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Original article

Quinone reductase induction activity of methoxylated analogues of resveratrol

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Abstract

Agents that induce the activity of phase II enzymes play an important role in intervening with the carcinogenic process at the initiation stage. Resveratrol is well known for its chemopreventive activity against major stages of carcinogenesis. In this study, several methoxylated analogues of resveratrol were synthesized and evaluated for their ability to induce the activity of the phase II enzyme quinone reductase (QR). Methoxy groups serve to increase lipophilicity and improve metabolic stability. Compared to resveratrol, analogues with *ortho*-methoxy substituents were found to be more potent inducers of QR and to exert their activity in a qualitatively different manner. The greater induction activities associated with these stilbenoids serve as a useful starting point for the design of improved chemopreventive agents.

Keywords: Methoxylated stilbenes; Resveratrol; Chemopreventive activity; Induction of quinone reductase

1. Introduction

The stilbene moiety is commonly encountered in natural products and many members are associated with therapeutically important pharmacological properties [1]. Resveratrol (*E*-3,4′,5-trihydroxystilbene) is probably the best-known stilbenoid. Its therapeutic potential is recognized in several areas like the chemoprevention of cancer, cardiovascular diseases and neurodegeneration [2,3]. Unfortunately, resveratrol is rapidly inactivated by phase II conjugation reactions [4] and it is generally a weak growth inhibitor (IC_{50} 40–200 μM) [5]. Novel stilbenoids based on the resveratrol structure may provide a solution to these limitations. Among naturally occurring stilbenoids, the biological potential of methoxylated stilbenes is particularly promising. For example, pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxystilbene), found in grapes and heartwood extracts of *Pterocarpus marsupium*, was almost as potent as resveratrol in terms of antioxidant activity and anticarcinogenic activity in a mouse mammary organ culture model [6]. Rhapontigenin (3,5,3′-trihydroxy-4′-methoxy-*trans*-stilbene), a component of Korean rhubarb (*Rheum undulatum*), was an inhibitor of human CYP1A1 [7] while another methoxy analogue (2′,3,4′,5-tetramethoxystilbene) was also a CYP inhibitor but selective for the CYP1B1 subtype [8]. The methoxylation of hydroxyl groups results in an increase in lipophilicity and a loss of hydrogen bond donor property. These changes will influence bioavailability, susceptibility to metabolism and possibly the pharmacological profile of the resulting analogue. A case in point is 3,4,5,4′-tetramethoxy-*trans*-stilbene, a synthetic methoxylated analogue of resveratrol which had an improved pharmacokinetic profile, selective growth inhibitory effect against cancer cells and a unique mode of action compared to resveratrol [9].

Resveratrol exerts many effects on cellular events associated with cancer initiation, promotion and progression. It is an antioxidant, antimutagen, induces apoptosis and moderates the activity of drug metabolizing enzymes [10–12]. Resveratrol is a moderate inducer of the phase II enzyme quinone

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reductase (NAD(P)H:quinone reductase, QR), with a CD (concentration required to double the specific activity of QR) of 21 μM [10]. It is also a monofunctional inducer in that it induces only phase II enzymes without affecting the activity of phase I enzymes [10]. QR induction is widely used as a biomarker for evaluating chemopreventive potential of compounds against cancer initiation [13].

In view of the biological potential of methoxylated stilbenes and the shortcomings associated with resveratrol, it is of interest to explore the potential of methoxylated derivatives of resveratrol as chemopreventive agents. For this purpose, a number of *E* and *Z* isomers of methoxylated stilbenes were synthesized and evaluated for QR induction activity. As QR also serves to maintain the antioxidant function of cells [14], antioxidants are often associated with QR induction activity [15], and the compounds were also screened for their antioxidant properties using an *in vitro* assay.

2. Chemistry

Table 1 lists the stilbenes (**3**–**15**) synthesized in this investigation. All the stilbenes have a 4′-methoxy group on ring B, with the exception of stilbene **15** which has a 2′-methoxy group on ring B. The substitution pattern on ring A is as follows: methoxy groups only (**3**–**10**, **15**), hydroxyl groups only (**11**–**13**) and one member (**14**) with both hydroxyl and methoxy groups. *Z* and *E* isomers were prepared for all members, except for stilbenes **9**, **11** and **15** which were synthesized as *E* isomers.

Stilbenes with 4′-methoxy substituent (**3**–**14**) were obtained by the Wittig reaction (Scheme 1). 4-Methoxybenzyltriphenylphosphonium bromide (**2**) was synthesized from 4-methoxybenzylalcohol by bromination, followed by reaction with triphenylphosphine. The phosphonium salt **2** was reacted with the methoxylated benzaldehyde in the presence of *n*-butyllithium as base to give the desired stilbene as a mixture of *E* and *Z* isomers. These were separated by column chromatography, with *Z* isomers obtained in higher yields. Stilbene **11** which has a 2-hydroxyl group on ring A was also synthesized by this route as protection of the 2-hydroxyl group was not necessary.

In the case of other stilbenes with hydroxyl groups on ring A (**12**–**14**), the phenolic hydroxyl group was protected by conversion to the *t*-butyldimethylsilyloxy ether before reacting with **2** (Scheme 2). The silyloxystilbenes **19** and **20** were obtained exclusively as *Z* isomers. Conversion to the *E* isomer was effected by refluxing in heptane in the presence of a catalytic amount of iodine. On the other hand, the siloxystilbene **21** was obtained as a mixture of *E* and *Z* isomers. The mixture was separated by column chromatography, after which the siloxyl protecting function was removed by reaction with tetrabutylammonium fluoride to give the final compounds (**12**–**14**).

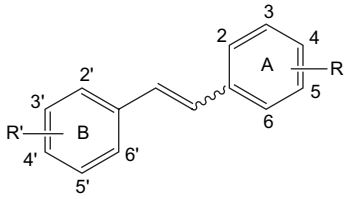
Unlike the other members, stilbene **15** was obtained by McMurry coupling of 2-methoxyaldehyde under Mukaiyama conditions [16] (Scheme 3).

3. Biological screening

3.1. Antioxidant activity

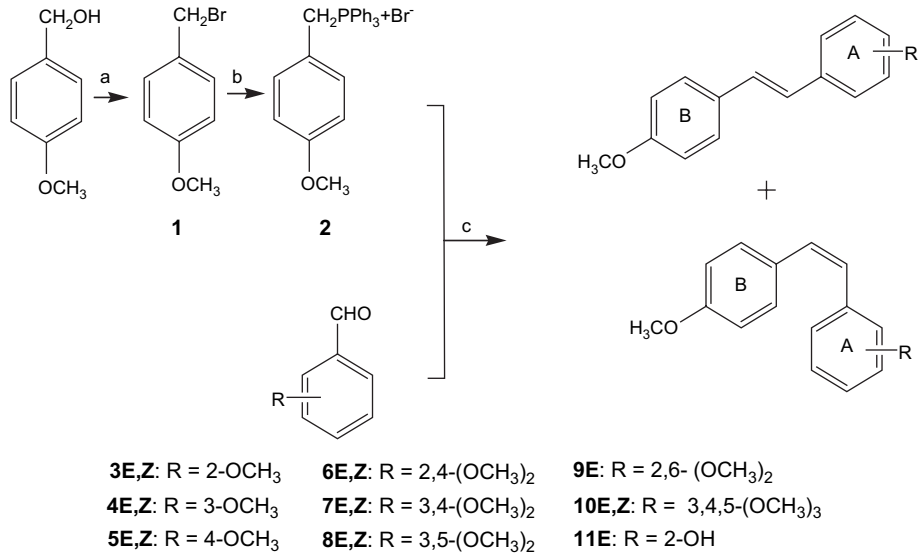
Antioxidant activity was evaluated by the ABTS [2,2′-azino-3-ethylbenzo-thiazoline-6-sulfonic acid] radical cation decolorisation assay [17]. This method is based on the ability of a potential antioxidant to scavenge the stable radical cation of ABTS ($ABTS^{\bullet+}$). The nitrogen radical of this species imparted a deep purple color at 734 nm that was lost when quenched by an antioxidant. The loss in absorbance of the ABTS radical cation was monitored at different concentrations of the test compound after a fixed time period of 5 min. A plot of the reduction in absorbance against different concentrations of the test compound gave a straight line, the gradient of which was correlated to the antioxidant activity of the test compound.

