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Synthesis and Evaluation of Cytotoxicity of Stilbene Analogues

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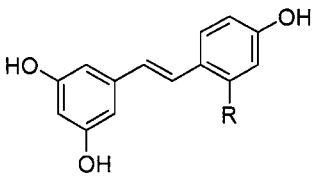
Resveratrol analogs were newly synthesized and evaluated for cytotoxicity in cultured human lung and colon cancer cells. 3,5,4-Trimethoxy-*trans*-stilbene and 3,5,2',4'-tetramethoxy-*trans*-stilbene were found to be more potent rather than resveratrol. 3,4,5-Trimethoxy-4'-bromo-*cis*-stilbene was the most active among the test compounds.

Key words: Cytotoxicty, Stilbene analogues, Resveratrol analogues

INTRODUCTION

Resveratrol (3,5,4-trihydroxy-*trans*-stilbene, **1**, Fig. 1), a naturally occurring phytoalexin present in grapes and other plants, has been reported to exhibit a variety of important biological effects including protective role in atherosclerosis and colonary heart diseases (Frankel *et al.*, 1993; Pace-Asciak *et al.*, 1995). Recently, various studies have demonstrated the potential of resveratrol to mediate the strong antioxidant, antimutagenic, antiinflammatory, or potent cancer chemopreventive effects toward the carcinogenic progress (Jang *et al.*, 1997; Uenobe *et al.*, 1997). In addition, resveratrol showed the growth inhibitory effect toward several cancer cell lines, suggesting the compound may have a modulation of cancer promotion/progression (Mgbonyebi *et al.*, 1998; Fontecave *et al.*, 1998; Park *et al.*, 2001). Based on the inhibitory potential of resveratrol on carcinogenesis, in this report, we studied the cytotoxic activities of newly synthesized resveratrol analogs on human lung (A549) and colon (Col2) cancer cells to discover more potent cancer chemo preventive or chemotherapeutic agents.



(**1**) R = H resveratrol
(**2**) R = OH oxyresveratrol

Fig. 1. Naturally Occuring phytoalexin, resveratrol (**1**) and oxyresveratrol (**2**)

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MATERIALS AND METHODS

Chemistry

^{1}H-NMR (300 MHz) spectra were measured on a Varian instrument and chemical shifts were recorded in δ. units relative to internal tetramethyl silane. IR spectra were measured on Fourier Transform Infrared spectrometer. All nonaqueous reactions were carried out under an argon or nitrogen atmosphere in dry solvents, unless otherwise noted. All reactions were monitored by thin-layer chromatography (E. Merck silica gel plate 60 F254). The spots were detected under UV light (254 nm and 366 nm). Flash column chromatography was conducted on E. Merck silica gel 60 (0.040-0.063 mm) and all chromatographic solvents were distilled before use.

(*Z*)-3-[2-(3,4,5-Trimethoxy-phenyl)-vinyl]-furan (24)

General procedure : To a solution of **3b** (0.76 mmol) in CH_2Cl_2 (4 mL) was added aldehyde **8** (0.76 mmol). Then, a powdered KOH (1.52 mmol) and 18-crown-6 (0.076 mmol) were added to the mixture. It was stirred at room temperature. After the mixture was stirred for 2 h, the resulting material was extracted with CH_2Cl_2 (×2). The combined organic layers were washed with brine, dried

($MgSO_4$) and concentrated *in vacuo*. Purification of the residue was performed by flash column chromatography on silica gel. Yield 67%, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.79 (6H, s), 3.87 (3H, s), 6.25 (1H, m), 6.35 (1H, d, *J* = 12.3 Hz), 6.47 (1H, d, *J* = 12.3 Hz), 6.58 (2H, s), 7.27 (1H, m), 7.40 (1H, m). EIMS (*m/z*) : 260 $[M]^+$.

(*E*)-3-[2-(3,4,5-Trimethoxyphenyl)-vinyl]-furan (25)

This compound was obtained from **3b** by the same procedure described for **24**. Yield 22%, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.86 (3H, s), 3.89 (6H, s), 6.64 (1H, m), 6.67 (2H, s), 6.74 (1H, d, *J* = 16.2 Hz), 6.89 (1H, d, *J* = 16.2 Hz), 7.40 (1H, m), EIMS (*m/z*) : 260 $[M]^+$.

(*Z*)-3-[2-(3,4,5-Trimethoxyphenyl)-vinyl]-thiophene (26)

This compound was obtained from **3b** by the same procedure described for **24**. Yield 70%, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.73 (6H, s), 3.86 (3H, s), 6.47 (1H, d, *J* = 12.3 Hz), 6.52 (2H, s) 6.53 (1H, d, *J* = 12.3 Hz), 6.95 (1H, dd, *J* = 1.8, 1.5 Hz) 7.16 (2H, m), EIMS (*m/z*) : 276 $[M]^+$.

