Synthesis and Biological Evaluation of Resveratrol and Analogues as Apoptosis-Inducing Agents

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Resveratrol **1** (3,4′,5-trihydroxy-*trans*-stilbene), a phytoalexin present in grapes and other food products, has recently been suggested as a potential cancer chemopreventive agent based on its striking inhibitory effects on cellular events associated with cancer initiation, promotion, and progression. This triphenolic stilbene has also displayed in vitro growth inhibition in a number of human cancer cell lines. In this context, a series of *cis*- and *trans*-stilbene-based resveratrols were prepared with the aim of discovering new lead compounds with clinical potential. All the synthesized compounds were tested in vitro for cell growth inhibition and the ability to induce apoptosis in HL60 promyelocytic leukemia cells. The tested *trans*-stilbene derivatives were less potent than their corresponding cis isomers, except for *trans*-resveratrol, whose cis isomer was less active. The best results were obtained with compounds **11b** and **7b**, the *cis*-3,5-dimethoxy derivatives of rhapontigenin **10a** (3,5,3′-trihydroxy-4′methoxy-*trans*-stilbene) and its 3′-amino derivative **10b**, respectively, which showed apoptotic activity at nanomolar concentrations. The corresponding trans isomers **12b** and **8b** were less active both as antiproliferative and as apoptosis-inducing agents. Of interest, **11b** and **7b** were active toward resistant HL60R cells and their activity was higher than that of several classic chemotherapeutic agents. The flow cytometry assay showed that at 50 nM compounds **7b** or **11b** were able to recruit almost all cells in the apoptotic sub-G_0-G_1 peek, thus suggesting that the main mechanism of cytotoxicity of these compounds could be the activation of apoptosis. These data indicate unambiguously that structural alteration of the stilbene motif of resveratrol can be extremely effective in producing potent apoptosis-inducing agents.

Introduction

Stilbene-based compounds are widely represented in nature and have become of particular interest to chemists and biologists because of their wide range of biological activities.[1,2] Stilbene itself does not occur in nature, but hydroxylated stilbenes have been found in a multitude of medicinal plants. Resveratrol **1** (3,4′,5-trihydroxy-*trans*-stilbene, Figure 1), a phytoalexin present in grapes and other food products,[2−4] has been reported to play a role in the prevention of heart diseases associated with red wine consumption because it inhibits platelet aggregation,[5] alters eicosanoid synthesis,[5,6] and modulates lipid and lipoprotein metabolism.[7,8] Resveratrol has recently been suggested as a potential cancer chemopreventive agent based on its striking inhibitory effects on cellular events associated with cancer initiation, promotion, and progression.[9] This triphenolic stilbene possesses strong antioxidative[10] and antiinflammatory[6] activities that may contribute to its chemopreventive/chemoprotective properties. In addition to the anticancer-promoting activity, **1** has displayed in vitro growth inhibition in a number of human cancer cell lines.[11]

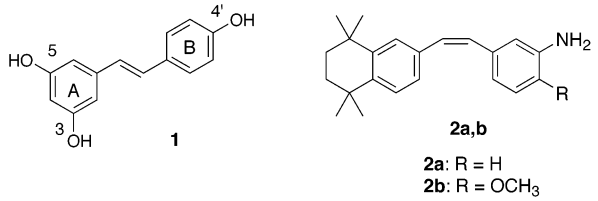

Figure 1. Resveratrol (**1**) and stilbene-related proapoptotic compounds.

The simplicity of resveratrol, associated with its interesting anticancer activity, offers promises for the rational design of new chemotherapeutic agents, and in this context, efforts have recently been devoted to the detailed study of structure−activity relationships (SAR) of this type of substituted stilbene derivative.[12−15] Resveratrol has also been shown to induce apoptosis in different cancer cell lines.[16−18] Since reduced apoptosis (programmed cell death) has been implicated in the development and progression of malignant tumors[19,20] and in the occurrence of chemoresistant phenotypes,[21−24] resveratrol-induced apoptosis might therefore contribute to its antitumor activity.

Because we recently started a study aimed at evaluating the apoptotic activity of a novel class of stilbene compounds structurally related to vitamin A[25,26] and because some derivatives (**2a**,**b**; Figure 1) were found to be endowed with potent apoptotic activity in both

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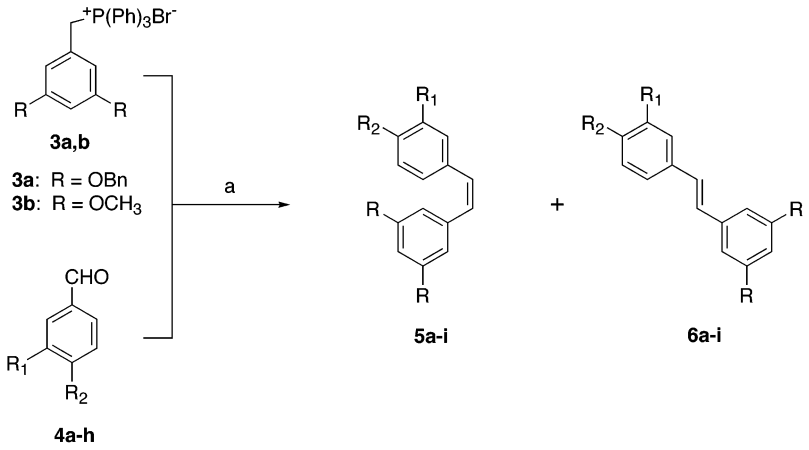
Scheme 1[a]



4a: $R_1 = H$, $R_2 = OBn$
4b: $R_1 = OBn$, $R_2 = OCH_3$
4c: $R_1 = NO_2$, $R_2 = OCH_3$
4d: $R_1 = H$, $R_2 = OTBDMS$
4e: $R_1 = OTBDMS$, $R_2 = OCH_3$
4f: $R_1 = OCH_3$, $R_2 = OTBDMS$
4g: $R_1 = R_2 = OTBMDS$
4h: $R_1 = H$, $R_2 = NO_2$

5a,**6a**: $R = R_2 = OBn$, $R_1 = H$
5b,**6b**: $R = R_1 = OBn$, $R_2 = OCH_3$
5c,**6c**: $R = OBn$, $R_1 = NO_2$, $R_2 = OCH_3$
5d,**6d**: $R = R_2 = OCH_3$, $R_1 = NO_2$
5e,**6e**: $R = OCH_3$, $R_1 = H$, $R_2 = OTBDMS$
5f,**6f**: $R = R_2 = OCH_3$, $R_1 = OTBDMS$
5g,**6g**: $R = R_1 = OCH_3$, $R_2 = OTBDMS$
5h,**6h**: $R = OCH_3$, $R_1 = R_2 = OTBDMS$
5i,**6i**: $R = OCH_3$, $R_1 = H$, $R_2 = NO_2$

[a] Reagents and conditons: (a) *n*-BuLi, THF, −78 °C.

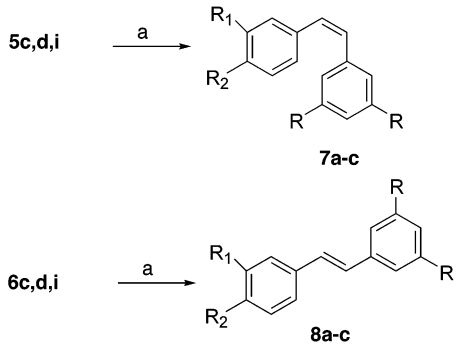
normal and MDR cell lines,[27,28] we believed that it would be further informative to deeply explore other classes of related stilbenes as a logical starting point in the quest of novel anticancer chemotherapeutics. Therefore, on the bases of these premises, we prepared various derivatives in order to explore new areas of structural alteration of resveratrol. A variety of substituents were introduced at positions 3′ and 4′ (OH, NH_2, OCH_3), and the replacement of the 3,5-hydroxy groups with methoxy functions was also investigated in this series, together with the determination of the effects of double bond isomerization (*E* and *Z* geometries).

All the synthesized compounds were tested in vitro for cell growth inhibition and the ability to induce apoptosis in HL60 premyelocytic leukemia cells. Two of them (**7b** and **11b**) showed an activity comparable to that of daunorubicin and proved to be potent apoptosis-inducing agents also active in multidrug-resistant (MDR) cell lines, in particular resistant to the apoptotic effects of several chemoterapeutic drugs such as daunorubicin, etoposide, and citarabine.

Chemistry

Preparation of the stilbene derivatives **5a**−**i** and **6a**−**i** was accomplished by means of the Wittig reaction between the appropriate aromatic aldehydes **4a**−**h** and the suitable aromatic ylides, in turn obtained from the phosphonium salts **3a**,**b** (Scheme 1). The reaction produced a mixture of *E* and *Z* isomers (7:3) purified and separated by flash chromatography. Spectral data of known and new compounds were consistent with those of stilbene derivatives previously described in the literature. Bond geometry of compounds was established by comparison of the ^1H NMR of the isomeric pairs. *Z* isomers showed the olefinic protons at 0.3−0.4 ppm higher field compared with the *E* isomers. The coupling constants of the vinylic protons of the *E* isomers were about 16 Hz, whereas the *Z* isomers showed coupling constants of 12 Hz. Nitro derivatives **5c**,**d**,**i** and the corresponding *E* isomers **6c**,**d**,**i** were then reduced with sodium dithionite to give the respective amino derivatives **7a**−**c** and **8a**−**c** in satisfactory yields (Scheme 2).