Table 1
Structures of stilbenes investigated for chemopreventive activity

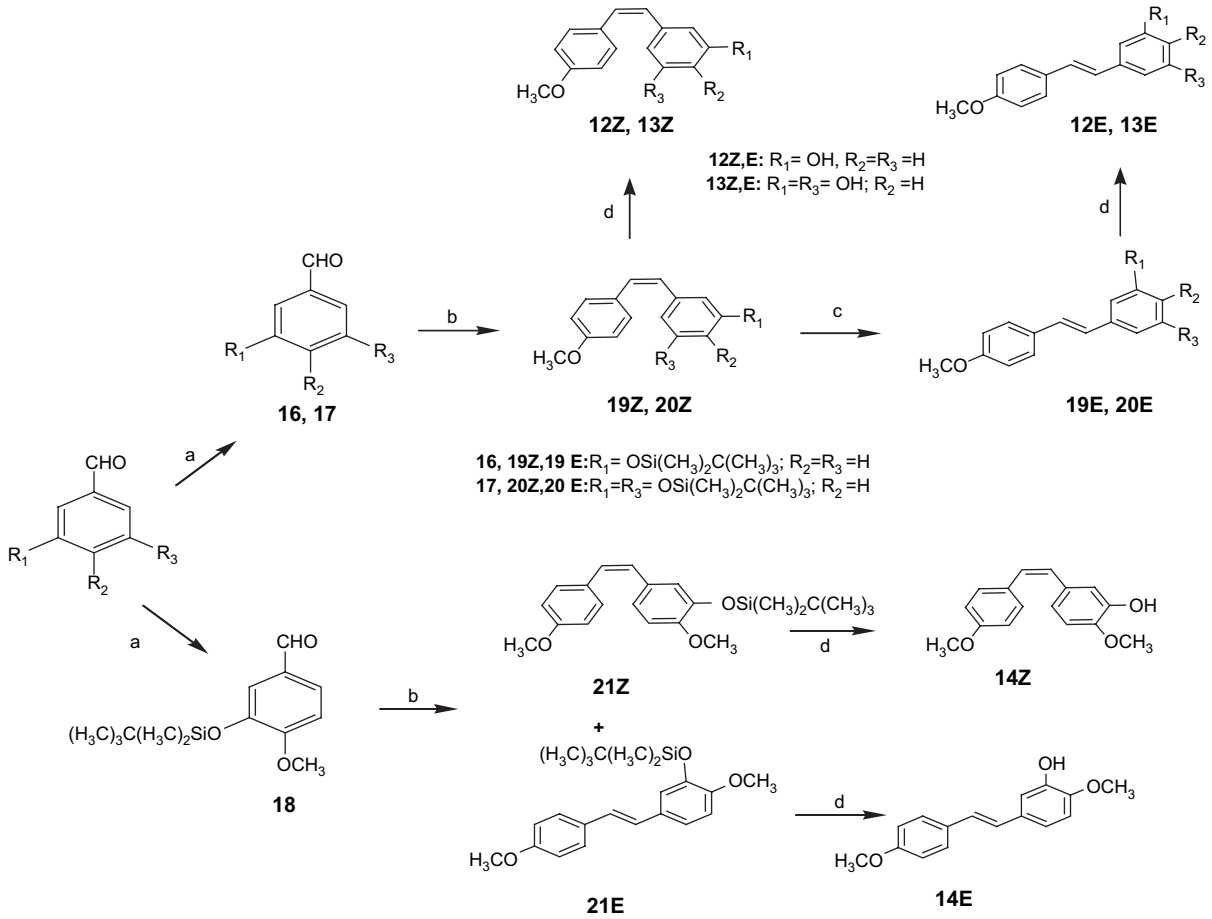


Compound	R	R′	Compound	R	R′
3*Z*, **3*E***	2-OCH_3	4′-OCH_3	**11*E***	2-OH	4′-OCH_3
4*Z*, **4*E***	3-OCH_3	4′-OCH_3	**12*Z***, **12*E***	3-OH	4′-OCH_3
5*Z*, **5*E***	4-OCH_3	4′-OCH_3	**13*Z***, **13*E***	3,5-OH	4′-OCH_3
6*Z*, **6*E***	2,4-OCH_3	4′-OCH_3	**14*Z***, **14*E***	3-OH, 4-OCH_3	4′-OCH_3
7*Z*, **7*E***	3,4-OCH_3	4′-OCH_3	**15*E***	2-OCH_3	2′-OCH_3
8*Z*, **8*E***	3,5-OCH_3	4′-OCH_3			
9*E*	2,6-OCH_3	4′-OCH_3	Resveratrol[a]	3,5-OH	4′-OH
10*Z*, **10*E***	3,4,5-OCH_3	4′-OCH_3	Pinosylvin[a]	3,5-OH	H

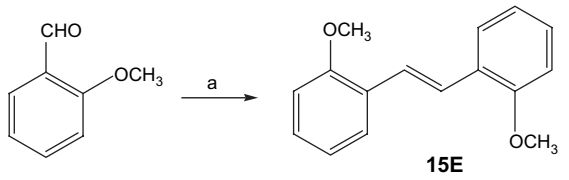
[a] Purchased from commercial sources.



Scheme 1. Synthetic pathway for stilbenes **3**–**11**. Reagents and conditions: (a) PBr_3, CH_2Cl_2, 0 °C; (b) PPh_3, toluene, reflux; (c) *n*-BuLi, THF, −78 °C. Only *E* isomers of compounds **9** and **11** were synthesized.



Scheme 2. Synthetic pathways for stilbenes **12**–**14**. Reagents and conditions: (a) *t*-Bu$(CH_3)_2$SiCl, DIEA, THF; (b) *n*-BuLi, THF, −78 °C, 4-methoxybenzyltriphenylphosphonium bromide **3**; (c) I_2, heptane, 12 h reflux; (d) Bu_4NF, THF. Compound **21** was obtained as a mixture of *Z* and *E* isomers. These were separated by column chromatography and then individually reacted via (d).



Scheme 3. Synthetic pathway for stilbene **15E**. Reagents and conditions: (a) $TiCl_4$, zinc powder, THF, 12 h reflux.

