Design, Synthesis, and Discovery of Novel *trans*-Stilbene Analogues as Potent and Selective Human Cytochrome P450 1B1 Inhibitors

Sanghee Kim,*,† Hyojin Ko,† Jae Eun Park,† Sungkyu Jung,† Sang Kwang Lee,‡ and Young-Jin Chun‡

Natural Products Research Institute, College of Pharmacy, Seoul National University, 28 Yungun, Jongro, Seoul 110-460, Korea, and College of Pharmacy, Chungang University, 221 Huksuk, Dongjak, Seoul 156-756, Korea

Received June 28, 2001

A series of *trans*-stilbene derivatives containing a 3,5-dimethoxyphenyl moiety were prepared through a new efficient solution phase synthetic pathway, and their inhibitory activities were evaluated on human cytochrome P450s (CYP) 1A1, 1A2, and 1B1 to find a potent and selective CYP1B1 inhibitor. We found that a substituent at the 2-position of the stilbene skeleton plays a very important role in discriminating between CYP1As and CYP1B1. Among the compounds tested, the most selective and potent CYP1B1 inhibitor was 2,3′,4,5′-tetramethoxystilbene. Compound **7j**, 2-[2-(3,5-dimethoxy-phenyl)vinyl]thiophene, showed greater inhibitory activities but had a lower selectivity toward all of the CYP1s tested.

Introduction

The cytochrome P450s (CYP) catalyze phase I oxidative metabolisms of a number of xenobiotics (e.g., drugs, carcinogens, and pesticides) and endogenous compounds (e.g., steroids and eicosanoids).[1] The CYP1 subfamily including 1A1, 1A2, and 1B1 has long been of interest to researchers because of its relevance to chemical carcinogenesis. CYP1B1 is expressed preferentially in steroidogenic tissues such as the adrenal, testis, and ovary and in steroid-sensitive tissues such as the breast, prostate, testis, and embryonic cells.[2,3] Like CYP1A1, CYP1B1 is involved in the metabolic activation of polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene, dibenzo[*a*,*l*]pyrene, 7,12-dimethylbenz[*a*]anthracene (DMBA), and 5-methylchrysene.[4] CYP1B1 is more active than CYP1A1 in converting DMBA to the carcinogenic metabolite 3,4-dihydrodiol 1,2-epoxide. CYP1B1 expression is regulated by the Ah receptor-mediated signal pathway, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent agonist of the Ah receptor, activates the transcription of CYP1B1.[5]

CYP1B1 is known to be a major 17β-estradiol (E2) 4-hydroxylase to produce 4-hydroxyestradiol (4-OHE2). Although the contribution of CYP1B1 and 4-OHE2 in estrogen-induced cancer is still speculative, increasing evidences for the carcinogenicity of estrogens suggest that 4-OHE2 is involved in the development of breast cancer in humans.[6−8] 4-OHE2 is also known to be a long-acting estrogen.[9] Cavalieri et al. have offered an explanation for the role of 4-OHE2 in tumor initiation.[10] 4-OHE2 can be oxidized to E2−3,4-semiquinone and E2−3,4-quinone, which binds to the N^7-guanine in DNA to form depurinating DNA adducts. The resulting apurinic sites in DNA can result in mutations in critical genes.[11] Moreover, the semiquinone free radical generated during redox cycling of catechol estrogens may react with molecular oxygens to form superoxide and other hydroxy radicals.[12,13] The formation of 8-hydroxylated guanines by the reaction of guanine bases of DNA with the resulting hydroxy radicals is considered to be an important indicator of oxidative injury to DNA and is increased in the liver and kidney of hamsters exposed to E2 or 4-OHE2.[14] E2 treatment also enhances lipid hydroperoxide-induced DNA adduct and malondialdehyde−DNA adduct formations by free radical generation.[15−17]

Because hydroxylation of E2 by CYP1B1 at the 4-position has been postulated to be important in estrogen carcinogenesis, selective inhibitors of CYP1B1 may prevent mammary tumor formation, especially in the breast. Selective inhibitors may also be very useful as pharmacological tools to elucidate the functions of CYPs. To find the selective inhibitors of CYP1s, many types of compounds have been tested to date.[18,19] However, compounds having a strong potency and selectivity for specific P450 isozymes are not characterized well.

Various *trans*-stilbene compounds were reported to be inhibitors of CYP. Resveratrol (**1a**, Figure 1) showed an inhibitory effect on human CYP1A1 and CYP1B1.[20,21] Recently, rhapontigenin (**2**), a natural hydroxystilbene, showed a strong selectivity of CYP1A1 inhibition[19] and it was found that the selectivities and inhibitory potency of stilbene compounds tested against CYP1s were sensitive to the substitution patterns on the *trans*-stilbene template. On the basis of these studies, we investigated the synthesis and biological evaluation of various *trans*-stilbene analogues in order to find a potent and selective CYP1B1 inhibitor. We designed and prepared a series of compounds of general structure (**3**, Figure 1) in which the phenyl ring on site A contains dimethoxy groups on the 3- and 5-positions instead of the corresponding dihydroxyl groups of the natural products **1** and **2**. We envisioned that the substitution by dimethoxy groups could enhance the activity since previously reported potent CYP1 inhibitors are generally lipophilic compounds. Several structural changes were made on site B in order to obtain compounds with the high selectivity that we were seeking. We report here the CYP1 inhibition of synthesized *trans*-stilbene analogues and our

* To whom correspondence should be addressed. Tel: 82-2-740-8913. Fax: 82-2-762-8322. E-mail: pennkim@snu.ac.kr.
† Seoul National University.
‡ Chungang University.

