



# Inhibition of Carcinogen-Activating Cytochrome P450 Enzymes by Xenobiotic Chemicals in Relation to Antimutagenicity and Anticarcinogenicity

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A variety of xenobiotic chemicals, such as polycyclic aromatic hydrocarbons (PAHs), aryl- and heterocyclic amines and tobacco related nitrosamines, are ubiquitous environmental carcinogens and are required to be activated to chemically reactive metabolites by xenobiotic-metabolizing enzymes, including cytochrome P450 (P450 or CYP), in order to initiate cell transformation. Of various human P450 enzymes determined to date, CYP1A1, 1A2, 1B1, 2A13, 2A6, 2E1, and 3A4 are reported to play critical roles in the bioactivation of these carcinogenic chemicals. *In vivo* studies have shown that disruption of *Cyp1b1* and *Cyp2a5* genes in mice resulted in suppression of tumor formation caused by 7,12-dimethylbenz[*a*]anthracene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, respectively. In addition, specific inhibitors for CYP1 and 2A enzymes are able to suppress tumor formation caused by several carcinogens in experimental animals *in vivo*, when these inhibitors are applied before or just after the administration of carcinogens. In this review, we describe recent progress, including our own studies done during past decade, on the nature of inhibitors of human CYP1 and CYP2A enzymes that have been shown to activate carcinogenic PAHs and tobacco-related nitrosamines, respectively, in humans. The inhibitors considered here include a variety of carcinogenic and/or non-carcinogenic PAHs and acetylenic PAHs, many flavonoid derivatives, derivatives of naphthalene, phenanthrene, biphenyl, and pyrene and chemopreventive organoselenium compounds, such as benzyl selenocyanate and benzyl selenocyanate; *o*-XSC, 1,2-, 1,3-, and 1,4-phenylenebis(methylene)selenocyanate.

**Key words:** Cytochrome P450, Metabolic activation, Chemical carcinogenesis, Enzyme inhibition, Polycyclic aromatic hydrocarbons, Tobacco-related nitrosamines

## INTRODUCTION

Rendic and Guengerich have recently summarized the roles of human xenobiotic metabolizing enzymes in the activation of a variety of environmental carcinogens and

mutagens to chemically reactive metabolites by searching more than 500 literatures reported until 2012 (1). Cytochrome P450 (P450 or CYP), sulfotransferase, aldo-keto reductase, *N*-acetyltransferase, cyclooxygenase, and flavon-containing monooxygenase are important enzymes involved

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Abbreviations: P450 or CYP, cytochrome P450; EROD, 7-ethoxyresorufin *O*-deethylation; PAH, polycyclic aromatic hydrocarbon; B[*a*]P, benzo[*a*]pyrene; B[*e*]P, benzo[*e*]pyrene; 3-MC, 3-methylcholanthrene; 7,12-DMBA, 7,12-dimethylbenzo[*a*]anthracene; DB[*a*,*f*]P, dibenzo[*a*,*f*]pyrene (or dibenzo[*def,p*]chrysene); ANF,  $\alpha$ -naphthoflavone; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; 1PP, 1-(1-propynyl)pyrene; 1EP, 1-ethynylpyrene; 4EP, 4-ethynylpyrene; 4Pbi, 4-propynyl biphenyl; BSC, benzyl selenocyanate; *o*-, *m*-, and *p*-XSC, 1,2-phenylenebis(methylene)selenocyanate, 1,3-phenylenebis(methylene)selenocyanate, 1,4-phenylenebis(methylene)selenocyanate, respectively.

in the metabolic activation of many carcinogens and their contributions to the activation of procarcinogens and promutagens have been estimated to be about 66%, 13%, 8%, 7%, 2%, and 1%, respectively (1). P450 enzymes have been shown to play major roles in activating these carcinogens, based on the analysis of formation of chemically reactive metabolites, DNA adduct and damage, chromosomal abbreviation, and bacterial mutagenicity and genotoxicity assays such as Ames and *umu* test systems (2-8). Our previous studies using *umu* genotoxicity assay with human P450 enzymes in conjunction with the results obtained from Ames mutagenicity assay and other detection systems reported so far (6,7-19) have suggested that human CYP1A1, 1A2, 1B1, 2A6, 2A13, 2E1, and 3A4 are major enzymes involved in the activation of various environmental carcinogens including PAHs and tobacco-related nitrosamines (Table 1). In this review, we first describe *in vivo* studies on the roles of CYP1 and 2A enzymes in the formation of tumors caused by various chemical carcinogens; these are reported using

gene-knockout mice and specific P450 inhibitors. Then, we summarize recent progress, mainly our *in vitro* studies done during the past decade, on the nature of chemical inhibitors of human P450 enzymes that participate in carcinogen activation (20-31).

***In vivo* studies of suppression of tumor formation caused by procarcinogens in gene knockout mice.**

Buters *et al.* (32) have first reported that disruption of *Cyp1b1* gene in mice causes suppression of formation of malignant lymphomas and other tumors induced by 7,12-DMBA as well as decreases in metabolizing 7,12-DMBA to a proximate carcinogenic 3,4-diol metabolite in primary embryonic stem cells (isolated from *Cyp1b1* null mice) that had been treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Table 2). These cell lines express Cyp1a1 protein at significant levels, but not Cyp1b1 protein, indicating that Cyp1b1 has a major role in activating 7,12-DMBA *in vivo* (32). The *Cyp1b1*-null mice have also been reported to be reduced in

**Table 1.** Major human P450 enzymes involved in the bioactivation of chemical carcinogens

P450	Group of carcinogen	Carcinogens activated by P450s
CYP1A1 CYP1A2 CYP1B1	PAH	Benzo[ <i>a</i> ]pyrene (B[ <i>a</i> ]P), 7,12-dimethylbenz[ <i>a</i> ]anthracene (7,12-DMBA), benz[ <i>a</i> ]anthracene (B[ <i>a</i> ]A), benzo[ <i>c</i> ]phenanthrene, 5-methylchrysene, dibenzo[ <i>a,l</i> ]pyrene (DB[ <i>a,l</i> ]P), 3-methylcholanthrene (3-MC), fluoranthene, and other PAHs, and their dihydrodiol derivatives
	Arylamine	2-Acetylaminofluorene, 2-aminofluorene, 2-aminoanthracene, 6-aminochrysene
	Heterocyclic amine	2-Amino-3-methylimidazo[4,5- <i>f</i> ]quinoline (IQ), 2-amino-3,5-dimethylimidazo[4,5- <i>f</i> ]quinoline (MeIQ), 2-amino-6-methyldipyrido[1,2- <i>α</i> :3',2'- <i>d</i> ]imidazole (Glu-P-1), 3-amino-1,4-dimethyl-5 <i>H</i> -pyrido[4,3- <i>b</i> ]indole (Trp-P-1), 2-amino-1-methyl-6-phenylimidazo[4,5- <i>b</i> ]pyridine (PhIP), and others
	Nitroarene	1-Nitropyrene, 2-nitropyrene, 6-nitrochrysene
	Estrogen	17β-estradiol, estrone
CYP2A6 CYP2A13	Nitrosamine	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), <i>N</i> -nitrosomnicotine (NNN)
	Mycotoxin	Aflatoxin B <sub>1</sub> (AFB <sub>1</sub> )
	Arylamine	2-Aminofluorene, 2-aminoanthracene
CYP2E1	Nitrosamine	Dimethylnitrosamine, diethylnitrosamine, NNK, NNN
	Arylhydrocarbon	Styrene
CYP3A4	Mycotoxin	Aflatoxin B <sub>1</sub> , aflatoxinG <sub>1</sub> , sterigmatocystin, dihydrodiol derivatives of PAHs

**Table 2.** Suppression of tumor formation caused by chemical carcinogens in gene knockout mice *in vivo*

Disruption of gene	Carcinogen administered	Suppression of tumor formation in organs	Reference
<i>Cyp1b1</i>	7,12-DMBA	Lymphoid tissue	Buters <i>et al.</i> (32)
	DB[ <i>a,l</i> ]P	Ovary, skin, lymphoid tissue	Buters <i>et al.</i> (34)
	7,12-DMBA	Ovary	Buters <i>et al.</i> (33)
	7,12-DMBA	Spleen (immunotoxicity)	Gao <i>et al.</i> (39)
	dibenzo[ <i>def,p</i> ]chrysene	Skin	Siddens <i>et al.</i> (36)
<i>Cyp2a5</i>	NNK	Lung	Megaraj <i>et al.</i> (47)
<i>Cyp2abfgs</i>	NNK	Lung	Li <i>et al.</i> (50)
<i>Cyp2e1</i>	Dimethylnitrosamine	Liver	Kang <i>et al.</i> (52)
<i>Epoxide hydrolase</i>	7,12-DMBA	Skin	Miyata <i>et al.</i> (40)
<i>Arylhydrocarbon receptor</i>	B[ <i>a</i> ]P	Skin	Shimizu <i>et al.</i> (41)

formation of ovarian cancers at a low dose of 7,12-DMBA (33), ovarian and skin tumors caused by DB[a,l]P (34,35), and skin tumors by dibenzo[def,p]chrysene (DB[a,l]P) (36). Disruption of *Cyp1b1*-null mice has been shown to be reduced in pre-B cell apoptosis (37), bone marrow cytotoxicity (38), and spleen cell immunotoxicity (39) by treatment with 7,12-DMBA. Epoxide hydrolase and arylhydrocarbon hydroxylase have also been shown to play important roles in the formation of skin tumor caused by 7,12-DMBA and B[a]P, respectively, in gene knockout mice (40,41).

