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Metabolic Activation of Polycyclic Aromatic Hydrocarbons and Aryl and Heterocyclic Amines by Human Cytochromes P450 2A13 and 2A6

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Abstract

Human cytochrome P450 (P450) 2A13 was found to interact with several polycyclic aromatic hydrocarbons (PAHs) to produce Type I binding spectra, including acenaphthene, acenaphthylene, benzo[*c*]phenanthrene, fluoranthene, fluoranthene-2,3-diol, and 1-nitropyrene. P450 2A6 also interacted with acenaphthene and acenaphthylene, but not with fluoranthene, fluoranthene-2,3-diol, or 1-nitropyrene. P450 1B1 is well known to oxidize many carcinogenic PAHs, and we found that several PAHs (i.e., 7,12-dimethylbenz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene-5,6-diol, benzo[*c*]phenanthrene, fluoranthene, fluoranthene-2,3-diol, 5-methylchrysene, benz[*a*]pyrene-4,5-diol, benzo[*a*]pyrene-7,8-diol, 1-nitropyrene, 2-aminoanthracene, 2-aminofluorene, and 2-acetylaminofluorene) interacted with P450 1B1, producing Reverse Type I binding spectra. Metabolic activation of PAHs and aryl- and heterocyclic amines to genotoxic products was examined in *Salmonella typhimurium* NM2009, and we found that P450 2A13 and 2A6 (as well as P450 1B1) were able to activate several of these procarcinogens. The former two enzymes were particularly active in catalyzing 2-aminofluorene and 2-aminoanthracene activation, and molecular docking simulations supported the results with these procarcinogens, in terms of binding in the active sites of P450 2A13 and 2A6. These results suggest that P450 2A enzymes, as well as P450 Family 1 enzymes including P450 1B1, are major enzymes involved in activating PAHs and aryl- and heterocyclic amines, as well as tobacco-related nitrosamines.

Introduction

Human P450s 2A13 and 2A6 have been shown to metabolize several xenobiotics—including coumarin, phenacetin, *p*-nitrophenol, and nicotine—and to activate tobacco-related nitrosamines—including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)—to reactive products that initiate cell transformation.[1–4] Several studies have also shown that chemical inhibitors can inhibit catalytic activity of P450 2A13 and 2A6, and most of these

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inhibitors induce spectral changes with these two P450 enzymes, producing Type I binding spectra.[5–8] However, little is known about how these two P450s catalyze the oxidations of other chemicals, as well as the above substrates.[9] Recent studies by Fukami et al.[10] reported that P450 2A13 is able to efficiently metabolize the air pollutants naphthalene, styrene, and toluene better than P450 2A6, indicating that P450 2A enzymes may be involved in the oxidation of a number of environmental chemicals, including carcinogenic polycyclic aromatic hydrocarbons (PAHs) and aryl- and heterocyclic amines.

The work in the acompanying paper[11] was focused on the interactions of P450 2A13 with a number of chemicals, of various types. In this study, we analyzed how P450 2A13 and 2A6 interact spectrally with a series of PAH compounds including (parent) PAHs, PAH-diols, and aryl- and heterocyclic amines and compared the results with those for P450 1B1. Metabolic activation of these procarcinogens by P450s 2A13, 2A6, and 1B1 was determined using the tester strain *Salmonella typhimurium* NM 2009, which is based on the expression of the *umu* gene in the bacteria.[12,13] Molecular docking simulation of the interaction of selected chemicals with active sites of P450s is also reported.

Experimental Procedures

Chemicals

Coumarin, 7-hydroxycoumarin, 7-ethoxyresorufin, and resorufin were purchased from SigmaAldrich (St. Louis, MO). *Escherichia coli* DH5α cells were purchased from Invitrogen (Carlsbad, CA).

Benzo[*a*]pyrene (B[*a*]P) and benz[*a*]anthracene (B[*a*]A) were purchased from SigmaAldrich Chemical Co. (St. Louis, MO). 7,8-Dihydroxy-7,8-dihydrobenzo[*a*]pyrene (B[*a*]P-7,8-diol) [(±)] and 3,4-dihydroxy-3,4-dihydrobenz[*a*]anthracene (B[*a*]A-*trans*-3,4-diol) were obtained from the National Cancer Institute Chemical Carcinogen Repository/Midwest Research Institute (Kansas City, MO). 7,12-Dimethylbenz[*a*]anthracene (DMBA), 3,4-dihydroxy-3,4-dihydro-7,12-dimethylbenz[*a*]anthracene (DMBA-*trans*-3,4-diol), 5,6-dihydroxy-5,6-dihydro-7,12-dimethylbenz[*a*]anthracene (DMBA-*cis*-5,6-diol), 1,2-dihydroxy-1,2-dihydrochrysene (chrysene-1,2-diol), 1,2-dihydroxy-1,2-dihydro-5-methylchrysene (5MeCh-1,2-diol), 11,12-dihydroxy-11,12-dihydrobenzo[*g*]chrysene (B[*g*]C-11,12-diol), and 3,4-dihydroxy-3,4-dihydrobenzo[*c*]phenanthrene (B[*c*]Phe-3,4-diol) were kindly donated by Dr. S. S. Hecht (University of Minnesota, Minneapolis, MN). Other carcinogens were obtained from the National Cancer Institute Chemical Carcinogen Repository/Midwest Research Institute (Kansas City, MO) or Toronto Research Chemicals (Toronto, Ontario, Canada). Other chemicals and reagents used in this study were obtained from sources described previously or were of the highest quality commercially available.[5,14,15]

Enzymes

The expression and purification of P450 2A6 and 2A13 enzymes were carried out using previously described methods, with some modifications.[16] The expression vector pKK322-2/2A13 containing CYP2A13 cDNA was kindly provided by Dr. E. E. Scott (University of Kansas). The 2A13 insert was used to replace the 2A6 insert in the "bicistronic" vector (with human NADPH-P450 reductase) previously constructed.[16] The *E. coli* strains DH5α (containing pCW/2A6) and TOPP-3 (containing pKK322-2/2A13) were inoculated into Luria-Bertani (LB) medium containing ampicillin (50 μg mL^{-1}) and incubated overnight at 37 °C. LB cultures were then seeded into 1 liter of Terrific Broth (TB) expression medium containing ampicillin (50 μg mL^{-1}). The expression cultures were grown at 37 °C with shaking at 250 rpm. When the OD_{600} of the cultures reached 0.5, supplements were added (0.5 mM 5-aminolevulinic acid, 1.0 mM isopropyl β-D-

thiogalactoside, 1.0 mM thiamine, and trace elements[17]) and the expression cultures were grown further at 30 °C with shaking at 200 rpm for 24 h.

