Resveratrol Derivatives and Their Role as Potassium Channels Modulators

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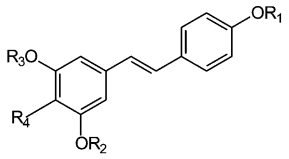
A series of stilbenoid analogues of resveratrol (*trans*-3,4′,5-trihydroxystilbene) with a stilbenic or a bibenzylic skeleton have been prepared by partial synthesis from resveratrol and dihydroresveratrol. The synthesized compounds have been evaluated for their ability to modulate voltage-gated channels.

Stilbenes, produced by several plants in response to pathogen attacks, regulate many biological functions. In agreement with their role as phytoalexins, antifungal, antibacterial, and cytotoxic activities have been reported.[1] Some of them, such as resveratrol (*trans*-3,4′,5-trihydroxystilbene), exert antioxidant activity;[2] modulate the synthesis of lipids;[3] inhibit ribonucleotide reductase[4] and DNA polymerase;[5] increase the activity of Map-kinase,[6] an enzyme potentially related to neurodegenerative deseases such as Alzheimer's and Parkinson's; inhibit platelet aggregation[7] and alter the eicosanoid synthesis,[8] both effects probably related to the inhibition of the cyclooxygenase and hydroperoxidase activities.[9] These findings have stimulated the study of these compounds as antiinflammatory, cardiotonic, and antiplatelet aggregating agents.

In addition, some stilbenoids (stilbenes and bibenzyls) have shown properties more strictly related to cancer chemoprevention and treatment, such as inhibition of tubulin polymerization[10] and antiestrogenic activity (specially useful for treatment of hormone-dependent cancers).[11] Resveratrols have been found to inhibit the growth of different types of tumors, such as histiocytic lymphoma, prostate, colon, and breast cancers. They demonstrate this effect through distinct processes, inducing the expression of pro-apoptotic genes, inhibiting progression in the cell cycle, modulating NO production, and interacting with estrogen receptors.[12] In general, they inhibit cellular events associated with tumor initiation, promotion, and progression.[9,12]

Our interest in the field of polyhydroxylated phenols, in particular stilbenoids, previously led to the isolation of the 2′-*O*-*β*-D-glucoside of combretastatin A-1 (3,4,4′,5-tetramethoxy-2′,3′-dihydroxystilbene) and of combretastatin B-1 (3,4,4′,5-tetramethoxy-2′,3′-dihydroxydihydrostilbene),[10] the 3′-*O*-*β*-D-glucoside of resveratrol,[7b] and the total synthesis of the natural compounds as well as of several analogues that have been tested with respect to different biological activities, evidencing interesting behaviors.[7b,13] We observed that some polyhydroxystilbenes selectively and reversibly increase the duration of the action potential in neural cells, by inhibiting the repolarization of potassium channels,[14] a kind of activity already found in other tissues in connection with antitumor (tamoxifen) and cardiac antiarythmic (tedisamil) agents. The diffusion of potassium ions across cell membranes, through potassium channels, underlines many fundamental biological processes.



R4 = H

1	a) $R_1 = CH_3$, $R_2 = R_3 = H$	10%
	b) $R_1 = CH_2Ph$, $R_2 = R_3 = H$	10%
	c) $R_1 = CH_2CH=C(CH_3)_2$, $R_2 = R_3 = H$	20%
2	a) $R_2 = CH_3$, $R_1 = R_3 = H$	traces
	b) $R_2 = CH_2Ph$, $R_1 = R_3 = H$	5%
	c) $R_2 = CH_2CH=C(CH3)_2$, $R_1 = R_3 = H$	20%
3	a) $R_1 = R_2 = CH_3$, $R_3 = H$	10%
	b) $R_1 = R_2 = CH_2Ph$, $R_3 = H$	18%
	c) $R_1 = R_2 = CH_2CH=C(CH_3)_2$, $R_3 = H$	15%
4	a) $R_2 = R_3 = CH_3$, $R_1 = H$	20%
	b) $R_2 = R_3 = CH_2Ph$, $R_1 = H$	20%
5	a) $R_1 = R_2 = R_3 = CH_3$	37%
	b) $R_1 = R_2 = R_3 = CH_2Ph$	29%
	c) $R_1 = R_2 = R_3 = CH_2CH=C(CH_3)_2$	5%

R4 = CH_2=CHC(CH_3)$_2$

6	$R_1 = R_2 = R_3 = H$	20%

Figure 1. *Trans* and *cis* derivatives of resveratrol.

On the basis of the preliminary results, we have undertaken the synthesis of several resveratrol analogues, to study the mechanism of their modulatory action of sodium and two voltage-dependent potassium currents.

Results and Discussion

The analogues shown in Figures 1 and 2 and in Scheme 1 have been synthesized either by partial or by total synthesis, in the former case by modifying the structure of the parent compound resveratrol (*trans*-3,4′,5-trihydroxystilbene).

