Arch Pharm Res Vol 27, No 2, 199-205, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Potent Inhibition of Human Cytochrome P450 1 Enzymes by Dimethoxyphenylvinyl Thiophene

Sang-Kwang Lee, Yongmo Kim, Mie Young Kim, Sanghee Kim[1], and Young-Jin Chun

College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea and [1]Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 110-460, Korea

(Received December 7, 2003)

Cytochrome P450 (P450) 1 enzymes such as P450 1A1, 1A2, and 1B1 are known to be involved in the oxidative metabolism of various procarcinogens and are regarded as important target enzymes for cancer chemoprevention. Previously, several hydroxystilbene compounds were reported to inhibit P450 1 enzymes and were rated as candidate chemopreventive agents. In this study, we investigated the inhibitory effect of 2-[2-(3,5-dimethoxyphenyl)vinyl]-thiophene (DMPVT), produced from the chemical modification of oxyresveratrol, on the activities of P450 1 enzymes. The inhibitory potential by DMPVT on the P450 1 enzyme activity was evaluated with the *Escherichia coli* membranes of the recombinant human cytochrome P450 1A1, 1A2, or 1B1 coexpressed with human NADPH-P450 reductase. DMPVT significantly inhibited ethoxyresorufin O-deethylation (EROD) activities with IC_{50} values of 61, 11, and 2 nM for 1A1, 1A2, and 1B1, respectively. The EROD activity in DMBA-treated rat lung microsomes was also significantly inhibited by DMPVT in a dose-dependent manner. The modes of inhibition by DMPVT were non-competitive for all three P450 enzymes. The inhibition of P450 1B1-mediated EROD activity by DMPVT did not show the irreversible mechanism-based effect. The loss of EROD activity in P450 1B1 with DMPVT incubation was not blocked by treatment with the trapping agents such as glutathione, *N*-acetylcysteine, or dithiothreitol. Taken together, the results suggested DMPVT to be a strong noncompetitive inhibitor of human P450 1 enzymes that should be considered as a good candidate for a cancer chemopreventive agent in humans.

Key words: Cytochrome P450 1 enzyme, Enzyme inhibitor, Dimethoxyphenylvinyl thiophene

INTRODUCTION

Human cytochrome P450 (P450) 1 enzymes, including P450 1A1, P450 1A2, and P450 1B1, have been studied extensively due to their roles in chemical carcinogenesis. These P450 enzymes are well known to activate many procarcinogens such as benzo[*a*]pyrene and other environmental pollutants to be mutagenic or carcinogenic and are regarded as the target enzymes for blocking tumor initiation (Guengerich and Shimada, 1991). Therefore, potent inhibitors of P450 1 enzymes are good candidate chemopreventive agents against cancer in humans.

To find potently selective inhibitors of P450 1 enzymes, various chemicals isolated from natural sources have been evaluated. Recent studies reported potent and selective inhibition of P450s by various stilbene compounds. Resveratrol (3,4',5-trihydroxystilbene) can inhibit human P450 1A1 and 1B1 (Chun *et al.*, 1997; Chang *et al.*, 2000). Rhapontigenin (3,3',5-trihydroxy-4'-methoxystilbene) was found to be a mechanism-based inactivator of P450 1A1 with an IC_{50} value of 0.4 μM (Chun *et al.*, 2001b). Rhapontigenin showed 400-fold selectivity for P450 1A1 over P450 1A2 and 23-fold selectivity for P450 1A1 over P450 1B1. 3,4'-Dimethoxy-5-hydroxystilbene showed strong inhibition of P450 1A1 and 1B1 (IC_{50} = 0.1 μM) (Chun *et al.*, 2001b). We also found 2,4,3',5'-tetramethoxystilbene, a methoxy derivative of oxyresveratrol, to be one of the most potently selective competitive inhibitor of P450 1B1 with an IC_{50} value of 6 nM (Chun *et al.*, 2001a). Because we found that inhibition of P450 1 enzymes by stilbene analogs were sensitive to the substitution patterns on the stilbene structure, we synthesized 2-[2-(3,5-dimethoxy-

Correspondence to: Young-Jin Chun, College of Pharmacy, Chung-Ang University, 221 Huksuk-Dong, Dongjak-Gu, Seoul 156-756, Korea
E-mail: yjchun@cau.ac.kr

henyl) vinyl]-thiophene (DMPVT) having a 2-thiophenyl ring structure instead of a phenyl ring at the 2-position of the stilbene skeleton (Kim *et al.*, 2002a). In this study, we determined the inhibitory potential of DMPVT on P450 1 enzymes with the goal of identifying a new cancer chemopreventive agent.