The experiment was also carried out using a standard antioxidant, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The quotient of the gradients of the lines obtained with the test compound and Trolox gave the TEAC (Trolox equivalent antioxidant capacity) of the test compound. The TEAC indicates the number of times the test compound is more effective than Trolox in quenching the ABTS radical cation under similar experimental conditions. TEAC of stilbenes **9*E***, **11*E*** and **15*E*** were not determined.

3.2. Microculture tetrazolium assay for determining cell viability

The assay is based on the ability of the mitochondrial enzyme succinate dehydrogenase of viable cells to reduce the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to an insoluble purple substance called formazan. The formation of formazan can be quantified by spectrophotometry (λ_{max} 590 nm) and afforded a convenient and sensitive means of monitoring the effect of test compounds on cell viability [18]. In this investigation, the MTT assay was carried out on murine hepatoma cells (Hepa 1c1c7). These cells were used in the QR induction assay and the MTT assay served to determine the concentration of test compound to be used in that assay. The concentration of test compound should be one that did not adversely affect the viability of the Hepa 1c1c cells (≥55% viability at the test concentration).

3.3. Induction of QR activity

The murine hepatoma cell line Hepa 1c1c7 contains easily measurable inducible QR. The induction of QR has been shown to correlate with the elevation of other protective phase 2 enzymes. Thus it serves as a reliable biomarker for identifying potential cancer chemopreventive agents [13]. Briefly, the QR induction assay is based on the generation of NADPH when glucose-6-phosphate was reduced by glucose-6-phosphate dehydrogenase in the presence of its cofactor NADP. Once formed, NADPH served as an electron donor for the QR mediated reduction of menadione to menadiol. Menadiol reduced MTT to formazan which was measured at 590 nm. Both NADP and menadione were regenerated during the catalytic cycle and therefore need not be replenished during the course of the assay. An inducer of QR increases the rate at which menadiol is generated and this would in turn lead to more formation of formazan. In this investigation, the compounds were screened for QR induction at concentrations that maintained cell viability at ≥55%.

The QR induction assay can also be used to determine if an agent is monofunctional (induces phase II enzymes only) or bifunctional (induces both phase I and II enzymes) in nature. This is done by comparing the levels of induction in wild type Hepa 1c1c7 cells with that observed in a mutant cell line (c1) which lacks CYP1A1 dependent aryl hydrocarbon hydrolase (AHH) activity. Monofunctional inducers induce QR activity of wild type and mutant Hepa cell lines to similar extents but bifunctional inducers induce QR activity of wild type cells to a greater degree. This is because the mutant cell line lacks an inducible phase I enzyme which is required to convert the compound into an activated form for the induction of the phase II enzyme. As reported in the literature [20], the induction properties of sulphoraphane and β-naphthoflavone were found in our assays to be monofunctional and bifunctional, respectively, when tested on wild type and mutant cell lines.

4. Results and discussion

4.1. Antioxidant activity of stilbenes

The antioxidant activity of the stilbenes was assessed from their TEAC values, which compares the radical scavenging activity of the test compound to that of a standard antioxidant (Trolox). Under the present experimental conditions, the TEAC of resveratrol was found to be 2.6 (Table 2). Pinosylvin (*E*-3,5-dihydroxystilbene) which does not have a 4′-OH on ring B had a lower TEAC value of 2.0. Interestingly, **13*E*** which has 4′-methoxy on ring B but the same ring A substituents (3,5-dihydroxyl) as resveratrol had a TEAC of 2.5. Thus, the antioxidant activity of resveratrol owes little to the presence of the 4′-OH group on ring B, as this group can be methoxylated or omitted with no loss of antioxidant activity. In contrast, the dihydroxyl group on ring A of resveratrol contributed significantly to the antioxidant activity. Starting from **13*E*** which had almost the same TEAC as resveratrol, removal of one OH group from ring A to give **12*E*** caused a sharp drop in TEAC (1.1). Replacing both OH groups with methoxy

Table 2
Radical quenching ability of stilbenes measured in terms of TEAC values

Compound[a]	TEAC[b]
12*Z*	1.04 (0.05)
12*E*	1.11 (0.03)
13*Z*	1.86 (0.03)
13*E*	2.49 (0.06)
14*Z*	1.11 (0.02)
14*E*	1.29 (0.06)
Resveratrol	2.74 (0.09)
Pinosylvin	2.04 (0.06)

[a] Other stilbenes (**3*E***, **Z**−**8*E***, **Z**, **10*E***, **Z**, **12*E***, **Z**−**14*E***, **Z**) had no radical quenching properties in this assay. Compounds **9*E***, **11*E*** and **15*E*** were not evaluated.

[b] Trolox equivalent antioxidant capacity, given as mean (±SD) of three determinations.

groups (**8*E***) completely abolished radical scavenging activity. When one of the two hydroxyl groups on ring A was methoxylated to give **14*E***, TEAC was reduced to 1.3. Thus, antioxidant activity depends on the presence of at least one OH group on ring A. Stilbene **11E** which has a 2-OH group on ring A should also possess antioxidant activity but its TEAC was not evaluated in this investigation. Should future investigations show otherwise, this may be due to the anomalous effect of the *ortho*-OH group (''ortho-effect'').

No antioxidant activity was observed when only methoxy groups were present on ring A. In contrast, methoxy groups on ring B did not adversely affect the antioxidant activity. As for the antioxidant activity of *Z* and *E* isomers, a comparison of the TEAC values of three pairs of *Z*/*E* isomers (**12**, **13** and **14**) showed that the *E* isomer had greater antioxidant activity.

4.2. Induction of quinone reductase activity

Prior to carry out the assay on QR induction activity, the viability of the test compounds was evaluated at various concentrations to identify suitable concentrations that maintained viability of the Hepa 1c1c7 cells at 55% or more. Based on the results, the stilbenes were evaluated at 12.5 μM, 5 or 1 μM. Greater cytotoxicity was generally observed for the *Z* isomers.

The concentration of resveratrol required to double the specific activity of QR (CD value) was reported to vary between 21–25 μM [19,21]. In our study, resveratrol at 25 μM increased quinone reductase activity by 1.5 times. The viability of the cells exposed to 25 μM resveratrol was about 55%, and as this might affect induction activity, resveratrol was tested at a lower concentration of 12.5 μM. At this concentration, cell viability rose to 80% but induction of QR was only 1.2-fold.