Bacterial inner membrane fractions containing P450 2A6 and 2A13 were isolated and prepared from TB expression cultures using a method described previously.[16] Purification of P450 enzymes (from the "monocistronic" vector) using a Ni^{2+}-nitrilotriacetate column was also performed using a previously described method.[18] Briefly, the prepared membrane fractions were solubilized overnight at 4 °C in 100 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol (v/v), 0.1 mM EDTA, 10 mM β-mercaptoethanol, and 2% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPS) (Affymetrix, Maumee, OH). The solubilized fractions were then loaded onto a Ni^{2+}-nitrilotriacetate column (Qiagen, Valencia, CA) and the proteins were eluted with a buffer containing 300 mM imidazole. The eluted fractions containing P450 2A6 and 2A13 proteins were subsequently dialyzed at 4 °C against 100 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol (v/v) and 0.1 mM EDTA.

The P450 1B1*3 variant ($Arg^{48}Ala^{119}Val^{432}Asn^{453}$) was used in this study. A bacterial "bicistronic" P450 1B1 system was prepared as described.[19–22] Briefly, the plasmid for expression of P450 1B1 plus human NADPH-P450 reductase (using a single promoter) was introduced into *E. coli* DH5α cells by a heat shock procedure, and the transformants were selected in Luria-Bertani medium containing ampicillin (100 μg mL^{-1}). Bacterial membranes were prepared and suspended as described above. Yields of P450, as determined by the original spectral method,[23] ranged between 40 and 250 nmol (liter medium)$^{-1}$. P450 1B1 was purified (form monocistronic *E. coli* expression systems) as described.[24]

Enzyme Assays

P450-dependent activation of procarcinogens to reactive products that cause induction of *umu* gene expression in the tester strain *S. typhimurium* NM2009 was determined as described previously.[13,25] Standard incubation mixtures contained P450 (10 pmol) and 2.5 μM 5-MeCh-1,2-diol, (±)B[*a*]P-7,8-diol, DB[*a*,*l*]P-11,12-diol, or 2-amino-3,5-dimethylimidazo[4,5-*f*]quinoline (MeIQ), in a final volume of 1.0 mL of 100 mM potassium phosphate buffer (pH 7.4) containing the NADPH-generating system[26] and 0.75 mL of bacterial suspension. The induction of *umu* gene expression was monitored by measuring β-galactosidase activity, using *o*-nitrophenyl-β-D-galactopyranoside as a substrate, and is presented as units of β-galactosidase activity min^{-1} (nmol P450)$^{-1}$.[13,25]

Spectral Binding Titrations

E. coli-expressed P450s 1B1,[24] 2A6,[16] and 2A13[7] were purified to electrophoetic homogenity as described. Purified P450 enzymes were diluted to 1.0 μM in 0.10 M potassium phosphate buffer (pH 7.4) containing 20% glycerol (v/v), and binding spectra were recorded with subsequent additions of chemical inhibitors in a JASCO V-550 or OLIS-Aminco DW2a (OLIS, Bogart, GA) spectrophotometer as described previously.[24,27] Briefly, the chemical inhibitors were added to the buffer, with or without P450, and the spectra were recorded between 350 nm and 500 (or 700) nm. The substrate binding spectra were obtained by subtracting the blank spectra (in the absence of P450) from the P450 spectra (in the presence of P450). Spectral dissociation constants (K_s) were estimated using GraphPad Prism software (GraphPad Software, San Diego, CA) and either a hyperboic fit or (in the case of very tight binding) a quadratic equations to correct for the amount of bound ligands.

Other Assays

P450[23] and protein[28] concentrations were estimated by the methods described previously.

Docking Simulations into Human P450 Enzymes

The crystal structures of P450 1B1,[29] 2A6,[30] and 2A13[8,31] have recently been reported and were used as the basis for the docking. Simulation was carried out for P450 enzymes using the MMFF94x force field described in the MOE software (ver. 2011.10, Computing Group, Montreal, Canada).[5,26] Fighty solutions were generated for each docking experiment and ranked according to the total interaction energy (*U* value). Lower *U* values (ligand-interaction energy) are an indication of higher interaction between a chemical and P450.

Results

Binding Spectra of Human P450s 2A13, 2A6, and 1B1 with Procarcinogens

Purified P450 2A13, 2A6, and 1B1 were examined for spectral changes upon the addition of different concentrations of chemicals (Figure 1). P450 1B1 showed Reverse Type I binding spectra with FA; the Soret absorbance at 393 nm was shifted to 415 nm and distinct α- and β-bands appeared at 564 and 532 nm, respectively. In contrast, both P450 2A13 and 2A6 gave Type I binding spectra upon addition of different concentrations of FA-2,3-diol and acenaphthene, respectively (Figures 1B, 1C). The α- and β-bands at 567 and 533 nm coalesced upon addition of these chemicals (Figures 1c, 1d). Six of the 17 procarcinogens tested for spectral interaction with P450 2A13—acenaphthene, acenaphthylene, B[*c*]Phe, FA, FA-2,3-diol, and 1-NP—produced spectral shifts with this enzyme; the K_s values were smaller than those measured with P450 1B1 (Table 1). P450 2A6 was found to interact with acenaphthene and acenaphthylene.