MATERIALS AND METHODS

Materials

7-Ethoxyresorufin, resorufin, DMSO, thiamine, IPTG, δ-aminolevulinic acid, and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO). 7,12-Dimethylbenza [*a*]anthracene (DMBA) was obtained from Aldrich (Milwaukee, WI). Bactopeptone, yeast extract, and bacto-agar were obtained from Difco Lab. (Detroit, MI). All other chemicals used were of the highest grade commercially available. The preparation of DMPVT was carried out according to methods described previously (Kim *et al.*, 2002a).

Recombinant human P450s

Coexpression (bicistronic) plasmids for human P450s (1A1, 1A2, or 1B1) and NADPH-P450 reductase were transformed into *Escherichia coli* (*E. coli*) DH5α (Parikh *et al.*, 1997). A single ampicillin-resistant colony of transformed cells was selected and cultured overnight to saturation at 37°C in LB medium containing 100 μg/mL ampicillin. A 10-mL aliquot was used to inoculate each liter of Terrific Broth (TB) containing 0.2% bactopeptone (w/v), 100 μg/mL ampicillin, 1.0 mM thiamine, trace elements, 0.5 mM δ-aminolevulinic acid, and 1.0 mM IPTG. The cultures were grown at 30°C with shaking at 200 rpm for 48 h. Membrane fractions were prepared by differential centrifugation from bacteria and suspended in 10 mM Tris-acetate buffer (pH 7.4) containing 1.0 mM EDTA and 20% (v/v) glycerol (Guengerich *et al.*, 1996).

Preparation of rat lung microsomes

Sprague Dawley (SD) rats were purchased from the Hanrim Animal Research Center (Hwasung, Korea) and were acclimated for 1-week acclimation prior to the experiments. The rats were administered with DMBA (60 mg/kg body weight, p.o.) dissolved in sesame oil. After 48 h, rat lung samples were isolated, suspended in 0.1 M Tris acetate buffer (pH 7.4) containing 0.1 M KCl, 1.0 mM EDTA, and 20 μM BHT, and homogenized in a Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 × g for 20 min at 4°C, and the resulting supernatant was centrifuged for 60 min at 100,000 × g at 4°C. The microsomal pellets were resuspended in 10 mM Tris acetate buffer (pH 7.4) containing 1.0 mM EDTA and 20% (v/v) glycerol (Guengerich, 1994). Protein concentrations were estimated using the bichinchoninic acid method according to the suppliers recommendations (Pierce Chemical Co., Rockford, IL) using bovine serum albumin as a standard. The isolated microsomes were stored at -80°C.

EROD enzyme assay

EROD activity was determined to measure P450 1A1, 1A2, and 1B1 activities (Burke *et al.*, 1985). The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.4), 2 mg/mL bovine serum albumin (BSA), 2 μM ethoxyresorufin, and rat lung microsomes or *E. coli* membranes (60 nM P450 1A1, 10 nM P450 1A2, or 2 nM P450 1B1, respectively). The reaction mixtures were pre-incubated at 37°C for 3 min, and the reactions were initiated by adding 120 μM NADPH. Incubations were performed in a shaking water bath at 37°C for 20 min and were terminated by adding 1 mL of methanol. The formation of resorufin was determined fluorometrically with a Perkin-Elmer LS 5 spectrofluorimeter with excitation and emission wavelengths of 550 nm and 585 nm, respectively. The P450 content of the cells and membranes was determined by the spectral method of Omura and Sato (1964) using an extinction coefficient of 91 mM^{-1} cm^{-1} with a Shimadzu UV-160A spectrophotometer at ambient temperature.

NADPH dependence of inhibition

Bacterial membranes containing human P450 1A1, 1A2, or 1B1, and NADPH-P450 reductase were preincubated in 0.1 M potassium phosphate buffer (pH 7.4) containing a tested compound at 37°C for 10 min in the presence or absence of 1 mM NADPH (Chun *et al.*, 1999). At various times during the preincubation, an aliquot of the preincubation mixture was diluted 10-fold into the reaction mixture containing 0.1 M potassium phosphate (pH 7.4), 2 mg/mL BSA, 2 μM ethoxyresorufin, and 120 μM NADPH. The mixtures were further incubated at 37°C for 20 min. The product of 7-ethoxyresorufin was monitored fluorometrically as described above.

Data analysis

Kinetic parameters from individual experiments were calculated using a nonlinear regression analysis program (Prism, Graphpad Software, San Diego, CA).