Of the stilbenes tested at 12.5 μM, only five compounds induced QR to a greater extent than resveratrol, namely **3*E***, **4*E***, **9*E***, **14*E*** and **15*E*** (Table 3). Of these, the QR induction activity of **3*E***, **9*E*** and **15*E*** fell within a narrow range of 1.8–2.0 and were significantly greater than that of **4*E***, **14*E*** and resveratrol tested at similar concentrations. Compounds **3*E***, **9*E*** and **15*E*** share in common the presence of *ortho*-methoxy substituents on their stilbene framework. It is notable that the regioisomers of **3*E*** (2-OCH_3), namely **4*E*** (3-OCH_3) and **5*E*** (4-OCH_3), had significantly lower QR induction activities. This serves to underscore the importance of the *ortho*-methoxy substituent on ring A. A similar observation was made for **9*E*** (two *ortho*-methoxy groups on ring A) and **15*E*** (*ortho*-methoxy substituents on both rings A and B). The QR induction activity of **3*E*** was further determined at a lower concentration of 6.3 μM and found to be 1.8, which still exceeded that of resveratrol (1.1) tested at the same concentration. Although these observations suggest that *ortho*-methoxy groups have a special role in inducing QR activity, there are limitations that the compounds had variable effects on cell viability at the test concentration (12.5 μM) (Table 3). A better approach might be to determine the CD values of the promising compounds but this would still be difficult for **3*E*** whose IC_{50} was estimated to be near 12.5 μM. Notwithstanding this limitation, the association of *ortho*-methoxy groups with good QR induction activity in stilbenes has not been observed before. It is notable that none of these compounds have antioxidant properties. Most inducers of phase II enzymes activate the antioxidant response element (ARE) through Keap1 and Nrf2 [22]. ARE is activated by electrophiles and antioxidants, and this may be the means by which resveratrol, a known antioxidant, induced phase II enzymes. The QR induction activities of the methoxylated resveratrol analogues are likely to be

Table 3
Effect of test compounds on viability of Hepa 1c1c7 cells and QR induction activities

Compound	% Viability[a]	QR induction of Hepa 1c1c cells[b]		Compound	% Viability[a]	QR induction of Hepa 1c1c cells[b]	
		Wild type	Mutant (c1)			Wild type	Mutant (c1)
3*E*	58 (12.5 μM)[c]	2.04 (0.07)[c]	1.14 (0.06)	**10*E***	86 (1 μM)	<1.0	ND
3*Z*	56 (1 μM)	<1.0	ND[d]	**10*Z***	88 (1 μM)	<1.0	ND
4*E*	90 (12.5 μM)	1.44 (0.05)	<1.0	**11*E***	80 (12.5 μM)	1.13 (0.04)	ND
4*Z*	92 (1 μM)	<1.0	ND	**12*E***	81 (12.5 μM)	1.08 (0.03)	ND
5*E*	67 (12.5 μM)	1.14 (0.04)	ND	**12*Z***	90 (5 μM)	<1.0	ND
5*Z*	64 (12.5 μM)	1.06 (0.04)	ND	**13*E***	87 (12.5 μM)	1.14 (0.05)	ND
6*E*	58 (12.5 μM)	1.18 (0.05)	ND	**13*Z***	96 (12.5 μM)	1.04 (0.04)	ND
6*Z*	85 (1 μM)	<1.0	ND	**14*E***	77 (12.5 μM)	1.33 (0.05)	ND
7*E*	100 (1 μM)	<1.0	ND	**14*Z***	75 (5 μM)	<1.0	ND
7*Z*	86 (1 μM)	<1.0	ND	**15*E***	82 (12.5 μM)	1.81 (0.02)	<1.0
8*E*	94 (1 μM)	<1.0	ND	Resveratrol	80 (12.5 μM)	1.24 (0.03)	1.16 (0.04)
8*Z*	82 (1 μM)	<1.0	ND	Pinosylvin	61 (12.5 μM)	1.07 (0.04)	ND
9*E*	90 (12.5 μM)	1.92 (0.06)	1.05 (0.02)				

[a] Mean of three determinations on Hepa 1c1c7 cells by MTT assay. Values in bracket indicate concentration of compound tested.

[b] QR induction activity (mean ± SD, $n = 3$) was determined on wild type Hepa 1c1c7 cells at the same concentration used to determine % viability. Compounds with QR activity > 1.2 were also tested on the mutant Hepa 1c1c7c1 cell line. CD (concentration required to double QR activity) values of sulforaphane were 0.26 (±0.04) μM and 0.28 (±0.04) μM on Hepa 1c1c7 wild type and c1 mutant cells, respectively. CD value of β-napthoflavaone was 0.028 (±0.003) μM on Hepa 1c1c7 wild type cells. It had no activity on c1 mutant cells.

[c] When tested at 6.3 μM, viability of Hepa 1c1c7 cells was 73%. QR induction at this concentration was 1.8.

[d] ND = not determined.

mediated by mechanisms that do not involve their antioxidant properties.

The induction properties of the *ortho*-methoxylated stilbenes **3*E***, **4*E***, **9*E*** and **15*E*** were further investigated to determine if they were mono or bifunctional inducers of metabolizing enzymes. Bifunctional inducers induce activities of both phase I and II enzymes, unlike monofunctional inducers which elevate phase II enzymes selectively without inducing phase I enzymes [22]. Resveratrol induced QR activity of the wild type Hepa 1c1c7 cells to the same extent as the mutant c1 cells that lacked a phase I enzyme (Table 3). Thus it is a monofunctional inducer and its ability to induce QR did not depend on prior biotransformation to an active metabolite by oxidative metabolism. In contrast, the methoxylated analogs **3*E***, **4*E***, **9*E*** and **15*E*** were found to be bifunctional inducers. Their bifunctional status raised the question as to the likely active metabolite responsible for the induction activity. Because these compounds have methoxyl groups, it is possible that one or more of these groups are dealkylated by phase I enzymes to generate phenolic hydroxyl groups. This has been observed for the antioxidant 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) whose induction activity was traced for conversion to a 1,4-diphenol by *O*-dealkylases in the Hepa cells [23]. Taking **3*E*** as an example, dealkylation of the *ortho*-methoxy group on ring A would result in **11*E***, but since the latter had negligible QR induction activity (Table 3), this was not a likely option. Another possibility was the dealkylation of the 4′-methoxy of **3*E*** to give an active metabolite (2-methoxy, 4′-hydroxystilbene). This compound was not synthesized but a comparison of the induction activities of resveratrol (1.2 at 12.5 μM) and its 4′-methoxylated analogue (**13*E***, 1.1 at 12.5 μM) suggested that the state of the 4′-substituent was unlikely to affect QR induction activity. Other options are possible such as hydroxylation of the methoxylated analogues at either ring A or B. Notwithstanding these possibilities, it is clear that *ortho*-substituted methoxylated stilbenes induced QR activity to a greater extent and work through different mechanisms compared to resveratrol.

5. Conclusion

A library of *Z* and *E* methoxylated analogues of resveratrol were synthesized and investigated for their chemopreventive potential through QR induction and antioxidant assays. The introduction of methoxy groups generally diminished QR induction except in compounds with *ortho*-methoxy substituents. We found that the QR induction activities of these analogues exceeded that of resveratrol. The best compound identified in this study (**3*E***) was 1.8 times more effective than resveratrol as a QR inducer when tested at 6.3 μM. However, there was a qualitative difference in the way these compounds induced QR activity. Unlike resveratrol, the methoxylated analogues were bifunctional inducers, that is, they required prior activation by phase I enzymes before they can induce phase II enzymes. The nature of the active metabolite is yet to be determined but it is quite clear that dealkylation of the methoxy groups was not involved. The methoxylated analogues also lacked the antioxidant activity of resveratrol and thus may induce QR activity by a different mechanism. The methoxylation of hydroxyl groups impart greater lipophilicity and stability against metabolic inactivation. Methoxylated analogues of resveratrol that retain QR induction activity, as those identified in this study, provide a useful starting point for the rational design of chemopreventive agents with improved pharmacokinetic and pharmacodynamic properties.