Other procarcinogens that interacted with P450 1B1 producing Reverse Type I binding spectra included B[*a*]A-3,4-diol, 7,12-DMBA-3,4-diol, 7,12-DMBA-5,6-diol, 5MeCh, 1-NP, B[*a*]P-4,5-diol, B[*a*]P-7,8-diol, 2-AA, 2AF, and 2-AAF (Table 1 and Figure 2). B[*c*]Phe, FA, and 1-NP had relatively high affinities for P450 1B1 (Table 1).

Metabolic Activation of 24 Procarcinogens by P450 2A13, 2A6, and 1B1

Metabolic activation of procarcinogens by P450 2A13, 2A6, and 1B1 was examined using *S. typhimurium* strain NM2009 to measure *umu* gene expression (Table 2).[13,24] The procarcinogens tested in this study included eight PAHs, nine PAH diols, and seven aryl and heterocyclic amines.

The parent PAHs 7,12-DMBA, B[*a*]P, and B[*c*]Phe induced *umu* gene expression when activated by P450 1B1 (Table 2). Both P450 2A13 and 2A6 were found to induce *umu* gene expression only when B[*c*]Phe was used as a PAH, and the activity of P450 2A13 was roughly twice as high as with P450 2A6 or P450 1B1.

We also examined several diol derivatives of PAHs for activation by P450 2A13 and 2A6 (as well as P450 1B1) to genotoxic products in the *umu* tester strain and found that 7,12-DMBA-3,4-diol, B[*a*]P-7,8-diol, 5MeCh-1,2-diol, B[*c*]Phe-3,4-diol, and FA-2,3-diol induced *umu* gene expression, although the activities were lower than those of P450 1B1 except in the case of FA-2,3-diol (Table 2).

Metabolic activation of aryl- and heterocyclic amines by P450s 2A13, 2A6, and 1B1 was examined in the same tester strain, which is highly sensitive to detect activation of aryl-and heterocyclic amines because this bacterium contains a plasmid (pNM12) that encodes an *O*-acetyltransferase gene originally isolated from *S. typhimurium* TA1535.[13,32] Interestingly, P450 2A13 and 2A6 activated 2-AF and 2-aminoanthracene (2-AA), respectively, at high rates and the activities of these P450 2A enzymes were found to be higher than those of

P450 1B1 in the case of 2-AF (Table 2). MeIQ, IQ, MeIQx, and Trp-P-1 were also found to be activated by P450s 2A13 and 2A6, as well as with P450 1B1 (Table 2).

Concentration-dependent increases in induction of *umu* gene expression by P450s 2A13 and 2A6 were observed using B[*c*]Phe, FA-2,3-diol, 2-AA, 7,12-DMBA-3,4-diol, B[*a*]P-7,8-diol, 2-AF, and MeIQ as substrates (Figures 3A, 3B). Decreases in OD_{600} for bacterial growth were used as measures of cytotoxic responses by reactive metabolites of these chemicals (Figures 3D, 3E). P450 1B1 was also examined with regard to concentration-dependent effects of B[*a*]P-7,8-diol (Figures 3C, 3F). The results showed that P450 2A13 was highly efficient in the activation of 2-AF to products that induce *umu* gene expression and cause toxicity to bacterial cells. P450 2A6 was more active in activating 2-AA than was P450 2A13.

Docking Simulation of Procarcinogens into P450 Active Sites

2-AF docked well into the active site of P450 2A13 with a ligand-P450 interaction energy of −215.2 (Figure 4A). In addition, the distance between the H-10 atom of 2-AF and the NH of Asn297 in P450 2A13 was only 2.95Å, a value compatible with that obtained for NNK binding to P450 2A13.[8,30] The interaction between P450 2A13 and 2-AF was very high (low *U* value) in docking studies, and there was a very close distance between the atoms H-10 of 2-AF and the NH of Asn297. The ligand-P450 interaction energies (*U* values) obtained in the interaction of P450 2A13 with 2-AA and P450 2A6 with 2-AF or 2-AA were not so small (Figure 4B, 4C, and 4D).

Molecular docking simulation was also examined in the interaction of P450 1B1 with B[*c*]Phe, FA, FA-2,3-diol, 7,12-DMBA, 5MeCh, B[*a*]P, and B[*a*]P-7,8-diol (Figure 5). The results showed that these procarcinogens were docked well into the active site of P450 1B1, particularly when B[*a*]P-7,8-diol was used (Figure 5G).

B[*c*]Phe, FA, and FA-2,3-diol were docked into P450s 2A13 and 2A6 (Figure 6) and the results showed that ligand-P450 interaction energies between P450 2A6 and these three procarcinogens were always larger that those obtained with P450 2A13 (Figure 6). Docking of PAHs including 7,12-DMBA (Figure 7A), 5MeCh (Figure 7B), B[*a*]P (Figure 7C), and B[*a*]P-7,8-diol (Figure 7D) into P450 2A13 were also examined.

Discussion

P450 2A13 and 2A6 have been shown to be expressed mainly in the respiratory tract and liver, respectively.[33–36] Because the former enzyme has higher activity than P450 2A6 to activate tobacco-related nitrosamines, e.g. NNK[33] and *N*-nitrosonornicotime (NNN),[37] to reactive metabolites that initiate cell transformation,[1] P450 2A13 has been accepted to be one of the most important enzymes in the etiology of lung cancer in humans.[33,35,38,39] Because tobacco smoke (as well as charred food and other products of pyrrolysis) contains different types of carcinogens, including PAHs and aryl- and heterocyclic amines,[40–42] it is necessary to determine if these P450 2A enzymes catalyze the activation of these procarcinogens, as well as tobacco-related nitrosamines.[43–45]

In this study, we examined the interaction of PAHs and aryl- and heterocyclic amines with P450 2A13 and 2A6, as well as P450 1B1, and their activatation by these P450 enzymes to reactive metabolites in the *S. typhimurium* tester strain NM2009.[12,13] The spectral titration studies showed that acenaphthene, acenaphthylene, B[*c*]Phe, FA, FA-2,3-diol, and 1-NP induced Type I binding spectra with P450 2A13, although such spectral changes were only seen for P450 2A6 with acenaphthene and acenaphthylene. These latter two chemicals did not interact spectrally with P450 1B1, but 7,12-DMBA, 7,12-DMBA-5,6-diol, B[*c*]Phe, FA,