RESULTS

Inhibition of P450 1 enzymes by dimethoxyphenylvinyl thiophene

To examine the effects of DMPVT (Fig. 1) on the activities of human P450 1A1, 1A2, and 1B1, the changes in EROD activities were measured with the *E. coli* membranes expressed human recombinant P450 1A1, 1A2, or

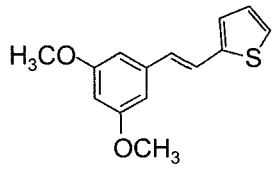

Fig. 1. Structure of 2-[2-(3,5-dimethoxyphenyl)vinyl]-thiophene (DMPVT)

1B1 with human NADPH-P450 reductase (Fig. 2). DMPVT exhibited a potent inhibition of P450 1 enzymes with IC_{50} values of 61, 11, and 2 nM for 1A1, 1A2, and 1B1, respectively. DMPVT showed 31-fold greater inhibi-tion of P450 1B1 over P450 1A1 and 6-fold greater inhibition of P450 1B1 over P450 1A2. As shown in Fig. 3, DMPVT also inhibited the EROD activity induced by DMBA in lung microsomes from SD rats in a dose-dependent manner. DMBA treatment significantly enhanced the EROD activity in rat lung microsomes *in vivo*. EROD activity is mainly caused by increasing P450 1A1 and 1B1 enzymes.

Mechanism of inhibition by dimethoxyphenylvinyl thiophene

To elucidate the mechanism of inhibition by DMPVT, kinetic studies were performed using recombinant human P450 1 enzymes. The modes of inhibition by DMPVT were noncompetitive for all three P450 1 enzymes (Fig. 4). To determine whether DMPVT is a mechanism-based inactivator of P450 1B1, P450 1B1 was preincubated with various concentrations of DMPVT in the presence of NADPH. As shown in Fig. 5, DMPVT did not follow pseudo-first-order kinetics when plotted as the log of the percentage of activity remaining versus time. To determine the possibilities of the reactive species escaping from the

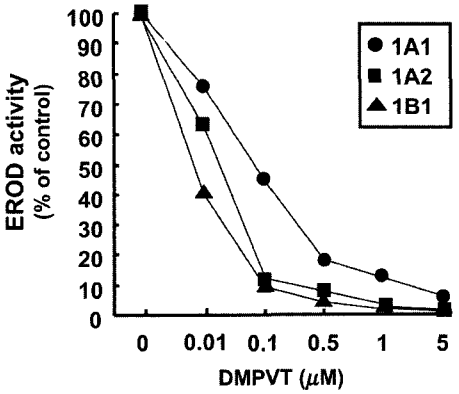

Fig. 2. Effect of DMPVT on P450 1A1, 1A2, and 1B1 activities in the P450-expression systems. Bacterial membranes coexpressing human P450s and NADPH-P450 reductase were incubated with DMPVT (0, 0.01, 0.1, 0.5, 1, or 5 μM) for 20 min at 37°C in the presence of 120 μM NADPH. Assays included EROD by P450 1A1 (●), P450 1A2 (■), or 1B1 (▲) in the presence of the indicated concentrations of DMPVT. Each data point represents the mean ± S.E.M. of three experiments.

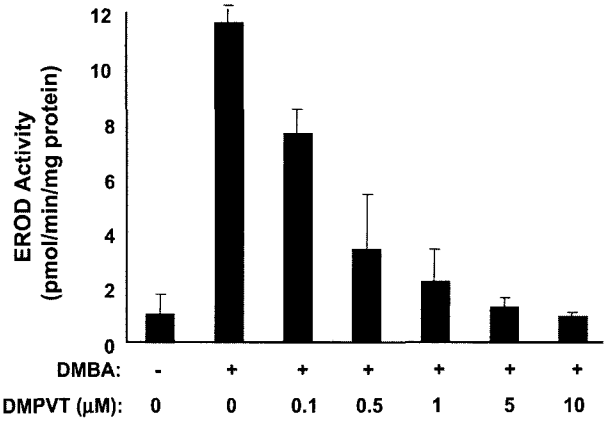

Fig. 3. Effect of DMPVT on EROD activity catalyzed by rat lung microsomes induced by DMBA. DMBA-induced rat lung microsomes were incubated with each concentration of DMPVT (0, 0.1, 0.5, 1, 5, or 10 μM) for 20 min at 37°C in the presence of 120 μM NADPH. EROD activities were determined as described under Materials and Methods. Each data point represents the mean ± S.E.M. of three experiments.