Scheme 2[a]

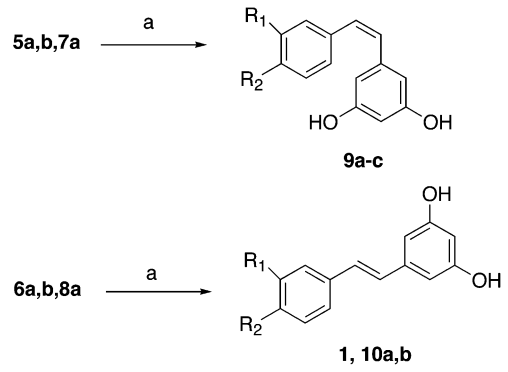


7a,**8a**: $R = OBn$, $R_1 = NH_2$, $R_2 = OCH_3$
7b,**8b**: $R = R_2 = OCH_3$, $R_1 = NH_2$,
7c,**8c**: $R = OCH_3$, $R_1 = H$, $R_2 = NH_2$

[a] Reagents and conditons: (a) $Na_2S_2O_4$, acetone/H_2O, 50 °C.

Finally, deprotection of the benzyloxy derivatives **5a**,**b**, **7a** and **6a**,**b**, **8a** performed with a complex prepared from aluminum chloride and *N*,*N*-dimethylaniline gave the desired compounds **9a**−**c** and **1**, **10a**,**b** (Scheme 3), whereas removal of the *tert*-butyldimethylsilyl (TBDMS) group from stilbenes **5e**−**h** and **6e**−**h** using tetrabutylammonium fluoride afforded compounds **11a**−**d** and **12a**−**d** in appreciable yields (Scheme 4).

Scheme 3[a]



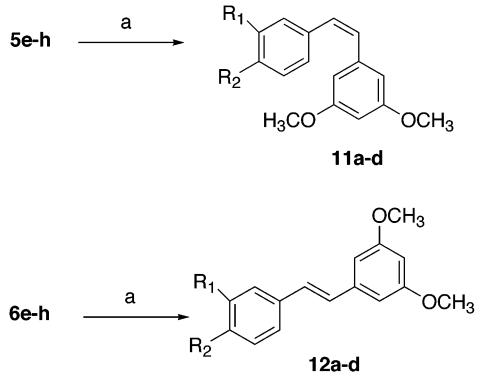
9a,10a: $R_1 = OH$, $R_2 = OCH_3$

9b,10b: $R_1 = NH_2$, $R_2 = OCH_3$

1,9c: $R_1 = H$, $R_2 = OH$

[a] Reagents and conditons: (a) $AlCl_3$, $(CH_3)_2NC_6H_5$, CH_2Cl_2, 0 °C.

Scheme 4[a]



11a,12a: $R_1 = H$, $R_2 = OH$

11b,12b: $R_1 = OH$, $R_2 = OCH_3$

11c,12c: $R_1 = OCH_3$, $R_2 = OH$

11d,12d: $R_1 = R_2 = OH$

[a] Reagents and conditons: (a) Bu_4NF, THF, room temp.

Results and Discussion

The interesting anticancer properties of resveratrol combined with our previous finding concerning the remarkable apoptotic activity associated with the stilbene motif of arotinoids[25−28] prompted us to investigate a series of resveratrol structural modifications as an extension of our quest for novel stilbene-based anticancer chemotherapeutics. The new resveratrol-based analogues were prepared with the aim of discovering new lead compounds with clinical potential. Particular attention was focused on the identification of new structural features for apoptotic activity with the main objective of investigating the importance of resveratrol's phenolic moieties in conferring cell death induction activity.

All the new synthesized compounds were assayed in vitro for cell growth inhibition and for their ability to induce apoptosis in HL60 cells. As shown in Table 1, *trans*-resveratrol **1** was effective as an antiproliferative agent in HL60 cells with an IC_{50} of 5 μM. In contrast, it was less effective as an apoptosis-inducing agent, showing an AC_{50} of only 50 μM. Quite surprisingly, the *cis*-resveratrol **9c** was scarcely active, being its IC_{50} was 10 times higher than that of *trans*-resveratrol and having its AC_{50} higher than 200 μM. This was somewhat surprising to us because the potent apoptotic activity of other *cis*-stilbenoids is well documented.[27,28] The natural rhapontigenin **10a** and its 3′-amino derivative **10b** had low cytotoxic activity and the respective cis isomers **9a** and **9b** were only slightly more active. Pterostilbene **12a**, the natural 3,5-dimethoxy analogue of resveratrol, was less active as antiproliferative and apoptosis-inducing agent than the parent compound. However, unlike the cis derivatives **9a**−**c** described above, the cis isomer **11a** showed interesting cytotoxicity associated with potent apoptotic activity ($IC_{50} = 2$ μM; $AC_{50} = 5$ μM). Indeed, although this compound had an antiproliferative activity of only 2.5-fold higher than *trans*-resveratrol, its ability to induce programmed cell death was 10-fold higher.

On the basis of these results, it appeared convenient to investigate the synthesis of new resveratrol analogues bearing the 3,5-dimethoxy motif at the A phenyl ring; moreover, amino, methoxy, and hydroxy moieties were considered at the 3′- and 4′-positions. The cytotoxic and the apoptotic activities were both diminished when the *cis*- and *trans*-pterostilbene analogues were tested as 4′-O-TBDMS (compounds **5e** and **6e**). When the 4′-OH group was replaced by the amino function, the activity of the trans isomer **8c** was higher than that of the parent compound **12a**, whereas the cis derivative **7c** showed an activity comparable to the *cis*-pterostilbene (**11a**). Additionally, it is worth noting that in switching the methoxy and hydroxy moieties as in compounds **11b** and **12b** vs **11c** and **12c**, we may greatly influence the activity, given that derivatives 3′-hydroxy-4′-methoxy (**11b** and **12b**) appear more active than the 3′-methoxy-4′-hydroxy compounds **11c** and **12c**. A positive effect if compared with pterostilbene **12a** was obtained by the introduction of a second hydroxy function at the 3′-position (compounds **11d** and **12d**), which resulted in about a 40- to 70-fold increase in both antiproliferative and apoptotic activity. The best results in this series were obtained with compounds **11b** and **7b**, the *cis*-3,5-dimethoxy derivatives of rhapontigenin **10a**, and its 3′-amino derivative **10b**. They were active at nanomolar concentrations (IC_{50} and AC_{50} of about 35 nM), whereas the corresponding trans isomers **12b** and **8b** were less active as antiproliferative and apoptosis-inducing agents.

As shown in Table 1, compounds **7b** and **11b** are the only compounds having similar IC_{50} and AC_{50}. This suggests that the main mechanism of cytotoxicity of these compounds could be the activation of apoptosis. The flow cytometry assay conducted in HL60 cells after staining with propidium iodide showed that at 50 nM compounds **7b** and **11b** were able to recruit almost all cells in the apoptotic sub-G_0-G_1 peak (Figure 2). The activity of these compounds was higher than several classic chemotherapeutic agents commonly used in clinical practice, such as etoposide, citarabine, 5-fluoruracil, and *cis*-platinum, but lower than that shown by daunorubicin (Table 2). However, unlike daunorubicin, etoposide, and citarabine, compounds **7b** and **11b** were also active in HL60R cells, a cell line derived from HL60 and expressing the multidrug-resistance phenotype (Table 3). It is worth noting that the classical MDR