The structure of resveratrol has been modified with the aim to vary (a) its lipophilicity in order to study the selectivity toward cell walls and, as a consequence, toward different tissues and (b) its geometry, with particular reference to the distance and the mutual orientation of the phenyl groups. The lipophilicity has been modified by the introduction of lipophilic chains in different positions of the molecule: several derivatives have been prepared that differ in the number and the position of the free hydroxyl groups and in the lipophilic chain used. The geometry of

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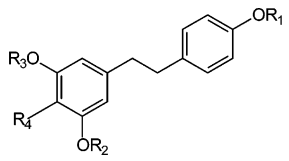

R4 = H

8 a) $R_1 = CH_3$, $R_2 = R_3 = H$ 15%
b) $R_1 = CH_2Ph$, $R_2 = R_3 = H$ 19%
c) $R_1 = CH_2CH{=}C(CH_3)_2$, $R_2 = R_3 = H$ 21%

9 a) $R_2 = CH_3$, $R_1 = R_3 = H$ 10%
b) $R_2 = CH_2Ph$, $R_1 = R_3 = H$ 11%
c) $R_2 = CH_2CH{=}C(CH_3)_2$, $R_1 = R_3 = H$ 24%

10 a) $R_1 = R_2 = CH_3$, $R_3 = H$ 24%
b) $R_1 = R_2 = CH_2Ph$, $R_3 = H$ 23%

11 a) $R_2 = R_3 = CH_3$, $R_1 = H$ 12%
b) $R_2 = R_3 = CH_2Ph$, $R_1 = H$ 14%

12 a) $R_1 = R_2 = R_3 = CH_3$ 28%
b) $R_1 = R_2 = R_3 = CH_2Ph$ 29%

R4 = CH_2=CHC(CH_3)2

13 $R_1 = R_2 = R_3 = H$ 15%

Figure 2. Bibenzyl derivatives obtained by modification of dihydroresveratrol.

the molecule can be significantly affected by the configuration (*trans* or *cis*) of the olefinic double bond. Reduction to give bibenzyls (α,*β*-dihydrostilbenes) was also considered to test whether the double bond is essential for activity or not.

To test the influence (in terms of number and position) of free phenolic groups on activity, the methyl derivatives **1a**−**5a** (Figure 1) were first synthesized by direct treatment of resveratrol with methyl iodide in dimethylformamide (acetone can be used as well) in the presence of potassium carbonate. This, among the proposed protocols, has been chosen because it is very simple and easy to scale-up and allows obtaining in one step all the isomers required for the biological evaluation. The protocol was then extended to the introduction of benzyl and dimethylallyl groups, the latter being encountered in natural prenylated stilbenoids as well as in other natural compounds where the prenyl moiety has been further elaborated. In all cases the *O*-alkylated products were generally obtained, except for the reaction with 3,3-dimethylallyl bromide, which yielded the C-alkylated product at C_4. The yields and the isomeric ratios can be modulated by changing the experimental conditions (reagent ratio, temperature, time), and the isomers can be separated by flash chromatography. Starting material was also recovered (5−25%) by flash chromatography and recycled.

To test the influence of the stilbenic double-bond configuration on the biological activity, the *cis*-derivative **7** was also synthesized (Scheme 1). Since *trans*−*cis* isomerization of the corresponding *trans*-isomer is not convenient, compound **7** was synthesized by reaction between the phosphonium salt of the commercially available 4-methoxybenzyl chloride and 3,5-bis(*tert*-butyldimethylsilyloxy)-benzaldehyde followed by desilylation with tetrabutylammonium fluoride, according to a reported protocol.[13] A 2.3/1 mixture of *trans*- and *cis*-4′-*O*-methylresveratrol **1a** and **7** (98% yield) was obtained and then divided in two portions. One was submitted to crystallization and flash chromatography, to give pure **1a** and **7.** The second portion was hydrogenated, in the presence of palladium on charcoal, to give the bibenzyl derivative **8a**. In fact, we wanted to test the influence of the double bond on activity and also to verify whether bibenzyls could take advantage of their greater conformational freedom and act as easier-to-prepare mimetics of either the corresponding *cis*- or *trans*-stilbenes, in case the only role of the double bond is to keep the phenyl rings in a proper mutual orientation, without a direct influence on activity. This assumption had been already confirmed by a molecular modeling study performed on *trans*- and *cis*-resveratrol as well as on dihydroresveratrol.[15]

Indeed, preliminary results obtained in the evaluation of the influence on potassium channels evidenced a greater activity of the bibenzyl **8a** (Scheme 1 and Figure 2) with respect to the corresponding *trans*-stilbene derivative **1a**, whereas **8a** and the *cis*-derivative **7** showed comparable activity. These observations were conclusive and shifted our attention to the derivatives with a bibenzyl skeleton.

Derivatives **8**−**13** were prepared from dihydroresveratrol (obtained by hydrogenation of resveratrol in the presence of palladium on charcoal) using benzyl, methyl, or 3,3-dimethylallyl bromide in dimethylformamide, according to the protocol optimized for the preparation of the derivatives **1**−**5** (Figure 2). Using 3,3-dimethylallyl bromide, the C_4-alkylated derivative **13** was also isolated, albeit in low yields (15% yield). A different protocol, using aqueous sodium hydroxide in dimethylformamide, was tested for the

Scheme 1. Total Synthesis of *cis*- and *trans*-4′-*O*-Methylresveratrol and 4′-*O*-Methyldihydroresveratrol