FA-3,4-diol, 5-methylchrysene, 1-NP, B[*a*]P, 4.5-diol, B[*a*]P-7,8-diol, 2-AA, 2-AF, and 2-AAF did induce Reverse Type I binding spectra with P450 1B1. The above results are of interest, in that P450 2A13—as well as P450 2A6—is able to interact with some of the PAHs. The Subfamily 2A P450s catalyzed the oxidation of the PAH B[*c*]Phe and a number of the PAH-diols to biologically inactive or chemically reactive metabolites, as in case of P450 1B1.[45–48]

There were several cases in which good substrates for P450s did not show spectral changes with these P450s. This is not surprising, in the context of earlier work with some of the rat P450s[49] and human P450 1A2. [50] Further, in comparing P450s 2A6 and 2A13, DeVore and Scott[8] reported that 2A6 bound NNK more tightly but that 2A13 is a much better catalyst of oxidation. Binding is a measure of inhibition of an enzyme but not necessarily of catalysis, in that the tighter binding may be non-productive.

Our study of the metabolic activation of PAHs and aryl- and heterocyclic amines in *S. typhimurium* strain NM2009 indicates that P450s 2A13 and 2A6, as well as P450 1B1, are highly active in activating these procarcinogens to genotoxic metabolites that cause both genotoxicity and death in the bacterial cells, depending on the chemical and P450 enzyme used. P450s 2A13 and 2A6 were found to be highly active in catalyzing the bioactivation of aryl- and heterocyclic amines; in particular, the former enzyme was prominent in catalyzing the activation of 2-AF and the latter activated 2-AA. P450s 2A13 and 2A6 also activated B[*a*]Phe and five of the PAH-diols, although the extent was less than that with P450 1B1.

Of the chemicals tested for activation to genotoxic products, 2-AF showed the highest activity (*umu* assay) with P450 2A13 (Table 2). In a strict sense, this is not an environmental chemical and is only used in experimental models. Although 2-AF and 2-AAF are often considered in the context of liver and bladder tumors,[51] 2-AF has also been reported to be activated in lung tumor cell lines.[52,53] Of the other compounds (Table 2) with high genotoxicity, the heterocyclic arylamines have been considered in the context of lung cancer, as well as other cancers, e.g., IQ causes lung tumors in mice[54] and also in a promotional model.[55] It should also be pointed out, in response to one of the reviewers, that FA is not classifed (IARC) as a human carcinogen, but there are a number of studies showing tumorigenicity in experimental animals.[56–61]

Molecular docking simulation studies supported the experimental results demonstrating that 2-AF can be docked well into active site of P450 2A13, showing that ligand-P450 interaction energy was very low ($U = -215.2$) and the distance between the H-10 atom of 2-AF and the NH of Asn297 in P450 2A13 was 2.97Å. These results are comparable with those obtained in a recent publication with a crystal structure of P450 2A13 bound to NNK.[8] Molecular docking studies also suggested that B[*c*]Phe, FA, and FA-2,3-diol, which showed spectral interaction with P450 2A13, but not P450 2A6, fit better into the active site of the former enzyme than the latter. The docking simulations of P450 1B1 with several procarcinogens tested supported the experimental evidence that P450 1B1 can metabolize these procarcinogens.[62,63]

In conclusion, we showed that P450 2A13 and 2A6, as well as P450 1B1, can interact with and metabolize various kinds of procarcinogens examined in this study. Of particular interest is the observation that P450 2A13 is known to be expressed in respiratory organs and has been reported to have roles in lung and other cancers caused by tobacco-related nitrosamines, e.g. NNK and NNN. Because tobaco smoke contains various kinds of procarcinogens such as PAHs and aryl- and heterocyclic amines as well as nitrosamines, it is necessary to consider the mechanisms of lung and other cancers for people exposed to numerous enviromental chemicals.

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Abbreviations

2-AA	2-aminoanthracene
2-AF	2-aminofluorene
2-AAF	2-acetamidofluorene
AFB_1	aflatoxin B_1
B[*a*]A	benz[*a*]anthracene
B[*a*]A-1,2-diol	1,2-dihydroxy-1,2-dihydrobenz[*a*]anthracene
B[*a*]A-*trans*-3,4-diol	3,4-dihydroxy-3,4-dihydrobenz[*a*]anthracene
B[*a*]A-*cis*-5,6-diol	5,6-dihydroxy-5,6-dihydrobenz[*a*]anthracene
B[*a*]A-8,9-diol	8,9-dihydroxy-8,9-dihydrobenz[*a*]anthracene
B[*g*]C-11,12-diol	11,12-dihydroxy-11,12-dihydrobenzo[g]chrysene
B[*a*]P	benzo[a]pyrene
B[*a*]P-4,5-diol	4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene
B[*a*]P-7,8-diol	7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene
chrysene-1,2-diol	1,2-dihydroxy-1,2-dihydrochryesene
7,12-DMBA	7,12-dimethylbenz[*a*]anthracene
7,12-DMBA-3,4-diol	3,4-dihydroxy-3,4-dihydro-7,12-dimethylbenz[*a*]anthracene
7,12-DMBA-5,6-diol	5,6-dihydroxy-5,6-dihydro-7,12-dimethylbenz[*a*]anthracene
B[*c*]Phe	benzo[*c*]phenanthrene
B[*c*]Phe-3,4-diol	3,4-dihydroxy-3,4-dihydrobenzo[*c*]phenanthrene
FA	fluoranthene
FA-2,3-diol	2,3-dihydroxy-2,3-dihydrofluoranthene
IQ	2-amino-3-methylimiidazo[4,5-*f*]quinoline
5MeCh	5-methylchrysene
5MeCh-1,2-diol	1,2-dihydroxy-1,2-dihydro-5-methylchrysene
MeIQ	2-amino-3,5-dimethylimiidazo[4,5-*f*]quinoline
MeIQx	2-amino-3,8-dimethylimiidazo[4,5-*f*]quinoxaline
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	*N*-nitrosonornicotime
1-NP	1-nitropyrene

PAHs	polycyclic aromatic hydrocarbons
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
Trp-P-1	3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole.