6. Experimental

6.1. Chemistry

Melting points were determined on a Buchi melting point apparatus (B540) in open glass capillary tubes and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF (DPX 300 MHz) spectrometer. ^{1}H and ^{13}C Chemical shifts were reported in δ (ppm), relative to tetramethylsilane as internal standard (for ^{1}H NMR only) and obtained in $CDCl_3$ as solvent, unless otherwise stated. Thin layer chromatography (TLC) was carried out on pre-coated plates (silica gel $60F_{254}$, Merck) and visualized with ultraviolet light. Flash column chromatography was performed with silica gel 60 (70–230 mesh) with hexane/ethyl acetate as eluting solvent. The purity of compounds **3–15** was assessed by reverse phase high-pressure column chromatography. Determinations were carried out on a Waters Delta 600 liquid chromatography system, using Zorbax Eclipse XDB-C_{18} column (4.6 mm × 150 mm, 5 μm) and UV detection (278 nm). The isocratic mode was employed using two solvent systems (methanol–water and acetonitrile–water in the ratio of 9:1). The area under the main peak was determined and expressed as a percentage of total peak area during a 15 min run. Compounds **3–15** were purified until their chromatograms showed a main peak with areas > 97% on both solvent systems.

6.1.1. 4-Methoxybenzyl bromide (*1*)

Phosphorus tribromide (3.1 ml) was slowly added to a solution of 4-methoxybenzylalcohol (12.79 ml) in dichloromethane (150 ml) at 0 °C, and stirring was continued for 12 h. The reaction mixture was poured into aqueous sodium bicarbonate and extracted with dichloromethane. Removal of the solvent *in vacuo* from the organic phase gave the product as a clear oil (19.1 g, 95%). ^{1}H NMR: δ 7.3 (2H, d, $J = 8.67$), 6.9 (2H, d, $J = 8.64$), 4.5 (2H, s, CH_2), 3.9 (3H, s, OCH_3).

6.1.2. 4-Methoxybenzyltriphenylphosphonium bromide (*2*)

Triphenylphosphine (25 g) was added to a solution of bromide **1** (19.1 g) in toluene (250 ml). The mixture was heated to reflux for 6 h and then cooled to room temperature. The product was collected, recrystallized from ethanol and obtained as a colorless solid (42.3 g, 96%), mp 234–235 °C. ^{1}H NMR: δ 7.7 (15H, m), 7.0 (2H, d, $J = 8.70$), 6.6 (2H, d, $J = 8.70$), 5.7 (2H, s), 3.7 (3H, s, OCH_3).

*6.1.3. General procedure for the synthesis of stilbenes (**3–11**)*

n-Butyllithium (1.6 M in hexane, 1 mmol) was added to 4-methoxybenzyltriphenylphosphonium bromide **2** (1.1 mmol) in anhydrous tetrahydrofuran (30 ml) at −78 °C, and the resulting red solution was stirred under nitrogen for 30 min. A solution of aldehyde (1 mmol) in anhydrous tetrahydrofuran was added dropwise over 30 min and the mixture was stirred for 16 h. The resulting suspension was poured into water and extracted with ethyl acetate. The organic phase was washed with brine, and removal of the solvent *in vacuo* gave a mixture of *cis* and *trans* isomers. The two isomers were separated by flash column chromatography (99:1 hexane/ethyl acetate), with the *Z* isomer eluted first from the column followed by the *E* isomer. The *Z* isomer was obtained as a clear oil, in contrast to the *E* isomer which was obtained as a colorless solid.

*6.1.3.1. 2,4′-Dimethoxystilbene (**3**).* Yield 60%, 0.72 g; **3Z** [24] (0.54 g): ^{1}H NMR: δ 7.22 (2H, d, $J = 7.2$ Hz), 7.17 (2H, d, $J = 8.67$), 6.90 (1H, d, $J = 8.3$), 6.70–6.78 (3H, m), 6.57 (2H, s), 3.77 (3H, s, OCH_3), 3.83 (3H, s, OCH_3).

Compound **3E** (0.18 g), mp 85–87 °C, lit. 85–86 °C [25]. ^{1}H NMR: δ 7.57 (1H, d, $J = 7.53$ Hz), 7.47 (2H, d, $J = 8.67$ Hz), 7.35 (1H, d, $J = 16.2$ Hz), 7.21 (1H, d, $J = 6.9$ Hz), 7.06 (1H, d, $J = 16.5$ Hz), 6.88–6.98 (4H, m), 3.83 (3H, s, OCH_3), 3.89 (3H, s, OCH_3).

*6.1.3.2. 3,4′-Dimethoxystilbene (**4**).* Yield 72%, 0.86 g; **4Z** [24] (0.64 g, oil): ^{1}H NMR: δ 7.15 (1H, d, $J = 7.9$ Hz), 7.2 (2H, d, $J = 8.67$ Hz), 6.83–6.88 (2H, m), 6.73–6.79 (3H, m), 6.54 (1H, d, $J = 12.42$ Hz), 6.48 (1H, d, $J = 12.42$ Hz), 3.79 (3H, s, OCH_3), 3.69 (3H, s, OCH_3). Compound **4E** (0.22 g), mp 106–109 °C, lit. 107–108 °C [26]. ^{1}H NMR: δ 7.45 (d, $J = 8.67$ Hz, 2H), 7.26 (t, $J = 8.3$ Hz, 1H), 7.04–7.10 (m, 3H), 6.89–6.97 (m, 3H), 6.80 (q, $J = 1.9$ Hz, 1H), 3.85 (3H, s, OCH_3), 3.83 (3H, s, OCH_3).

*6.1.3.3. 4,4′-Dimethoxystilbene (**5**).* Yield 72%, 0.99 g; **5Z** [27] (0.69 g, oil): ^{1}H NMR: δ 7.2 (4H, d, $J = 9$ Hz), 6.77 (4H, d, $J = 9.0$ Hz), 6.45 (s, 2H), 3.79 (6H, s, OCH_3). Compound **5E** (0.3 g), mp 214–215 °C, lit. 214–216 °C [28]. ^{1}H NMR: δ 7.43 (4H, d, $J = 9$ Hz), 6.93 (s, 2H), 6.89 (4H, d, $J = 9.0$ Hz), 3.83 (6H, s, OCH_3).

*6.1.3.4. 2,4,4′-Trimethoxystilbene (**6**).* Yield 84%, 1.03 g; **6Z** (0.7 g, oil): ^{1}H NMR: δ 7.1–7.2 (3H, m), 6.73 (2H, d, $J = 8.6$ Hz), 6.5 (2H, d, $J = 1.5$ Hz), 6.46 (1H, d, $J = 2.3$ Hz), 6.33 (1H, q, $J = 2.6$ Hz), 3.81 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.77 (3H, s, OCH_3). ^{13}C NMR (300 MHz): δ 160.2, 158.4, 158.3, 130.5, 130.2, 130.0, 128.6, 123.7, 119.1, 113.4, 104.3, 98.3, 55.5, 55.3, 55.1.