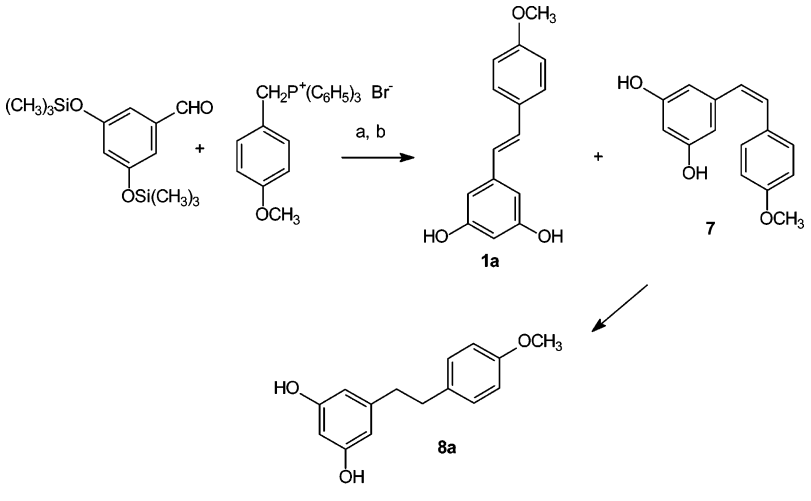

Table 1. Effects of *trans*-Resveratrol, Dihydroresveratrol, and Selected Analogues on Potassium Channels

compound	final concentration (μM)	I_{erg} blocked %; *n*	I_{DR} blocked %; *n*
	trans-Resveratrol and Dihydroresveratrol		
trans-resveratrol	90	28.9; 7	42.9; 6
dihydroresveratrol	90	39.2; 6	52.0; 6
	trans- and *cis*-Stilbene Analogues		
3a (*trans* 3,4′-di-*O*-methyl-)	30	11.5; 8	9.5; 7
7 (*cis* 4′-*O*-methyl-)	90	27.7; 9	70.4; 6
	Bibenzyl Analogues		
8a (4′-*O*-methyl-)	90	24.8; 8	89.4; 6
8b (4′-*O*-benzyl-)	90	18.0; 9	89.0; 5
9a (3-*O*-methyl-)	90	45.3; 7	69.0; 7
9b (3-*O*-benzyl)	90	68.0; 5	89.0; 6
9c[a] (3-*O*-dimethylallyl-)	36	53.7; 6	55.5; 7
10a+**11a** (1.7/1 molar ratio) (3,4′-di-*O*-methyl- and 3,5-di-*O*-methyl-)	90	41.2; 6	77.2; 5
10b (3,4′-di-*O*-benzyl-)	90	53.0; 5	50.0; 6
12a (3,4′,5-tri-*O*-methyl-)	90	11.3; 7	38.6; 8
12b (3,4′,5-tri-*O*-benzyl-)	90	no effect; 8	57.0; 6
13[b] (4-*C*-dimethylallyl-)	45	50.1; 6	74.0; 7

[a] This compound was also tested at 90 μM on I_{ERG} with blockade of 88.7%. [b] This compound was also tested at 90 μM on I_{ERG} with blockade of 88.2%.

reaction with benzyl bromide and afforded, after flash chromatography, 61% yield of the two bis-derivatives **10b** and **11b** and a mixture of the two mono-derivatives **8b** and **9b.** The tribenzyl derivative **12b** was not isolated.

The effects of some resveratrol derivatives were tested on the F-11 neuroblastoma cell line, which expresses a sodium current (I_{Na}) and two different types of voltage-dependent potassium currents: a rapidly inactivating inward-rectifying current (I_{ERG}) and a slowly inactivating delayed rectifier current (I_{DR}).[16] As is well known, sodium channels promote the action potential upstroke in excitable cells; on the contrary, delayed rectifier (DR) potassium channels counteract the role of sodium channels, inducing action potential repolarization.[17] The ERG channel, encoded by the human *ether-à-go-go*-related gene, belongs to a family of voltage-activated, outward-rectifying potassium channels. Gene mutations that cause an inherited cardiac disorder known as long QT syndrome (LQT) pointed out ERG channels function in repolarization of cardiac action potential.[18] Studies made in other tissues, such as nervous system, pituitary gland, and pancreas, revealed ERG implications also in neuronal excitability and in modulation of hormone release.[19] In addition, it has been suggested that ERG channels have a role in supporting neoplastic cell proliferation.[20] ERG channels are present in tumor cells of various histogenesis, where they establish a depolarized resting potential which is required for cell proliferation.[20] Also other potassium channels, different from ERG, seem to play an important role in tumor cell proliferation, and sodium channels were demonstrated to be able to enhance the metastatic potential of rat cancer prostate cells.[21]

To record currents flowing through potassium channels, we performed experiments by patch clamp technique in the whole-cell configuration. We tested resveratrol, dihydroresveratrol, and compounds from the three groups of resveratrol derivatives: *trans*- and *cis*-stilbene derivatives and bibenzyl derivatives. The data are summarized in Table 1.

All compounds affected potassium currents, revealing a low specificity of blockage. All compounds had less effect on ERG current than on the DR one; only derivatives **3a** and **10b** did not follow this pattern. Reduction of the stilbenic double bond in resveratrol to give dihydroresveratrol enhanced inhibitory activity on both I_{ERG} and I_{DR}. Activity enhancement following reduction of the double bond was confirmed by the comparison between 3,4′-di-*O*-methylresveratrol **3a** and 3,4′-di-*O*-methyldihydroresveratrol **10a** (the latter as a mixture with the corresponding 3,5-di-*O*-methyl derivative **11a**). Dihydroresveratrol, the mono 3-*O*-alkylated derivatives **9a**−**c**, and the 3,4′ dialkylated derivatives **10a**,**b** blocked both ERG and DR currents quite strongly. Dihydroresveratrol blocked 39.2% ERG and 52% DR; compound **9a** blocked 45.3% ERG and 69.0% DR. The different intensity of blockage between ERG and DR was more marked for some compounds, for example, the *cis*-stilbene derivative **7** (27.7% ERG and 70.4% DR) and the bibenzyl derivatives **8a** (24.8% ERG and 89.4% DR). The compounds **7** and **8a** blocked sodium current very strongly (data not shown). The obtained results would suggest that mono-*O*-alkylation of the phenolic function at C-4′ in dihydroresveratrol enhances inhibitory activity on the I_{DR} current and depresses activity on the I_{ERG} current, whereas mono-*O*-alkylation of the phenolic function at C-3 and mono-C-alkylation at C-4 enhance activity on both I_{ERG} and I_{DR} currents. Tri-*O*-alkylation depressed inhibitory activity on I_{ERG}.