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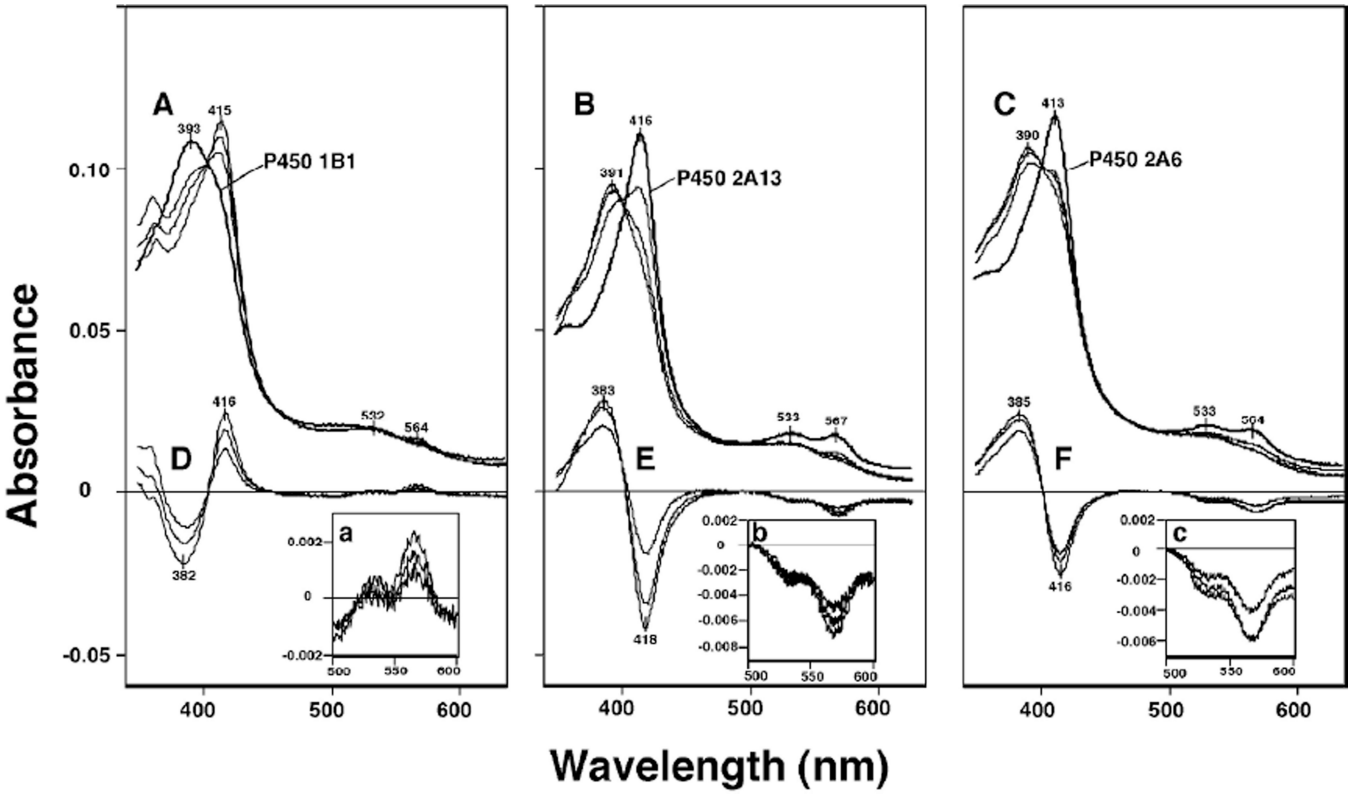

Figure 1.

Absolute (A, B, and C) and Reverse Type I difference spectra of P450 1B1 (D) and Type I difference spectra of P450 2A13 (E) and 2A6 (F) induced by different concentrations of FA (A, D, and a), FA-2,3-diol (B, E, and b), and acenaphthene (C, F, and c), respectively. Inserts a, b, and c show the difference spectra for the α and β bands of the P450 enzymes. P450 concentrations used were 1.0 μM in 100 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol (v/v). The concentration of chemicals added varied from 0.25–16 μM.