Thus, roles of CYP1B1 protein in the activation of various carcinogenic PAHs have been suggested in gene knockout mice *in vivo*. Of note, Luch *et al.* (42) have found that CYP1B1 plays a more important role than CYP1A1 in activating DB[a,l]P to highly reactive DB[a,l]P-11,12-diol-13,14-epoxides and our previous *in vitro* studies have shown that human CYP1B1 is more active in forming B[a]P-7,8-diol from B[a]P than CYP1A1 and 1A2 (43). Uno *et al.* have reported that CYP1A1 may be involved in detoxification and protection against oral B[a]P in mice, since CYP1A1 null mice died within 30 days after oral B[a]P (125 mg/kg), while wild-type mice did not show any signs of toxicity during the course of experiments (44). They also studied effects of oral B[a]P in *Cyp1a1*-, *1a2*-, and *1b1*-null mice and their double knockout mice and found that a balance of expression of *Cyp1a1* and *1b1* proteins in several organs is important to understand the basis of toxicity and carcinogenicity caused by oral administration of B[a]P (45,46).

Megaraj *et al.* have shown that *Cyp2A5*-null mice are reduced in the formation of lung tumor caused by NNK and that CYP2A13 is suggested to play roles in bioactivating NNK to initiate lung tumor in a humanized mouse model (47). CYP2A13 genetic polymorphisms may cause individual differences in susceptibilities towards tobacco-related cancers in humans (47-49). In mice, other *Cyp2*-family enzymes as well as *Cyp2a4* and *2a5* may be involved in NNK-induced tumor on analysis using *Cyp2abfgs*-null mice (49-51). *Cyp2e1* has been reported to play key roles in the formation of liver tumors by dimethylnitrosamine in the studies of gene knockout mice (52).

#### ***In vivo effects of P450 inhibitors on suppression of tumor formation caused by carcinogens in experimental animals.***

It has been reported that several PAH compounds suppress, prolong, or delay tumor formation caused by potent carcinogens such as 7,12-DMBA, B[a]P, dibenz[a,h]anthracene, and 3-MC in laboratory animals (Table 3) (53-58). Weak or non-carcinogen PAHs, such as B[e]P, have also been reported to reduce tumor formation caused by environmental carcinogens (59-64), and as described below, B[e]P has been determined to be a potent inhibitor for CYP1 family enzymes (20). CYP1 inhibitors such as ANF, 9-hydroxyellipticine, and 1-ethynylpyrene have also been reported to have anticarcinogenic activities

in mice treated with 7,12-DMBA and B[a]P (56,65,66). Furanocoumarin derivatives (such as imperatorin and bergamottin) and flavonoids (such as naringenin, apigenin, quercetin, and hesperidin), which have been reported to inhibit human CYP1, 2A, and/or 3A enzymes *in vitro* (23,24), have chemopreventive activities in experimental animals (67-73). 8-Methoxypsoralen and isothiocyanate derivatives, such as benzyl- and phenethyl isothiocyanates, which are the potent inhibitors of CYP2A6 and 2A13 (74,75), have chemopreventive activities in mice when these chemicals are administered before or just after the administration of NNK and azoxymethane (Table 3) (74-80).

Synthetic organoselenium compounds such as BSC, and *o*-, *m*- and *p*-XSC, which are recently reported by us to inhibit human CYP1 and 2A enzymes (25,81), have chemopreventive activities in mice administered 7,12-DMBA, B[a]P, and NNK (Table 3) (82-89). El-Bayoumy *et al.* (87) reported that *p*-XSC is active in preventing tumor formation caused by NNK when it is injected before the administration of NNK, indicating that the mechanism of action of *p*-XSC is due to inhibition of P450s that activate NNK to active metabolites (47-51).

#### ***In vitro inhibition of carcinogen-activating P450 enzymes.***

Extensive studies have shown that there is a variety of xenobiotic and endogenous chemicals that inhibit individual forms of human P450s (6,20-26,90-92). Historically, many researchers have studied and searched specific xenobiotic and endogenous inhibitors for P450 enzymes in order to examine roles of P450s in substrate oxidation reactions, to evaluate new drug development and drug-drug interaction in clinical trials, and to understand the basis of chemical toxicity and carcinogenesis (93-97). Following xenobiotic chemicals have been reported to be relatively specific inhibitors for individual human P450 enzymes; furafyllin, fluvoxamine, and a-naphthoflavone for CYP1 enzymes, methoxsalen, tranlycypromine, and tryptamine for CYP2A enzymes, ticlopine and thiotepa (triethylenethiophosphoramidate) for CYP2B6, sulphaphenazole, fluconazole, and omeprazole for CYP2C enzymes, quinidine, terbinafine, and fluoxetine for CYP2D6, disulfiram, pyridine, and diethyldithiocarbamate for CYP2E1, and ketoconazole, itraconazole, and retinavir for CYP3A enzymes (6-8,20, 91,92,94).

Since CYP1A1, 1A2, 1B1, 2A6, and 2A13 have been recognized to be key enzymes in understanding the basis of chemical carcinogenesis caused by a variety of carcinogenic PAHs and tobacco-related nitrosamines, we summarize, mainly our recent studies during the past decade, on the nature of numerous xenobiotic chemicals that inhibit these human P450 enzymes (20-31). Followings are described here that a) inhibition of CYP1 enzymes by a variety of PAHs and acetylenic PAH inhibitors, b) different mechanisms of inhibition of CYP1 enzymes by PAHs and acetylenic PAH

**Table 3.** Suppression by P450 inhibitors of tumor formation caused by chemical carcinogens *in vivo* by in laboratory animals (1)