Our data suggest that, despite their lack of target specificity among ion channels, resveratrol derivatives modulate voltage-gated potassium channels similarly to the parent compound, and in some cases they show better activity and higher specificity toward I_{DR} than I_{ERG} currents.

Recently, resveratrol has been found to directly stimulate large-conductance Ca^{2+}-activated potassium channel activity in vascular endothelial cells. These channels are believed to play an important role in controlling hormonal secretion in neurons or neuroendocrine cells.[22]

In view of recent findings, the action of resveratrol and its derivatives on ion channels, although not exhaustive, offers opportunities for discovering novel therapeutic agents as well as significant challenges in the form of tissue and organ specificity.

Experimental Section

General Experimental Procedures. Resveratrol was donated by Pharmascience Inc. (Montreal, Canada). Reagent grade tetrahydrofuran was refluxed over $LiAlH_4$ and distilled. Reagent grade dimethylformamide was distilled at reduced pressure under nitrogen and kept over 4 Å molecular sieves.

(*E*)-4′-*O*-Methylresveratrol,[13] (*Z*)-4′-*O*-methylresveratrol,[13] and dihydroresveratrol[13,23] have been synthesized according to the reported protocols.

^{1}H NMR and ^{13}C NMR spectra were recorded at 200 and 300 MHz on Bruker spectrometers. MS spectra were recorded with a VG7070 E9 spectrometer. Melting points were obtained by using a Buchi 535 apparatus. Flash-column chromatographies were performed on silica gel Merck Kieselgel 60 (230−400 mesh). Thin-layer chromatographies were performed on silica gel plates (60 F_{254}, Merck); spots were detected visually by ultraviolet irradiation (254 nm) or by spraying with methanol/H_2SO_4, 9:1, followed by heating at 100 °C.

Typical Procedure for the Preparation of Compounds 1−13. Dry potassium carbonate (0.68 g, 4.92 mmol) was added to a solution of resveratrol (0.56 g, 2.46 mmol) in dry dimethylformamide (8 mL). Methyl iodide (0.23 mL, 3.68 mmol) was added dropwise. The reaction was monitored by TLC (silica gel, eluting with *n*-hexane/ethyl acetate, 7:3). After 6 h, the reaction mixture was filtered. The filtrate was diluted with water (6 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude material was flash chromatographed (silica gel, eluting with *n*-hexane/ethyl acetate, 7:3) and afforded 3,4′,5-tri-*O*-methylresveratrol **5a** (37%), 3,4′-di-*O*-methylresveratrol **3a** (10%), 3,5-di-*O*-methylresveratrol **4a** (20%), 4′-*O*-methylresveratrol **1a**[13] (10%), and starting resveratrol (10%). 3-*O*-Methylresveratrol was obtained in traces.

1a:[13] colorless crystals (EtOAc/*n*-hexane); mp 176−178 °C; ^{1}H NMR ($CDCl_3$ + D_2O) δ 3.79 (3H, s, CH_3), 6.15 (1H, dd, J = 2.0, 2.0 Hz, H-4), 6.45 (2H, d, J = 2.0 Hz, H-2 and H-6), 6.90 and 7.01 (2H, AB system, J = 16.4 Hz, CH═CH), 6.93 (2H, d, J = 8.6 Hz, H-3′ and H-5′), 7.51 (2H, d, J = 8.6 Hz, H-2′ and H-6′); EIMS *m*/*z* 242 [M$^+$], 121 [C_8H_9O].

3a: colorless crystals (EtOAc/*n*-hexane); mp 115−117 °C; ^{1}H NMR ($CDCl_3$ + D_2O) δ 3.85 (3H, s, CH_3), 3.89 (3H, s, CH_3), 6.31 (1H, dd, J = 2.3, 2.3 Hz, H-4), 6.57 (1H, d, J = 2.3 Hz, H-2), 6.63 (1H, d, J = 2.3 Hz, H-6), 6.89 and 7.05 (2H, AB system, J = 15.8 Hz, CH═CH), 6.95 (1H, d, J = 8.8 Hz, H-3′ and H-5′), 7.45 (2H, d, J = 8.8 Hz, H-2′ and H-6′); EIMS *m*/*z* 256 [M$^+$]; 121 [C_8H_9O]; *anal*. C 74.58%, H 6.18%, calcd for $C_{16}H_{16}O_3$, C 75%, H 6.25%.

4a:[23] colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 3.85 (6H, s, CH_3), 6.4 (1H, dd, J = 2.0, 2.0 Hz, H-4), 6.7 (2H, d, J = 2.0 Hz, H-2 and H-6), 6.90 and 7.00 (2H, AB system, J = 15.8 Hz, CH═CH), 6.91 (1H, d, J = 8.8 Hz, H-3′ and H-5′), 7.45 (2H, d, J = 8.8 Hz, H-2′ and H-6′); EIMS *m*/*z* 256 [M$^+$], 121 [C_8H_9O].