10.1021/jm010298j CCC: $22.00
Published on Web 12/07/2001

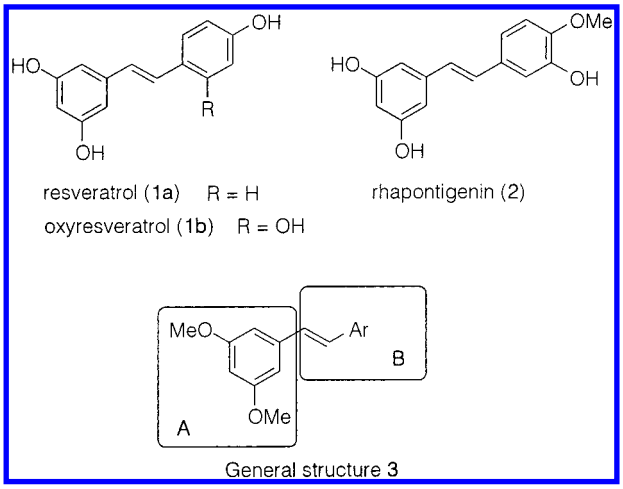


Figure 1. Structures of **1a**, **1b**, **2**, and general structure **3**.

discovery of 2,3′,4,5′-tetramethoxystilbene to be a selective and potent CYP1B1 inhibitor.

Chemistry

The preparation of 3,5-dimethoxy-*trans*-stilbene derivatives was carried out according to Scheme 1. The stilbene skeleton could be constructed by Wittig reactions between an aromatic aldehyde and an aromatic phosphonium ylide. However, semistabilized ylide, like benzyl ylide, usually provides a mixture of *Z* and *E* isomers[22,23] as well as triphenylphospine oxide as a byproduct that necessitates chromatography or crystallization for purification. For a practical and efficient synthesis of *trans*-stilbene, we employed a Honer−Wadsworth−Emmons reaction, which offers the advantage of generating the water soluble dimethyl phosphoric acid byproducts, instead of triphenylphospine oxide, as well as providing the desired trans isomer as the major product.[24] It was felt that this would remove one of the key obstacles in preparing the large number of *trans*-stilbene derivatives and could be utilized in a solution-phase stilbene library synthesis.

Honer−Wadsworth−Emmons reactions between phosphonate **4** and commercially available aromatic aldehydes **5** (1.1 equiv) in the presence of freshly powdered KOH and a catalytic amount of 18-crown-6 in CH_2Cl_2 yielded a mixture of olefins **6** with a *Z*/*E* ratio of ca. 1:3 to 1:5 by thin-layer chromatography (TLC) intensity (Scheme 1). After standard aqueous work up, the excess aldehydes of **5** could be removed from the mixture by treatment with Girard's reagent T ((carboxymethyl)-trimethylammonium chloride hydrazide) and acetic acid.[25] The products obtained after aqueous work up were very pure (>98% purity by nuclear magnetic resonance (NMR) analysis). These *Z*/*E* mixtures of **6** were efficiently converted to *E* isomers of **7** by heating with catalytic amounts of iodine in refluxing heptane.[26] The reaction mixtures were diluted with diethyl ether and washed with saturated aqueous sodium bisulfite and water to remove iodine from the *trans*-stilbene derivatives. The NMR spectra of synthesized *trans*-stilbenes showed that these are very clean and did not contain any notable impurities.

The amide analogue **9** was obtained by the condensation of acid with amine as depicted in Scheme 2. The commercially available 3,5-dimethoxybenzoic acid (**8**) was treated with trichloroacetonitrile and triphenylphosphine in CH_2Cl_2 at room temperature, and then, the aroyl chloride formed was converted to the amide **9** in good yields by adding, in situ, 2,4-dimethoxyaniline and triethylamine.[27] The corresponding imine analogue **11** was prepared by condensation of 3,5-dimethoxy benzaldehyde (**10**) and 2,4-dimethoxyaniline using a Dean and Stark apparatus (Scheme 2).