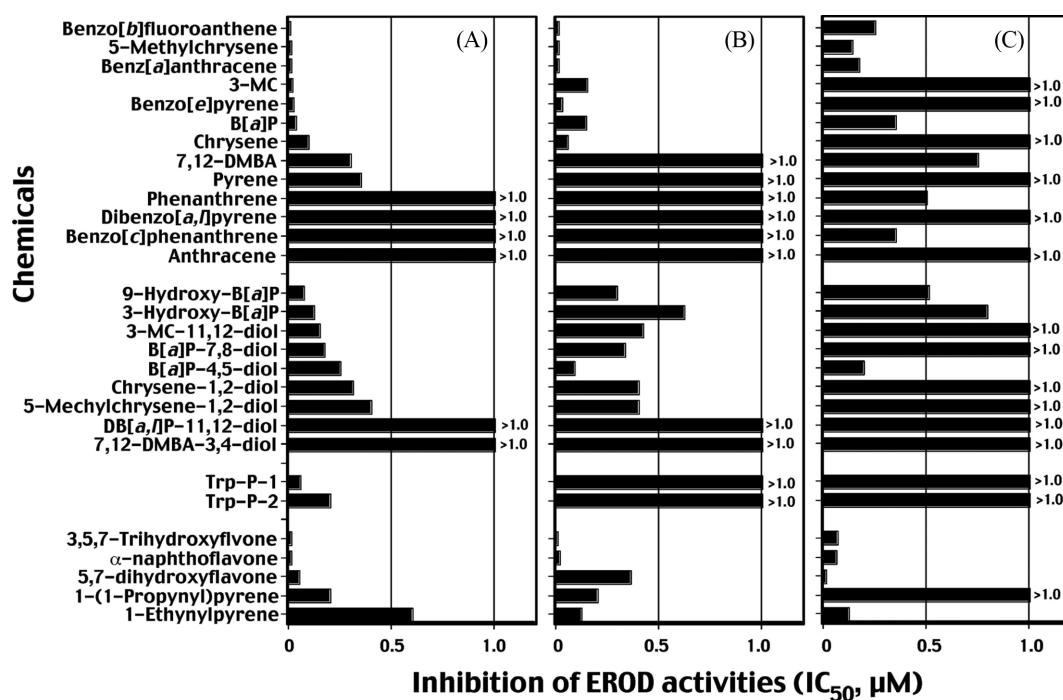
Inhibitor	Suggested P450 inhibition	Carcinogen administered	Suppression of tumor formation	Reference
<i>α</i> -Naphthoflavone	CYP1	7,12-DMBA, B[a]P	Skin	Gelboin and Kinoshita (53)
	CYP1	7,12-DMBA, B[a]P	Skin	Kinoshita and Gelboin (54)
	CYP1	7,12-DMBA	Skin	Slaga <i>et al.</i> (56)
9-Hydroxyellipticine	CYP1	7,12-DMBA	Skin	Lesca and Mansuy (65)
Benzo[ <i>e</i> ]pyrene	CYP1	7,12-DMBA	Skin	DiGiovanni <i>et al.</i> (60)
	CYP1	Dibenz[ <i>a,h</i> ]anthracene	Skin	DiGiovanni <i>et al.</i> (60)
1,2,5,6-Dibenzofluorene		3-MC	Skin	Riegel <i>et al.</i> (57)
		7,12-DMBA	Skin	Hill <i>et al.</i> (58)
1-Ethynylpyrene	CYP1	7,12-DMBA, B[a]P	Skin	Alworth <i>et al.</i> (66)
Imperatorin	CYP1, 2A, 3A	7,12-DMBA, B[a]P	Skin	Cai <i>et al.</i> (67)
Bergamottin	CYP1, 2A 3A	7,12-DMBA	Skin	Kleiner <i>et al.</i> (68)
Isopimpinellin	CYP1, 2A 3A	7,12-DMBA	Skin	Kleiner <i>et al.</i> (68)
Naringenin	CYP1, 2A	7,12-DMBA	Oral	Sulfikkarali <i>et al.</i> (70)
Apigenin	CYP1, 2A	7,12-DMBA	Oral	Silvan <i>et al.</i> (71)
Quercetin	CYP1, 2A	NNK, B[a]P	Lung	Kassie <i>et al.</i> (72)
Hesperidin	CYP2C, 3A	Azoxymethane	Colon	Tanaka <i>et al.</i> (73)
8-Methoxypsoralen	CYP2A	NNK	Lung	Takeuchi <i>et al.</i> (75)
	CYP2A	NNK	Lung	Miyazaki <i>et al.</i> (76)
	CYP2A	NNK	Lung	Takeuchi <i>et al.</i> (77)
	CYP2A	NNK	Lung	Takeuchi <i>et al.</i> (74)
Benzyl isothiocyanate Phenyl isothiocyanate	CYP1, 2A	B[a]P	Lung, stomach	Wattenberg <i>et al.</i> (78)
	CYP2A	NNK	Lung	Morse <i>et al.</i> (79)
	CYP2A	NNK	Lung	Morse <i>et al.</i> (80)
BSC	CYP1, 2A 3A	Azoxymethane	Colon	Fiala <i>et al.</i> (82)
	CYP1, 2A 3A	B[a]P	Stomach	El-Bayoumy (89)
<i>p</i> -XSC, BSC	CYP1, 2A 3A	7,12-DMBA	Mammary	El-Bayaoumy <i>et al.</i> (84)
<i>p</i> -XSC	CYP1, 2A 3A	7,12-DMBA	Lung	Prokopczyk <i>et al.</i> (85)
	CYP1, 2A 3A	B[a]P, NNK	Lung	Prokopczyk <i>et al.</i> (86)

inhibitors, c) inhibition of P450 enzymes by flavonoid derivatives, d) interaction of xenobiotic chemicals with CYP2A13 and 2A6, and e) inhibition of CYP1 and 2A enzymes by chemopreventive organoselenium compounds.

***In vitro* inhibition of CYP1A1, 1A2, and 1B1 by xenobiotic chemicals.** In humans, CYP1A1 and 1A2 share 80% amino acid sequence identity and are ~40% identical with CYP1B1 (98-101). cDNA clones and amino acid sequences of former two enzymes have been characterized in 1985-1986 (98-100), while a human CYP1B1 cDNA clone and amino acid sequence were reported in 1994 (101). The crystal structures of CYP1A2 (102), CYP1B1 (103), and CYP1A1 (104) all bound to ANF in the active site cavity of the enzymes have been reported and characterized.

A variety of chemical inhibitors for human CYP1A1 and 1A2 enzymes had been reported by many investigators (90,91,105-109). Since human CYP1B1 protein was not

expressed in yeast and *Escherichia coli* and characterized until 1994-1997 (16,104,110,111), studies on the comparison of selectivities of xenobiotic inhibitors for CYP1A1, 1A2, and 1B1 were examined in 1998 by us (112) and by other investigators (113-117). We first examined total of 24 polycyclic hydrocarbons, many containing acetylenic side chains for their abilities to inhibit 7-ethoxyresorufin *O*-deethylation activities catalyzed by human CYP1A1, 1A2, and 1B1 (112). We found that 1-(1-propynyl)pyrene and 2-(1-propynyl)phenanthrene nearly completely inhibited CYP1A1 at concentrations where no CYP1B1 inhibition was observed and that 2-ethynylpyrene and ANF nearly completely inhibited CYP1B1 at concentrations where no CYP1A1 inhibition was noted. All four of the above compounds also inhibited CYP1A2. We conclude that (i) several polycyclic hydrocarbons and their oxidation products are inhibitors of human CYP1A1, 1A2, and 1B1; (ii) of these inhibitors only some are mechanism-based inactiva-



**Fig. 1.** Inhibition ( $IC_{50}$  values) of 7-ethoxyresorufin *O*-deethylation (EROD) activities of CYP1B1 (A), 1A2, (B), and 1A1 (C) by PAHs, PAH metabolites, Trp-P-1 and Trp-P-2, and flavonoids and acetylenic PAHs.  $IC_{50}$  values exceeded over  $1.0 \mu\text{M}$  are indicated in the figure. Data are taken from Shimada and Guengerich (20) with modification.

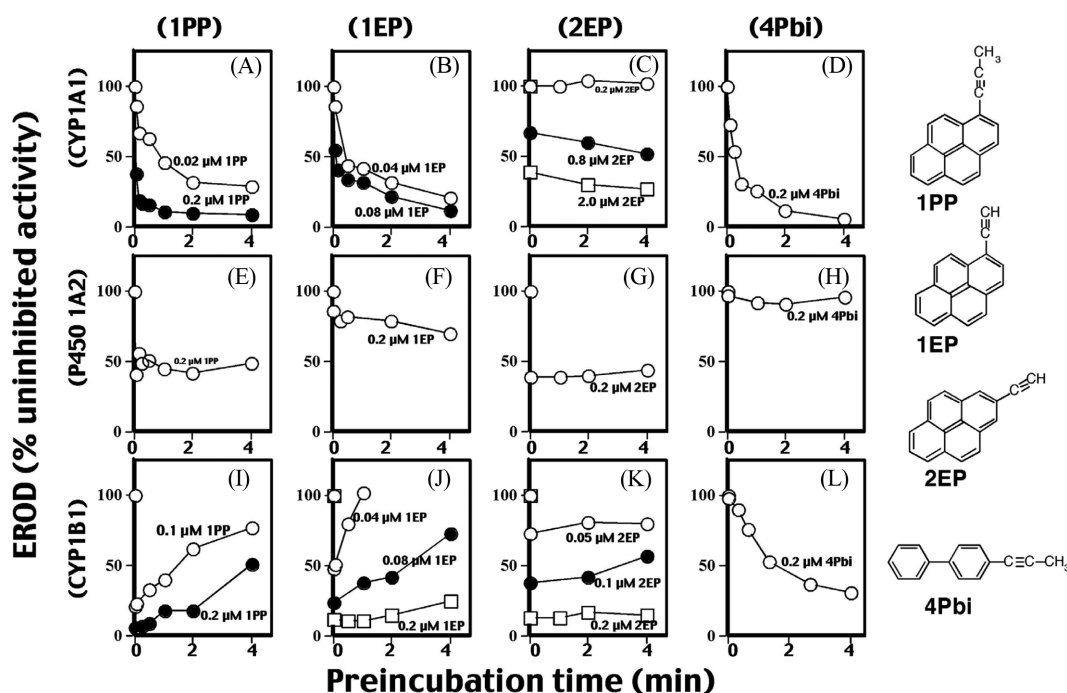
tors; and (iii) some of the inhibitors are potentially useful for distinguishing between human CYP1A1 and 1B1 (112).