Compound **6E** (0.33 g), mp 86–88 °C. ^{1}H NMR: δ 7.43–7.5 (3H, m), 7.25 (1H, d, $J = 16.2$ Hz), 6.95 (1H, d, $J = 16.6$ Hz), 6.88 (2H, d, $J = 8.67$ Hz), 6.52 (1H, q, $J = 2.3$ Hz), 6.47 (1H, d, $J = 2.3$ Hz), 3.86 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.82 (3H, s, OCH_3). ^{13}C NMR (300 MHz): δ 160.2, 158.8, 157.8, 131.2, 127.5, 126.9, 126.6, 121.2, 119.9, 114.0, 104.9, 98.5, 55.5, 55.4, 55.3.

*6.1.3.5. 3,4,4′-Trimethoxystilbene (**7**).* Yield 77%, 0.94 g; **7Z** [29] (0.66 g, oil): ^{1}H NMR: δ 7.21–7.26 (3H, m), 6.74–6.84 (4H, m), 6.48 (1H, d, $J = 12.03$ Hz), 6.43 (1H, d, $J = 12.06$ Hz), 3.87 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.65 (3H, s, OCH_3). Compound **7E** (0.28 g), mp 136–137 °C, lit. mp 136–138 °C [30]. ^{1}H NMR: δ 7.44 (2H, d, $J = 8.67$ Hz), 6.84–7.05 (7H, m), 3.95 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.83 (3H, s, OCH_3).

*6.1.3.6. 3,4′,5-Trimethoxystilbene (**8**).* Yield 60%, 0.74 g; **8Z** [31] (0.55 g): ^{1}H NMR: δ 7.21 (2H, d, $J = 8.67$ Hz), 6.77 (2H, d, $J = 8.67$ Hz), 6.53 (1H, d, $J = 12.42$ Hz), 6.44 (1H, d, $J = 12.03$ Hz), 6.43 (2H, d, $J = 2.25$ Hz), 6.32 (1H, t, $J = 2.25$ Hz), 3.78 (3H, s, OCH_3), 3.67 (6H, s, OCH_3). Compound **8E** (0.19 g), mp 49–54 °C, lit. mp 50 °C [32]. ^{1}H NMR: δ 7.45 (2H, d, $J = 8.64$ Hz), 7.04 (1H, d, $J = 16.2$ Hz), 6.902 (1H, d, $J = 16.56$ Hz), 6.90 (2H, d, $J = 9.06$ Hz), 6.65 (2H, d, $J = 2.28$ Hz), 6.38 (1H, t, $J = 2.28$ Hz), 3.83 (9H, s, OCH_3).

*6.1.3.7. 2,4′,6-Trimethoxystilbene (**9**).* Compound **9E** (0.65 g, 48% yield), mp 79–80 °C. ^{1}H NMR: δ 7.52 (1H, d, $J = 16.56$ Hz), 7.47 (2H, d, $J = 9.06$ Hz), 7.31 (1H, d, $J = 16.56$ Hz), 7.14 (1H, t, $J = 8.28$ Hz), 6.88 (2H, d, $J = 8.64$ Hz), 6.59 (2H, d, $J = 8.28$ Hz), 3.89 (6H, s, OCH_3), 3.82 (3H, s, OCH_3). ^{13}C NMR (300 MHz): δ 158.8, 158.5, 132.1, 131.9, 127.6, 127.5, 117.8, 115.1, 113.9, 104.0, 55.8, 55.3.

*6.1.3.8. 3,4,4′,5-Tetramethoxystilbene (**10**).* Yield 80%, 1.2 g; **10Z** [33] (0.8 g, oil): ^{1}H NMR: δ 7.24 (2H, d, $J = 8.67$ Hz), 6.79 (2H, d, $J = 8.67$ Hz), 6.52 (1H, d, $J = 12.06$ Hz), 6.50 (2H, s), 6.42 (1H, d, $J = 12.06$ Hz), 3.84 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.69 (6H, s, OCH_3). Compound **10E** (0.4 g), mp 158–159 °C, lit. mp 157 °C [34]. ^{1}H NMR: δ 7.44 (2H, d, $J = 8.64$ Hz), 6.98 (1H, d, $J = 16.2$ Hz), 6.90 (2H, d, $J = 9.39$ Hz), 6.89 (1H, d, $J = 16.2$ Hz), 6.72 (2H, s), 3.92 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.84 (3H, s, OCH_3).

*6.1.3.9. 2-Hydroxy-4′-methoxystilbene (**11**).* Compound **11E** (0.023 g, 2% yield), mp 141–143 °C, lit. mp 145–147 °C [35]. ^{1}H NMR (DMSO-d_6): δ 9.66 (1H, s, OH), 7.53 (1H, d, $J = 7.9$ Hz), 7.49 (2H, d, $J = 8.67$ Hz), 7.26 (1H, d, $J = 16.6$ Hz), 7.13 (1H, d, $J = 16.6$ Hz), 7.04–7.09 (1H, m), 6.94 (2H, d, $J = 8.67$ Hz), 6.78–6.86 (2H, m), 3.77 (3H, s, OCH_3).

6.1.4. General procedure for the protection of phenolic groups with tert-butyldimethylsilyl chloride

To a solution of the hydroxybenzaldehyde (5 mmol) in anhydrous tetrahydrofuran (30 ml) was added *N*,*N*-diisopropylethylamine (DIEA, 1.29 g, 2 equiv). The solution was stirred for 15 min, after which *tert*-butyldimethylsilyl chloride (1.79 g, 2 equiv) was added, and the solution was stirred for

12 h. The reaction mixture was poured into water and extracted with ethyl acetate. Removal of the solvent *in vacuo* from the organic phase provided a clear oil. The oil was separated by flash column chromatography (9:1 hexane/ethyl acetate) to give the product as a clear oil.

*6.1.4.1. 3-(tert-Butyldimethylsilyloxy)benzaldehyde (**16**).* Yield 70%, 0.93 g. ^{1}H NMR: δ 9.95 (1H, s, $J = 1$ Hz, CHO), 7.48 (1H, t, $J = 1.14$ Hz), 7.40 (1H, t, $J = 7.53$ Hz), 7.33 (1H, t, $J = 2.64$ Hz), 7.10 (1H, q, $J = 1.14$ Hz), 1.0 (9H, s, $C(CH_3)_3$), 0.23 (6H, s, $Si(CH_3)_2$).

*6.1.4.2. 3,5-Di(tert-butyldimethylsilyloxy) benzaldehyde (**17**).* Four equivalents (2.58 g) of DIEA and 2 equiv (3.58 g) of *tert*-butyldimethylsilyl chloride were reacted with 3,5-dihydroxybenzaldehyde (5 mmol) as described earlier. Yield 58%, 0.97 g. ^{1}H NMR: δ 9.86 (s, $J = 1$H, CHO), 6.96 (d, $J = 2.25$ Hz, 2H), 6.59 (t, $J = 2.28$ Hz, 1H), 0.99 (18H, s, $C(CH_3)_3$), 0.22 (12H, s, $Si(CH_3)_2$).

*6.1.4.3. 3-(tert-Butyldimethylsilyloxy)-4-methoxy benzaldehyde (**18**).* Two equivalents (1.29 g) of DIEA and 2 equiv (1.79 g) of *tert*-butyldimethylsilyl chloride were reacted with 3-hydroxy-4-methoxybenzaldehyde (5 mmol) as described earlier. Yield 80%, 1.07 g. ^{1}H NMR: δ 9.8 (s, $J = 1$H, CHO), 7.48 (q, $J = 1.86$ Hz, 1H), 7.37 (d, $J = 1.89$ Hz, 1H), 6.96 (d, $J = 8.31$ Hz, 2H), 3.9 (3H, s, OCH_3), 1.0 (9H, s, $C(CH_3)_3$), 0.17 (6H, s, $Si(CH_3)_2$).