Results and Discussion

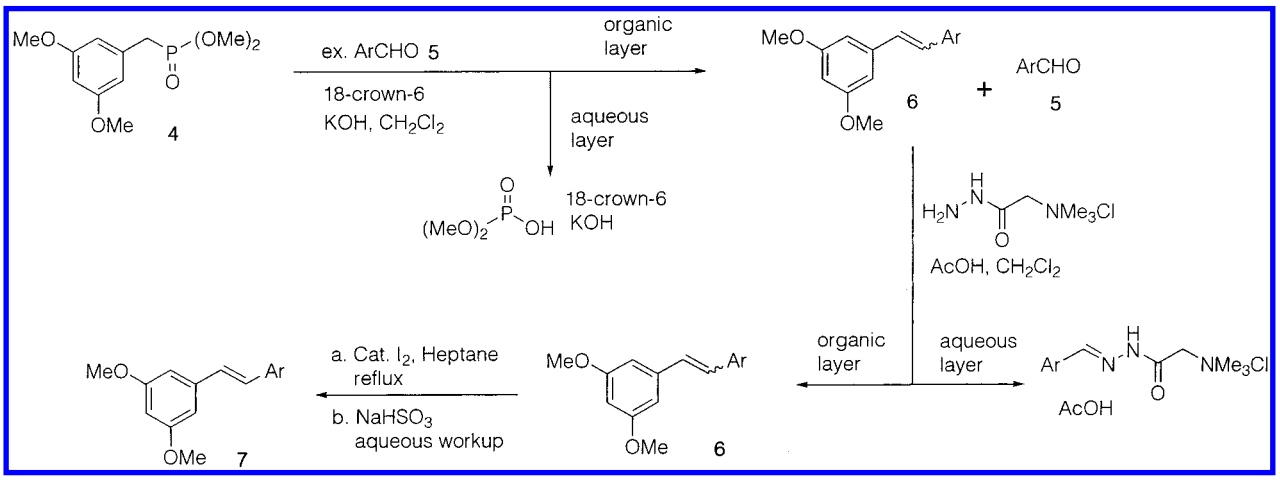
The inhibitory effects on ethoxyresorufin *O*-deethylation (EROD) by *trans*-stilbene compounds were determined in bicistronic bacterial membranes containing human CYP1A1, CYP1A2, and CYP1B1.[19,28] As indicated in Table 1, among the compounds tested, 2,3′,4,5′-tetramethoxystilbene (**7a**) was found to be the most selective and potent CYP1B1 inhibitor to date. Compound **7a** showed a potent inhibitory effect on CYP1B1 ($IC_{50} = 6 \pm 2$ nM) and, to a lesser extent, on CYP1A1 ($IC_{50} = 300 \pm 20$ nM) and CYP1A2 ($IC_{50} = 3100 \pm 880$ nM). Compound **7a** is a methylated derivative of naturally occurring oxyresveratrol (**1b**, Figure 1). Compound **1b** inhibited CYP1s having IC_{50} values of 15, 150, and 34 μM for 1A1, 1A2, and 1B1, respectively.[19] These results showed that replacement of four hydroxyl groups of **1b** with methoxy groups potentiated the inhibitory activity and caused profound changes in selectivity.

Recently, we investigated the mechanism of CYP1B1 inhibition by **7a**.[29] Compound **7a** is a competitive inhibitor of CYP1B1 with a K_i value of 3 nM. 4-Hydroxylation of E2 by CYP1B1-expressing membranes or purified CYP1B1 were strongly blocked by compound **7a**. However, **7a** does not cause a time-dependent inactivation of CYP1B1. It was also found that **7a** is relatively stable in the presence of CYP1B1.

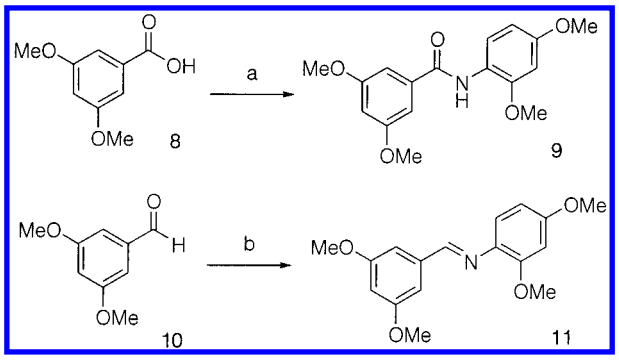
Modification of the phenyl ring on site B by the addition/deletion or change in the position of methoxy groups (compounds **7b**−**e**) resulted in decreased potency and selectivity. These observations indicated that the exact locations of methoxy groups are a very important feature for selectivity. In comparison with **7a**, compound **7e** differed structurally by only the methoxy group at the 2-position. The inhibitory activities of **7e** against 1A1 and 1A2 were only slightly decreased as compared to compound **7a**. However, **7e** was found to be about 130 times less active against 1B1 ($IC_{50} = 790 \pm 100$ nM) than **7a**. These results suggested that the presence of a 2-methoxy group may play a very important role in binding to the active site of CYP1B1. Therefore, we replaced the 2-methoxy group with an F or OH group (compounds **7f**,**g**). These compounds also preferentially inhibited 1B1 over 1A1 and 1A2 and exhibited better properties than compound **7e**. However, the inhibitory potentials and selectivities were diminished substantially as compared to **7a**. When the ethylene bridge of compound **7a** was replaced with an amide or an imine linkage (compounds **9** and **11**), none showed significant activities, but the selectivity between 1As and 1B1 was shown as to be expected.

In another modification, we replaced the phenyl ring on site B with 4-pyridyl, 3-furanyl, and 2-thiophenyl rings (compounds **7h**−**j**). However, none of them was as selective as **7a**. Whereas compounds **7h**,**i** had weak inhibitory effects on CYP1s, compound **7j** containing a

Scheme 1



Scheme 2[a]



[a] Reagents: (a) i. CCl_3CN, Ph_3P, CH_2Cl_2, room temperature; ii. 2,4-dimethoxyaniline, Et_3N. (b) 2,4-Dimethoxyaniline, toluene, reflux.