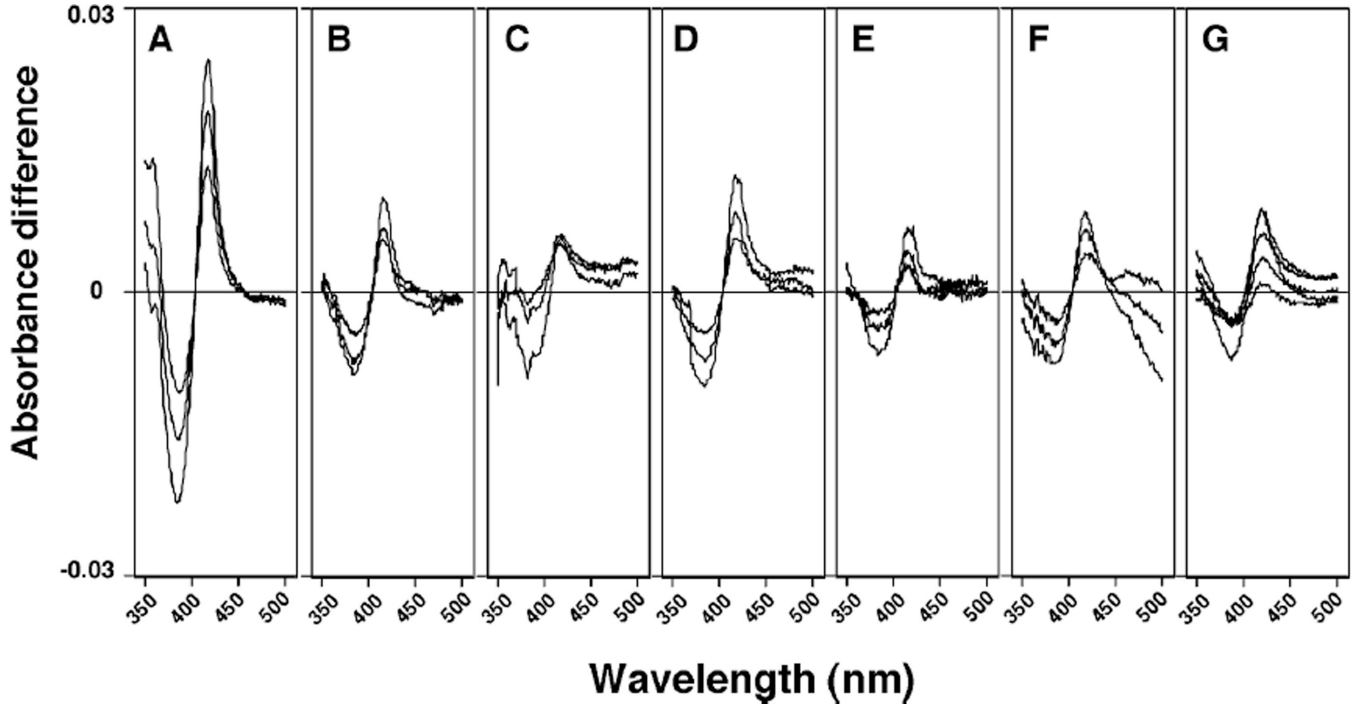

Figure 2.

Reverse Type I binding spectra for P450 1B1 with different concentrations of (A) FA, (B) B[*c*]Phe, (C) 7,12-DMBA, (D) 7,12-DMBA-5,6-diol, (E) B[*a*]P-4,5-diol, (F) 1-NP, and (G) 2-AF. Experimental details are the same as in the legend to Figure 1.

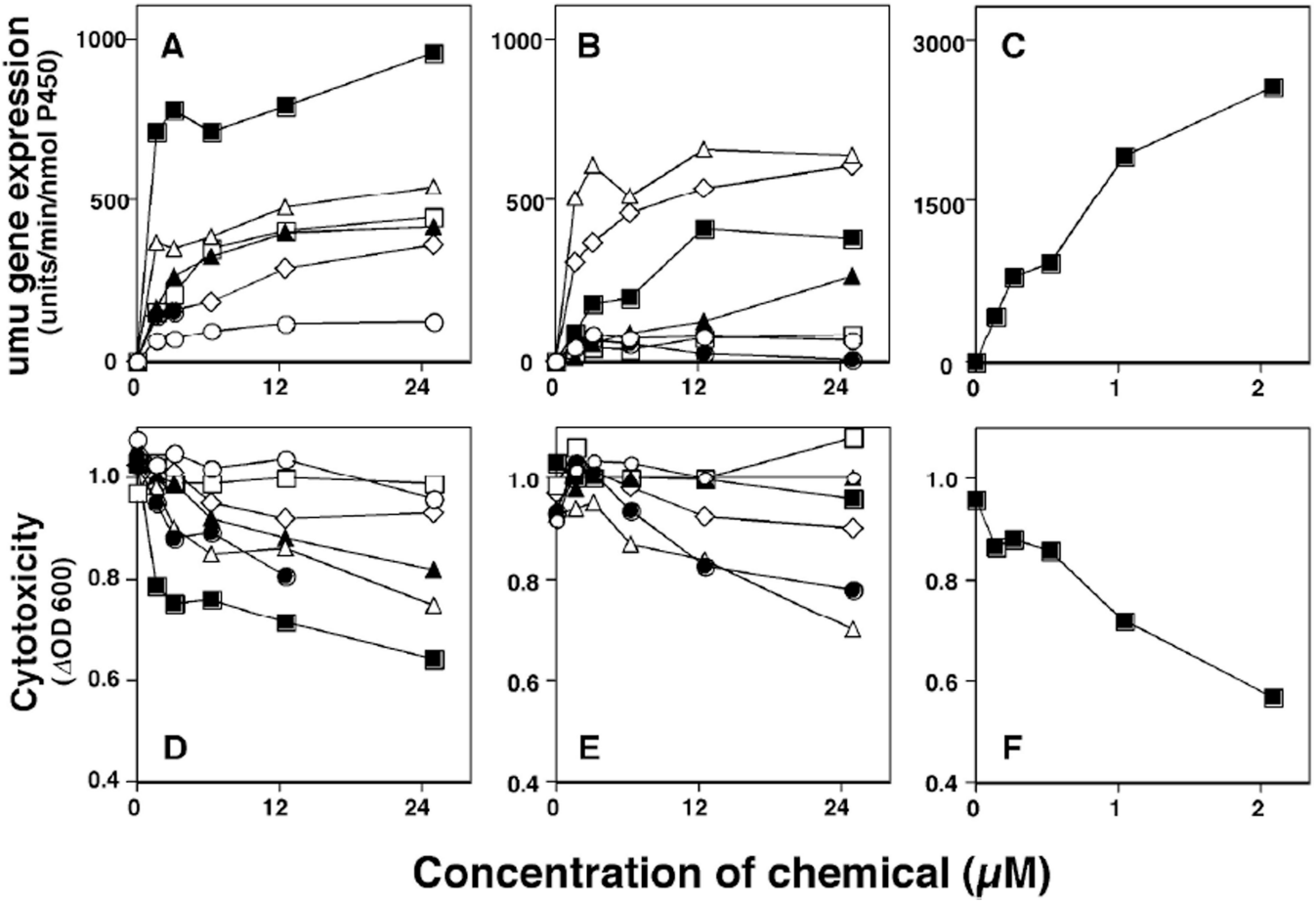

Figure 3.