(*E*)-3-[2-(3,4,5-Trimethoxyphenyl)-vinyl]-thiophene (27)

This compound was obtained from **3b** by the same procedure described for **24**. Yield 20%, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.87 (3H, s), 3.91 (6H, s), 6.70 (2H, s), 6.88 (1H, d, *J* = 16.2 Hz), 7.04 (1H, d, *J* = 16.2 Hz), 7.26 (1H, m) 7.32 (2H, m), EIMS (*m/z*) : 276 $[M]^+$.

(*Z*)-1-(3,4,5-Trimethoxyphenyl)-2-(4-Bromophenyl)-ethene (28)

This compound was obtained from **3b** by the same procedure described for **24**. Yield 65%, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.61 (6H, s), 3.77 (3H, s), 6.37 (2H, s), 6.41 (1H, d, *J* = 12.0 Hz), 6.48 (1H, d, *J* = 12.0 Hz) 7.10 (2H, d, *J* = 8.4 Hz), 7.31 (2H, d, *J* = 8.4 Hz), EIMS (*m/z*) : 350 $[M+1]^+$.

(*E*)-1-(3,4,5-Trimethoxyphenyl)-2-(4-Bromophenyl)-ethene (29)

E,Z-mixture compound were obtained from **3b** by the same procedure described for **24.** To a solution of *E,Z*-mixture (0.4 mmol) in heptane (20 mL) was added a catalytic amount of iodine. The reaction mixture was refluxed for 2 h. The solution was then quenched with a saturated solution of $NaHSO_3$ (2 mL). The resulting material was extracted with CH_2Cl_2 (×2). The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the residue was performed by flash column chromatography on silica gel. Yield 85%, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.80 (3H, s), 3.85 (6H, s), 6.66 (2H, s), 6.87 (1H, d, *J* = 16.2 Hz), 6.96 (1H, d, *J* = 16.2 Hz), 7.30 (2H, d, *J* = 8.7 Hz) 7.41 (2H, d, *J* = 8.7 Hz), EIMS (*m/z*) : 350 $[M+1]^+$.

3,5-Dimethoxy-*N*-(4-methoxyphenyl)-benzamide (30)

General procedure: To a mixture of 3,5-dimethoxybenzoic acid (**12**) (1 mmol) and trichloroacetonitrile (2.0 mmol) in CH_2Cl_2 (2 mL) was added Ph_3P (2.0 mmol) in CH_2Cl_2 (1 mL) under argon at room temperature. After stirred 1 h, the reaction mixture was treated with amine **13** (1 mmol) followed by triethylamine (3 mmol), and the mixture was stirred for 1 h. The reaction mixture was poured into water and extracted with EtOAc (x 2). The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue was performed by flash column chromatography on silica gel. Yield 90%, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.74 (3H, s), 3.76 (6H, s), 6.53 (1H, t, *J* = 2.4 Hz), 6.83 (2H, s), 7.67 (1H, brs), EIMS (*m/z*): 304 $[M+1]^+$.

Evaluation of cytotoxic potential with cultured human cancer cells

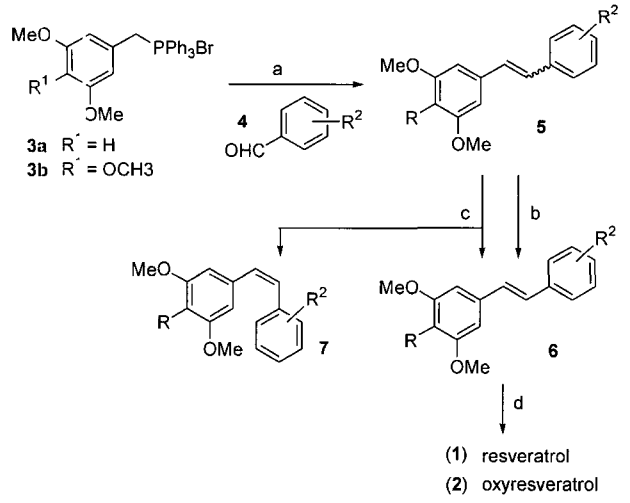
Cytotoxic potential was determined as described previously (Lee *et al.*, 1998). Briefly, cells (A549, human lung cancer cells; Col2, human colon cancer cells, in log growth phase) were counted, diluted to 5×10^4 cells/mL with fresh medium, and added to 96-well microtiter plates (190 μL/well) containing test materials (10 μL in 10% aqueous DMSO). Test plates were incubated for 3 days at 37°C in CO_2 incubator. After treatment, the cells were fixed with TCA and viability was determined with a sulforhodamine B (SRB) protein staining method. The results were expressed as a percentage, relative to solvent-treated control incubations, and IC_{50} values were calculated using non-linear regression analyses (percent survival versus concentration).