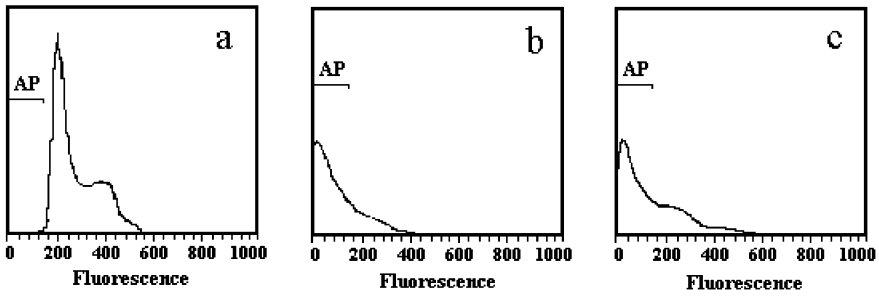
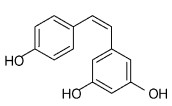
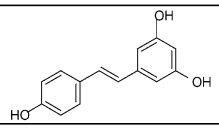
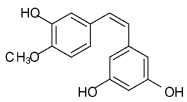
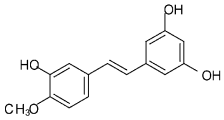
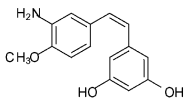
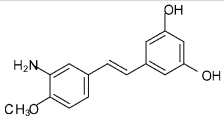
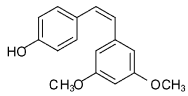
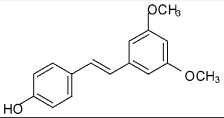
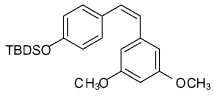
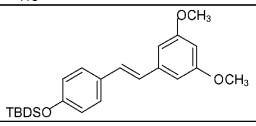
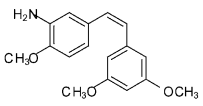
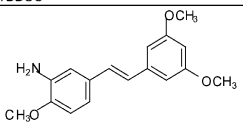
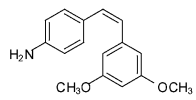
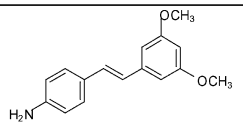
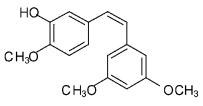
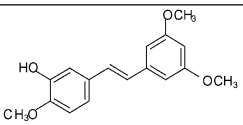
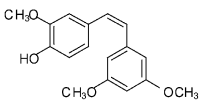
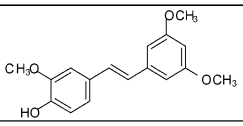
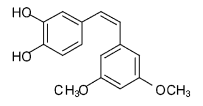
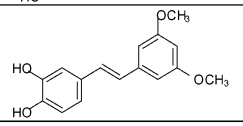


Figure 2. Flow cytometry determination of apoptosis. HL60 cells were exposed to 50 nM of **7b** (b) or **11b** (c). After 48 h, the cells were collected and stained with PI. Apoptosis was determined by FACscan flow cytometry assay using the CellQuest software. The hypodiploid sub-G_0-G_1 peak, designed as AP, represents cells undergoing apoptosis. The data for the control are shown in (a).

Table 1. Antiproliferative (IC_{50}) and Apoptotic-Inducing Activity (AC_{50}) of *trans*-Resveratrol **1** and Its Derivatives in the HL60 Cell Line: Comparison of Cis and Trans Compounds

N.	Formula	IC_{50} μM	AC_{50} μM	N.	Formula	IC_{50} μM	AC_{50} μM
9c		42±6.4	>200	**1**		5 ± 0.9	50 ± 8.2
9a		40 ± 5.3	70 ± 7.6	**10a**		48 ± 8.8	> 200
9b		40 ± 6.1	68 ± 5.9	**10b**		80 ± 11	> 200
11a		2 ± 0.4	5 ± 0.8	**12a**		35 ± 6	70 ± 8.2
5e		50 ± 11	> 200	**6e**		48 ± 7.7	> 200
7b		0.03±0.005	0.04±0.0012	**8b**		4±0.8	8±1.5
7c		2.5±0.2	8±0.8	**8c**		4±0.3	15±2
11b		0.03±0.0012	0.04±0.0045	**12b**		0.7±0.09	0.9±0,1
11c		20±4	38±2.5	**12c**		25±8	40±10
11d		0.05±0.008	0.1±0.003	**12d**		0.8±0.1	1±0.09

reversing agent verapamil was unable to completely reverse the resistance of HL60R cells toward drugs such as daunorubicin and etoposide (Table 4). On the contrary, the IC_{50} and AC_{50} of compounds **7b** and **11b** evaluated in resistant HL60R cells were almost similar to those obtained in sensitive parental cells, also without use of verapamil. Resistance to chemotherapeutic agents is a major problem in cancer therapy. This phenomenon

Table 2. IC_{50} and AC_{50} of Daunorubicin, Etoposide, Citarabine, 5-Fluorouracil, and *cis*-Platinum in the HL60 Cell Line: Comparison with **7b** and **11b**

compd	IC_{50} (μM)	AC_{50} (μM)
daunorubicin	0.005 ± 0.0017	0.04 ± 0.006
etoposide	0.123 ± 0.07	0.45 ± 0.08
citarabine	0.08 ± 0.01	0.15 ± 0.05
5-fluorouracil	1.5 ± 0.3	6.5 ± 0.8
cis-platinum	0.6 ± 0.08	1.2 ± 0.1
7b	0.03 ± 0.005	0.04 ± 0.0012
11b	0.03 ± 0.0012	0.04 ± 0.0045

Table 3. IC_{50} and AC_{50} of **7b** and **11b** in the HL60R Cell Line: Comparison with Daunorubicin, Etoposide, and Citarabine

compd	IC_{50} (μM)	AC_{50} (μM)
7b	0.035 ± 0.002	0.05 ± 0.0012
11b	0.025 ± 0.003	0.03 ± 0.0045
daunorubicin	1.5 ± 0.2	10 ± 8
etoposide	6.5 ± 0.9	15 ± 3.2
citarabine	0.35 ± 0.04	8 ± 12

Table 4. IC_{50} of **7b**, **11b**, Daunorubicin, and Etoposide in Combination with 5 μM Verapamil in the HL60R Cell Line

compd	IC_{50} (μM)
7b	0.032 ± 0.004
11b	0.02 ± 0.003
daunorubicin	0.04 ± 0.003
etoposide	0.5 ± 0.008

has often been ascribed to the overexpression of multidrug transporters on the surface membrane of cancer cells such as P-glycoprotein (which confer the classic MDR phenotype) or to qualitative and quantitative alterations in anticancer drug targets such as topoisomerases and thimidylate synthase.[29−31]

Several MDR-reversing agents are known, and they are currently investigated in various stages of clinical development. However, despite the interesting results obtained in vitro with MDR modulators such as calcium channel blockers, antimalarials, antibiotics, cyclosporins, hormones, and others and despite occasional responses in patients with hematologic malignancies or non-small-cell lung cancer,[32−34] several limitations to the use of these modulators are well-known, including multiple cellular mechanisms of resistance, alterations in pharmacokinetics of cytotoxic agents, and clinical toxicities.[35] Therefore, the development of compounds active toward sensitive and MDR cells such as **7b** and **11b** could be a useful tool for the treatment of malignancies expressing the MDR phenotype.

Additionally, a general conclusion arising from the SAR study of resveratrol analogues based on their apoptotic activity on the HL60 cell line can be offered. All the tested *trans*-stilbene analogues were less potent than their corresponding cis isomers excepting *trans*-resveratrol, whose cis counterpart was inactive. Therefore, although we recently hypothesized that apoptotic activity may be associated with the cis stereochemistry of stilbenoids,[25,27] this cannot be considered an absolute prerequisite. However, the cis configuration of the stilbene architecture seems to be at least an important feature in conferring apoptotic activity. The low activity of compounds **9a**−**c**, compared with the good activity shown by the corresponding 3,5-dimethoxy derivatives **11a**,**b** and **7b**, suggests that introduction of the 3,5-dimethoxy motif may be required for good proapoptotic action. Moreover, the greater activity of 3′,4′-dihydroxy analogue **11d** with respect to the monohydroxylated derivative **11a** pointed out the importance of a hydroxy function at the C-3′ position. This finding was also supported by comparing the activity of compound **11b** (AC_{50} = 0.04 μM) and its isomer **11c** (AC_{50} = 38 μM) in which the 3′-hydroxy function was switched with the methoxy group at C-4′. Finally, replacement of a 3′-hydroxy group with the amino function did not affect the proapoptotic activity. Thus, the introduction of either a hydroxy or an amino group at the C-3′ position and a methoxy group at C-3, C-4′, and C-5 produces the greatest proapoptotic effects as seen in compounds **7b** and **11b**.

In summary, structural alteration of the natural *trans*-resveratrol offered the discovery of two potent apoptosis-inducing agents. The activity of these compounds was higher than that of several classic chemotherapeutic agents, and they could be useful for treatment of malignancies expressing the MDR phenotype. The cis stereochemistry of the novel stilbenoids seems to be associated with their potent apoptotic activity. Consequently, because the *cis*-resveratrol does not possess activity, the interpretation of these data does not appear straightforward. One possibility is that the activity of **7b** and **11b** could be related, in some ways, to their structural similarity to other known natural stilbenes such as combretastatine A4 and its analogues.[36] However, of note, our data suggest unambiguously that structural alteration of the stilbene motif of resveratrol can be extremely effective in producing potent apoptosis-inducing agents, and our work is continuing in the exploration of structurally restricted derivatives.

Materials and Methods

All solvents were redistilled prior to use. All reactions were carried out under an inert atmosphere. Solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Reactions were monitored by thin-layer chromatography (TLC) on precoated silica gel plates (Kieselgel 60 F_{254}, Merck). Flash column chromatographies were performed on silica gel (particle size of 40−63 μM, Merck). Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian VXR 300 instruments with $CDCl_3$ (TMS as internal reference) as solvent unless otherwise noted. IR spectra were obtained on a Nicolet Avatar 320 E.S.P. instrument; ν_{max} is expressed in cm^{-1}. The elemental composition of compounds agreed to within ±0.4% of the calculated value. When the elemental analysis is not included, crude compounds were used in the next step without further purification.