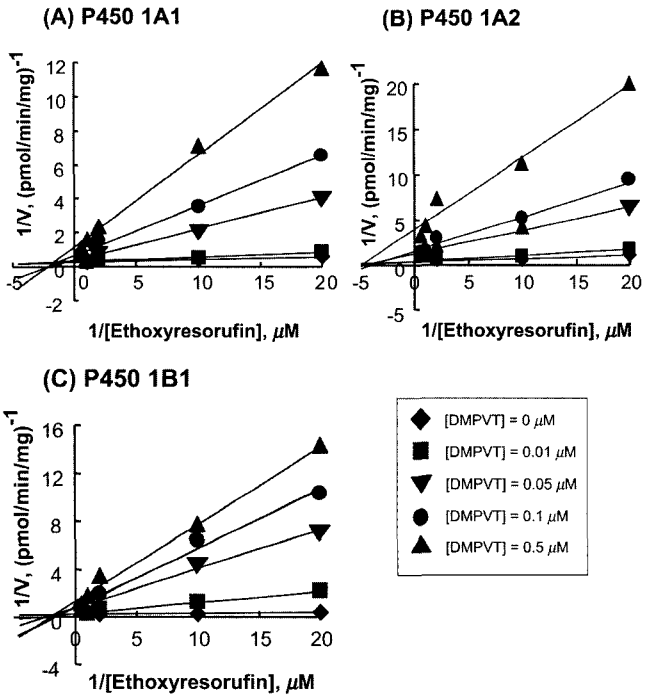

Fig. 4. Kinetic analysis of P450 1A1, 1A2, and 1B1 inhibition by DMPVT. EROD activity was determined with *E.coli* membrane coexpressed human P450s and NADPH-P450 reductase in the presence of DMPVT (0, 0.01, 0.05, 0.1, or 0.5 μM). Lineweaver-Burk plot was plotted and the kinetic parameters were calculated using non-linear regression methods. (A) P450 1A1, (B) P450 1A2, (C) P450 1B1. Each data point represents the mean ± S.E.M. of three experiments.

P450 active site and binding to nucleophilic sites in the vicinity of the active site to form protein adducts, the effects of the exogenous nucleophiles, such as glutathione,

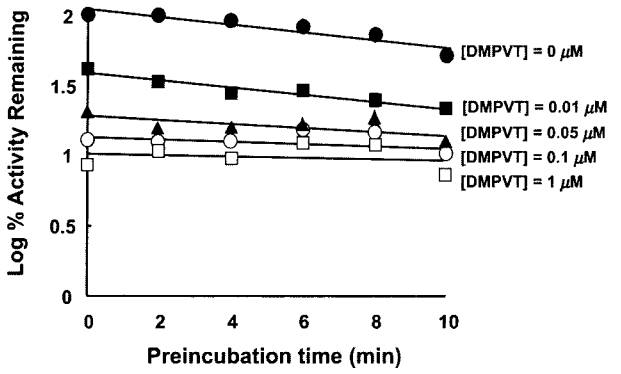

Fig. 5. Time-dependent effect of P450 1B1 inhibition after incubation with DMPVT and NADPH. P450 1B1 was first incubated with each concentration of DMPVT (0, 0.01, 0.05, 0.1, or 1 μM) for 10 min at 37 °C, and aliquots were removed at the time points indicated and assayed for residual P450 1B1 activity as described under Materials and Methods. Each data point represents the mean ± S.E.M. of three experiments.

N-acetylcysteine, or dithiothreitol, on the inhibition of EROD activity was investigated by coincubating P450s with 50 nM DMPVT. No protection against P450 1B1 inhibition by DMPVT was observed in the presence of 2 mM glutathione, *N*-acetylcysteine, or dithiothreitol (Fig. 6).

DISCUSSION

Currently, the three P450 1 enzymes P450 1A1, 1A2, and 1B1 have been identified as effective molecular targets for cancer chemopreventive agents. Previously we found that resveratrol, a trans-stilbene compound, inhibits recombinant human P450 1A1 and 1B1 at micromolar concentrations (Chun *et al.*, 1999). A number of trans-stilbene compounds nave been examined to determine the potency of P450 inhibition. Rhapontigenin (3,3',5-trihydroxy-4'-methoxystilbene) exhibited a potent and selective inhibition of P450 1A1 with an IC_{50} value of 0.4 μM and 3,5-dihydroxy-4'-methoxystilbene also showed strong inhibition of P450 1A1 activity (Chun *et al.*, 2001).