2-thiophenyl ring showed remarkably greater inhibitory activities against all of the CYP1s tested, which had IC_{50} values of 61 ± 21, 11 ± 2, and 2 ± 1 nM for 1A1, 1A2, and 1B1, respectively. Compound **7j** exhibited a 30-fold greater selective inhibition of 1B1 over 1A1, but the selectivity between 1B1 and 1A2 was much lower than that of **7a** (about 5-fold as compared to a 500-fold selectivity).

In conclusion, a series of lipophilic stilbene derivatives have been prepared and evaluated as to their effects on the activity of CYP1A1, CYP1A2, and CYP1B1 with the goal of identifying a potent and selective CYP1B1 inhibitor. From our results, the most selective and potent CYP1B1 inhibitor was **7a** and the most active CYP1B1 inhibitor was compound **7j**. We found that the presence of a substituent at the 2-position of the stilbene skeleton plays a very important role in discriminating between CYP1As and CYP1B1 and influencing the pharmacological effect. Our results are of interest for establishing the preliminary structure−activity relationships of stilbenes as CYP1s inhibitors and allowing the design and synthesis of a stilbene derivative that has a superior selectivity and activity. In addition, the active *trans*-stilbene analogue **7a** can be a useful compound for characterizing the enzymatic properties of CYP1B1 because of its strong selectivity. It may also be valuable for the development of a chemopreventive or therapeutic agent for cancer by protecting the CYP1B1-dependent E2 metabolism.

Experimental Section

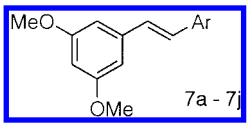
General. Except where indicated, materials and reagents were used as supplied by the manufacturer. Melting points were determined with a Bûchi melting point B-540 apparatus and were not corrected. NMR spectra were recorded at 300 MHz using a Varian Gemini 2000 spectrometer with tetramethylsilane as an internal standard. Electron impact mass spectra were taken on a HP 5989B mass spectrometer at 70 eV. Elemental analyses were performed on an EA1110 elemental analyzers. The results were within 0.4% of calculated values. Reactions were monitored by TLC analysis using E. Merck silica gel 60 F-254 thin-layer plates. Flash chromatography was carried out on E. Merck Kieselgel 60 (230−400 mesh) silica gel.

Biological Assay. Bacterial coexpression plasmids for human CYP1A1, 1A2, 1B1, or NADPH-P450 reductase were kindly provided by Dr. F. Peter Guengerich (Vanderbilt University, Nashville, TN). Bicistronic bacterial membranes were prepared in accordance with Guengerich et al.[33] Protein concentrations were determined using the bichinchoninic acid method according to the supplier's recommendations (Pierce Chemical Co., Rockford, IL). To measure CYP1A1, 1A2, or 1B1 enzyme activities, EROD was determined. Bicistronic membranes containing 5 nM of CYP1A1, 1A2, or 1B1 were added to 0.1 M potassium phosphate buffer (pH 7.4) containing 2 μM ethoxyresorufin and varying concentrations of inhibitors.[19] The reaction mixtures were preincubated at 37 °C for 3 min. The reactions were initiated by the addition of an NADPH-generating system consisting of 5 mM glucose 6-phosphate, 0.5 unit mL^{-1} glucose 6-phosphate dehydrogenase, and 0.5 mM $NADP^+$. After 10 min of incubation at 37 °C, the reactions were terminated by the addition of 1 mL of MeOH. The formation of resorufin was determined fluorometrically using a Perkin-Elmer LS 5 spectrofluorometer at excitation and emissions wavelengths of 550 and 585 nm.

(3,5-Dimethoxy-benzyl)phosphonic Acid Dimethyl Ester (4). A mixture of 3,5-dimethoxy-benzyl bromide (2.31 g, 10 mmol) and trimethyl phosphite (3.75 g, 30 mmol) in a sealed tube was heated at 180 °C in an oil bath for 8 h. After the mixture was cooled, the excess trimethyl phosphite was removed in vacuo. Purification of the residue by short flash column chromatography on silica gel (eluent: EtOAc) gave 2.42 g (93%) of **4** as an oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.45 (t, J = 2.4 Hz, 2H), 6.35 (dd, J = 4.2, 2.1 Hz, 1H), 3.77 (s, 6H), 3.70 (s, 3H), 3.66 (s, 3H), 3.10 (d, J = 21.6 Hz, 2H).

General Procedure for the Preparation of (*E*)-Stilbenes (7). To a well-stirred suspension of phosphonate **4** (1.0 mmol), freshly powdered KOH (2.0 mmol), and 18-crown-6 (0.1 mmol) in 2 mL of CH_2Cl_2 was added commercially available aromatic aldehydes **5** (1.1 mmol) at room temperature. After the mixture was additionally stirred for 3−6 h, the mixture was diluted with 15 mL of CH_2Cl_2 and washed with water (10 mL) and brine (2 × 10 mL). The organic layer was dried over

Table 1. Effects of Stilbene Analogues on EROD Catalyzed by Human Cytochrome P450s[a]