In 2006, we studied if carcinogenic or non- or weak carcinogenic PAHs as well as acetylenic PAHs, inhibit CYP1-catalytic activities (20), because some of these PAH compounds such as B[e]P and 1,2,5,6-dibenzofluorene prevented tumor formation caused by carcinogenic PAH compounds as described above (57-64). We examined following chemicals as benzo[b]fluoranthene, 5-methylchrysene, B[a]A, 3-MC, B[a]P, B[e]P, chrysene, 7,12-DMBA, pyrene, phenanthrene, DB[a,h]P, benzo[c]phenanthrene, anthracene, pyrene, and phenanthrene and several PAH metabolites, Trp-P-1, Trp-P-2, and flavonoids (Fig. 1). In the figure, inhibition of EROD activities are shown as  $IC_{50}$  values within  $1.0 \mu\text{M}$  chemical concentration. Interestingly, B[a]A, benzo[b]fluoranthene, and 5-methylchrysene inhibited CYP1B1- and 1A2-dependent EROD activities with  $IC_{50}$  values of below  $0.01 \mu\text{M}$ . The  $IC_{50}$  values obtained with CYP1A1-dependent EROD activities were always higher than those with CYP1A2 and 1B1. Our results also showed that B[e]P which have been reported to be weak or non-carcinogens (59-64), very strongly inhibited CYP1B1 and 1A2 but not CYP1A1 at  $1 \mu\text{M}$  concentration. Conversely, potent carcinogens such as benzo[c]phenanthrene and DB[a,h]P did not show significant inhibition of EROD activities by P450s, except that the former PAH inhibited CYP1A1-dependent EROD activity with an  $IC_{50}$  of  $0.33$

$\mu\text{M}$ . Metabolites of PAHs (e.g., 3-OH and 9-OH B[a]P and dihydrodiol derivatives of PAHs) were rather weak inhibitors of P450-dependent EROD activities as compared with the parent PAHs. As suggested by us and other investigators, 3,5,7-trihydroxyflavone (galangin), 5,7-dihydroxyflavone (chrysin), and ANF were potent inhibitors for three CYP1 enzymes (20,105-108). Trp-P-1 and Trp-P-2 inhibited more strongly CYP1B1 than CYP1A1 and 1A2 (Fig. 1).

We also found that 5-methylchrysene, B[a]P, B[a]A, and DB[a,h]P inhibited metabolic activation of 5-methylchrysene-1,2-diol, ( $\pm$ )B[a]P-7,8-diol, and DB[a,h]P-11,12-diol to genotoxic metabolites catalyzed by CYP1B1 and 1A1 by measuring induction of *umu* gene expression in *S. typhimurium* NM2009 (20). The results suggest that these PAHs inhibit second step of metabolic activation of these dihydrodiols to DNA-damaging products as well as first step of metabolism (by measuring inhibition of EROD activity) (20). Thus, individual PAHs may affect their own and metabolism of other carcinogens catalyzed by CYP1A1, 1A2, and 1B1, and these phenomena may cause alteration in their ability to transform cells when single or complex PAH mixtures are ingested by mammals, influencing risk assessment (113-117).

**Different mechanisms of inhibition of P450 1A1-, 1A2-, and 1B1 by PAHs and acetylenic PAH inhibitors.** Since reports have shown that many acetylenic PAH inhibitors



**Fig. 2.** Effects of preincubation time on inhibition of CYP1A1 (A-D), CYP1A2 (E-H), and CYP1B1 (I-L) dependent EROD activities by 1PP (A, E, and I), 1EP (B, F, and J), 2-EP (C, G, and K), and 4Pbi (D, H, and L). P450 (50 pmol) was pre-incubated with different concentrations of 1PP, 1EP, 2EP, and 4Pbi in the presence of 1 mM NADPH during indicated periods of time, and then the reactions were started by the addition of 5  $\mu$ M 7-ethoxyresorufin to determine EROD activities. The reactions were monitored at 25°C. Data are taken from Shimada *et al.* (21) with modification.

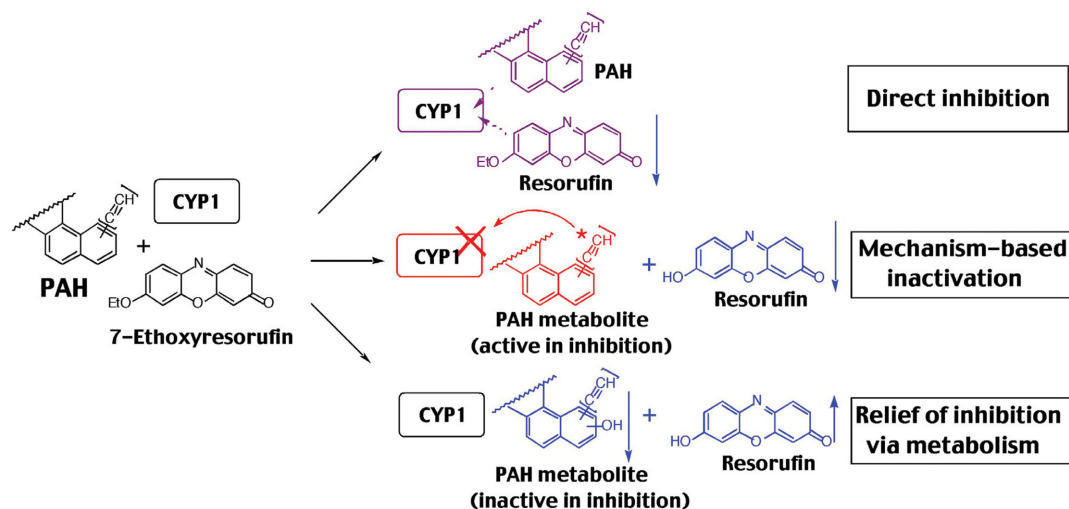
inhibit P450-catalytic activities by mechanism-based manner (20,66,97,118-120), we have studied mechanisms of inhibition of CYP1-dependent EROD activities by PAHs used in this study (Fig. 2) (20-23). Our initial experiments show that preincubation of 1PP, 1EP, and 4Pbi with CYP1A1 for 0-4 min in the presence of NADPH caused inhibition of EROD activities in a time-dependent manner, indicating inhibition by a mechanism-based manner (Fig. 2A, 2B, 2D). However, 2EP inhibits P450 1A1 directly (preincubation does not affect the activities) (Fig. 2C) (21). CYP1B1-dependent EROD activity was inhibited by 1PP and 1EP without metabolism, and such decreases in activities were reversed with increasing pre-incubation time, indicating that CYP1B1 is able to metabolize 1PP and 1EP to products that loose inhibitory activity (relief of inhibition via metabolism) (Fig. 2I, 2J, 3). 4Pbi inhibited CYP1B1 in a mechanism-based manner similar to CYP1A1, although such inactivation in CYP1B1 ( $t_{1/2} = 3.4$  min) was slower than that of the CYP1A1 ( $t_{1/2} = 15$  s) (Fig. 2L, 2D). 2EP inhibited CYP1B1 directly. Four chemicals inhibited CYP1A2 directly (Fig. 2E-2H). These results indicated that there are three different mechanisms of inhibition of CYP1-enzymatic activities; a) direct inhibition, b) mechanism-based inhibition (competitive inhibition), and c) relief of inhibition via metabolism as seen in 1PP and 1EP with CYP1B1 (Fig. 3). The mechanism namely, relief of inhibition via

metabolism, was also observed in B[a]A, B[a]P, B[e]P, 5-methylchrysene, and 7,12-DMBA with CYP1B1, although chrysene and 3-MC inhibited CYP1B1 by competitive manner (21). Interestingly, these PAHs as B[a]A, benzo[b]fluoranthene, benzo[j]fluoranthene, B[a]P, chrysene, 5-methylchrysene, B[e]P, dibenz[a,j]acridine, and 7,12-DMBA inhibited CYP1A2 by mechanism-based manner and inhibited CYP1A1 by a competitive manner (20). Thus, these PAHs may modify the biological activities of their own and other PAH compounds through inhibition of CYP1-catalytic activities by different mechanisms (20,21).

#### **Inhibition of P450 enzymes by flavonoid derivatives.**

A variety of plant flavonoids are found in the environment and these natural products are shown to have various biological properties, e.g. anti-oxidative and anti-mutagenic activities, thus preventing cancer, heart disease, bone loss, and a number of diseases (121-123). These biological activities are reported to vary with the number and substitution positions of hydroxyl and/or methoxy groups in the flavonoid molecules (124-126). Inhibition of P450 enzymes by diverse flavonoid derivatives has been extensively studied in several laboratories (127-137).

In 2009, we have reported that various chemicals including flavonoid, stilbene, pyrene, naphthalene, and biphenyl and their derivatives interact with CYP1B1 inducing reverse