Metabolic activation (A, B, and C) and cytotoxicity (D, E, and F) of procarcinogens by P450 2A13 (A and D), P450 2A6 (B and E), and P450 1B1 (C and F) in *S. typhimurium* NM2009. Procarcinogens used were B[*c*]Phe (○), FA-2,3-diol (●), 2-AA (△), 7,12-DMDA-3,4-diol (▲), B[*a*]P-7,8-diol (□), 2-AF (■), and MeIQ (◇) in Parts A, B, D, and E and B[*a*]P-7,8-diol (■) in Parts C and F. Metabolic activation of procarcinogens by P450 enzyme system was determined by induction of *umu* gene expression in *S. typhimurium* NM2009 and cytotoxicity was determined by measuring decreased bacterial OD_{600}. Data are means of duplicate determinations.

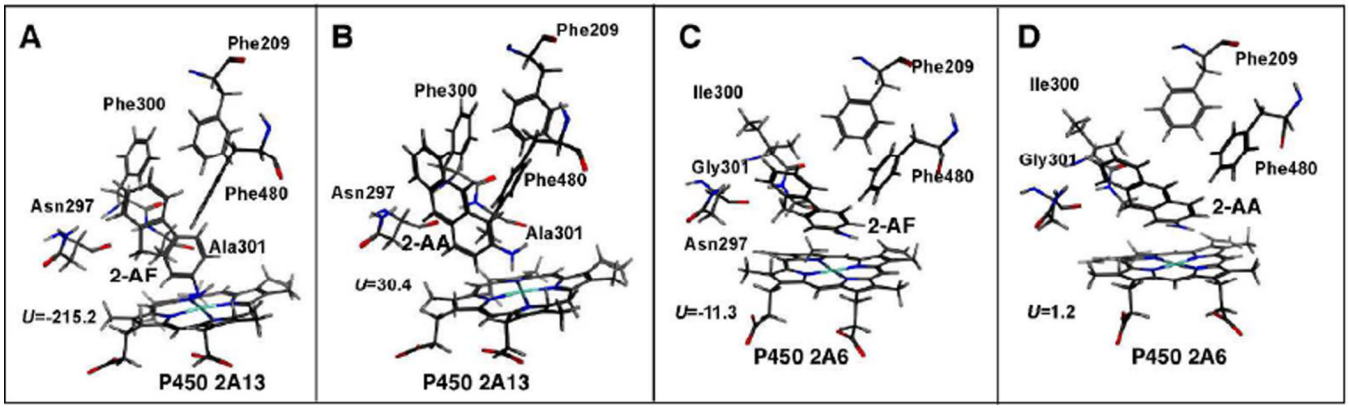

Figure 4.

Docking simulations of interaction of 2-AF (A, C) and 2-AA (B, D) with P450 2A13 (A , B) and P450 2A6 (C, D). The distance (2.95 Å) between the atom in the H-10 moiety of 2-AF and the NH of Asn297 in P450 2A13 is shown in Part A. *U* values indicate the interaction energy.

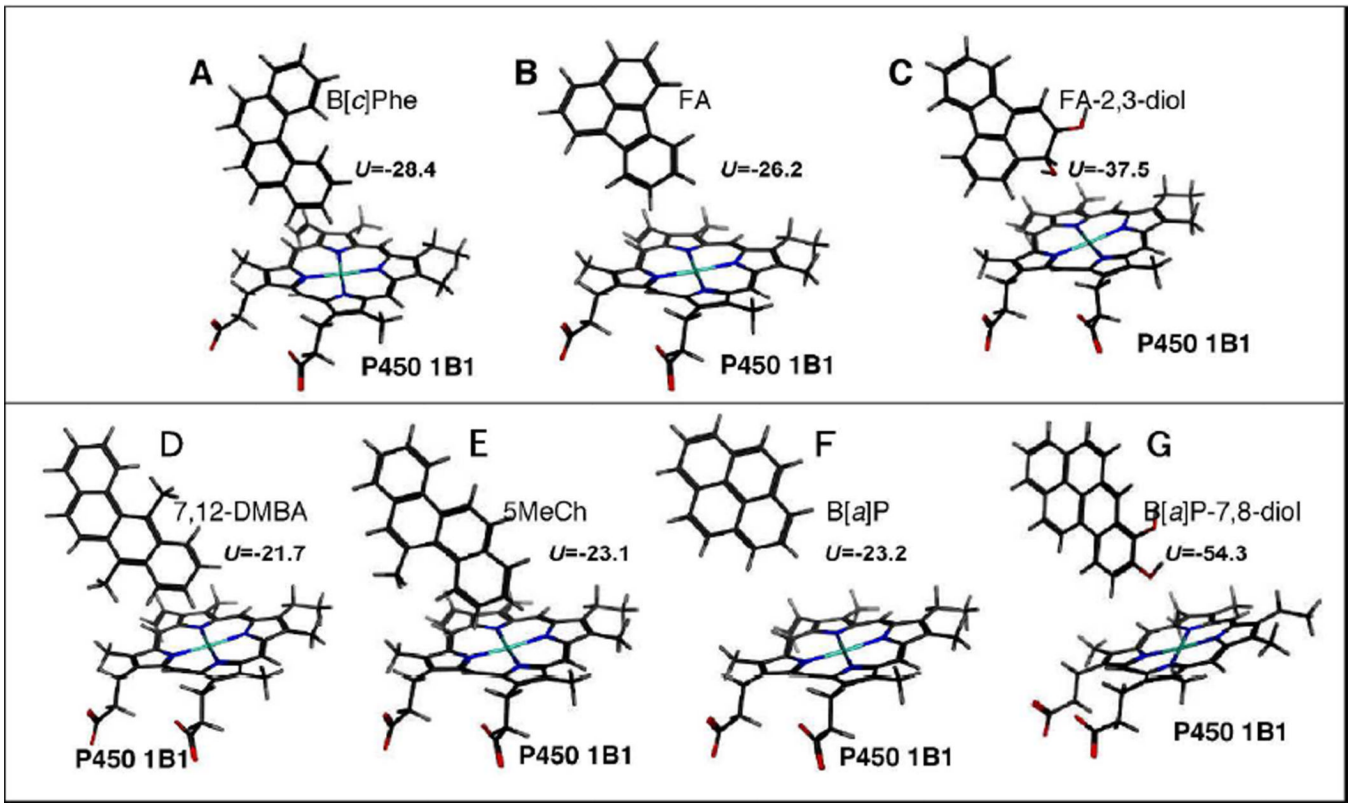

Figure 5.