5a:[23] colorless crystals (EtOAc/*n*-hexane); mp 57 °C; ^{1}H NMR ($CDCl_3$);[23] EIMS *m*/*z* 270 [M$^+$], 121 [C_8H_9O].

1b: colorless crystals (EtOAc/*n*-hexane); mp 114−117 °C; ^{1}H NMR ($CDCl_3$ + D_2O) δ 5.18 (2H, s, CH_2), 6.25 (1H, dd, J = 2.2, 2.2 Hz, H-4), 6.58 (2H, d, J = 2.2 Hz, H-2 and H-6), 6.83 (2H, d, J = 8.3 Hz, H-3′ and H-5′), 6.90 and 7.05 (2H, AB system, J = 15.0 Hz, CH═CH), 7.08 (2H, d, J = 8.3 Hz, H-2′ and 6′), 7.5−7.2 (5H, m); EIMS *m*/*z* 318 [M$^+$]; *anal*. C 78.99%, H 5.64%, calcd for $C_{21}H_{18}O_3$, C 79.25%, H 5.66%.

2b: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 5.18 (2H, s, CH_2), 6.25 (1H, dd, J = 2.0, 2.0 Hz, H-4), 6.55 (1H, d, J = 2.0 Hz, H-2), 6.7 (1H, d, J = 2.0 Hz, H-6), 6.90 and 7.08 (2H, AB system, J = 15.4 Hz, CH═CH), 6.9 (1H, d, J = 8.2 Hz, H-3′ and H-5′), 7.45 (1H, d, J = 8.2 Hz, H-2′ and H-6′), 7.5−7.2 (5H, m); EIMS *m*/*z* 318 [M$^+$].

3b: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 5.09 (2H, s, CH_2), 5.18 (2H, s, CH_2), 6.25 (1H, dd, J = 1.5, 1.5 Hz, H-4), 6.55 (1H, d, J = 1.5 Hz, H-2), 6.7 (1H, d, J = 1.5 Hz, H-6), 6.8 (2H, d, J = 8.3 Hz, H-3′ and H-5′), 6.9 and 7.08 (2H, AB system, J = 15.4 Hz, CH═CH), 7.1 (2H, d, J = 8.3 Hz, H-2′ and H-6′), 7.5−7.2 (10H, m); EIMS *m*/*z* 408 [M$^+$]; *anal*. C 82.55%, H 5.80%, calcd for $C_{28}H_{24}O_3$ C 82.35%, H 5.88%.

4b: colorless crystals (EtOAc/*n*-hexane); mp 123−127 °C; ^{1}H NMR ($CDCl_3$ + D_2O) δ 5.09 (2H, s, CH_2), 5.11 (2H, s, CH_2), 6.22 (1H, dd, J = 1.5, 1.5 Hz, H-4), 6.55 (2H, d, J = 1.5 Hz, H-2 and H-6), 6.87 and 7.08 (2H, AB system J = 15.0 Hz, CH═CH), 6.76 (2H, d, J = 8.3 Hz, H-3′ and H-5′), 7.05 (2H, d, J = 8.3 Hz, H-2′ and H-6′), 7.5 (10H, m); EIMS *m*/*z* 408 [M$^+$]; *anal*. C 82.10%, H 5.84%, calcd for $C_{28}H_{24}O_3$, C 82.35%, H 5.88%.

5b: colorless crystals (EtOAc/*n*-hexane); mp 161 °C; ^{1}H NMR ($CDCl_3$) δ 5.08 (4H, s, 2 × CH_2), 5.10 (2H, s, CH_2), 6.45 (1H, dd, J = 1.8, 1.8 Hz, H-4), 6.60 (2H, d, J = 8.4 Hz, H-3′ and H-5′), 6.75 (2H, d, J = 1.8 Hz, H-2 and H-6), 6.93 and 7.04 (2H, AB system, J = 15.0 Hz, CH═CH), 7.6−7.2 (15H, m), 7.10 (2H, d, J = 8.4 Hz, H-2′ and H-6′), 7.2−7.6 (15 H); EIMS *m*/*z* 498 [M$^+$]; *anal*. C 84.16%, H 5.94%, calcd for $C_{35}H_{30}O_3$ C 84.34%, H 6.02%.

1c: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 1.75 (3H, s, CH_3), 1.85 (3H, s, CH_3), 4.50 (2H, m, CH_2), 5.5 (1H, dd, J = 7.0, 7.0 Hz, CH═C), 6.20 (1H, s, H-4), 6.58 (2H, s, H-2 and H-6), 6.80 (2H, d, J = 8.3 Hz, H-3′ and H-5′), 6.90 and 7.05 (2H, AB system, J = 15.7 Hz, CH═CH), 7.10 (2H, d, J = 8.3 Hz. H-2′ and H-6′); EIMS *m*/*z* 296 [M$^+$]; *anal*. C 77.35%, H 6.80%, calcd for $C_{19}H_{20}O_3$, C 77.03%, H 6.70%.