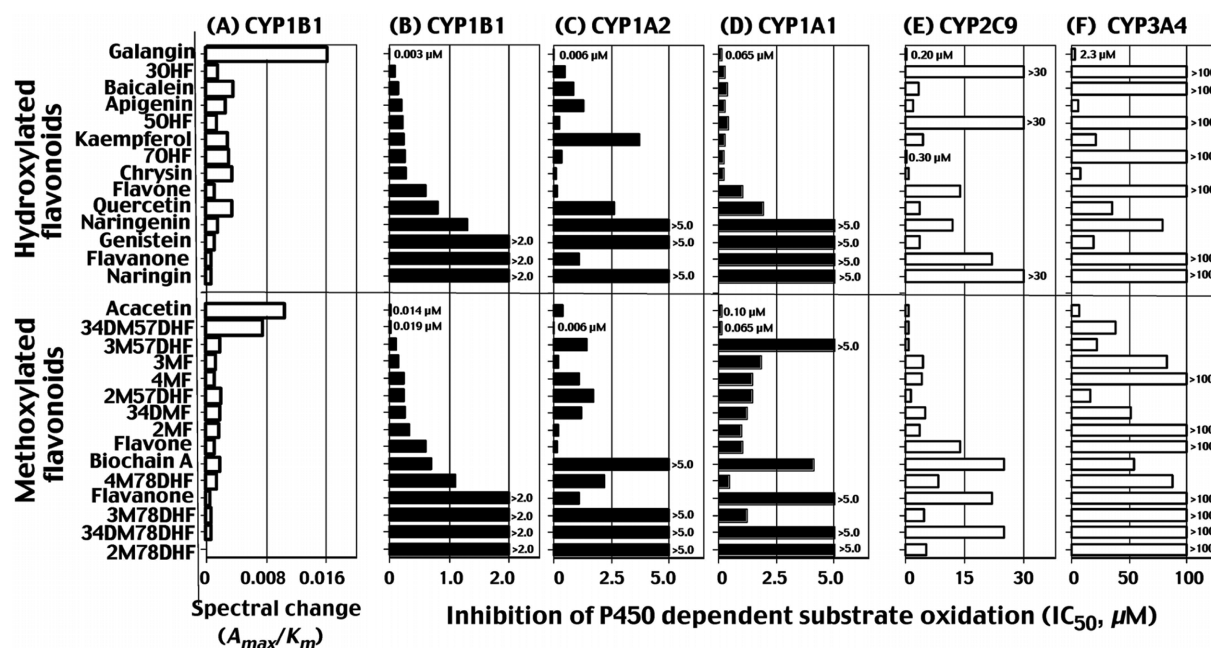
**Fig. 3.** Three different mechanisms of inhibition of CYP1 enzymes by PAHs and acetylenic PAHs. Data are from Shimada *et al.* (21).

type I binding spectra and that these spectral changes are correlated with abilities to inhibit CYP1B1-dependent EROD activities (23). We further examined the relationship between spectral interaction of CYP1B1, 1A1, 1A2, 2C9, and 3A4 with total of 33 flavonoid derivatives and their potencies ( $IC_{50}$  values) to inhibit P450 catalytic activities by measuring EROD activities for CYP1B1, 1A1, and 1A2, flurbinoprofen 4'-hydroxylation activities for CYP2C9, and midazolam 4-hydroxylation activities for CYP3A4 (Fig. 4) (24). In the figure, results with selected 27 flavonoid derivatives are shown and the scale of  $IC_{50}$  values vary with 1~2.0  $\mu$ M for CYP1B1, 0~5.0  $\mu$ M for CYP1A2 and 1A1, 0~30  $\mu$ M for CYP2C9, and 0~100  $\mu$ M for CYP3A4 (Fig. 4). The potencies of spectral binding of CYP1B1 were found to correlate with the abilities to inhibit 7-ethoxyresorufin *O*-deethylation activity catalyzed by CYP1B1 ( $r = 0.92$ ). The presence of a hydroxyl group in flavone, e.g. 3-, 5-, and 7-monohydroxy- and 5,7-dihydroxyflavone (chrysin), decreased the 50% inhibition concentration ( $IC_{50}$ ) of CYP1B1 from 0.6  $\mu$ M (with flavone) to 0.09, 0.21, 0.25, and 0.27  $\mu$ M, respectively, and 3,5,7-trihydroxyflavone (galangin) was the most potent, with an  $IC_{50}$  of 0.003  $\mu$ M. The introduction of a 4'-methoxy- or 3',4'-dimethoxy group into 5,7-dihydroxyflavone yielded other active inhibitors of CYP1B1 with  $IC_{50}$  values of 0.014 and 0.019  $\mu$ M, respectively. The above hydroxyl- and/or methoxy- groups in flavone molecules also increased the inhibition activity with CYP1A1 but not always towards CYP1A2, where 3-, 5-, or 7-hydroxyflavone, and 4'-methoxy-5,7-dihydroxyflavone were less inhibitory than flavone itself, although CYP1A1 and 1A2 did not show spectral changes with these compounds. CYP2C9, which was also negative in inducing spectral changes with flavonoids, was more inhibited by 7-hydroxy-, 5,7-dihydroxy-, and 3,5,7-trihydroxyflavones than by flavone but was weakly inhibited by 3- and 5-hydroxyfla-

none. Flavone and several other flavonoids produced type I binding spectra with CYP3A4, but such binding was not always related to the inhibitory activities towards CYP3A4 (24). The  $IC_{50}$  values with flavonoids to inhibit CYP2C9 and 3A4 were higher than those to inhibit CYP1B1, 1A2, and 1A1 (Fig. 4). These results indicate that there are different mechanisms of inhibition for CYP1A1, 1A2, 1B1, 2C9, and 3A4 by various flavonoid derivatives and that the number and position of hydroxyl and/or methoxy groups highly influence the inhibitory actions of flavonoids towards these enzymes.

Our molecular docking analysis supported that there are different orientations of interaction of various flavonoids with active sites of P450 enzymes examined, thus causing differences in inhibition potencies observed in these P450s (24).

**Interaction of xenobiotic chemicals with human CYP2A13 and 2A6.** CYP2A6 and 2A13 are expressed mainly in the liver and respiratory tract, respectively, in humans (4,138,139). CYP2A6 is active in catalyzing metabolism of several drugs, e.g. coumarin and phenacetin, and also metabolic activation of tobacco-related nitrosamines (including NNK and NNN) to carcinogenic metabolites (140,141). However, CYP2A13 is shown to be more active than CYP2A6 in activating NNK and NNN (140,141) and these findings are of interest because the latter enzyme is mainly expressed in respiratory organs, the sites of exposure to numerous environmental chemicals including NNK, NNN, and PAHs (4,138,139). As described above, several chemicals that inhibit CYP2A13 and 2A6 enzymes suppress tumor formation caused by NNK, 7,12-DMBA, B[a]P, and azoxymethane (Table 3) (47-51), it is interesting to examine whether various xenobiotic chemicals interact with and inhibit CYP2A13 and 2A6-dependent catalytic activi-



**Fig. 4.** Intensities of reverse type I binding spectra of CYP1B1 with 27 flavonoids (A) and inhibition by these flavonoids of EROD activities catalyzed by CYP1B1 (B), 1A1 (C), and 1A2 (D), flurbiprofen 4'-hydroxylation activities catalyzed by CYP2C9 (E), midazolam 4-hydroxylation activities catalyzed by CYP3A4 (F). The spectral changes are shown as spectral binding efficiency ( $\Delta A_{max}/K_m$  values).  $IC_{50}$  values are shown to be 0~1.0  $\mu M$  for CYP1B1, 1A2, and 1A1, and 0~30  $\mu M$  for CYP2C9 and 3A4. Abbreviations used; 3HF, 3-hydroxyflavone; 5HF, 5-hydroxyflavone; 7HF, 7-hydroxyflavone; 57DHF, 5,7-dihydroxyflavone; 357THF, 3,5,7-trihydroxyflavone; 4'57THF, 4',5,7-trihydroxyflavone; 4'57THIF, 4',5,7-trihydroxyisoflavone; 4'57THFva, 4',5,7-trihydroxyflavanone; 4'57THFvaG, 4',5,7-trihydroxyflavanone glycoside; 567THF, 5,6,7-trihydroxyflavone; 34'57TetraHF, 3,4',5,7-tetrahydroxyflavone; 33'4'5'7PHF, 3,3',4',5',7-pentahydroxyflavone; 4M57DHF, 4'-methoxy-5,7-dihydroxyflavone; 4'M57DHisoF, 4'-methoxy-5,7-dihydroxyisoflavone; 2MF, 2'-methoxyflavone; 3MF, 3'-methoxyflavone; 4MF, 4'-methoxyflavone; 3'4'DMF, 3',4'-dimethoxyflavone; 2'M57DHF, 2'-methoxy-5,7-dihydroxyflavone; 3'M57DHF, 3'-methoxy-5,7-dihydroxyflavone; 3'4'M57DHF, 3',4'-dimethoxy-5,7-dihydroxyflavone; 2'M78DHF, 2'-methoxy-7,8-dihydroxyflavone; 3'M78DHF, 3'-methoxy-7,8-dihydroxyflavone; 4'M78DHF, 4'-methoxy-7,8-dihydroxyflavone; and 3'4'M78DHF, 3',4'-dimethoxy-7,8-dihydroxyflavone. Data are taken from Shimada *et al.* (30) with modification.