		IC_{50} (nM)[c]				
compd[b]	Ar	1A1	1A2	1B1	ratio (1A1/1B1)	ratio (1A2/1B1)
7a	2,4-dimethoxyphenyl	300 ± 20	3100 ± 880	6 ± 2	50	520
7b	3,4,5-trimethoxyphenyl	140 ± 30	930 ± 120	3200 ± 560	0.044	0.29
7c	3,5-dimethoxyphenyl	920 ± 40	198 000 ± 14 000	17 600 ± 480	0.05	11
7d	3,4-dimethoxyphenyl	750 ± 80	570 000 ± 1500	3000 ± 740	0.25	190
7e	4-methoxyphenyl	830 ± 120	6200 ± 960	790 ± 100	1.1	7.9
7f	2-hydroxy-4-methoxyphenyl	980 ± 220	31 100 ± 2900	390 ± 90	2.5	80
7g	2-fluoro-4-methoxyphenyl	610 ± 40	5800 ± 1300	97 ± 30	6.3	60
7h	4-pyridyl	1100 ± 480	290 ± 110	460 ± 80	2.4	0.63
7i	3-furanyl	6600 ± 2000	740 ± 100	2100 ± 260	3.1	0.35
7j	2-thiophenyl	61 ± 21	11 ± 2	2 ± 1	31	5.5
9	see Scheme 2	1500 ± 230	64 000 ± 5500	670 ± 120	2.2	96
11	see Scheme 2	300 000 ± 6300	$>2 \times 10^6$	4900 ± 1300	61	>400
1b[d]	oxyresveratrol	15 000	150 000	34 000	0.44	4.4

[a] Enzyme activities were measured as described in the Experimental Section. [b] Compounds **7a**,[30] **7b**,[22,31] **7c**,[30,31] **7d**,[22,31] **7e**,[30,31] and **7h**[32] are previously known. [c] The IC_{50} values were means ± range of two separate experiments determined using a quadratic expression of nonlinear regression methods with Graph-Pad Prism software (San Diego, CA). [d] Taken from ref 19.

$MgSO_4$ and concentrated in vacuo. The residue was dissolved in 2 mL of CH_2Cl_2. To this solution were added Girard's reagent T ((carboxymethyl)trimethylammonium chloride hydrazide, 0.5 mmol) and AcOH (5 mmol), and the resulting mixture was stirred for 2 h at room temperature. The insoluble material was filtered off, the filtrate was concentrated in vacuo, and the residue was dissolved in 15 mL of EtOAc. The solution was washed with brine (3 × 10 mL) and dried over $MgSO_4$, and the solvent was removed in vacuo to yield the desired stilbene (ca. 0.9−0.95 mmol) as a mixture of *E* and *Z* isomers. To the solution of this mixture in heptane (5 mL) was added a catalytic amount of iodine (1 crystal) and then heated to reflux for 12 h. The reaction mixture was diluted with 20 mL of ether and washed with saturated aqueous sodium bisulfite (10 mL) and brine (2 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to provide the desired *E*-stilbene **7** (ca. 0.9−0.95 mmol).

(*E*)-1-(2,4-Dimethoxyphenyl)-2-(3,5-dimethoxyphenyl)-ethene (7a). Yield = 95%; mp = 78−79 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49 (d, J = 8.7 Hz, 1H), 7.36 (d, J = 16.5 Hz, 1H), 6.94 (d, J = 16.5 Hz, 1H), 6.67 (d, J = 2.1 Hz, 2H), 6.50 (m, 2H), 6.36 (t, J = 2.4 Hz, 1H), 3.87 (s, 3H), 3.83 (s, 3H), 3.82 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.88, 160.61, 158.10, 140.37, 127.35, 126.98, 123.85, 119.33, 107.08, 104.99, 104.38, 99.41, 98.50, 97.50, 55.51, 55.40, 55.36, 55.22. EIMS *m*/*z*: 300 (M^+, 100%). Anal. ($C_{18}H_{20}O_4$) C, H.

(*E*)-1-(3,5-Dimethoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene (7b). Yield = 95%; mp = 136−138 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.95 (d, J = 16.2 Hz, 1H), 6.86 (d, J = 16.2 Hz, 1H), 6.67 (s, 2H), 6.59 (d, J = 2.1 Hz, 2H), 6.33 (dd, J = 2.1, 2.1 Hz, 1H), 3.85 (s, 6H), 3.80 (s, 3H), 3.77 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.00, 153.41, 139.22, 132.85, 130.42, 129.14, 106.70, 104.51, 99.99, 60.96, 56.14, 55.36. EIMS *m*/*z*: 330 (M^+, 100%). Anal. ($C_{19}H_{22}O_5$) C, H.

(*E*)-1,2-Di(3,5-dimethoxyphenyl)ethene (7c). Yield = 94%; mp = 129−135 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.93 (s, 2H), 6.58 (d, J = 2.1 Hz, 4H), 6.32 (t, J = 2.1 Hz, 2H), 3.75 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.99, 139.16, 129.20, 104.65, 100.14, 55.38. EIMS *m*/*z*: 300 (M^+, 100%). Anal. ($C_{18}H_{20}O_4$) C, H.

(*E*)-1-(3,4-Dimethoxyphenyl)-2-(3,5-dimethoxyphenyl)-ethene (7d). Yield = 92%; mp = 66−67 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.99−6.94 (m, 3H), 6.83 (d, J = 16.5 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 6.59 (d, J = 2.1 Hz, 2H), 6.31 (t, J = 2.1 Hz, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.76 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.99, 149.44, 149.07, 139.57, 130.28, 129.00, 126.81, 120.00, 107.90, 106.60, 101.10, 55.40, 55.30. EIMS *m*/*z*: 300 (M^+, 100%). Anal. ($C_{18}H_{20}O_4$) C, H.