To identify new compounds that can inhibit P450 1 enzymes more efficiently and at lower concentrations, we synthesized new stilbene derivatives based on oxyresveratrol as a lead structure (Kim *et al.*, 2002a). Oxyresveratrol is a relatively weak inhibitor of P450 1 enzymes with IC_{50} values of 15, 150, and 34 μM for 1A1, 1A2, and 1B1, respectively. DMPVT, a dimethoxy derivative of oxyresveratrol containing a 2-thiophenyl ring instead of the phenyl ring, strongly inhibited P450 1 enzymes with IC_{50} values of 61, 11, and 2 nM for 1A1, 1A2, and 1B1, respectively. The result that DMPVT also significantly inhibited the EROD activity in lung microsomes from DMBA-treated Sprague Dawley rats suggested that administration of DMPVT may

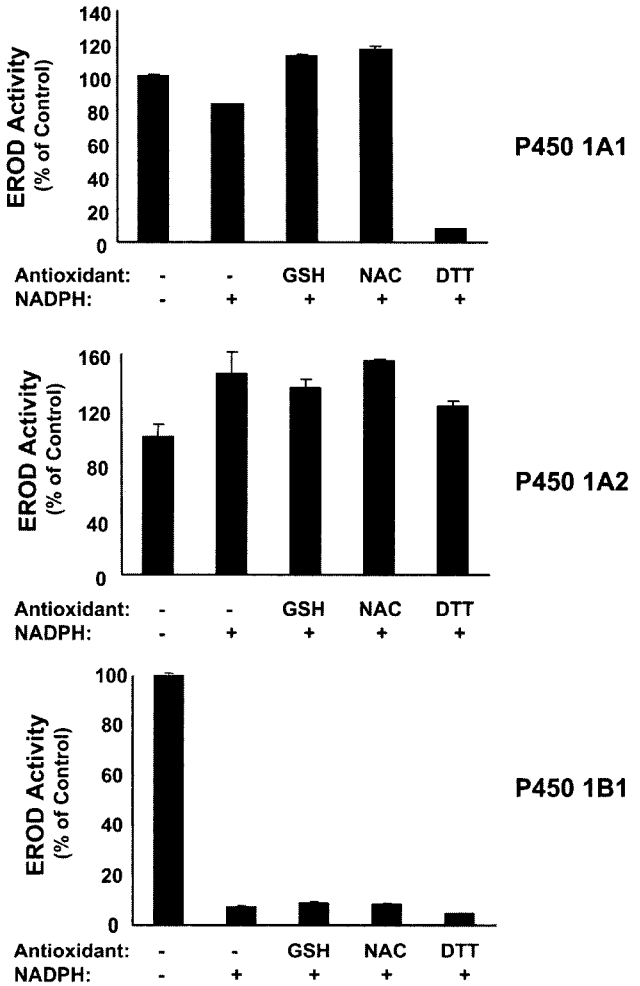

Fig. 6. Effects of exogenous nucleophiles on the inhibition of P450 enzymes by DMPVT. The *E.coli* membranes expressing human P450s and NADPH-P450 reductase were preincubated for 10 min with NADPH (1 mM), DMPVT (50 nM) and the indicated exogenous nucleophiles (2 mM) or water. At the end of the incubation, aliquots were assayed for EROD activity. Each data point represents the mean ± S.E.M. of three experiments.

also decrease the P450 activity *in vivo*. From the kinetic studies with an inhibitor, the modes of inhibition by DMPVT were found to be noncompetitive for all three P450 1 enzymes. These results were noteworthy because all previously studied P450 inhibitors with *trans*-stilbene structure, such as resveratrol, rhapontigenin, and 2,4,3', 5'-tetramethoxystilbene, showed competitive inhibition. Thus, substitution of the phenyl ring with the 2-thiophenyl ring can significantly modify the mode of enzyme kinetics although we do not know how the 2-thiophenyl ring interacts with the active site moieties of the P450 enzymes. Recently ODonnell *et al.* (2003) reported that suprofen, a nonsteroidal anti-inflammatory agent having a thiophene ring structure, showed the mechanism-based inactivation of P450 2C9 *via* the formation of a reactive

intermediate(s) derived from the bioactivation of its thiophene ring. P450 2C9-catalyzed thiophene epoxidation may lead to the formation of the pyrazine adduct or P450 2C9 adduct. Tienilic acid, the uricosuric diuretic agent with a thiophene ring, also showed the suicide inactivation of P450 2C9 (Jean *et al.*, 1996; Koenigs *et al.*, 1999). We determined whether DMPVT is a mechanism-based inactivator of P450 1B1 to investigate the mechanism of inhibition. However, inhibition of P450 1B1 by DMPVT did not follow the pseudo-first-order kinetics. Moreover, the results that the trapping agents such as glutathione, N-acetylcysteine, and dithiothreitol could not prevent the inactivation of P450 1B1 by DMPVT suggest that DMPVT is a reversible noncompetitive inhibitor of P450 1B1. Interestingly, we found that preincubation with NADPH caused notably different effects on the inhibition of each P450 1 enzyme. These results also suggest that the environments of the active sites in three P450 1 enzymes are quite different and that DMPVT may be useful as a tool to investigate the structures of P450 active sites.