RESULTS AND DISCUSSION

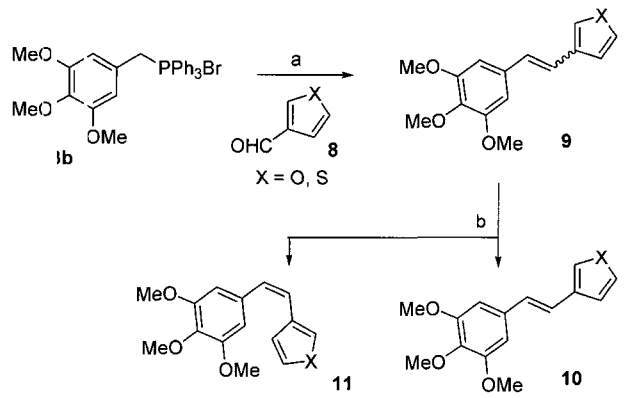
Chemistry

In the synthesis of resveratrol related compounds, three structural modifications have been considered primarily: (a) replacement of hydroxyl group of resveratrol to methoxy group; (b) substitution of the aromatic rings of stilbene to various substituted benzene or heteroaromatic rings; (c) replacement of the stilbene double bond to amide bond.

Naturally occurring resveratrol (**1**) and oxyresveratrol (**2**) were prepared from the corresponding methoxy stilbene **6** by full demethylation (Alonso *et al.*, 1997; Wilds *et al.*, 1948) with an excess of methylmagnesium iodide under heating at 100-160°C. The synthesis of methoxylated stilbene analogs of resveratrol was carried out employing the Wittig reaction between an aromatic aldehyde and an



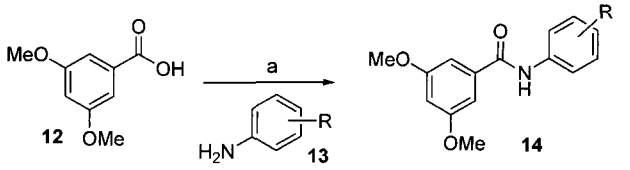
Scheme 1. Reagents and conditions: (a) aldehyde **4**, KOH, cat.18-crown-6, CH_2Cl_2, 2 h, rt , 80-90%; (b) I_2, heptane, 2 h, reflux, >95%; (c) silicagel chromatography; (d) exc. MeMgI, 0.5 h, 100-160°C, 50-60%



Scheme 2. Reagents and conditions: (a) aldehyde **8**, KOH, cat.18-crown-6, CH_2Cl_2, 2 h, rt , 80-90%; (b) silica gel chromatography

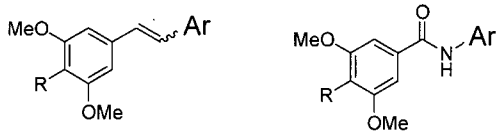
aromatic phosphonium ylide as shown in Scheme 1 (Zhang *et al.*, 1998; Orsini *et al.*, 1997; Kucerovy *et al.*, 1997). Condensation of the commercially available aryl aldehydes **4** and benzyl triphenylphosphonium halide **3** in the presence of potassium hydroxide and a catalytic amount of 18-crown-6 provided the expected olefin **5** with a mixture of *E*- and *Z*-isomers in an approximately 1:3 to 1:5 ratio depending on substituted groups (Bellucci *et al.*, 1996). This mixture was efficiently converted to *E*-isomer **6** by heating with a catalytic amount of iodine in refluxing heptane (Zhang *et al.*, 1998). In some cases, the mixture **5** was separated by silica gel chromatography to afford the major *Z*-isomer **7** and the minor *E*-isomer **6**. The five membered heteroaromatic ring-containing analogs were prepared in a similar manner as illustrated in Scheme 2. Wittig reactions between aldehyde **8** and **3b**, followed by silica ge chromatography of an *E/Z* mixture **9**, provided the minor *E*-isomer **10** and the major *Z*-isomer **11**.