2c: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 1.75 (3H, s, CH_3), 1.85 (3H, s, CH_3), 4.5 (2H, m, CH_2CH_2), 5.5 (1H, dd, J = 7.0, 7.0 Hz), 6.32 (1H, s, H-4), 6.58 (1H, s, H-6), 6.65 (1H, s, H-2), 6.82 and 6.98 (2H, AB system, J = 15.8 Hz, CH═CH), 6.90 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.11 (2H, d, J = 8.0 Hz, H-2′ and H-6′); EIMS *m*/*z* 296 [M$^+$]; *anal*. C 77.38%, H 6.58%, calcd for $C_{19}H_{20}O_3$ C 77.03%, H 6.70%.

3c: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 1.71 (6H, s, 2 × CH_3), 1.82 (6H, s, 2 × CH_3), 4.50 (2H, d, J = 6.8 Hz, CH_2), 4.53 (2H, d, J = 6.8 Hz, CH_2), 5.5 (2H, dd, J = 6.8, 6.8 Hz, CH═C), 5.51 (2H, dd, J = 6.8, 6.8 Hz, CH═C), 6.32 (1H, dd, J = 1.8, 1.8 Hz, H-4), 6.58 (1H, d, J = 1.8 Hz, H-6), 6.65 (1H, d, J = 1.8 Hz, H-2), 6.84 and 6.98 (2H, AB system, J = 15.8 Hz, CH═CH), 6.90 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.11(2H, d, J = 8.0 Hz, H-2′ and H-6′); EIMS *m*/*z* 364 [M$^+$], 107, 69; *anal*. C 79.48%, H 7.58%, calcd for $C_{24}H_{28}O_3$, C 79.12%, H 7.69%.

5c: colorless syrup; ^{1}H NMR ($CDCl_3$) δ 1.78 (6H, s, 2 × CH_3), 1.88 (12H, s, 3 × CH_3), 4.48 (2H, d, J = 6.9 Hz, CH_2), 4.51 (4H, d, J = 6.9 Hz, CH_2), 5.5 (3H, t, J = 6.9, 6.9 Hz, CH═C), 6.41(1H, dd, J = 2.0, 2.0 Hz, H-4), 6.88 and 7.01 (2H, AB system, J = 16.0 Hz, CH═CH), 6.89 (2H, d, J = 2.0 Hz, H-2 and H-6), 6.89 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.01 (2H, d, J = 8.0 Hz, H-2′ and H-6′); EIMS *m*/*z* 432 [M$^+$], 107, 69.

6: colorless syrup; ^{1}H NMR ($CDCl_3$) δ 1.75 (3H, s, CH_3), 1.85 (3H, s, CH_3), 3.38 (2H, d, J = 6.9 Hz, CH_2), 5.5 (1H, d, J = 6.9 Hz, CH═C), 6.7 (2H, s, H-2 and H-6), 6.95 (2H, d, J = 8.4 Hz, H-3′ and H-5′), 6.95 and 7.05 (2H, AB system, J = 15.8 Hz, CH═CH), 7.48 (2H, d, J = 8.4 Hz, H-2′ and H-6′); EIMS *m*/*z* 296 [M$^+$]; *anal*. C 77.38%, H 6.58%, calcd for $C_{19}H_{20}O_3$, C 77.03%, H 6.76%.

8a: colorless crystals (EtOAc/*n*-hexane); mp 61 °C; ^{1}H NMR-(DMSO-d_6 + D_2O) δ 2.68 (4H, m, CH_2CH_2), 3.67 (3H, s, CH_3), 6.03 (1H, dd, J = 2.0, 2.0 Hz, H-4), 6.08 (2H, d, J = 2.0 Hz, H-2 and H-6), 6.85 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.15 (2H, d, J = 8.0 Hz, H-2′ and H-6′); EIMS *m*/*z* 244 [M$^+$], 123, 121; *anal*. C 61.30%, H 6.66%, calcd for $C_{15}H_{16}O_3$, C 61.48%, H 6.56%.

9a: colorless syrup; ^{1}H NMR (DMSO-d_6) δ 2.7 (4H, m, CH_2-CH_2), 3.65 (3H, s, CH_3), 6.12 (1H, dd, J = 2.0, 2.0 Hz, H-4), 6.2 (2H, d, J = 2.0 Hz, H-2 and H-6), 6.64 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 6.99 (2H, d, J = 8.5 Hz, H-2′ and H-6′), 9.1 (1H, bs, OH, exchanges with D_2O), 9.2 (2H, bs, 2 × OH, exchanges with D_2O); EIMS *m*/*z* 244 [M$^+$], 123, 121; *anal*. C 61.32%, H 6.60%, calcd for $C_{15}H_{16}O_3$, C 61.48%, H 6.56%.

10a: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 2.80 (4H, m, CH_2CH_2), 3.21 (3H, s, CH_3), 3.28 (3H, s, CH_3), 6.23 (2H, s, H-2 and H-6), 6.28 (1H, s, H-4), 6.82 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 7.08 (2H, d, J = 8.5 Hz, H-2′ and H-6′); EIMS *m*/*z* 258 [M$^+$]; *anal*. C 74.58%, H 6.68%, calcd for $C_{16}H_{18}O_3$, C 74.42%, H 6.98%.

11a: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 2.8 (4H, m, CH_2CH_2), 3.75 (3H, s, CH_3), 3.76 (3H, s, CH_3), 6.33 (2H, s, H-2 and H-6), 6.34 (1H, s, H-4), 6.73 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.34 (2H, d, J = 8.0 Hz, H-2′ and H-6′); EIMS *m*/*z* 258 [M]$^+$; *anal*. C 74.58%, H 6.68%, calcd for $C_{16}H_{18}O_3$, C 74.42%, H 6.98%.