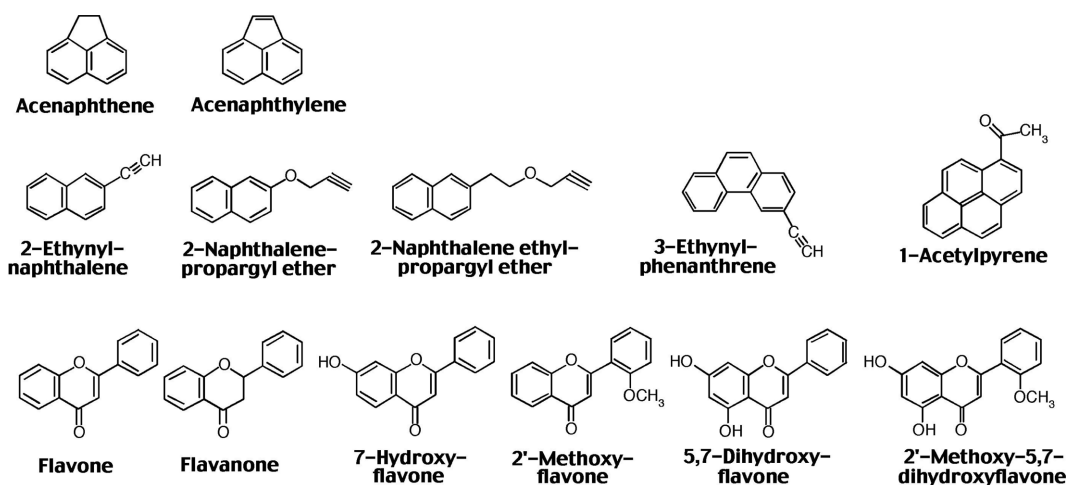
ties and are metabolized by these P450 enzymes (26,27).

A total of 68 chemicals including acenaphthene, acenaphthylenes, derivatives of naphthalene, phenanthrene, fluoranthene, pyrene, biphenyl, and flavone have been examined for their abilities to interact with human CYP2A13 and 2A6 (Fig. 5) (26). Fifty-one of these 68 chemicals induced stronger type I binding spectra (iron low- to high-spin state shift) with CYP2A13 than those seen with CYP2A6, i.e. the spectral binding intensities ( $\Delta A_{max}/K_s$  ratio) determined with these chemicals were always higher for CYP2A13. In addition, benzo[*c*]phenanthrene, fluoranthene, 2,3-dihydroxy-2,3-dihydrofluoranthene, pyrene, 1-hydroxypyrene, 1-nitropyrene, 1-acetylpyrene, 2-acetylpyrene, 2,5,2',5'-tetrachlorobiphenyl, 7-hydroxyflavone, 5,7-dihydroxyflavone (chrysin), and 3,5,7,-dihydroxyflavone (galangin) were found to induce a type I spectral change only with CYP2A13. Coumarin 7-hydroxylation, catalyzed by CYP2A13, was strongly inhibited by acenaphthene, acenaphthylene, 2-ethynylnaphthalene, 2-naphthalene propargyl ether, 2-naphthalene ethyl propargyl ether, 3-ethynylphenanthrene, 1-acetylpyrene, flavone, flavanone, 7-hydroxyflavone, 2'-methoxyflavone, 5,7-

dihydroxyflavone, and 2'-methoxy-5,7-dihydroxyflavone; these chemicals induced type I spectral changes with low  $K_s$  values (Fig. 5). Among various chemicals tested, benzo[*c*]phenanthrene, fluoranthene, pyrene, 1-hydroxypyrene, 1-nitropyrene, 1- and 2-acetylpyrene, 2,5,2',5'-tetrachlorobiphenyl, 7-hydroxyflavone, 5,7-dihydroxyflavone (chrysin), 3,5,7-trihydroxyflavone (galangin), and ANF did not induce spectral changes with CYP2A6 (26). These chemicals were also found to be non-inhibitory or weak inhibitors of CYP2A6-dependent coumarin 7-hydroxylation activity. Thus, different selectivities of several chemicals in inducing spectral changes with these CYP2A enzymes were found, although it should be noted that 2-ethynylnaphthalene, naphthalene, 1-(1-propynyl)pyrene, 1-ethynylpyrene, 2-ethynylnaphthalene, phenanthrene, acenaphthene, acenaphthylene, biphenyl, and resveratrol had relatively similar tendencies to induce spectra with CYP2A13 and 2A6 (26).

Twenty four chemicals including naphthalene, phenanthrene, biphenyl and their derivatives have been determined and compared to induce type I spectral changes

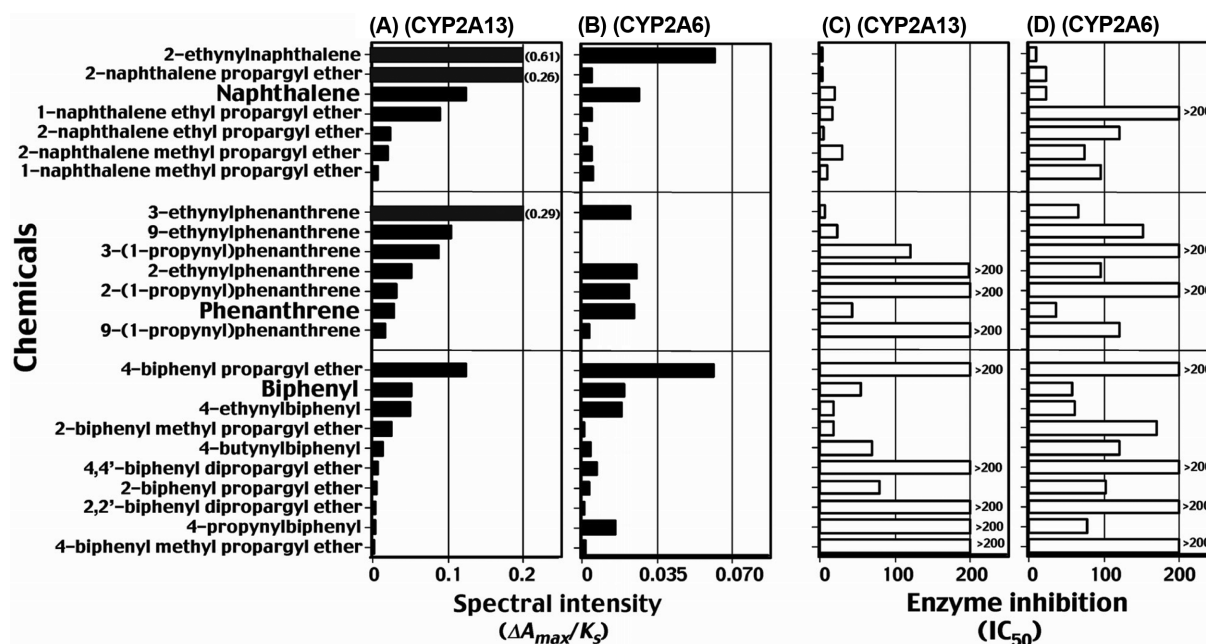




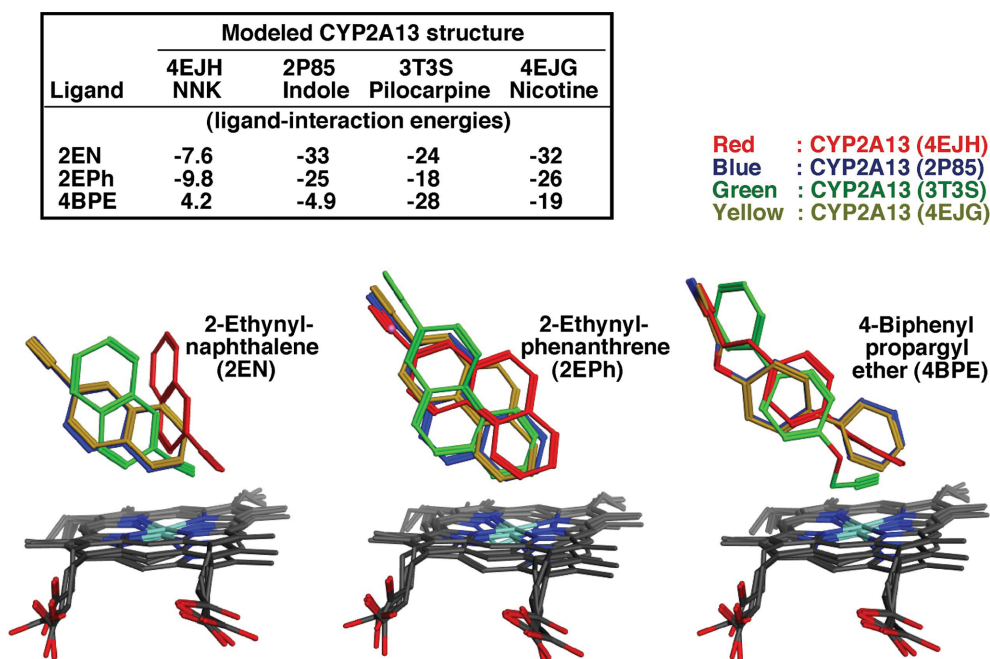
**Fig. 5.** Compounds that show strong inhibition of CYP2A13-dependent coumarin 7-hydroxylation activities. Data are taken from Shimada *et al.* (26) with modification.