Compounds **4a** and **4b** were obtained by benzylation of commercially available 4-hydroxybenzaldehyde and 3-hydroxy-4-methoxybenzaldehyde, respectively. Compounds **4d**,[12] **4e**,[37] **4f**,[38] and **4g**[38] were obtained from the commercially available 4-hydroxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, and 3,4-dihydroxybenzaldehyde, respectively, by protection of phenols with the *tert*-butyldimethylsilyl group (TBDMS) following standard procedure.

General Procedure for Preparation of Stilbenes 5a−i and 6a−i. To the phosphonium bromide salt **3a**,**b** (1.0 equiv) in anhydrous tetrahydrofuran at −78 °C was added *n*-butyllithium (2 M in hexanes, 1.0 equiv), and the resulting red solution was stirred under nitrogen for 2 h. A solution of aldehydes **4a**−**h** (1.0 equiv) in tetrahydrofuran was added dropwise over 30 min, and the mixture was stirred for 2−6 h

at room temperature. The resulting suspension was poured into water and extracted with dichloromethane. The organic phase was washed with brine, and removal of the solvent in vacuo afforded a mixture of the *cis*/*trans*-stilbenes **5a**−**i** and **6a**−**i** that were separated by flash chromatography (9.7:0.3 petroleum ether/ethyl acetate). The *cis*-stilbenes were eluted first, followed by the trans isomers.

3,4′,5-Tri(benzyloxy)stilbenes (5a, 6a). Reaction of 3,5-di(benzyloxy)benzyl]triphenylphosphonium bromide **3a**[39] and 4-benzyloxybenzaldehyde **4a** (1.2 g, 5.65 mmol) gave a mixture of *cis*-stilbene **5a** and the *trans*-isomer **6a**. **5a**: 0.72 g (15% yield); light-yellow oil; ^{1}H NMR δ 4.96 (s, 4H), 5.10 (s, 2H), 6.62 (m, 5H), 6.94 (m, 2H), 7.27−7.46 (m, 17H). **6a**: 0.43 g (26% yield); light-yellow oil; ^{1}H NMR δ 5.07 (s, 2H), 5.15 (s, 4H), 6.30−6.40 (m, 2H), 6.50 (s, 1H), 6.65 (s, 1H), 6.85 (m, 1H), 6.95−7.13 (m, 4H), 7.44−7.53 (m, 15H). Anal. ($C_{35}H_{30}O_3$) C, H.

3,3′,5-Tri(benzyloxy)-4′-methoxystilbenes (5b, 6b). Reaction of [3,5-di(benzyloxy)benzyl]triphenylphosphonium bromide **3a** and 3-benzyloxy-4-methoxybenzaldehyde **4b** (1.2 g, 4.95 mmol) gave a mixture of *cis*-stilbene **5b** and the *trans*-isomer **6b**. **5b**: 0.50 g (19% yield); white powder; mp 134 °C; ^{1}H NMR δ 3.89 (s, 3H), 4.95 (s, 6H), 6.45−6.65 (m, 5H), 6.8−7 (m, 3H), 7.32−7.38 (m, 15H). **6b**: 1.41 g (54% yield); white powder; mp 49−52 °C; ^{1}H NMR δ 3.93 (s, 3H), 5.1 (s, 4H), 5.22 (s, 2H), 6.56 (t, 1H, J = 2.2 Hz), 6.76 (m, 1H), 6.82 (d, 1H, J = 16.6 Hz), 6.91 (m, 2H), 6.99 (d, 1H, J = 16.2 Hz), 7.06−7.10 (m, 2H), 7.33−7.52 (m, 15H). Anal. ($C_{36}H_{32}O_4$) C, H.

3,5-Di(benzyloxy)-3′-nitro-4′-methoxystilbenes (5c, 6c). Reaction of [3,5-di(benzyloxy)benzyl]triphenylphosphonium bromide **3a** and the commercially available 3-nitro-4-methoxybenzaldehyde **4c** (1 g, 5.52 mmol) gave a mixture of *cis*-stilbene **5c** and the *trans*-isomer **6c**. **5c**: 0.42 g (16% yield); yellow oil; ^{1}H NMR δ 3.94 (s, 3H), 4.98 (s, 4H), 6.51−6.60 (m, 5H), 6.88−6.92 (m, 2H), 7.37−7.44 (m, 10 H), 7.80−7.81 (m, 1H). **6c**: 0.68 g (26% yield); yellow powder; mp 111 °C; ^{1}H NMR δ 4.01 (s, 3H), 5.10 (s, 4H), 6.78 (m, 2H), 7.00 (m, 2H), 7.28−7.46 (m, 13H), 8.02 (m, 1H). Anal. ($C_{29}H_{25}NO_5$) C, H, N.

3,4′,5-Trimethoxy-3′-nitrostilbenes (5d, 6d). Reaction of 3,5-dimethoxybenzyltriphenylphosphonium bromide **3b**[12] and 3-nitro-4-methoxybenzaldehyde **4c** (0.7 g, 3.86 mmol) gave a mixture of *cis*-stilbene **5d** and the *trans*-isomer **6d**. **5d**: 0.33 g (27% yield); yellow crystals; mp 49−52 °C; ^{1}H NMR δ 3.67 (s, 6H), 3.90 (s, 3H), 6.34 (m, 3H), 6.44 (d, 1H, J = 12.3 Hz), 6.57 (d, 1H, J = 12 Hz), 6.89 (m, 1H), 7.38 (m, 1H), 7.74 (m, 1H). **6d**: 0.32 g (32% yield); yellow crystals; mp 107−110 °C; ^{1}H NMR δ 3.83 (s, 6H), 3.98 (s, 3H), 6.41 (t, 1H, J = 2.4 Hz), 6.65 (m, 2H), 6.98 (m, 2H), 7.07 (m, 1H), 7.65 (m, 1H), 7.99 (m, 1H). Anal. ($C_{17}H_{17}NO_5$) C, H, N.

3,5-Dimethoxy-4′-*tert*-butyldimethylsilyloxystilbenes (5e, 6e). Reaction of 3,5-dimethoxybenzyltriphenylphosphonium bromide **3b** and 4-(*tert*-butyldimethylsilyloxy)benzaldehyde **4d** (1.3 g, 5.7 mmol) gave a mixture of *cis*-stilbene **5e** and the *trans*-isomer **6e**. **5e**: 0.54 g (25% yield); yellow oil; IR ν_{max} (Nujol) cm^{-1} 3380, 1593, 1506, 1454, 1255, 1148, 1065, 907, 835, 784, 682; ^{1}H NMR δ 0.22 (s, 6H), 1.02 (s, 9H), 3.70 (s, 6H), 6.36 (t, 1H, J = 2.4 Hz), 6.50 (m, 4H), 6.74−6.77 (m, 2H), 7.19−7.21 (m, 2H); ^{13}C NMR δ −4.4, 18.3, 25.7, 55.2, 99.8, 106.5, 119.7, 128.7, 130.1, 139.3, 154.8, 160.4. **6e**: 0.32 g (15% yield); yellow oil; IR ν_{max} (Nujol) cm^{-1} 3385, 3298, 3180, 1670, 1588, 1506, 1265, 1204, 1147, 1060, 958, 912, 840; ^{1}H NMR δ 0.26 (s, 6H), 1.04 (s, 9H), 3.87 (s, 6H), 6.43 (t, 1H, J = 2.4 Hz), 6.69−6.70 (m, 2H), 6.87−6.89 (m, 2H), 6.94 (d, 1H, J = 16.2), 7.08 (d, 1H, J = 16.2), 7.42−7.45 (m, 2H); ^{13}C NMR δ −4.3, 18.3, 25.7, 55.3, 99.5, 104.3, 107.0, 120.2, 126.6, 127.6, 128.7, 130.4, 139.6, 155.4, 160.8. Anal. ($C_{22}H_{30}SiO_3$) C, H.