*6.1.5. General procedure for the synthesis of silyloxystilbenes (**19–21**)*

Equimolar quantities (5 mmol) of benzaldehyde (**16**, **17** or **18**) and 4-methoxybenzyltriphenyl phosphonium bromide **2** were reacted in the presence of *n*-butyllithium and tetrahydrofuran as solvent as described earlier. Stilbenes **19** and **20** were obtained as *Z* isomers. The *E* isomer was obtained by refluxing a solution of *Z* isomer (5 mmol) in heptane (20 ml) in the presence of a catalytic amount of iodine (one crystal) for 16 h. The reaction mixture was diluted with 20 ml ether and washed with saturated aqueous sodium bisulfite (40 ml) and brine (2×10 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give the *trans* isomer in 90–92% yields. Stilbene **21** was obtained as a mixture of *Z* and *E* isomers that were separated by column chromatography.

*6.1.5.1. 3-(tert-Butyldimethylsilyloxy)-4′-methoxystilbene (**19**).* Compound **19*Z***: clear oil, 76% yield. ^{1}H NMR: δ 7.18 (d, $J = 9.06$ Hz, 2H), 7.09 (d, $J = 7.92$ Hz, 2H), 6.82–6.99 (m, 3H), 6.75 (d, $J = 9.03$ Hz, 1H), 6.52 (d, $J = 12.06$ Hz, 1H), 6.45 (d, $J = 12.06$ Hz, 1H), 3.78 (3H, s, OCH_3), 0.93 (9H, s, $C(CH_3)_3$), 0.093 (6H, s, $Si(CH_3)_2$).

Compound **19*E***: ^{1}H NMR: δ 7.45 (d, $J = 8.67$ Hz, 2H), 7.19 (d, $J = 15.8$ Hz, 1H), 7.08 (d, $J = 15.8$ Hz, 1H), 6.88–6.99 (m, 5H), 6.72 (q, $J = 2.28$ Hz, 1H), 3.83 (3H, s, OCH_3), 1 (9H, s, $C(CH_3)_3$), 0.22 (6H, s, $Si(CH_3)_2$).

*6.1.5.2. 3,5-Di-(tert-butyldimethylsilyloxy)-4′-methoxystilbene (**20**).* Compound **20*Z***: clear oil, 68% yield. ^{1}H NMR: δ 7.17 (d, $J = 8.67$ Hz, 2H), 6.75 (d, $J = 8.64$ Hz, 2H), 6.5 (d, $J = 12.42$ Hz, 1H), 6.4 (d, $J = 12.06$ Hz, 1H), 6.36 (d, $J = 1.89$ Hz, 2H), 6.19 (t, $J = 2.25$ Hz, 1H), 3.77 (3H, s, OCH_3), 0.93 (18H, s, $C(CH_3)_3$), 0.097 (12H, s, $Si(CH_3)_2$).

Compound **20*E***: ^{1}H NMR: δ 7.44 (d, $J = 8.67$ Hz, 2H), 6.97 (d, $J = 16.56$ Hz, 1H), 6.89 (d, $J = 8.64$ Hz, 2H), 6.83 (d, $J = 16.2$ Hz, 1H), 6.6 (d, $J = 2.25$ Hz, 2H), 6.24 (t, $J = 2.28$ Hz, 1H), 3.83 (3H, s, OCH_3), 0.99 (18H, s, $C(CH_3)_3$), 0.21 (12H, s, $Si(CH_3)_2$).

*6.1.5.3. 3-(tert-Butyldimethylsilyloxy)-4,4′-dimethoxystilbene (**21**).* Yield 71%, 1.32 g; **21*Z*** (0.8 g): ^{1}H NMR: δ 7.19 (d, $J = 8.67$ Hz, 2H), 6.7–6.83 (m, 5H), 6.44 (d, $J = 12.06$ Hz, 1H), 6.39 (d, $J = 12.06$ Hz, 1H), 3.78 (6H, s, OCH_3), 0.93 (9H, s, $C(CH_3)_3$), 0.06 (6H, s, $Si(CH_3)_2$). **21*E***: (0.52 g). ^{1}H NMR: δ 7.42 (d, $J = 8.67$ Hz, 1H), 7.03 (d, $J = 7.89$ Hz, 2H), 6.81–6.9 (m, 5H), 3.83 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 1.02 (9H, s, $C(CH_3)_3$), 0.18 (6H, s, $Si(CH_3)_2$).

*6.1.6. General procedure for deprotection of silyoxystilbenes (**19–21**)*

Tetrabutylammonium fluoride was added to a solution of the *E* or *Z* silyloxy-protected stilbene in anhydrous tetrahydrofuran (20 ml). The pale yellow solution was stirred for 45 min, poured into water, and extracted with dichloromethane, from which removal of the solvent *in vacuo* provided a clear oil in 80–90% yield. The oil was separated by gravity column chromatography (9:1 hexane/ethyl acetate) to give the product.

*6.1.6.1. 3-Hydroxy-4′-methoxystilbene (**12**).* Compound **12*Z*** (80% yield). ^{1}H NMR: δ 7.19 (d, $J = 8.67$ Hz, 2H), 7.1 (d, $J = 7.53$ Hz, 1H), 6.84 (d, $J = 7.53$ Hz, 1H), 6.73–6.78 (m, 3H), 6.68 (q, $J = 2.64$ Hz, 1H), 6.52 (d, $J = 12.42$ Hz, 1H), 6.44 (d, $J = 12.06$ Hz, 1H), 3.78 (3H, s, OCH_3). ^{13}C NMR (300 MHz): δ 158.6, 155.4, 139.2, 130.2, 130.0, 129.7, 129.6, 128.4, 121.4, 115.4, 114.1, 113.6, 55.2.

Compound **12*E*** (91% yield), mp 157–160 °C, lit. mp 159–160 °C [36]. ^{1}H NMR (DMSO-d_6): δ 9.39 (1H, s, OH), 7.5 (d, $J = 8.64$ Hz, 2H), 7.16 (d, $J = 7.5$ Hz, 1H), 7.1 (d, $J = 15.5$ Hz, 1H), 7.0 (d, $J = 15.8$ Hz, 1H), 6.93–7.0 (m, 4H), 6.65 (q, $J = 1.89$ Hz, 1H), 3.34 (3H, s, OCH_3).

*6.1.6.2. 3,5-Dihydroxy-4′-methoxystilbene (**13**).* Compound **13*Z*** [37] (94% yield). ^{1}H NMR: δ 7.2 (2H, d, $J = 8.67$ Hz), 6.76 (2H, d, $J = 8.67$ Hz), 6.5 (1H, d, $J = 12.03$ Hz), 6.37 (1H, d, $J = 12.03$ Hz), 6.31 (2H, d, $J = 2.25$ Hz), 6.22 (1H, t, $J = 2.25$ Hz), 3.78 (3H, s, OCH_3). **13*E*** (82% yield), mp 164–167 °C, lit. mp 158–166 °C [38]. ^{1}H NMR (DMSO-d_6): δ 9.21 (2H, s, OH), 7.52 (2H, d, $J = 8.67$ Hz), 6.86–7.01 (4H, m), 6.4 (2H, d, $J = 1.89$ Hz), 6.1 (1H, t, $J = 3.69$ Hz), 3.34 (3H, s, OCH_3).