12a: colorless crystals (EtOAc/*n*-hexane); mp 61−63 °C; ^{1}H NMR ($CDCl_3$) δ 2.87 (4H, m, CH_2CH_2), 3.8 (6H, s, 2 × CH_3), 3.91 (3H, s, CH_3), 6.34−6−37 (3H, H-2, H-4, H-6), 6.85 (2H, d, J = 8.3 Hz, H-3′ and H-5′), 7.11 (2H, d, J = 8.3 Hz, H-2′ and H-6′); EIMS *m*/*z* 272 [M$^+$], 151, 121; *anal*. C 75.23%, H 7.2%, calcd for $C_{17}H_{20}O_3$, C 75.0%, H 7.35%.

8b: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 2.87 (4H, bs, CH_2CH_2), 5.0 (2H, s, CH_2), 6.19 (1H, dd, J = 2.0, 2.0 Hz, H-4), 6.24 (2H, d, J = 2.0 Hz, H-2 and H-6), 6.90 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 7.09 (2H, d, J = 8.5 Hz, H-2′ and H-6′), 7.35−7.45 (5H, m); EIMS *m/z* 320 [M^+]; *anal*. C 78.58%, H 7.58%, calcd for $C_{21}H_{20}O_3$, C 78.75%, H 7.94%.

9b: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 2.79 (4H, bs, CH_2CH_2), 5.0 (2H, s, CH_2), 6.24 (1H, d, J = 2.3 Hz, H-2), 6.34 (1H, dd, J = 2.3, 2.3 Hz, H-4), 6.41 (1H, d, J = 8.5 Hz, H-3′ and H-5′), 6.42 (1H, d, J = 2.3 Hz, H-2), 7.01 (2H, d, J = 8.0 Hz, H-2′ and H-6′), 7.35−7.5 (5H, m); EIMS *m/z* 320[M^+]; *anal*. C 78.35%, H 7.90%, calcd for $C_{21}H_{20}O_3$, C 78.75%, H 7.94%.

10b: colorless syrup; ^{1}H NMR ($CDCl_3$ + D_2O) δ 2.85 (4H, s, CH_2CH_2), 5.02 (2H, s, CH_2), 5.05 (2H, s, CH_2), 6.29 (1H, d, J = 2.3 Hz, H-6), 6.35 (1H, dd, J = 2.3, 2.3 Hz, H-4), 6.45 (1H, d, J = 2.3 Hz, H-2), 6.93 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.12 (2H, d, J = 8.0 Hz, H-2′ and H-6′), 7.35−7.5 (10H, m); EIMS *m/z* 410 [M^+], 197; *anal*. C 81.58%, H 6.18%, calcd for $C_{28}H_{26}O_3$, C 81.95%, H 6.34%.

11b: colorless syrup; ^{1}H NMR ($CDCl_3$) δ 2.85 (4H, s, CH_2-CH_2), 5.02 (2H, s, CH_2), 5.05 (2H, s, CH_2), 6.45 (2H, d, J = 2.2 Hz, H-2 and H-6), 6.5 (1H, dd, J = 2.2, 2.2 Hz, H-4), 6.76 (2H, d, J = 8.4 Hz, H-3′ and H-5′), 7.05 (2H, d, J = 8.4 Hz, H-2′ and H-6′), 7.35−7.5 (10H, m); EI MS *m/z* 410 [M^+], 197; *anal*. C 81.55%, H 6.20%, calcd for $C_{28}H_{26}O_3$, C 81.95%, H 6.34%.

12b: colorless crystals (EtOAc/*n*-hexane); mp 81−83 °C; ^{1}H NMR ($CDCl_3$ + D_2O) δ 2.85 (4H, s, CH_2CH_2), 5.0 (4H, s, 2 × CH_2), 5.4 (2H, s, CH_2), 6.43 (2H, d, J = 2.2 Hz, H-2 and H-6), 6.46 (1H, dd, J = 2.2, 2.2 Hz, H-4), 6.9 (2H, d, J = 8.4 Hz, H-3′ and H-5′), 7.07 (2H, d, J = 8.4 Hz, H-2′ and H-6′), 7.35−7.5 (15H, m); EIMS *m/z* 500 [M^+], 303, 197; *anal*. C 83.97%, H 6.38%, calcd for $C_{35}H_{32}O_3$, C 84.0%, H 6.4%.

8c: colorless syrup; ^{1}H NMR ($CDCl_3$): δ 1.79 (3H, s, CH_3), 1.81 (3H, s, CH_3), 2.78 (2H, s, disappears with D_2O), 2.86 (4H, s, CH_2CH_2), 4.5 (2H, d, J = 7.0 Hz), 5.0 (1H, dd, J = 7.0, 7.0 Hz), 6.12 (1H, dd, J = 2.0, 2.0 Hz, H-4), 6.15 (2H, d, J = 2.0 Hz, H-2 and H-6), 6.7 (2H, d, J = 8.1 Hz, H-3′ and H-5′), 7.0 (2H, d, J = 8.1 Hz, H-2′ and H-6′); EIMS *m/z* 298 [M^+], 107, 69; *anal*. C 76.28%, H 7.18%, calcd for $C_{19}H_{22}O_3$, C 76.51%, H 7.38%.