3,4′,5-Trimethoxy-3′-*tert*-butyldimethylsilyloxystilbenes (5f, 6f). Reaction of 3,5-dimethoxybenzyltriphenylphosphonium bromide **3b** and 3-(*tert*-butyldimethylsilyloxy)-4-methoxybenzaldehyde **4e** (0.92 g, 3.46 mmol) gave a mixture of *cis*-stilbene **5f** and the *trans*-isomer **6f**. **5f**: 0.33 g (24% yield); yellow oil; ^{1}H NMR δ 0.10 (s, 6H), 0.97 (s, 9H), 3.71 (s, 6H), 3.81 (s, 3H), 6.35 (t, 1H, J = 2.6 Hz), 6.46−6.50 (m, 4H), 6.73−6.90 (m, 3H); ^{13}C NMR δ −4.8, 18.3, 25.7, 55.1, 55.4, 99.5, 106.6, 111.6, 121.3, 122.8, 128.7, 129.9, 130.2, 139.5, 144.4, 150.2, 160.5. **6f**: 0.24 g (18% yield); yellow powder; mp 55 °C; ^{1}H NMR δ 0.25 (s, 6H), 1.10 (s, 9H), 6.43 (t, 1H, J = 2.2 Hz), 6.71−6.72 (m, 1H), 6.87 (m, 1H), 6.92 (d, 1H, J = 16.6 Hz), 6.95 (m, 2H), 7.08−7.13 (m, 2H); ^{13}C NMR δ −4.7, 18.4, 25.7, 29.6, 55.1, 99.5, 104.1, 111.8, 118.5, 120.4, 126.4, 128.6, 130.1, 139.4, 144.9, 150.7, 160.7. Anal. ($C_{23}H_{32}SiO_4$) C, H.

3,3′,5-Trimethoxy-4′-*tert*-butyldimethylsilyloxystilbenes (5g, 6g). Reaction of 3,5-dimethoxybenzyltriphenylphosphonium bromide **3b** and 4-(*tert*-butyldimethylsilyloxy)-3-methoxybenzaldehyde **4f** (0.74 g, 2.77 mmol) gave a mixture of *cis*-stilbene **5g** and the *trans*-isomer **6g**. **5g**: 0.46 g (41% yield); yellow oil; ^{1}H NMR δ 0.13 (s, 6H), 0.98 (s, 9H), 3.59 (s, 3H), 3.66(s, 6H), 6.31 (m, 2H), 6.46 (m, 3H), 6.75 (m, 3H); ^{13}C NMR δ −4.6, 18.5, 25.7, 55.2, 99.7, 106.5, 107.0, 112.6, 120.5, 122.0, 128.7, 130.4, 130.6, 139.4, 144.2, 150.3, 160.4. **6g**: 0.39 g (35% yield); yellow oil; ^{1}H NMR δ 0.21 (s, 6H), 1.04 (s, 9H), 3.85 (s, 6H), 3.89 (s, 3H), 6.40 (t, 1H, J = 2.2 Hz), 6.67 (m, 2H), 6.87 (m, 2H), 7.04 (m, 3H); ^{13}C NMR δ −4.7, 18.5, 25.6, 55.2, 55.3, 99.4, 104.1, 109.6, 119.7, 120.8, 126.5, 129.0, 130.8, 139.4, 144.9, 150.8, 160.7. Anal. ($C_{23}H_{32}SiO_4$) C, H.

3,5-Dimethoxy-3′,4′-di-(*tert*-butyldimethylsilyloxy)stilbenes (5h, 6h). Reaction of 3,5-dimethoxybenzyltriphenylphosphonium bromide **3b** and 3,4-di(*tert*-butyldimethylsilyloxy)-benzaldehyde **4g** (1.35 g, 3.68 mmol) gave a mixture of *cis*-stilbene **5h** and the *trans*-isomer **6h**. **5h**: 0.46 g (25% yield); colorless oil; ^{1}H NMR δ 0.10 (s, 6H), 0.22 (s, 6H), 0.96 (s, 9H), 1.01 (s, 9H), 3.71 (s, 6H), 6.35 (t, 1H, J = 2.2 Hz), 6.47 (m, 4H), 6.77 (m, 3H). ^{13}C NMR δ −4.0, 4.3, 18.4, 18.5, 25.9, 26.0, 55.1, 99.6, 106.5, 120.5, 121.3, 122.5, 128.6, 130.2, 130.4, 139.5, 146.1, 146.3, 160.5. **6h**: 0.37 g (20% yield); oil; ^{1}H NMR δ 0.25 (s, 6H), 0.26 (s, 6H), 1.02 (s, 9H), 1.04 (s, 9H), 3.80 (s, 3H), 3.86 (s, 3H), 6.38 (m, 2H), 6.67 (m, 2H), 6.86 (m, 1H), 7.01 (m, 2H), 7.28 (m, 1H). ^{13}C NMR δ −4.0, 18.5, 21.9, 26.0, 55.2, 55.4, 97.4, 99.6, 104.3, 107.0, 119.2, 120.0, 121.1, 126.6, 128.9, 130.7, 139.6, 146.9, 160.8. Anal. ($C_{28}H_{44}SiO_4$) C, H.

3,5-Dimethoxy-4′-nitrostilbenes (5i, 6i). Reaction of 3,5-dimethoxybenzyltriphenylphosphonium bromide **3b** and the commercially available 4-nitrobenzaldehyde **4h** (0.4 g, 2.65 mmol) gave a mixture of *cis*-stilbene **5i** and the *trans*-isomer **6i**.[35] **5i**: 0.25 g (33% yield); yellow solid; mp 72 °C; ^{1}H NMR δ 3.66 (s, 3H), 6.33 (m, 3H), 6.58 (d, 1H, J = 12 Hz), 6.73 (d, 1H, J = 12.3), 7.38 (d, 2H, J = 9 Hz), 8.07 (d, 2H, J = 8.7). **6i**: 0.35 g (46% yield); yellow solid; mp 134−136 °C; ^{1}H NMR δ 3.83 (s, 6H), 6.44 (t, 1H, J = 2.1), 6.68 (d, 2H, J = 1.8 Hz), 7.09 (d, 1H, J = 16.2 Hz), 7.19 (d, 1H, J = 16.2 Hz), 7.61 (d, 2H, J = 8.7), 8.20 (d, 2H, J = 9 Hz). Anal. ($C_{16}H_{15}NO_4$) C, H, N.

General Procedure for the Reduction of Nitro Derivatives 5c,d,i and 6c,d,i. The nitrostilbene derivatives **5c**,**d**,**i** or **6c**,**d**,**i** (1.0 equiv) was dissolved in acetone/water (10:5 mL), and the mixture was heated to 50 °C. After 30 min, sodium dithionite (25.0 equiv) was slowly added, and the mixture was heated to reflux (1−4 h) and then cooled to room temperature. Water was added, and the product was isolated by extraction with ethyl acetate. The organic phase was washed with brine, and the solvent was removed in vacuo. The crude products were used in a further reaction without purification.

***cis*-3,5-Di(benzyloxy)-3′-amino-4′-methoxystilbene (7a).** Nitro derivative **5c** (0.37 g, 0.8 mmol) led to **7a** as a yellow oil (0.32 g, 92% yield); ^{1}H NMR δ 3.85 (s, 3H), 4.94 (s, 4H), 6.42 (d, 1H, J = 12.2 Hz), 6.52 (t, 1H, J = 2.6 Hz), 6.51 (d, 1H, J = 12.2 Hz), 6.62 (m, 2H), 6.70 (m, 3H), 7.39 (m, 10 H).

***trans*-3,5-Di(benzyloxy)-3′-amino-4′-methoxystilbene (8a).** Nitro derivative **6c** (0.53 g, 1.1 mmol) led to **8a** as a yellow oil at room temperature (0.29 g, 42% yield); ^{1}H NMR δ 3.89 (s, 3H), 5.07−5.11 (m, 4H), 6.58−6.99 (m, 8H), 7.40−7.49 (m, 10H).

***cis*-3,4′,5-Trimethoxy-3′-aminostilbene (7b).** Nitro derivative **5d** (0.13 g, 0.4 mmol) led to **7b** as a yellow oil (0.04 g, 32% yield); ^{1}H NMR δ 3.70 (s, 6H), 3.85, (s, 3H), 6.35 (t, 1H, J = 2.2), 6.45−6.54 (m, 4H), 6.70 (m, 3H); ^{13}C NMR δ 55.2, 55.5, 99.6, 106.6, 109.9, 115.3, 119.5, 128.3, 129.8, 130.6, 135.6, 139.5, 146.6, 160.3. Anal. ($C_{17}H_{19}NO_3$) C, H, N.

***trans*-3,4′,5-Trimethoxy-3′-aminostilbene (8b).** Nitro derivative **6d** (0.2 g, 0.6 mmol) led to **8b** as a yellow oil (0.09 g, 50% yield); ^{1}H NMR δ 3.85 (s, 6H), 3.90 (s, 3H), 6.41 (t, 1H, J = 2.2 Hz), 6.68 (m, 2H), 6.89 (d, 1H, J = 16.0 Hz), 6.90−6.95 (m, 3H), 7.02 (d, 1H, J = 16.2); ^{13}C NMR δ 55.3, 55.5, 99.5, 104.3, 110.3, 112.3, 117.8, 126.2, 129.2, 130.2, 136.2, 139.7, 147.4, 160.8. Anal. ($C_{17}H_{19}NO_3$) C, H, N.