*6.1.6.3. 3-Hydroxy-4,4′-dimethoxystilbene (**14**).* Compound **14*Z*** (94% yield), mp 77–79 °C. ^{1}H NMR: δ 7.2 (2H, d, $J = 8.67$ Hz), 6.86 (1H, d, $J = 1.89$ Hz), 6.7–6.79 (4H, m), 6.45 (1H, d, $J = 12.03$ Hz), 6.39 (1H, d, $J = 12.06$ Hz), 5.5 (1H, s, OH), 3.87 (3H, s, OCH_3), 3.79 (3H, s, OCH_3). ^{13}C

NMR (300 MHz): δ 158.6, 145.6, 145.2, 131.0, 130.1, 129.8, 128.8, 128.3, 120.9, 114.9, 113.6, 110.4, 55.9, 55.2.

Compound **14*E*** [39] (92% yield), mp 192–193 °C. ^{1}H NMR (DMSO-d_6): δ 8.95 (1H, s, OH), 7.49 (2H, d, $J = 8.67$ Hz), 7.0 (1H, d, $J = 1.86$ Hz), 6.88–6.96 (6H, m), 3.78 (3H, s, OCH_3), 3.77 (3H, s, OCH_3).

*6.1.7. E-2,2′-dimethoxystilbene (**15**)*

Two equivalents of titanium tetrachloride in dichloromethane (1.5 M) was added dropwise to powdered zinc (2.5 equiv) in a round bottom flask that was charged with N_2 gas and cooled in an ice water bath [16]. About 50 ml of THF was then added and the mixture was brought to reflux for 2 h. It was then cooled to room temperature and a solution of 2-methoxybenzaldehyde (5 mmol) in THF was added dropwise. The mixture was then refluxed for 12 h. The reaction mixture was extracted with dichloromethane (3 times) and the organic layer was washed with brine and dried over anhydrous $MgSO_4$. The organic solvent was removed *in vacuo* and the residue was purified by column chromatography (99% hexane, 1% ethyl acetate). The product was obtained as a mixture of *Z* and *E* isomers, and converted to the *E* isomer by refluxing in heptane in the presence of iodine. Compound **15*E*** was obtained in 10% yield, mp 135–136 °C, lit. mp 135–136 °C [40]. ^{1}H NMR: δ 7.63–7.66 (2H, m), 7.47 (2H, s), 7.2–7.23 (2H, m), 6.87–6.98 (4H, m), 3.88 (6H, s, OCH_3).

6.2. Determination of quinone reductase activity

Activity of quinone reductase was determined in Hepa 1c1c7 murine hepatoma cells (American Type Culture Collection) grown in 96-well microtitre plates according to a described procedure [19]. Briefly, cells (10^4 per well) were grown for 24 h in α-minimal essential medium (without ribonucleosides or deoxyribonucleosides) containing 10% (v/v) fetal calf serum, 0.01% penicillin G, 0.15% sodium bicarbonate, 0.01% streptomycin sulfate in an humidified atmosphere of 5% CO_2 at 37 °C. The cells were then exposed to a known concentration of the test compound (stock solution prepared in DMSO) for 24 h. The final concentration of DMSO in each well was kept at 0.5% v/v or lower. After this time, the media was decanted and the cells lysed by a solution containing 0.8% w/v digitonin and 2 mM EDTA with agitation (10 min). An aliquot (200 μl) of a solution (''complete reaction mixture'') was then added to each well. This solution was freshly prepared just prior to use and consisted of 7.5 ml 0.5 M Tris-Cl (pH = 7.4), 100 mg bovine serum, 1 ml 1.5% Tween-20, 0.1 ml 7.5 mM FAD, 1 ml 150 mM glucose-6-phosphate, 90 μl 50 mM NADP, 300 units yeast glucose-6-phosphate dehydrogenase, 45 mg MTT and deionized water to a final volume of 150 ml. Menadione (0.2 μl of a 50 mM solution prepared in acetonitrile) was added just before the mixture was dispensed into the wells. After addition, the plate was gently agitated for 5 min, after which the reaction was quenched by adding a solution (50 μl) of 0.3 mM dicoumarol in 0.5% DMSO and 5 mM potassium phosphate, pH = 7.4. A blue color due to the formation of formazan was observed in each well, the absorbance of which was measured at 590 nm on a plate reader. Blank wells contained no cells and control wells contain Hepa 1c1c7 cells treated with medium containing 0.5% DMSO but without the test compound. QR induction activity of a compound tested at a given concentration was determined from the ratio of absorbances obtained in the presence of test compound and the control well, after subtracting the absorbance of the blank well. QR induction activity was also determined in the same manner on a mutant Hepa 1c1c7 cell line (c1, ATCC CRL-2716) for selected target compounds. In addition, the QR induction activities of other compounds were concurrently determined: resveratrol and sulforaphane (Sigma–Aldrich Chemical Company), pinosylvin (Sequoia Research Products), β-naphthoflavone (Fluka Chemical Company). Experiments were carried out at least three times on separate occasions.

6.3. Determination of antioxidant activity

The ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorisation assay was carried out according to a reported method [17]. Briefly, a solution of ABTS free radical ($ABTS^{\bullet+}$) was prepared by reacting ABTS (Sigma–Aldrich) with potassium persulfate. It was then diluted with PBS (pH 7.4) to give an absorbance of 0.700 (±0.02) at 734 nm, 25 °C. The absorbance of the diluted solution remained unchanged for up to 15 min. An aliquot of the test compound prepared from a stock solution in ethanol was added to the diluted $ABTS^{\bullet+}$ solution (1 ml) and the decrease in absorbance was monitored at 1, 3 and 5 min. The percentage reduction in absorbance at 5 min was plotted as a function of concentration (2.5–25 μM) of test compound. A straight line was obtained, the gradient of which reflected the scavenging ability of the test compound. The experiment was repeated with the known antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma–Aldrich Company). The ratio of the gradients of the lines obtained from test compound and Trolox was used to determine the TEAC (Trolox equivalent antioxidant capacity) of the test compound.

$TEAC = Gradient_{Test\ compound}/Gradient_{Trolox}$.

Experiments were carried out at least three times, on separate occasions.

6.4. Microculture tetrazolium assay for determining cell viability

The cytotoxicity of stilbenes **3–15** was determined on Hepa 1c1c7 cells using the microculture tetrazolium assay [18]. Stock solutions of the test compounds were prepared in DMSO and serially diluted to give final concentrations of 1–25 μM. Not more than 1% DMSO (final concentration) was present in each well. The test compounds were incubated with the cells for 24 h, after which MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide), Sigma–Aldrich] was added for 3 h and the cells lysed to release the formazan product. The latter was dissolved in DMSO

(150 μl) and the absorbance was determined within 30 min at 590 nm on a microtitre plate reader. The absorbance values from no less than three independent determinations were averaged, adjusted by subtraction of blank values (wells without cells) and expressed as a percentage of the average absorbance of control wells (wells with untreated cells).

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