(*E*)-1-(4-Methoxyphenyl)-2-(3,5-dimethoxyphenyl)-ethene (7e). Yield = 94%; mp = 55−57 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.4−7.35 (m, 2H), 6.97 (d, J = 16.5 Hz, 1H), 6.83 (d, J = 16.2 Hz, 1H), 6.84−6.81 (m, 2H), 6.58 (d, J = 2.1 Hz, 2H), 6.30 (t, J = 2.1 Hz, 1H), 3.76 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.98, 159.41, 139.71, 129.95, 128.75, 127.79, 126.59, 114.15, 104.35, 99.65, 55.36, 55.33. EIMS *m*/*z*: 270 (M^+, 100%). Anal. ($C_{17}H_{18}O_3$) C, H.

(*E*)-2-[2-(3,5-Dimethoxy-phenyl)vinyl]-5-methoxyphenol (7f). Yield = 90%; oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 (d, J = 8.7 Hz, 1H), 7.20 (d, J = 17.1 Hz, 1H), 6.85 (d, J = 16.2 Hz, 1H), 6.58 (d, J = 2.1 Hz, 2H), 6.43 (dd, J = 2.4, 8.4 Hz, 1H), 6.30−6.29 (m, 2H), 3.74 (s, 6H), 3.69 (s, 3H). EIMS *m*/*z*: 286 (M^+, 100%). Anal. ($C_{17}H_{18}O_4$) C, H.

(*E*)-1-(3,5-Dimethoxyphenyl)-2-(2-fluoro-4-methoxyphenyl)ethene (7g). Yield = 90%; mp = 51−55 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43 (t, J = 8.7 Hz, 1H), 7.10 (d, J = 16.5 Hz, 1H), 6.91 (d, J = 16.5 Hz, 1H), 6.64 (dd, J = 2.7, 8.7 Hz, 1H), 6.59 (d, J = 2.1 Hz, 2H), 6.56 (dd, J = 2.7, 12.6 Hz, 1H), 6.32 (t, J = 2.1 Hz, 1H), 3.76 (s, 6H), 3.75 (s, 3H). EIMS *m*/*z*: 288 (M^+, 100%). Anal. ($C_{17}H_{17}FO_3$) C, H.

(*E*)-4-[2-(3,5-Dimethoxy-phenyl)vinyl]pyridine (7h). Yield = 90%; mp = 139−144 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.51 (d, J = 5.7 Hz, 2H), 7.39−7.33 (m, 2H), 7.19 (d, J = 16.5 Hz, 1H), 6.93 (d, J = 16.2 Hz, 1H), 6.63 (d, J = 2.1 Hz, 2H), 6.40 (t, J = 2.1 Hz, 1H), 3.77 (s, 6H). EIMS *m*/*z*: 241 (M^+, 77%). Anal. ($C_{15}H_{15}NO_2$) C, H, N.

(*E*)-3-[2-(3,5-Dimethoxy-phenyl)vinyl]furan (7i). Yield = 91%; oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (s, 1H), 7.4 (m, 1H), 6.95 (d, J = 16.2 Hz, 1H), 6.74 (d, J = 16.2 Hz, 1H), 6.65 (m, 1H), 6.61 (d, J = 2.1 Hz, 2H), 6.38 (t, J = 2.1 Hz, 1H), 3.82 (s, 6H). EIMS *m*/*z*: 230 (M^+, 100%). Anal. ($C_{14}H_{14}O_3$) C, H.

(*E*)-2-[2-(3,5-Dimethoxy-phenyl)vinyl]thiophene (7j). Yield = 91%; oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.13 (m, 2H), 7.00 (br d, J = 3.0 Hz, 1H), 6.93 (dd, J = 3.3, 5.1 Hz, 1H), 6.78 (d, J = 16.2 Hz, 1H), 6.55 (d, J = 2.4 Hz, 2H), 6.31 (t, J = 2.4 Hz, 1H), 3.75 (s, 6H). EIMS *m*/*z*: 246 (M^+, 100%). Anal. ($C_{14}H_{14}O_2S$) C, H, S.

***N*-(2,4-Dimethoxy-phenyl)-3,5-dimethoxy-benzamide (9).** To a mixture of 3,5-dimethoxybenzoic acid (182 mg, 1 mmol) and trichloroacetonitrile (288 mg, 2.0 mmol) in CH_2Cl_2 (2 mL) was added Ph_3P (524 mg, 2.0 mmol) in CH_2Cl_2 (1 mL) under argon at room temperature. After the mixture was stirred for 1 h, the reaction mixture was treated with 2,4-dimethoxyaniline (153 mg, 1 mmol) followed by triethylamine (0.42 mL, 3 mmol), and the mixture was stirred for 1 h. The reaction mixture was poured into water and extracted with EtOAc. The

extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography on silica gel (eluent: CH_2Cl_2/MeOH = 40/1) gave 270 mg (85%) of **9** as a white solid; mp = 87−89 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.32 (d, J = 9.3 Hz, 1H), 8.21 (s, 1H), 6.94 (d, J = 2.4 Hz, 2H), 6.54 (t, J = 2.4 Hz, 1H), 6.46 (m, 1H), 6.44 (s, 1H), 3.82 (s, 3H), 3.79 (s, 6H), 3.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.76, 160.96, 156.55, 149.50, 137.71, 121.33, 120.67, 105.02, 103.85, 103.41, 99.66, 55.82, 55.61, 55.57. EIMS m/z: 317 (M^+, 75%). Anal. ($C_{17}H_{19}NO_5$) C, H, N.