Various trans-stilbene compounds isolated from natural sources or synthesized chemically were identified as having many pharmacological effects. The diverse beneficial effects of resveratrol are well documented. Resveratrol shows cancer chemopreventive, cardioprotective, and neuroprotective activities through the suppression of cellular proliferation, promotion of differentiation, induction of apoptosis, scavenging of reactive oxygen species, down-regulation of carcinogen metabolizing enzymes and proinflammatory proteins, and inhibition of estrogenic activity (Fremont, 2000; Pervaiz, 2003). Oxyresveratrol is known to inhibit the dopa oxidase activity of tyrosinase (Shin *et al.*, 1998a; Kim *et al.*, 2002b), cyclooxygenase (Shin *et al.*, 1998b), rat liver mitochondrial ATPase (Nimmanpisut *et al.*, 1976), and P450 enzymes (Chun *et al.*, 2001). The inhibitory effect on the release of β-hexaminidase from cultured RBL-2H3 cells and P450 1A1 of rhapontigenin has been reported (Cheong *et al.*, 1999; Chun *et al.*, 2001). These hydroxystilbenes are also known to suppress ovine cyclooxygenase-1 activity (Shin *et al.*, 1998b).

A number of natural products and their derivatives have been studied to evaluate their effect on P450 1 enzymes. Flavonoids such as α-naphthoflavone, galangin, acacetin, diosmetin, eriodictyol, hesperetin, homoeriodictyol, and naringenin have been reported as strong inhibitors of P450 enzymes (Shimada *et al.*, 1998; Zhai *et al*, 1998; Doostar *et al.*, 2000). Naturally occurring coumarins such as xanthotoxin, imperatorin, isopimpinellin, bergamottin, and coriandrin have also shown potent inhibition of P450 1 activities (Mays *et al.*, 1990; Kleiner *et al.*, 2002; Cai *et al.*, 1996). Recently St. Johns wort (*Hypericum perforatum*) preparation and their major constituents such as hypericin, pseudohypericin, and hyperforin potently inhibited P450 1A1 activity (Schwarz *et al.*, 2003). Moreover, extensive studies on P450 inhibition by stilbenes such as resveratrol, oxyresveratrol, rhapontigenin, 2,4,3',5'-tetramethoxystilbene, and DMPVT have been performed by our research group.

The mechanism by which DMPVT inhibits P450 1 enzymes is not clearly determined. We showed that DMPVT acts as a noncompetitive inhibitor of P450 1 enzymes including P450 1A1, 1A2, and 1B1. These data suggested that DMPVT binds to a region of P450 enzymes that does not participate in substrate binding. Various noncompetitive inhibitors of P450 1 enzymes have been reported. Quercetin has shown noncompetitive inhibition of P450 1A2 with K_i of 3.3 μM (Obach, 2000). Hypericin has exhibited noncompetitive inhibition of P450 1A1 with a K_i of 0.6 μM (Schwarz *et al.*, 2003). Galangin and diosmetin have inhibited P450 1A1 activity in a noncompetitive manner (Ciolino and Yeh, 1999; Ciolino *et al.*, 1998). 5,8-Dihydroxynaphthoquinone and 5-hydroxy-naphthoquinone showed noncompetitive inhibition of P450 1A1 activity (Inouye *et al.*, 2000). Recently Dawling *et al.* (2003) reported that methoxyestrogens, such as 2-methoxyestradiol, 2-hydroxy-3-methoxyestradiol, or 4-methoxyestradiol, act as noncompetitive feedback inhibitors on P450 1A1 and P450 1B1-mediated estradiol hydroxylation. Since noncompetitive inhibitors can bind to the regulatory region, DMPVT may be useful for determining the unknown mechanisms of the enzymatic regulation of P450 1B1.