(intensities,  $A_{max}/K_s$  ratio) with CYP2A13 (Fig. 6A) and 2A6 (Fig. 6B) and to inhibit coumarin 7-hydroxylation catalyzed by CYP2A13 (Fig. 6C) and 2A6 (Fig. 6D) (26,28-30). All of these chemicals induce type I binding spectra with CYP2A13 having high affinities with 2-ethynyl-naphthalene, 2-naphthalene propargyl ether, naphthalene, 1-naphthalene ethylpropargyl ether, 2-naphthalene ethylpropargyl ether, 2-naphthalene methyl propargyl ether, 1-naphthalene methyl propargyl ether, 3-ethynylphenanthrene, 9-ethynylphenanthrene, 3-(1-propynyl)phenanthrene, 2-ethynylphenanthrene, 2-(1-propynyl)phenanthrene, phenanthrene, 9-(1-propynyl)phenanthrene, 4-biphenyl propargyl ether, biphenyl, 4-ethynylbiphenyl, 2-biphenyl methyl propargyl ether, 4-butynylbiphenyl, 4,4'-biphenyl dipropargyl ether, 2-biphenyl propargyl ether, 2,2'-biphenyl dipropargyl ether, 4-propynylbiphenyl, 4-biphenyl methyl propargyl ether

ether, biphenyl, and 4-ethynylbiphenyl (Fig. 6A). These spectral intensities in CYP2A13 tended to relate to the potencies of these chemicals to inhibit coumarin 7-hydroxylation activities catalyzed by this enzyme (Fig. 6C). All of these 24 chemicals also interacted with CYP2A6, however, spectral intensities and inhibition of coumarin 7-hydroxylation activities found in CYP2A6 were lesser than those in CYP2A13, except that 4-propynylbiphenyl inhibited CYP2A6 ( $IC_{50} = 70 \mu M$ ) more than CYP2A13 ( $IC_{50} > 200 \mu M$ ); this compound was less active in inducing type I binding spec-



**Fig. 6.** Type I binding spectra of interaction of naphthalene, phenanthrene, biphenyl, and their derivatives with CYP2A13 (A) and 2A6 (B) and inhibition of coumarin 7-hydroxylation activities of CYP2A13 (C) and 2A6 (D) by these chemicals. Data are taken from Shimada *et al.* (26,28-30) with modification.



**Fig. 7.** Molecular docking analysis of ligand-interaction energies ( $U$  values) of 2-ethynyl-naphthalene, 2-ethynyl-phenanthrene, and 4-biphenyl propargyl ether obtained using reported crystal structures of CYP2A13 (4EJH), 2A13 (2P85), 2A13 (3T3S), and 2A13 (4EJG) bound to NNK, indole, pilocarpine, and nicotine, respectively. Data are from Murayama, N., Shimada, T. and Yamazaki, H. (unpublished results).

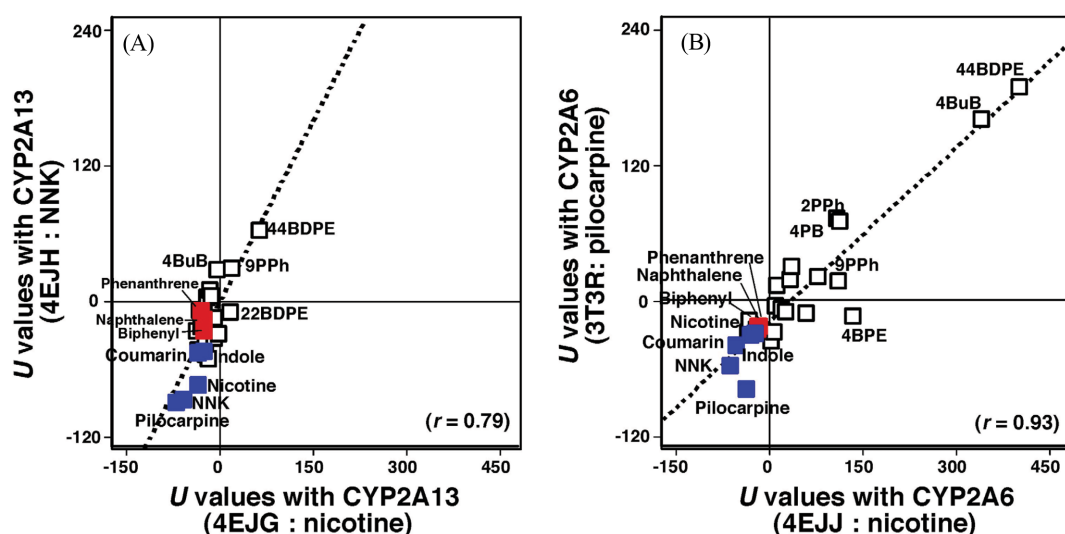
tra with CYP2A13 (Fig. 6C, 6D).

Since molecular docking analysis has been shown to be a useful tool for the studies of the interactions of various ligands with active sites of enzymes, such as P450s, we examined and compared the ligand-interaction energies ( $U$  values) with these 24 chemicals using reported crystal structures of CYP2A13 (4EJH), 2A13 (2P85), 2A13 (3T3S), and 2A13 (4EJG) (142-144) bound to NNK, indole, pilocarpine, and nicotine, respectively, and CYP2A6 (1Z10), 2A6 (3T3R), and 2A6 4EJJ) (145,146) bound to coumarin, pilocarpine, and nicotine, respectively (30). We first determined the  $U$  values of interaction of 2-ethynyl-naphthalene, 2-ethynyl-phenanthrene, and 4-biphenylpropargyl ether with CYP2A13 (4EJH), CYP2A13 (2P85), CYP2A13 (3T3S), and CYP2A13 (4EJG) (Fig. 7) and obtained optimal  $U$  values on analysis with MMFF94x force field (30). The  $U$  values are somewhat different when different crystal structures of CYP2A13 were used (Fig. 7).

In order to examine structure-function relationships of the interactions of above 24 chemicals with active sites of CYP2A13 and CYP2A6, we compared the  $U$  values obtained with CYP2A13 4EJG (nicotine type) and CYP2A13 4EJH (NNK type) (Fig. 8A) and CYP2A6 4EJJ (nicotine type) and CYP2A6 3T3R (pilocarpine type) (Fig. 8B) (30). There were good correlations between  $U$  values of CYP2A13 4EJG (nicotine-type) and 4EJH (NNK-type) ( $r = 0.79$ ,  $p < 0.01$ ) and of CYP2A6 4EJJ (nicotine-type) and 2A6 3T3R (pilocarpine-type) ( $r = 0.93$ ,  $p < 0.01$ ) with these 24 chemi-

cals and also with NNK, indole, pilocarpine, and nicotine as standards for CYP2A substrates (Fig. 8) (140-144). It was also found that the parent compounds, naphthalene, phenanthrene, and biphenyl had  $U$  values comparable to those of NNK, indole, pilocarpine, nicotine, and coumarin (Fig. 8). The results support the usefulness of molecular docking analysis in understanding the basis of molecular interaction of xenobiotic chemicals with the active sites of P450 proteins and possibly other enzymes.

Very recently, we carried out *in vitro* studies if these chemicals that interact with and inhibit CYP2A13 and 2A6 are oxidized by these enzymes (26,28-31). The results obtained showed that CYP2A13 is the major enzyme in 1-hydroxylation of pyrene, 8-hydroxylation of 1-hydroxypyrene (to form 1,8-dihydroxypyrene), hydroxylation of 1-nitropyrene and 1-actylpyrene (26). CYP2A13 also oxidized naphthalene, phenanthrene, and biphenyl to 1-naphthol, 9-hydroxyphenanthrene, and 2- and/or 4-hydroxybiphenyl, respectively (30). Our results also showed that acetylenic PAH compounds such as 2-ethynyl-naphthalene, 1-naphthalene ethyl propargyl ether, 2-naphthalene propargyl ether, 2-ethynyl-phenanthrene, 3-ethynylphenanthrene, 2-(1-propynyl)phenanthrene, 3-(1-propynyl)phenanthrene, and 4-biphenyl propargyl ether which interact highly with CYP2A13 were found to be metabolized by this enzyme (30). In contrast, 2,5,2',5'-tetrachlorobiphenyl was found to be oxidized by CYP2A6 to form 4-hydroxylated metabolite at a much higher rate than by CYP2A13 (31).



**Fig. 8.** Correlations of ligand-interaction energies ( $U$  values) of interaction of 24 chemicals (and also NNK, indole, pilocarpine, nicotine, and coumarin) with crystal structures of CYP2A13 4EJG (nicotine-type) and 4EJH (NNK-type) (A) and of CYP2A6 4EJJ (nicotine-type) and 3T3R (pilocarpine-type) (B). Points obtained with naphthalene, phenanthrene, and biphenyl are shown in red, other 21 chemicals in open square, and coumarin, indole, NNK, nicotine, and pilocarpine in blue. Abbreviations used in this figure: 4-biphenyl propargyl ether (4BPE), 9-(1-propynyl)phenanthrene (9PPH), 4-butynylbiphenyl (4BuB), 2,2'-biphenyl dipropargyl ether (22BDPE), and 4,4'-biphenyl dipropargyl ether (44BDPE). Data are taken from Shimada *et al.* (30) with modification.