***cis*-3,5-Dimethoxy-4′-aminostilbene (7c).** Nitro derivative **5i** (0.14 g, 0.5 mmol) led to **7c** as a yellow powder (0.06 g, 48% yield); mp 44−47 °C; ^{1}H NMR δ 3.57 (s, 6H), 6.20 (t, 1H, J = 2.4 Hz), 6.25 (m, 1H), 6.34−6.43 (m, 5H), 6.99 (m, 2H); ^{13}C NMR δ 55.2, 99.5, 106.6, 114.6, 115.1, 127.4, 130.2, 130.6, 139.8, 145.5, 160.4. Anal. ($C_{16}H_{17}NO_2$) C, H, N.

***trans*-3,5-Dimethoxy-4′-aminostilbene (8c).**[40] Nitro derivative **6i** (0.15 g, 0.5 mmol) led to **8c** as yellow solid (0.05 g, 37% yield); mp 86−90 °C; ^{1}H NMR δ 3.85 (s, 6H), 6.38 (m, 1H), 6.66 (m, 4H), 6.86 (d, 1H, J = 16.2 Hz), 7.03 (d, 1H, J = 16.2 Hz), 7.33 (m, 2H); ^{13}C NMR δ 54.3, 98.1, 102.9, 113.9, 123.0, 126.7, 128.3, 138.8, 146.0, 159.7. Anal. ($C_{16}H_{17}NO_2$) C, H, N.

General Procedure for the Cleavage of the Benzyloxy Group of Compounds 5a,b, 7a and 6a,b, 8a. Freshly distilled *N*,*N*-dimethylaniline (3.0 equiv) was added to a well-stirred solution of the benzyloxy derivative **5a**,**b**, **7a** or **6a**,**b**, **8a** (1.0 equiv) in dry methylene chloride (10 mL) under a nitrogen atmosphere at 0 °C. After 5 min, anhydrous $AlCl_3$ (4.0 equiv) was added in one portion. After 2−8 h, the reaction mixture was cooled and 1.0 M HCl (10 mL) was added. The resulting mixture was extracted with ethyl acetate, and the combined extracts were washed with brine. Removal of solvent in vacuo from the organic phase yielded the crude hydroxystilbene, which was purified by flash chromatography.

***cis*-3,4′,5-Trihydroxystilbene (9c). 9c** was prepared from **5a** (0.56 g, 1.1 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 6:4) gave **9c** as a white powder (0.062 g, 24% yield): mp 169−170 °C (lit.[41] mp 170−174 °C). Anal. ($C_{14}H_{12}O_3$) C, H.

***trans*-3,4′,5-Trihydroxystilbene (Resveratrol) (1). 1** was prepared from **6a** (0.42 g, 0.9 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 7:3) gave **1** as a white powder (0.07 g, 36% yield): mp 258 °C (lit.[41] mp 260 °C). Anal. ($C_{14}H_{12}O_3$) C, H.

***cis*-3,3′,5-Trihydroxy-4′-methoxystilbene (9a). 9a** was prepared from **5b** (0.50 g, 0.95 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 8:2) gave **9a** as a light-yellow oil (0.038 g, 15% yield): ^{1}H NMR (CD_3COCD_3) δ 3.84 (s, 3H), 6.24 (t, 1H, J = 2.2 Hz), 6.31−6.41 (m, 4H), 6.77−6.85 (m, 3H), 7.3 (br, 1H), 8.16 (s, 2H); ^{13}C NMR (CD_3COCD_3) δ 56.1, 102.3, 107.8, 111.9, 116.2, 121.5, 129.5, 130.4, 131.0, 140.3, 146.8, 147.5, 159.1. Anal. ($C_{15}H_{14}O_4$) C, H.

***trans*-3,3′,5′-Trihydroxy-4-methoxystilbene (Rhapontigenin) (10a). 10a** was prepared from **6b** (0.50 g, 0.90 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 7:3) gave **10a** as a white powder (0.040 g, 16% yield): mp 195°C (lit.[42] mp 195 °C). Anal. ($C_{15}H_{14}O_4$) C, H.

***cis*-3,5-Dihydroxy-3′-amino-4′-methoxystilbene (9b). 9b** was prepared from **7a** (0.32 g, 0.7 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 7:3) gave **9b** as a yellow powder (0.11 g, 58% yield): mp 65 °C; ^{1}H NMR (CD_3COCD_3) δ 3.15 (br, 1H), 3.82 (s, 3H), 4.30 (br, 1H), 6.23 (t, 1H, J = 2.2 Hz), 6.32 (d, 1H, J = 12.3 Hz), 6.35 (m, 2H), 6.41 (d, 1H, J = 12.3 Hz), 6.58−6.61 (m, 1H), 6.70−6.73 (m, 2H), 8.15 (br, 2H); ^{13}C NMR (CD_3COCD_3) δ 55.7, 102.3, 108.0, 110.7, 115.5, 118.9, 129, 130.8, 131.1, 137.8, 140.5, 147.1, 159.1. Anal. ($C_{15}H_{15}NO_3$) C, H, N.

***trans*-3,5-Dihydroxy-3′-amino-4′-methoxystilbene (10b). 10b** was prepared from **8a** (0.37 g, 0.8 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 7.5:2.5) gave **10b** as a red powder (0.09 g, 42% yield): mp 163 °C; ^{1}H NMR (CD_3COCD_3) δ 3.86 (s, 3H), 6.28 (t, 1H, J = 2.2 Hz), 6.54−6.55 (m, 2H), 6.81 (s, 1H), 6.80−6.92 (m, 3H), 7.00 (m, 2H), 8.20 (br, 2H); ^{13}C NMR (CD_3COCD_3) δ 55.8, 102.5, 105.5, 111.1, 112.3, 117.5, 126.8, 129.6, 131.2, 138.3, 140.8, 148.0, 159.4. Anal. ($C_{15}H_{15}NO_3$) C, H, N.

General Procedure for the Silyloxy Deprotection of Compounds 5e−h and 6e−h. To a solution of the silyloxy-protected stilbene **5e**−**h** or **6e**−**h** (1 equiv) in anhydrous tetrahydrofuran (10 mL) tetrabutylammonium fluoride (1 M in THF, 3 equiv) was added. The pale-yellow solution was stirred for 45 min, poured into water, and extracted with dichloromethane. Removal of solvent in vacuo from the organic phase yielded the crude hydroxystilbenes **11a**−**d** and **12a**−**d**, which were purified by flash chromatography.

***cis*-3,5-Dimethoxy-4′-hydroxystilbene (11a). 11a** was obtained from **5e** (0.43 g, 1.2 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 7.5:2.5) gave **11a** (0.07 g, 23% yield) as a light-yellow oil.[12] Anal. ($C_{16}H_{16}O_3$) C, H.

***trans*-3,5-Dimethoxy-4′-hydroxystilbene (Pterostilbene) (12a). 12a** was obtained from **6e** (0.24 g, 0.7 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 8:2) gave **12a** (0.04 g, 24% yield) as a white powder: mp 88°C (lit.[43] mp 87−88 °C). Anal. ($C_{16}H_{16}O_3$) C, H.

***cis*-3,4′,5-Trimethoxy-3′-hydroxystilbene (11b). 11b** was obtained from **5f** (0.33 g, 0.8 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 9:1) gave **11b** (0.21 g, 89% yield) as a yellow oil: ^{1}H NMR δ 3.70 (s, 6H), 3.85 (s, 3H), 6.36 (t, 1H, J = 2.2 Hz), 6.50 (m, 4H), 6.71−6.80 (m, 2H), 6.92 (m, 1H); ^{13}C NMR δ 55.1, 55.8, 99.6, 106.6, 110.2, 115.0, 121.0, 128.8, 130.0, 130.3, 139.1, 145.0, 145.7, 160.3. Anal. ($C_{17}H_{18}O_4$) C, H.

***trans*-3,4′,5-Trimethoxy-3′-hydroxystilbene (12b). 12b** was obtained from **6f** (0.24 g, 0.6 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 9:1) gave **12b** (0.12 g, 70% yield) as a white powder: mp 90−91 °C (lit.[44] mp 89−90 °C). Anal. ($C_{17}H_{18}O_4$) C, H.

***cis*-3,3′,5-Trimethoxy-4′-hydroxystilbene (11c). 11c** was obtained from **5g** (0.37 g, 0.9 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 9:1) gave **11c** (0.23 g, 87% yield) as a yellow oil: ^{1}H NMR δ 3.69 (s, 3H), 3.71 (s, 6H), 6.36 (t, 1H, J = 2.2), 6.51 (m, 4H), 6.84 (m, 3H); ^{13}C NMR δ 55.1, 55.6, 99.4, 106.5, 111.2, 113.9, 122.5, 128.3, 128.9, 130.2, 139.4, 144.8, 145.8, 160.4. Anal. ($C_{17}H_{18}O_4$) C, H.

***trans*-3,3′,5-Trimethoxy-4′-hydroxystilbene (12c). 12c** was obtained from **6g** (0.30 g, 0.75 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 9:1) gave **12c** (0.19 g, 88% yield) as a white solid: mp 82−84 °C (lit.[44] mp 85−86 °C). Anal. ($C_{17}H_{18}O_4$) C, H.