In conclusion, the results of this study suggest that DMPVT, a dimethoxy derivative of oxyresveratrol containing a 2-thiophenyl ring, potently inhibits P450 1 enzyme activities. DMPVT inhibited P450 1B1 more strongly than it inhibited P450 1A1 or 1A2, and it is one of the strongest noncompetitive inhibitors of P450 1B1 although the selectivity is relatively weaker than 2,4,3',5'-tetramethoxystilbene, a previously reported specific P450 1B1 inhibitor (Chun *et al.*, 2001). On the basis of these data, we suggest that the 2-position of the stilbene skeleton on naturally occurring stilbenes may play an important role in inhibiting P450 activities. Future studies should focus on the *in vivo* potential of DMPVT as a strong cancer chemopreventive agent.

ACKNOWLEDGEMENT

This work was supported by the Chung-Ang University Research Grant in 2002.

REFERENCES

Burke, M. D., Thompson, S., Elcombe, C. R., Halpert, J., Haaparanta, T., and Mayer, R. T., Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450.

Biochem. Pharmacol., 34, 3337-3345 (1985).

Cai, Y., Baer-Dubowska, W., Ashwood-Smith, M. J., Ceska, O., Tachibana, S., and DiGiovanni, J., Mechanism-based inactivation of hepatic ethoxyresorufin O-dealkylation activity by naturally occurring coumarins. *Chem. Res. Toxicol.*, 9, 729-736 (1996).

Chang, T. K., Lee, W. B., and Ko, H. H., *Trans*-resveratrol modulates the catalytic activity and mRNA expression of the procarcinogen-activating human cytochrome P450 1B1 *Can. J. Physiol. Pharmacol.*, 78, 874-881 (2000).

Cheong, H., Ryu, S. Y., and Kim, K. M., Anti-allergic action of resveratrol and related hydroxystilbenes. *Planta Med.*, 65, 266-268 (1999).

Chun, Y. J., Kim, M. Y., and Guengerich, F. P., Resveratrol is a selective human cytochrome P450 1A1 inhibitor. *Biochem. Biophys. Res. Commun.*, 262, 20-24 (1999).

Chun, Y. J., Kim, S., Kim, D., Lee, S. K., and Guengerich, F. P., A new selective and potent inhibitor of human cytochrome P450 1B1 and its application to antimutagenesis. *Cancer Res.*, 61, 8164-8170 (2001a).

Chun, Y. J., Ryu, S. Y., Jeong, T. C., and Kim, M. Y., Mechanism-based inhibition of human cytochrome P450 1A1 by rhapontigenin. *Drug Metab. Dispos.*, 29, 389-393 (2001b).

Ciolino, H. P., Wang, T. T., and Yeh, G. C., Diosmin and diosmetin are agonists of the aryl hydrocarbon receptor that differentially affect cytochrome P450 1A1 activity. *Cancer Res.*, 58, 2754-2760 (1998).

Ciolino, H. P. and Yeh, G. C., The flavonoid galangin is an inhibitor of CYP1A1 activity and an agonist/antagonist of the aryl hydrocarbon receptor. *Br. J. Cancer,* 79, 1340-1346 (1999).

Dawling, S., Roodi, N., and Parl, F. F., Methoxyestrogens exert feedback inhibition on cytochrome P450 1A1 and 1B1. *Cancer Res.*, 63, 3127-3132 (2003).

Doostdar, H., Burke, M. D., and Mayer, R. T., Bioflavonoids: selective substrates and inhibitors for cytochrome P450 CYP1A and CYP1B1. *Toxicology,* 144, 31-38 (2000).

Fremont, L., Biological effects of resveratrol. *Life Sci.*, 66, 663-673 (2000).

Guengerich, F. P., Analysis and characterization of enzymes, In Hayes, A.W. (Ed). Principles and Methods of Toxicology. Raven Press, New York, pp. 1259-1313, (1994).

Guengerich, F. P., Martin, M. V., Guo, Z., and Chun, Y. J., Purification of functional recombinant P450s from bacteria. *Methods Enzymol.*, 272, 35-44 (1996).

Guengerich, F. P. and Shimada, T., Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.*, 4, 391-407 (1991).

Inouye, K., Saito, A., Orita, M., Tonomura, B., Imaishi, H., and Ohkawa, H., Inhibitory effects of 1,4-naphthoquinone derivatives on rat cytochrome P4501A1-dependent monooxygenase activity in recombinant yeast microsomes. *J. Biochem.*, 127, 1041-1046 (2000).

Jean, P., Lopez-Garcia, P., Dansette, P., Mansuy, D., and Goldstein, J. L., Oxidation of tienilic acid by human yeast-expressed cytochromes P-450 2C8, 2C9, 2C18 and 2C19. Evidence that this drug is a mechanism-based inhibitor specific for cytochrome P-450 2C9. *Eur. J. Biochem.*, 241, 797-804 (1996).