9c: colorless syrup; ^{1}H NMR ($CDCl_3$) δ 1,79 (3H, s), 1.8 (3H, s, CH_3), 2.80 (4H, m, CH_2CH_2), 4.5 (2H, d, J = 7.3 Hz, CH_2), 5.5 (1H, dd, J = 7.3, 7.3 Hz, CH═C), 6.28 (1H, dd, J = 2.1, 2.1 Hz, H-4), 6.3 (1H, d, J = 2.1 Hz, H-6), 6.4 (1H, d, J = 2.1 Hz, H-2), 6.75 (2H, d, J = 8.7 Hz, H-3′ and H-5′), 7.04 (2H, d, J = 8.7 Hz, H-2′ and H-6′); EIMS *m/z* 298 [M^+], 107, 69; *anal*. C 76.58%, H 7.38%, calcd for $C_{19}H_{22}O_3$, C 76.51%, H 7.38%.

13: colorless syrup; ^{1}H NMR ($CDCl_3$) δ 1.83 (6H, s, CH_3), 2.80 (4H, m, CH_2CH_2), 3.31 (2H, d, J = 7.4 Hz, CH_2), 5.10 (1H, dd, J = 7.4, 7.4 Hz, CH═C), 6.20 (2H, s, H-2 and H-6), 6.75 (2H, d, J = 8.7 Hz, H-3′ and H-5′), 7.04 (2H, d, J = 8.7 Hz, H-2′ and H-6′); EIMS *m/z* 298 [M^+], 107, 69; *anal*. C 76.38%, H 7.58%, calcd for $C_{19}H_{22}O_3$, C 76.51%, H 7.38%.

Biological Tests. Cell Cultures. The F11 cell line[24] (mouse neuroblastoma N18TG-2 x rat DRG; Platika et al., 1985) was cultured in Dulbecco's modified Eagle's medium (Hyclone), containing 4.5 g L^{-1} of glucose and 10% of fetal calf serum (Hyclone). The cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2. Cells were plated onto 35 mm Petri dishes (Costar) and then used for patch clamp experiments.

The standard pipet solution with a $[Ca^{2+}]_i$ of 10^{-7} M (*p*Ca 7) was composed of (mM) potassium aspartate 130, NaCl 10, $MgCl_2$ 2, $CaCl_2$ 1.3, EGTA 10, Hepes 10, MgATP 1.

I_{DR} recordings were made in a standard extracellular solution containing (mM) NaCl 130, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, Hepes 10, glucose 5, pH 7.3. Tetraethylammonium chloride (TEA-Cl, 20 mM) was added to standard extracellular solution to block DR current; to respect the osmolarity, an equimolar amount of NaCl was removed from the solution. I_{ERG} was evoked with an external high potassium solution ($[K^+]_o$ = 40 mM), in which NaCl was replaced by an equimolar amount of KCl. The class III antiarhythmic agent WAY 123.398, a selective blocker of erg current, was kept as a 1 mM stock solution in distilled water and applied at a final concentration of 1 μM.

Resveratrol derivatives were prepared from 300 mM stock solutions in DMSO. In the final solution DMSO was present in the ratio of 1:2000, and it did not affect the currents. For the final concentration of the single compounds, see Table 1.

Cells plated on dishes were incubated at 37 °C for about 24 h. Patch clamp experiments were performed at room temperature with an amplifier MultiClamp 700A (Axon Instruments, CA) and 1200 Digidata (Axon Instruments, CA). The whole cell configuration of the patch clamp technique was employed using pipets (borosilicate glass; Clark Instruments UK) whose resistance was in the range 2−4 MΩ. Extracellular solutions were delivered through a 9-hole (0.6 mm) remote-controlled linear perfusion manifold placed near the cell under study. Cell capacitance and series resistance were compensated (85−95%) before each protocol run. I_{DR} were recorded using a depolarizing stimulation at 60 mV from a holding potential of −60 mV; for I_{DR} the cells were perfused with a physiologic low potassium solution. DR traces were TEA-isolated (data not shown). ERG tail currents were elicited at a voltage of −120 mV after 15 s preconditioning at 0 mV; ERG traces were recorded using an external high potassium solution and were WAY 123.398-isolated (data not shown). For data acquisition and analysis, pClamp 8 software (Axon Instruments, CA) and Origin 6 (Microcal Software, Northampton, MA) were routinely used.

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(15) Analysis of the potential energy surface (using the AM1 Hamiltonian[25] as implemented in MOPAC[26]) evidenced an overall quasi coplanar topography for the lowest energy conformation of *trans*-resveratrol; however, different orientations of the phenyl rings were accessible at a low energy cost (Δ*E* ca. 0.1−1.8 kcal/mol) also including the perpendicular ones with respect to the stilbenic double bond. The *cis*-configuration of the stilbenic double bond destabilizes the coplanar arrangement and forces one of the phenyl rings in an almost perpendicular orientation with respect to the stilbenic double bond. Analysis of dihydroresveratrol evidenced the presence of several *quasi*-transoid conformations of comparable energy (Δ*E* between 0.5 and 1.7 kcal/mol) which were different with respect to the orientation of the phenyl rings. *Cisoid* conformations for dihydroresveratrol were about 5−9 kcal/mol less stable, depending on the orientation of the phenyl rings. Dihydroresveratrol could therefore be able to mimic *trans*-resveratrol at a very low energy cost. Higher energy (5−9 kcal/mol in the AM1 scale) would be required to mimic *cis*-resveratrol, a value, however, that, at least for some orientations of the phenyl rings, should not prevent the molecule from adopting this conformation if required for activity.

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