#### Inhibition of human P450s by chemopreventive organoselenium compounds.

We have previously shown that BSC and *o*-, *m*-, and *p*-XSC induce reverse type I binding spectra with CYP1A1, 1A2, and 1B1 and inhibit EROD activities catalyzed by these P450 enzymes (81). The affinities of four selenium compounds in interactions with P450 family 1 enzymes were not very different; the  $K_s$  values obtained in the spectral interactions of four selenium compounds with CYP1A1, 1A2, and 1B1 were 19~30  $\mu\text{M}$ , 6.3~13  $\mu\text{M}$ , and 3.6~5.7  $\mu\text{M}$ , respectively, and the  $\text{IC}_{50}$  values for these chemicals were 0.10~0.45  $\mu\text{M}$  for CYP1A1, 0.20~1.3  $\mu\text{M}$  for CYP1A2, and 0.13~0.27  $\mu\text{M}$  for CYP1B1 (Table 4). However, these organoselenium compounds were found to have relatively higher affinities for CYP1B1 than

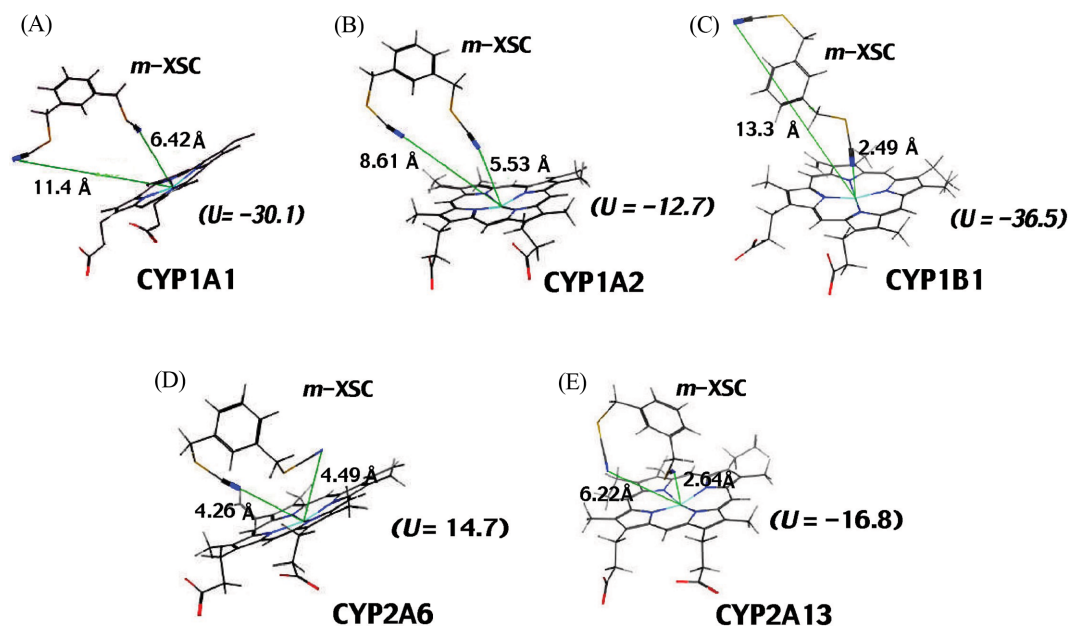
CYP1A1 and 1A2, because the  $K_s$  values in CYP1B1 were lower and the  $\Delta A_{\text{max}}/K_s$  values in CYP1B1 were higher than those in the cases of the latter two enzymes (81).

These four organoselenium compounds also induce type I binding spectra with CYP2A13 and 2A6 and are able to inhibit coumarin 7-hydroxylation activities by these enzymes (Table 4) (25). We concluded that i) four selenium compounds bind to CYP2A6 and 2A13 to induce type I binding spectra (25), ii) both CYP2A13 and 2A6-dependent coumarin 7-hydroxylation activities are significantly inhibited by these selenium compounds (Table 4), and iii) the spectral changes and catalytic inhibition by these chemicals are more profoundly observed with CYP2A13 than CYP2A6 (25). Other human P450 enzymes, such as CYP2C9, 2E1,

**Table 4.** Inhibition of CYP1A1-, 1A2, and 1B1-dependent EROD activities and CYP2A6- and 2A13-dependent coumarin 7-hydroxylation activities by organoselenium compounds

(Chemical) (P450)	BSC	<i>o</i> -XSC	<i>m</i> -XSC	<i>p</i> -XSC
	$\text{IC}_{50}$ ( $\mu\text{M}$ ) for inhibition of EROD activity			
CYP1A1	0.45 $\pm$ 0.038	0.11 $\pm$ 0.021	0.10 $\pm$ 0.013	0.26 $\pm$ 0.031
CYP1A2	1.3 $\pm$ 0.22	0.39 $\pm$ 0.042	0.20 $\pm$ 0.021	0.63 $\pm$ 0.059
CYP1B1	0.27 $\pm$ 0.031	0.14 $\pm$ 0.027	0.13 $\pm$ 0.011	0.16 $\pm$ 0.009
(Chemical) (P450)	BSC	<i>o</i> -XSC	<i>m</i> -XSC	<i>p</i> -XSC
	$\text{IC}_{50}$ ( $\mu\text{M}$ ) for inhibition of coumarin 7-hydroxylation activity			
CYP2A6	4.3 $\pm$ 0.36	2.7 $\pm$ 0.34	2.4 $\pm$ 0.19	6.2 $\pm$ 0.55
CYP2A13	1.2 $\pm$ 0.19	1.2 $\pm$ 0.13	0.22 $\pm$ 0.031	1.4 $\pm$ 0.21

$\text{IC}_{50}$  values were obtained by measuring EROD activities for CYP1A1, 1A2, and 1B1 and coumarin 7-hydroxylation activities for CYP2A6 and 2A13. Data for  $\text{IC}_{50}$  values represent means  $\pm$  SE. Data are taken from Shimada *et al.* (25) with modification.



**Fig. 9.** Molecular docking analysis of interaction of *m*-XSC with CYP1A1 (A), 1A2 (B), 1B1 (C), 2A6 (D), and 2A13 (E). The ligand-P450 interaction energies (*U* values) and distances between the N-atom in one of the  $-\text{CH}_2\text{SeCN}$  moieties of *m*-XSC and the Fe-atom (calculated using *in silico* analysis) in these P450s are shown in the figure.

and 3A4, do not show any apparent spectral changes with these selenium compounds tested. Thus, one of the mechanisms underlying prevention of cancers caused by PAHs and tobacco-related carcinogens with these selenium compounds is suggested to be due to the results of inhibition of P450 family 1 and 2A enzymes.

Molecular docking analysis was done to see interaction of *m*-XSC with CYP1A1, 1A2, 1B1, 2A6, and 2A13 (Fig. 9). The distances between the N-atom in one of the  $-\text{CH}_2\text{SeCN}$  moieties of *m*-XSC and the Fe-atom in CYP1A1, 1A2, and 1B1 were calculated *in silico*. By comparing the distances in CYP1A1, 1A2, and 1B1, it was found that one of the selenium moieties was closely oriented in the active sites of CYP1B1 (2.49 Å); these distances were 5.53 Å and 6.42 Å in CYP1A1 and 1A2, respectively. In contrast to the cases in P450 family 1 enzymes, both selenium moieties at 1- and 3-positions of *m*-XSC were docked near the heme of CYP2A13 and 2A6 (25). The distance between N-atom of *m*-XSC and the Fe-atom of CYP2A13 (2.64 Å) (Fig. 9C) was also close as compared with CYP2A6 (4.26 or 4.49 Å) (Fig. 9D, 9E).

## CONCLUSIONS

Mouse Cyp1b1 and 2a5 have been shown to be important enzymes in initiating cell transformation caused by environmental carcinogens such as 7,12-DMBA, B[a]P, DB[a,l]P, and NNK based on the effects of disruption of respective P450 genes and specific chemical P450 inhibitors on the suppression of tumor formation caused by car-

cinogens *in vivo*. Because human CYP1B1 (and also CYP1A1 and 1A2) and CYP2A13 (and CYP2A6) have been shown to be the major enzymes involved in the activation of these carcinogenic PAHs and tobacco-related nitrosamines *in vitro*, it is interesting to determine what kinds of xenobiotic chemicals inhibit individual forms of human P450 enzymes. In this review, we have described the nature of various xenobiotic chemicals that inhibit human CYP1 and 2A enzymes; these chemicals include carcinogenic and non- or weak carcinogenic PAHs, arylacetylenes, plant flavonoid derivatives, organoselenium compounds, and other chemicals. Many chemical inhibitors induce type I, type II, and reverse type I spectral changes with specific form(s) of P450 and these spectral intensities often, but not all, relate to the abilities to inhibit and/or to be metabolized by these P450 enzymes. Molecular docking analysis is a useful tool in examining the interactions of chemical inhibitors with P450 enzymes and determining how these chemicals are metabolized by P450 enzymes. Dietary consumption of chemical inhibitors for P450 enzymes and polymorphisms of various P450 genes may affect differences in cancer susceptibilities caused by a variety of environmental carcinogens in humans.

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