Kim, S., Ko, H., Park, J. E., Jung, S., Lee, S. K., and Chun, Y. J., Design, synthesis, and discovery of novel trans-stilbene analogues as potent and selective human cytochrome P450 1B1 inhibitors. *J. Med. Chem.*, 45, 160-164 (2002a).

Kim, Y. M., Yun, J., Lee, C. K., Lee, H., Min, K. R., and Kim, Y., Oxyresveratrol and hydroxystilbene compounds. Inhibitory effect on tyrosinase and mechanism of action. *J. Biol. Chem.*, 277, 16340-16344 (2002b).

Kleiner, H. E., Vulimiri, S. V., Reed, M. J., Uberecken, A., and DiGiovanni, J., Role of cytochrome P450 1a1 and 1b1 in the metabolic activation of 7,12-dimethylbenz[*a*]anthracene and the effects of naturally occurring furanocoumarins on skin tumor initiation. *Chem. Res. Toxicol.*, 15, 226-235 (2002).

Koenigs, L. L., Peter, R. M., Hunter, A. P., Haining, R. L., Rettie, A. E., Friedberg, T., Pritchard, M. P., Shou, M., Rushmore, T. H., and Trager, W. F., Electrospray ionization mass spectrometric analysis of intact cytochrome P450: identification of tienilic acid adducts to P450 2C9. *Biochemistry,* 38, 2312-2319 (1999).

Mays, D., Hilliard, J., Wong, D., Chambers, M., Park, S., Gelboin, H., and Gerber, N., Bioactivation of 8-methoxypsoralen and irreversible inactivation of cytochrome P-450 in mouse liver microsomes: modification by monoclonal antibodies, inhibition of drug metabolism and distribution of covalent adducts. *J. Pharmacol. Exp. Ther.*, 254, 720-731 (1990).

Nimmanpisut, S., Chudapongse, P., and Ratanabanangkoon, K., Effects of 2,4,3',5'-tetrahydroxystilbene on oxidative phosphorylation by rat liver mitochondria. *Biochem. Pharmacol.*, 25, 1245-1248 (1976).

Obach, R. S., Inhibition of human cytochrome P450 enzymes by constituents of St. John's Wort, an herbal preparation used in the treatment of depression. *J. Pharmacol. Exp. Ther.*, 294, 88-95 (2000).

O'Donnell, J. P., Dalvie, D. K., Kalgutkar, A. S., and Obach, R. S., Mechanism-based inactivation of human recombinant P450 2C9 by the nonsteroidal anti-inflammatory drug suprofen. *Drug Metab. Dispos.*, 31, 1369-1377 (2003).

Omura, T. and Sato, R., The carbon-monoxide binding pigment of liver microsomes. *J. Biol. Chem.*, 239, 2370-2378 (1964).

Parikh, A., Gillam, E. M., and Guengerich, F. P., Drug metabolism by *Escherichia coli* expressing human cytochromes P450. *Nat. Biotechnol.*, 15, 784-788 (1997).

Pervaiz, S., Resveratrol: from grapevines to mammalian biology. *FASEB J.*, 17, 1975-1985 (2003).

Schwarz, D., Kisselev, P., and Roots, I., St. John's wort extracts and some of their constituents potently inhibit ultimate carcinogen formation from benzo[*a*]pyrene-7,8-dihydrodiol

by human CYP1A1. *Cancer Res.*, 63, 8062-8068 (2003).

Shimada, T., Yamazaki, H., Foroozesh, M., Hopkins, N. E., Alworth, W. L., and Guengerich, F. P., Selectivity of polycyclic inhibitors for human cytochrome P450s 1A1, 1A2, and 1B1. *Chem. Res. Toxicol.,* 11, 1048-1056 (1998).

Shin, N. H., Ryu, S. Y., Choi, E. J., Kang, S. H., Chang, I. M., Min, K. R., and Kim, Y., Oxyresveratrol as the potent inhibitor on dopa oxidase activity of mushroom tyrosinase. *Biochem. Biophys. Res. Commun.,* 243, 801-803 (1998a).

Shin, N. H., Ryu, S. Y., Lee, H., Min, K. R., and Kim, Y., Inhibitory effects of hydroxystilbenes on cyclooxygenase from sheep seminal vesicles. *Planta Med.,* 64, 283-284 (1998b).

Zhai, S., Dai, R., Friedman, F. K., and Vestal, R. E., Comparative inhibition of human cytochromes P450 1A1 and 1A2 by flavonoids. *Drug Metab. Dispos.,* 26, 989-992 (1998).