The amide analogs of resveratrol were obtained by the



Scheme 3. Reagents and conditions: (a) CCl_3CN, PPh_3, CH_2Cl_2, 1 h, rt; then **13**, Et_3N, 1 h, rt, 80-90%

Table I. Cytotoxic activities[a] of stilbene analogs



Compds	R	Ar	E/Z	A549[b] (IC_{50}: g/mL)	Col2[b] (IC_{50}: g/mL)
1	Resveratrol		E	6.9	18.7
2	Oxyresveratrol		E	>20	>20
15	H	Ph(4-methoxy)	E	0.8	0.9
16	H	Ph(2,4-dimethoxy)	E	0.8	0.8
17	H	Ph(3,5-dimethoxy)	E	>20	>20
18	H	Ph(3,4,5-trimethoxy)	E	>20	>20
19	H	Ph(3,4-dimethoxy)	E	16.2	15.8
20	H	Ph(2,4,5-trimethoxy)	Mix	>20	>20
21	H	Ph(2,4,6-trimethoxy)	Mix	3.2	7.2
22	OMe	Ph(2,3-diimethoxy)	Mix	>20	>20
23	H	Furan-3-yl	Mix	17.0	15.6
24	OMe	Furan-3-yl	Z	0.2	0.3
25	OMe	Furan-3-yl	E	>20	>20
26	OMe	Thiophen-3-yl	Z	2.1	2.6
27	OMe	Thiophen-3-yl	E	>20	>20
28	OMe	Ph(4-bromo)	Z	0.01	0.01
29	OMe	Ph(4-bromo)	E	4.7	1.6
30	H	Ph(4-methoxy)	-	2.6	5.1
31	H	Ph(2,4-dimethoxy)	-	>20	>20

[a]Cytotoxicity determination: SRB assay as described previously (Skehan *et al.*, 1990; Lee *et al.*, 1998).

[b]Human cancer cell lines: A549 (non-small cell lung carcinoma from ATCC) and Col2 (human colon carcinoma from the Department of Surgical Oncology, University of Illinois at Chicago, USA).

condensation of acid chlorides with amine as depicted in Scheme 3. The commercially available 3,5-dimethoxybenzoic acid (**12**) was treated with trichloroacetonitrile and triphenylphosphine in CH_2Cl_2 at room temperature, and then the aroyl chloride formed was converted to the amide **14** in good to modest yields by adding, *in situ*, the amine **13** and triethylamine (Jang *et al.*, 1999).

Cytotoxic Activities

The cytotoxic activity of compounds **1-2** and **15-31** in

cultured human lung (A549) and colon (Col2) cancer cells was determined by the sulforhodamine B (SRB) assay as described previously (Skehan *et al.*, 1990; Lee *et al.*, 1998). As indicated in Table I, among the compounds tested, nine compounds showed more potent cytotoxic activities than resveratrol against cancer cells. Activities of the compounds **15**, **16**, **24**, and **28** were highly potent with the IC_{50} values in the range of less than 1.0 μg/mL. Especially, the compound **28** exhibited approximately 600 times more potent cytotoxicity than resveratrol against A549, and approximately 1800 times against Col2, respectively. In terms of structure-activity relationship (SAR), substitution of hydroxyl group of resveratrol (**1**) and oxyresveratrol (**2**) to methoxy group, as exemplified by compounds **15** and **16**, potentiated the cytotoxic activity by approximately ten times. Further, the *cis*-configured stilbenes were more potent than their corresponding *trans* isomers as shown in **24-29**. For example, compound **28**, a brominated stilbene with *cis*-configuration, the most potent one among the synthesized compounds, showed 150 times more potent cytotoxicity compared to the corresponding *trans*-isomer **29**. Replacing the *p*-bromobenzene of **28** with 5-membered heteroaromaticring, such as furan and thiofuran **24-27**, abolished or decreased the activity. Substitution of the double bond with an amide bond (**30-31**) of methoxylated resve-ratrol analogs **15-16**, resulted in a loss of activity.

From the point of view of SAR, the substitution with methoxy groups at certain position and the *cis*-form of stilbene backbone, exemplified by **28**, enhanced the cytotoxic activity. Recently, the similar results were shown with combretastatin A-4 (3,4,5-trimethoxy-3-hydroxy-4-methoxy-*cis*-stilbene) relating to the potentiated cytotoxic activity of the *cis* isomer compared to the corresponding *trans* isomer (Pettit *et al.*, 2000; Pettit *et al.*, 1995; Ohsumi *et al.*, 1998). Further elucidation of the cytotoxic mechanisms was conducted with the compound **16**. The results indicated that the induction of apoptosis was one possible way (data not shown).

In conclusion, these results are of interest for establishing the SAR of the stilbenes, and the active novel stilbene analogs might be candidates for development of potential cancer chemotherapeutic or cancer chemopreventive agents.

ACKNOWLEDGEMENTS

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