allowed to cool to rt and stirred overnight. The mixture was then filtered and the residue dissolved in $CHCl_3$, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 1:1), affording **44** (92 mg, 0.30 mmol, 49% yield) as a solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.87 (s, 3H), 3.88 (s, 3H), 4.03 (s, 3H), 6.54 (d, 1H, J = 2.2 Hz), 6.91 (d, 1H, J = 2.2 Hz), 6.96 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 8.01 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.6 Hz).

4.1.28. 2-(*p*-Hydroxyphenyl)-4,6-hydroxybenzothiazole (9)

A solution of **44** (92 mg, 0.30 mmol) in 3.3 mL of anhydrous CH_2Cl_2 was cooled to −78 °C under nitrogen flux and treated with a 1 M solution of BBr_3 in Et_2O (3.2 mL, 3.20 mmol). The mixture was stirred at rt overnight, then quenched with water and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by column chromatography (*n*-hexane/ethyl acetate 1:1), affording **9** (52 mg, 0.20 mmol, 66% yield) as a solid. ^{1}H NMR (acetone-d_6) δ (ppm): 6.50 (d, 1H, J = 2.2 Hz), 6.88 (d, 1H, J = 2.2 Hz), 6.96 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.88 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 8.76 (br, 3H). MS *m/z* 259 (M^+, 100).

4.2. Antiproliferative assays

All drugs were dissolved in absolute ethanol (EtOH) before use.

4.2.1. Cell lines

Human breast cancer cell line MDA-MB-231 (American Type Culture Collection, Rockville, MD, USA), was maintained at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium (EuroClone Ltd, Wetherby West Yorkshire, UK), supplemented with 5% fetal bovine serum (EuroClone Ltd, Wetherby West Yorkshire, UK) and 100 ng/mL each of penicillin and streptomycin (Invitrogen, San Giuliano Milanese, Italy).

4.2.2. Cell proliferation by sulforhodamine B (SRB) assay

Cells were seeded in 96-wells tissue culture plates at 1.3×10^3 cells/well and were allowed to adhere for 24 h before treatment. Cells were grown in the presence of vehicle (EtOH or DMSO) (five wells) or drugs at a indicated concentrations (five wells for each treatment). Cellular growth was assessed after 6 days by SRB assay. Briefly, proteins were precipitated with 10% (final concentration) trichloroacetic acid for 1 h at 4 °C and stained for 30 min with SRB dye 0.4% w/v in acetic acid 1% v/v. Finally, precipitated proteins were washed and solubilized in Tris buffer 10 mM. Absorbance (optical density, OD) was measured at 540 nm by using a microplate reader. For each treatment the proliferation index was calculated as OD TC t_x/OD TC t_0 where 'OD TC t_x' is the mean optical density of treated cells at time x and 'OD TC t_0' is the value at time zero.

4.3. Vascular assays

All the experimental procedures were carried out following the guidelines of the European Community Council Directive 86-609. To determine a possible vasodilator mechanism of action, the compounds were tested on isolated thoracic aortic rings of male normotensive Wistar rats (250–350 g). The rats were sacrificed by cervical dislocation under light ether anesthesia and bled. The aortae were immediately excised and freed of extraneous tissues. The endothelial layer was removed by gently rubbing the intimal surface of the vessels with a hypodermic needle. Five millimeter wide aortic rings were suspended, under a preload of 2 g, in 20 mL organ baths, containing Tyrode solution (composition of saline in mM: NaCl 136.8; KCl 2.95; $CaCl_2$ 1.80; $MgSO_4 \cdot 7H_2O$ 1.05; NaH_2PO_4 0.41; $NaHCO_3$ 11.9; Glucose 5.5), thermostated at 37 °C and continuously gassed with a mixture of O_2 (95%) and CO_2 (5%). Changes in tension were recorded by means of an isometric transducer (Grass FTO3), connected with a preamplifier (Buxco Electronics) and with a software of data acquisition (BIOPAC Systems Inc., MP 100). After an equilibration period of 60 min, the endothelial removal was confirmed by the administration of acetylcholine (ACh) (10 μM) to KCl (30 mM)-precontracted vascular rings. A relaxation <10% of the KCl-induced contraction was considered representative of an acceptable lack of the endothelial layer, while a relaxation $\geqslant$70% of the KCl-induced contraction was considered representative of an acceptable integrity of the endothelium. Forty-five minutes after the confirmation of the endothelium removal/integrity, the aortic preparations were contracted by treatment with a single concentration of KCl (30 mM) and when the contraction reached a stable *plateau*, threefold increasing concentrations of the reference drug resveratrol were added cumulatively. Preliminary experiments showed that the KCl (30 mM)-induced contractions remained in a stable tonic state for at least 40 min. When required, in some sets of experiments, the non-selective potassium channel blocker Tetraethylammonium chloride (TEA 10 mM) or the BK-selective blocker Iberiotoxin (IbTX, 100 nM) were added to aortae, after the KCl (30 mM)-induced contraction, followed by the administration of tested compounds. In another set of experiments, the behavior of compound 6 in high depolarizing conditions was observed, replacing the standard KCl concentration of 30 mM with a 60 mM KCl concentration. The reference drug resveratrol (Sigma) was dissolved (10 mM) in DMSO and further diluted in Tyrode solution. Acetylcholine chloride (Sigma) was dissolved (100 mM) in EtOH 95% and further diluted in bidistilled water whereas KCl, TEA, and IbTX were both dissolved in Tyrode solution. All the solutions were freshly prepared immediately before the pharmacological experimental procedures. Previous experiments showed a

complete ineffectiveness of the administration of the vehicle. The vasorelaxing efficacy was evaluated as maximal vasorelaxing response, expressed as a percentage (%) of the contractile tone induced by KCl 30 mM (or KCl 60 mM). When the limit concentration 30 μM (the highest concentration, which could be administered) of resveratrol or test compounds did not reach the maximal effect, the parameter of efficacy represented the vasorelaxing response, expressed as a percentage (%) of the contractile tone induced by KCl 30 mM, evoked by this limit concentration. The parameter of potency was expressed as pIC_{50}, calculated as negative logarithm of the molar concentration of the tested compounds, evoking a half reduction of the contractile tone induced by KCl 30 mM. The pIC_{50} could not be calculated for those compounds showing an efficacy parameter lower than 50%. The parameters of efficacy and potency were expressed as mean ± standard error, for 5–10 experiments. Student *t* test was selected as statistical analysis, $P < 0.05$ was considered representative of a significant statistical difference. Experimental data were analyzed by a computer fitting procedure (software: GraphPad Prism 4.0).

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