(3,5-Dimethoxy-benzylidene)-(2,4-dimethoxy-phenyl)-amine (11). A solution of 3,5-dimethoxy benzaldehyde (153 mg, 1 mmol) and 2,4-dimethoxyaniline (153 mg, 1 mmol) in toluene (5 mL) was heated to reflux in a Dean and Stark apparatus for 16 h. After the solvent was removed in vacuo, the crude product was recrystallized from MeOH to give 151 mg (50%) of **11** as a light brown solid; mp = 70−72 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.43 (s, 1H), 7.06 (d, J = 2.4 Hz, 2H), 7.2 (d, J = 8.4 Hz, 1H), 6.57−6.54 (m, 2H), 6.50 (dd, J = 2.7, 8.7 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 6H), 3.83 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.5, 138.6, 134.8, 120.7, 115.1, 107.1, 107.0, 116.2, 104.3, 104.1, 103.8, 99.4, 99.3, 55.8, 55.7, 55.5, 55.4. EIMS m/z: 301 (M^+, 100%). Anal. ($C_{17}H_{19}NO_4$) C, H, N.

Acknowledgment. This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (HMP-00-CH-15-0014).

Supporting Information Available: ^{1}H NMR spectra and mass spectra of **7a**, **7f**, **7g**, **7i**, **7j**, **9**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ortiz de Montellano, P. R. *Cytochrome P450*: *Structure, Mechanism, and Biochemistry*, 2nd ed.; Plenum Press: New York, 1995.
(2) Savas, U.; Christou, M.; Jefcoate, C. R. Mouse endometrium stromal cells express a polycyclic aromatic hydrocarbon-inducible cytochrome P450 that closely resembles the novel P450 in mouse embryo fibroblasts. *Carcinogenesis* **1993**, *14*, 2013−2018.
(3) McKay, J. A.; Melvin, W. T.; Ah-See, A. K.; Ewen, S. W. B.; Greenlee, W. F.; Marcus, C. B.; Burke, M. D.; Murray, G. I. Expression of CYP1B1 in breast cancer. *FEBS Lett.* **1995**, *374*, 270−272.
(4) Shimada, T.; Hayes, C. L.; Yamazaki, H.; Amin, S.; Hecht, S. S.; Guengerich, F. P.; Sutter, T. R. Activation of chemically diversed procarcinogens by human cytochrome P-450 1B1. *Cancer Res.* **1996**, *56*, 2979−2984.
(5) Christou, M.; Savas, U.; Schroeder, S.; Shen, X.; Thompson, T.; Gould, M. N.; Jefcoate, C. R. Cytochromes CYP1A1 and 1B1 in the rat mammary gland: cell-specific expression and regulation by polycyclic aromatic hydrocarbons and hormones. *Mol. Cell. Endocrinol.* **1995**, *115*, 41−50.
(6) Liehr, J. G.; Ricci, M. J. 4-Hydroxylation of estrogens as marker of human mammary tumors. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3294−3296.
(7) Spink, D. C.; Spink, B. C.; Cao, J. Q.; Gierthy, J. F.; Hayes, C. L.; Li, Y.; Sutter, T. R. Induction of cytochrome P450 1B1 and catechol estrogen metabolism in ACHN human renal adenocarcinoma cells. *J. Steroid Biochem. Mol. Biol.* **1997**, *62*, 223−232.
(8) Spink, D. C.; Eugster, H. P.; Lincoln, D. W.; Schuetz, J. D.; Schuetz, E. G.; Johnson, J. A.; Kaminsky, L. S.; Gierthy, J. F. 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1A1: a comparison of the activities induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in MCF-7 cells with those from heterologous expression of the cDNA. *Arch. Biochem. Biophys.* **1992**, *293*, 342−348.
(9) Barnea, E. R.; MacLusky, N. J.; Naftolin, F. Kinetics of catechol estrogen-estrogen receptor dissociation: a possible factor underlying differences in catechol estrogen biological activity. *Steroids* **1983**, *41*, 643−656.
(10) Cavalieri, E. L.; Stack, D. E.; Devanesan, P. D.; Todorovic, R.; Dwivedy, I.; Higginbotham, S.; Johansson, S. L.; Patil, K. D.; Gross, M. L.; Gooden, J. K.; Ramanathan, R.; Cerny, R. L.; Rogan, E. G. Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10937−10942.
(11) Stack, D. E.; Byun, J.; Gross, M. L.; Rogan, E. G.; Cavalieri, E. L. Molecular characteristics of catechol estrogen quinones in reactions with deoxyribonucleosides. *Chem. Res. Toxicol.* **1996**, *9*, 851−859.
(12) Roy, D.; Liehr, J. G. Temporary decrease in renal quinone reductase activity induced by chronic administration of estradiol to male Syrian hamsters. *J. Biol. Chem.* **1988**, *263*, 3646−3651.
(13) Bolton, J. L.; Trush, M. A.; Penning, T. M.; Dryhurst, G.; Monks, T. J. Role of quinones in toxicology. *Chem. Res. Toxicol.* **2000**, *13*, 135−160.