***cis*-3,5-Dimethoxy-3′,4′-dihydroxystilbene (11d). 11d** was obtained from **5h** (0.37 g, 0.7 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 8:2) gave **11d** (0.04 g, 20% yield) as a yellow oil (lit. data in ref 45): ^{1}H NMR δ 3.68 (s, 6H), 5.28 (br, 2H), 6.33 (t, 1H, J = 2.2 Hz), 6.46 (m, 4H), 6.78 (m, 3H); ^{13}C NMR δ 55.3, 99.7, 106.8, 115.0, 115.7, 122.3, 128.6, 129.9, 130.1, 139.4, 143.0, 143.1, 160.3. Anal. ($C_{16}H_{16}O_4$) C, H.

***trans*-3,5-Dimethoxy-3′,4′-dihydroxystilbene (12d). 12d** was obtained from **6h** (0.25 g, 0.5 mmol). Flash chromatography of the final reaction mixture (petroleum ether/ethyl acetate, 9:1) gave **12d** (0.04 g, 29% yield) as a white solid: mp 112−115 °C (lit.[46] mp 72−74 °C); ^{1}H NMR δ 3.85 (s, 6H), 5.70 (br, 2H), 6.42 (m, 1H), 6.66 (m, 2H), 6.90 (m, 4H), 7.08 (m, 1H). Anal. ($C_{16}H_{16}O_4$) C, H.

Biology. Cell Culture. Cells were grown in RPMI 1640 (Gibco Grand Island, NY) containing 10% FCS (Gibco), 100 U/mL penicillin (Gibco), 100 μg/mL streptomycin (Gibco), and 2 mM l-glutamine (Sigma Chemical Co., St. Louis, MO) in a 5% CO_2 atmosphere at 37 °C.

Sample Preparation. Each compound was dissolved in DMSO or PBS. They were diluted to the appropriate experimental concentrations in tissue medium and protected from light. In each experiment, DMSO never exceeded 0.1%, and this percentage did not interfere with cell growth.

Cytotoxicity Assays. To evaluate the number of live and dead cells, cells were stained with trypan blue and counted

on a hemocytometer. To determine the cytotoxic activity of the drugs tested, 2×10^5 cells were plated into 25 mm wells (Costar, Cambridge, U.K.) in 1 mL of complete medium and treated with different concentrations of each drug. After 48 h of incubation, the number of viable cells was determined and expressed as a percent of control proliferation.

Evaluation of Apoptosis by Annexine V. Apoptosis was detected after 48 h of cell culture using Annexine-V-Fluos staining kit (Roche Molecular Biochemicals, Mannheim, Germany). Cells (10^6) were washed with PBS and centrifuged at 200*g* for 5 min. The cell pellet was suspended in 100 μL of staining solution containing annexine-V-fluorescein labeling reagent and propidium iodide and was incubated for 15 min at 20 °C. Annexine V propidium iodide negative cells were evaluated by flow cytometry (Becton Dickinson) and fluorescence microscopy.

Flow Cytometric Determination of Apoptosis. Cell cycle progression and apoptosis were analyzed by quantitating DNA content after staining with propidium iodide (PI). Untreated or drug-treated cells were collected by centrifugation, suspended in PBS, and fixed in FACS permeabilizing solution 2 (Becton-Dickinson) (ratio 1:10) for 10 min at 20 °C. After centrifugation at 500*g* for 5 min, cells were suspended in 1 mL of PBS containing PI (50 mg/mL) and RNAse (0.2 mg/mL) in polypropylene tubes and incubated at room temperature for 30 min. The tubes were then placed at 4 °C in the dark until use in flow cytometry analysis using an FACscan flow cytometer (Becton-Dickinson) with CellQuest software. The data were registered on a linear scale. Apoptotic cells were evaluated as a sub-G0-G1 hypodiploid peak.

Morphological Evaluation of Apoptosis and Necrosis. Drug-induced apoptosis and necrosis were determined morphologically after labeling with acridine orange and ethidium bromide.[22] Cells (2×10^5) were centrifuged (300*g*), and the pellet was resuspended in 25 μL of the dye mixture. An amount of 10 μL of the mixture was examined in oil immersion with a 100× objective using a fluorescence microscope. Live cells were determined by the uptake of acridine orange (green fluorescence) and exclusion of ethidium bromide (red fluorescence) stain. Live and dead apoptotic cells were identified by perinuclear condensation of chromatin stained by acridine orange and ethidium bromide, respectively, and by the formation of apoptotic bodies. Necrotic cells were identified by uniform labeling of the cells with ethidium bromide. Morphological evaluation of apoptosis assay was used to determine the percentage of apoptotic cells reported in all tables in this study. Flow cytometry determination of apoptosis and evaluation of apoptosis by annexine were used to confirm the percentage of apoptotic cells obtained by the morphological assay.

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References

(1) Hart, J. H. Role of Phytostilbenes in Decay and Disease Resistance. *Annu. Rev. Phytopathol.* **1991**, *19*, 437−458.
(2) Burns, J.; Yokota, T.; Ashihara, H.; Lean, M. E. J.; Crozier, A. Plants Foods and Herbal Sources of Resveratrol. *J. Agric. Food Chem.* **2002**, *50*, 3337−3340.
(3) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Resveratrol: A Molecule Whose Time Has Come? And Gone? *Clin. Biochem.* **1997**, *30*, 91−113.
(4) Fremont, L. Biological Effects of Resveratrol. *Life Sci.* **2000**, *66*, 663−673.
(5) Pace-Asciak, C. R.; Hahn, S.; Diamandis, E. P.; Soleas, G.; Goldberg, D. M. The Red Wine Phenolics *trans*-Resveratrol and Quercetin Block Uman Platelet Aggregation and Eicosanoid Synthesis: Implications for Protection Against Coronary Heart Disease. *Clin. Chem. Acta* **1995**, *235*, 207−219.
(6) Kimura, Y.; Okuda, H.; Arichi, S. Effects of Stilbenes on Arachidonate Metabolism in Leukocytes. *Biochim. Biophys. Acta* **1985**, *834*, 275−278.
(7) Belguendouz, L.; Fremont, L.; Gozzellino, M. T. Interaction of trans Resveratrol with Plasma Lipoproteins. *Biochem. Pharmacol.* **1998**, *55*, 811−816.
(8) Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. Inhibition of Human LDL Oxidation by Resveratrol. *Lancet* **1993**, *341*, 1103−1104.
(9) Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H. S.; Farnworth, R. N.; Kinghorn, A. D.; Metha, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer Chemopreventive Activity of Resveratrol, a Natural Product Derived from Grapes. *Science* **1997**, *275*, 218−220.
(10) Stivala, L. A.; Savio, M.; Carafoli, F.; Perucca, P.; Bianchi, L.; Maga, G.; Forti, L.; Pagoni, U. M.; Albini, A.; Prosperi, E.; Vannini, V. Specific Structural Determinants Are Responsible for the Antioxidant Activity and the Cell Cycle Effects of Resveratrol. *J. Biol. Chem.* **2001**, *276*, 22586−22594.
(11) Mgbonyebi, O.; Russo, J.; Russo, I. Antiproliferative Effect of Synthetic Resveratrol on Human Breast Epithelial Cells. *Int. J. Oncol.* **1998**, *12*, 865−869.
(12) Pettit, G. R.; Grealish, M. P.; Jung, M. K.; Hamel, E.; Pettit, R. K.; Chapuis, J. C.; Schmidt, J. M. Antineoplastic Agents. 465. Structural Modification of Resveratrol: Sodium Resveratrin Phosphate. *J. Med. Chem.* **2002**, *45*, 2534−2542.
(13) Kim, S.; Ko, H.; Park, J. E.; Jung, S.; Lee, S. K.; Chun, Y. J. Design, Synthesis, and Discovery of Novel *trans*-Stilbene Analogues as Potent and Selective Human Cytochrome P450 1B1 Inhibitors. *J. Med. Chem.* **2002**, *45*, 160−164.
(14) Thakkar, K.; Geahlen, R. L.; Cushman, M. Synthesis and Protein−Tyrosine Kinase Inhibitory Activity of Polyhydroxylated Stilbene Analogues of Piceatannol. *J. Med. Chem.* **1993**, *36*, 2950−2955.
(15) Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H. M.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of Analogues of (*Z*)-1-(4-Methoxyphenyl)-2-(3,4,5-Trimethoxyphenyl)Ethene as Potential Cytotoxic and Antimitotic Agents. *J. Med. Chem.* **1992**, *35*, 2293−2306.
(16) Clement, M. V.; Hirpara, J. L.; Chawdhury, S. H.; Pervaiz, S. Chemopreventive Agent Resveratrol, a Natural Product Derived from Grapes, Triggers CD95 Signaling-Dependent Apotosis in Human Tumor cells. *Blood* **1998**, *92*, 996−1002.
(17) Huang, C.; Ma, W. Y.; Goranson, A.; Dong, Z. Resveratrol Suppresses Cell Transformation and Induces Apoptosis through a p53-Dependent Pathway. *Carcinogenesis* **1999**, *20*, 237−242.
(18) Surh, Y. J.; Hurh, Y. J.; Kang, J. Y.; Lee, E.; Kong, G.; Lee, S. J. Resveratrol, an Antioxidant Present in Red Wine, Induces Apoptosis in Human Promyelocytic Leukemia (HL-60) Cells. *Cancer Lett.* **1999**, *140*, 1−10.
(19) Arens, M. J.; Wyllie, A. H. Apoptosis: Mechanism and Roles in Pathology. *Int. Rev. Exp. Pathol.* **1991**, *32*, 223−254.
(20) Daniel, P. T. Dissecting the Pathways to Death. *Leukemia* **2000**, *14*, 2035−2044.
(21) Hickman, J. A. Apoptosis and Chemotherapy Resistance. *Eur. J. Cancer* **1996**, *32A*, 921−926.
(22) Hannun, Y. A. Apoptosis and the Dilemma of Cancer Chemotherapy. *Blood* **1997**, *89*, 1845−1853.
(23) Prokop, A.; Wieder, T.; Sturm, I.; Essman, F.; Seeger, K.; Wutcher, C.; Ludwig, W.-D. Henze, G.; Dörken, B.; Daniel, P. T. Relapse in Childhood Acute Lymphoblastic Leukemia Is Associated with a Decrease of the Bax/Bcl-2-Ratio and Loss of Spontaneous Caspase-3 Processing *in Vivo*. *Leukemia* **2000**, *14*, 1606−1613.
(24) Raisova, M.; Bektas, M.; Wieder, T.; Daniel, P.; Eberle, J.; Orfanos, C. E.; Geilen, C. C. Resistance to CD95/Fas-Induced and Ceramide-Mediated Apoptosis of Human Melanoma Cells Is Caused by a Defective Mitochondrial Cytochrome C Release. *FEBS Lett.* **2000**, *473*, 27−32.
(25) Simoni, D.; Tolomeo, M. Retinoids, Apoptosis and Cancer. *Curr. Pharm. Des.* **2001**, *7*, 1823−1837 and references therein.
(26) Tolomeo, M.; Simoni, D. Drug Resistance and Apoptosis in Cancer Treatment: Development of New Apoptosis-Inducing Agents Active in Drug Resistant Malignancies. *Curr. Med. Chem.: Anti-Cancer Agents* **2002**, *2*, 387−406 and references therein.
(27) Simoni, D.; Roberti, M.; Invidiata, F. P.; Rondanin, R.; Baruchello, R.; Malagutti, C.; Mazzali, A.; Rossi, M.; Grimaudo, S.; Dusonchet, L.; Meli, M.; Raimondi, M. V.; D'Alessandro, N.; Tolomeo, M. Programmed Cell Death (PCD) Associated with the Stilbene Motif of Arotinoids: Discovery of Novel Apoptosis Inducer Agents Possessing Activity on Multidrug Resistant Tumor Cells. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2669−2673.
(28) Simoni, D.; Roberti, M.; Invidiata, F. P.; Rondanin, R.; Baruchello, R.; Malagutti, C.; Mazzali, A.; Rossi, M.; Grimaudo, S.; Capone, F.; Dusonchet, L.; Meli, M.; Raimondi, M. V.; Landino, M.; D'Alessandro, N.; Tolomeo, M.; Arindam, D.; Lu, S.; Benbrook, D. M. Heterocycle-Containing Retinoids. Discovery of a Novel Isoxazole Arotinoid Possessing Potent Apoptotic Activity in Multidrug and Drug-Induced Apoptosis-Resistant Cells. *J. Med. Chem.* **2001**, *44*, 2308−2318.
(29) Tan, B.; Piwnica-Worms, D.; Ratner, L. Multidrug Resistance Transporters and Modulation. *Curr. Opin. Oncol.* **2000**, *12*, 450−458.