(14) Han, X.; Liehr, J. G. 8-Hydroxylation of guanine bases in kidney and liver DNA of hamsters treated with estradiol: role of free radicals in estrogen-induced carcinogenesis. *Cancer Res.* **1994**, *54*, 5515−5517.
(15) Wang, M. Y.; Liehr, J. G. Induction by estrogens of lipid peroxidation and lipid peroxide-derived malonaldehyde-DNA adducts in male Syrian hamsters: role of lipid peroxidation in estrogen-induced kidney carcinogenesis. *Carcinogenesis* **1995**, *16*, 1941−1945.
(16) Wang, M. Y.; Liehr, J. G. Lipid hydroperoxide-induced endogenous DNA adducts in hamsters: possible mechanism of lipid hydroperoxide-mediated carcinogenesis. *Arch. Biochem. Biophys.* **1995**, *316*, 38−46.
(17) Winter, M. L.; Liehr, J. G. Free radical-induced carbonyl content in protein of estrogen-treated hamsters assayed by sodium boro-[^{3}H]-hydrine reduction. *J. Biol. Chem.* **1991**, *266*, 14446−14450.
(18) Shimada, T.; Yamazaki, H.; Foroozesh, M.; Hopkins, N. E.; Alworth, W. L.; Guengerich, F. P. Selectivity of polycyclic inhibitors for human cytochrome P450s 1A1, 1A2, and 1B1. *Chem. Res. Toxicol.* **1998**, *11*, 1048−1056 and references therein.
(19) Chun, Y. J.; Ryu, S. Y.; Jeong, T. C.; Kim, M. Y. Mechanism-based inhibition of human cytochrome P450 1A1 by rhapontigenin. *Drug Metab. Dispos.* **2001**, *29*, 389−393.
(20) Chun, Y. J.; Kim, M. Y.; Guengerich, F. P. Resveratrol is a selective human cytochrome P450 1A1 inhibitor. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 20−24.
(21) Chang, T. K.; Lee, W. B.; Ko, H. H. Trans-resveratrol modulates the catalytic activity and mRNA expression of the procarcinogen-activating human cytochrome P450 1B1. *Can. J. Physiol. Pharmacol.* **2000**, *78*, 874−881.
(22) Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. Synthesis and evaluation of stilbene and dihydrostilbene derivatives as potential anticancer agents that inhibit tubulin polymerization. *J. Med. Chem.* **1991**, *34*, 2579−2588.
(23) Yamataka, H.; Nagareda, K.; Ando, K.; Hanafusa, T. Relative reactivity and stereoselectivity in the Wittig reactions of substituted benzaldehydes with benzylidenetriphenylphosphorane. *J. Org. Chem.* **1992**, *57*, 2865−2869.
(24) Kucerovy, A.; Li, T.; Prasad, K.; Repič, O.; Blacklock, T. J. An efficient large-scale synthesis of methyl 5-[2-(2,5-dimethoxyphenyl)ethyl]-2-hydroxybenzoate. *Org. Process Res. Dev.* **1997**, *1*, 287−293.
(25) Hughes, I. Application of polymer-bound phosphonium salts as traceless supports for solid-phase synthesis. *Tetrahedron Lett.* **1996**, *37*, 7595−7598.
(26) Zhang, J.-T.; Dai, W.; Harvey, R. G. Synthesis of higher oxidized metabolites of dibenz[*a*,*j*]anthracene implicated in the mechanism of carcinogenesis. *J. Org. Chem.* **1998**, *63*, 8125−8132.
(27) Jang, D. O.; Park, D. J.; Kim, J. A mild and efficient procedure for the preparation of acid chlorides from carboxylic acids. *Tetrahedron Lett.* **1999**, *40*, 5323−5326.
(28) Burke, M. D.; Thompson, S.; Elcombe, C. R.; Halpert, J.; Haaparanta, T.; Mayer, R. T. Ethoxy-, pentoxy-, and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem. Pharmacol.* **1985**, *34*, 3337−3345.
(29) Chun, Y.-J.; Kim, S.; Kim, D.; Lee, S.-K.; Guengerich, F. P. A new selective and potent inhibitor of human cytochrome P450 1B1 and its application to antimutagenesis. *Cancer Res.* **2001**, *61*, 8164−8170.
(30) Ali, M. A.; Kondo, K.; Tsuda, Y. Studies on crude drugs effective on visceral larva migrans. XV. Synthesis and nematocidal activity of hydroxystilbenes. *Chem. Pharm. Bull.* **1992**, *40*, 1130−1136.
(31) Ghai, G.; Ho, C.-T.; Chen, H. Y.; Rosen, R. T.; Wang, M.; Telang, N.; Lipkin, M. Resveratrol analogues for prevention of cancer. PCT Application WO 0121165, March 29, 2001.
(32) Donnio, B.; Bruce, D. W. Liquid-crystalline, polycatenar complexes of silver(I): dependence of the mesomorphism on the ligand and anion. *New J. Chem.* **1999**, *23*, 275−286.
(33) Guengerich, F. P.; Martin, M. V.; Guo, Z.; Chun, Y. J. Purification of functional recombinant P450s from bacteria. *Methods Enzymol.* **1996**, *272*, 35−44.

JM010298J