(30) Deffie, A. M.; Batra, J. K.; Goldenberg, G. J. Direct Correlation between DNA Topoisomerase II Activity and Cytotoxicity in Adriamycin-Sensitive and -Resistant P388 Leukemia Cell Lines. *Cancer Res*. **1989**, *49*, 58−62.
(31) Sanguedolce, M. R.; Alessandro, R.; De Leo, G.; Gullotti, L.; Sanguedolce, F.; Vultaggio, G.; Diana, G.; Cirello, B.; Rausa, L. Failure of Detection of the Tyrosine to Histidine Substitution at the Residue 33 of Thymidilate Synthase in Human Colorectal Cancer. A Preliminary Study. *Anticancer Res.* **2000**, *20*, 4347−4350.
(32) Dalton, W. S.; Crowley, J. J.; Salmon, S. S.; Grogan, T. M.; Laufman, L. R.; Weiss, G. R.; Bonnet, J. D. A Phase-III Randomized Study of Oral Verapamil as a Chemosensitizer To Reverse Drug Resistance in Patients with Refractory Myeloma. A Southwest Oncology Group Study. *Cancer* **1995**, *75*, 815−820.
(33) Milroy, R. A Randomized Clinical Study of Verapamil in Addition to Combination Chemotherapy in Small Cell Lung Cancer. West of Scotland Lung Cancer Research Group and the Aberdeen Oncology Group. *Br. J. Cancer* **1993**, *68*, 813−818.
(34) Wishart, G. C.; Bissett, D.; Paul, J.; Jodrell, D.; Harnett, A.; Habeshaw, T.; Kerr, D. J.; Macham, M. A.; Soukop, M.; Leonard, R. C. J. Quinidine as a Resistance Modulator of Epirubicin in Advanced Breast Cancer: Mature Results of a Placebo-Controlled Randomized Trial. *Clin. Oncol*. **1994**, *12*, 1771−1777.
(35) Covelli, A. Modulation of Multidrug Resistance (MDR) in Hematological Malignancies. *Ann. Oncol*. **1999**, *10* (Suppl. 6), 53−59.
(36) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, F.; Schmidt, J. M. Isolation, Structure, and Synthesis of Combretastatins A-1 and B-1, Potent New Inhibitors of Microtubule Assembly, Derived from *Combretum Coffrum*. *J. Nat. Prod*. **1987**, *50* (1), 119−131.
(37) Orsini, F.; Pelizzoni, F.; Bellini, B.; Miglierini, G. Synthesis of Biologically Active Polyphenolic Glycosides (Combretastatin and Resveratrol Series). *Carbohydr. Res.* **1997**, *301*, 95−109.
(38) Cardona, M. L.; Fernandez, M. I.; Garcia, M. B.; Pedro, J. R. Synthesis of Natural Polhydroxystilbenes. *Tetrahedron* **1986**, *42*, 2725−2730.
(39) Reimann, E. Natürliche Stilbene. Synthese von Polyhydroxystilbenäthern durch Wittig-Reaktion (Natural Stilbenes. Synthesis of Polyhydroxystilbene Ethers through the Wittig Reaction). *Chem. Ber*. **1969**, *102*, 2881−2888.
(40) Chiaki, T.; Masaomi, S. Manufacture of Nitro and Amino Compounds Used for Electrophotographic Photoreceptor. *Jpn. Kokai Tokkyo Koho* **1995** (Application JP 94-59659 19940330).
(41) Jayatilake, G. S.; Jayasuriya, H.; Lee, E. S.; Koonchanok, N. M.; Geahlen, R. L.; Ashendel, C. L.; McLaughlin, J. L.; Chang, C. J. Kinase Inhibitors from Polygonum Cuspidatum. *J. Nat. Prod*. **1993**, *56*, 1805−1810.
(42) Kashiwada, Y.; Nonaka, G.; Nishioka, I. Studies on Rhubarb (Rhei Rhizoma). VI. Isolation and Characterization of Stilbenes. *Chem. Pharm. Bull*. **1984**, *32*, 3501−3517.
(43) Späth, E.; Schläger, J. Über die Inhaltsstoffe des roten Sandelholzels. II. Mitteil.: Die Konstitution des Pterostilbens (On the Contents of Red Sandalwood. Part 2. The Constitution of Pterostilbene). *Chem. Ber*. **1940**, *73*, 881−884.
(44) Gill, M. T.; Bajaj, R.; Chang, C. J.; Nichols, D. E.; McLaughlin, J. L. 3,3′,5′-Tri-*O*-methylpiceatannol and 4,3′,5′-Tri-*O*-methylpiceatannol: Improvements over Piceatannol in Bioactivity. *J. Nat. Prod*. **1987**, *50*, 36−40.
(45) Pettit, G. R.; Singh, S. B.; Isolation and Synthesis of Combretastatins as Neoplasm Inhibitors. *Eur. Pat. Appl*. **1998** (Application: EP 88-300064 19880106).
(46) Ma, Z.; Li, X.; Li, N.; Wang, J. Stilbenes from *Sphaerophysa salsula*. *Fitoterapia* **2002**, *73*, 313−315.

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