Docking simulations of interaction of (A) B[*c*]Phe, (B) FA, and (C) FA-2,3-diol (D), 7,12-DMBA, (E) 5-MeCh, (F) B[*a*]P, and (G) B[*a*]P-7,8-diol with P450 1B1. The heme group of the P450 is shown in the lower part of each part. *U* values indicate the interaction energy.

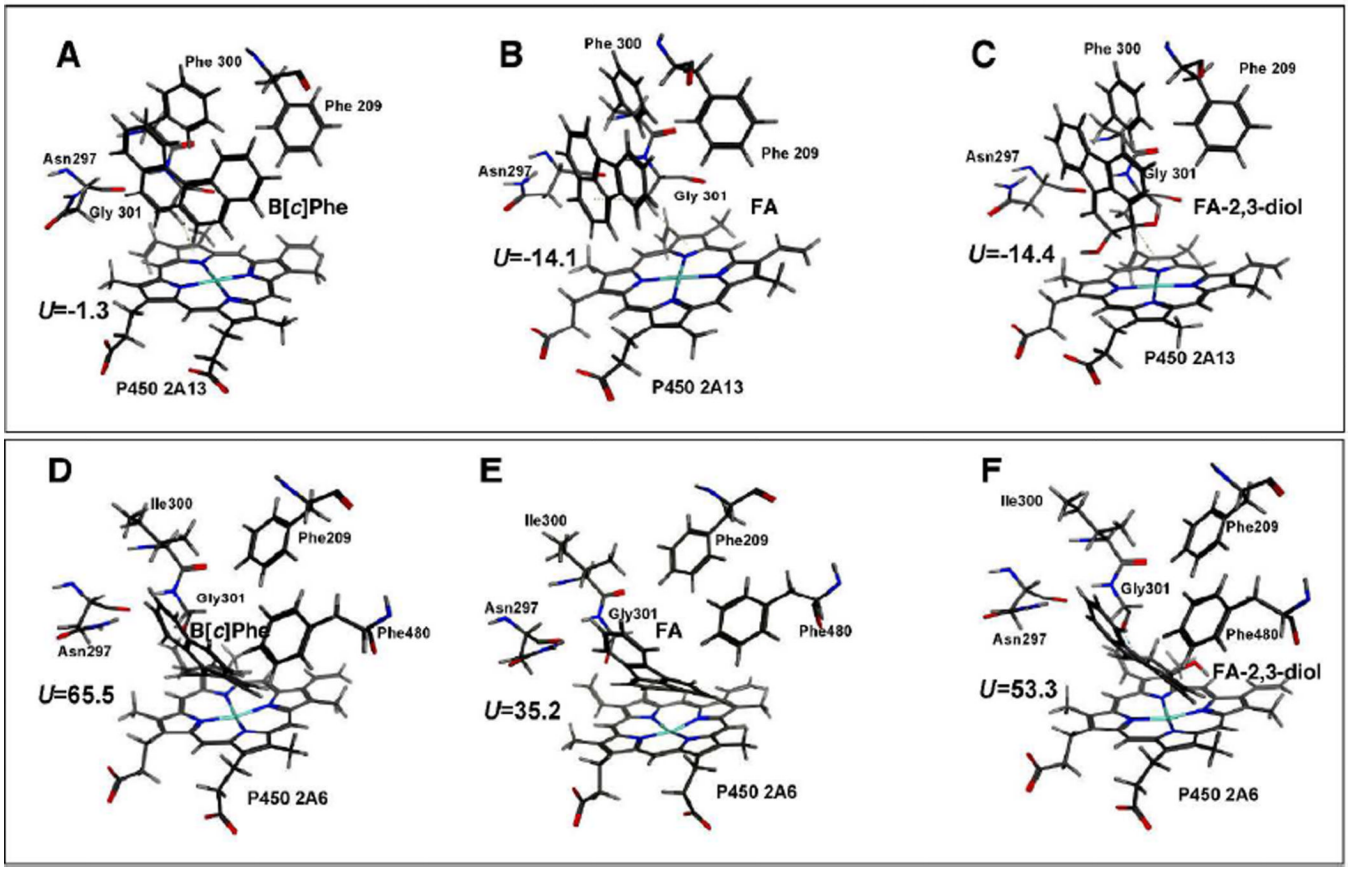

Figure 6.
Docking simulation of interaction of (A) B[*c*]Phe, (B) FA, and (C) FA-2,3-diol with P450 2A13 and (D) B[*c*]Phe, (E) FA, and (F) FA-2,3-diol with P450 2A6. *U* values indicate the interaction energy.

Table 1

Spectral interaction of PAH and other compounds with P450s 2A13, 2A6, and 1B1

chemicals	P450 2A13			P450 2A6			P450 1B1		
	Type I spectra			Type I spectra			Reverse Type I spectra		
	K_s (μM)	ΔA_{max}	$\Delta A_{max}/K_s$	K_s (μM)	ΔA_{max}	$\Delta A_{max}/K_s$	K_s (μM)	ΔA_{max}	$\Delta A_{max}/K_s$
acenaphthene	0.48 ± 0.10	0.074 ± 0.005	0.15	1.2 ± 0.21	0.068 ± 0.002	0.057	>20		
acenaphthylene	0.61 ± 0.19	0.050 ± 0.004	0.082	4.4 ± 0.52	0.076 ± 0.52	0.017	>20		
B[*a*]A	>20						>20		
B[*a*]A-*trans*-3,4-diol							>20		
7,12-DMBA	>20						8.8 ± 1.3	0.039 ± 0.002	0.0044
7,12-DMBA-3,4-diol	>20						>20		
7,12-DMBA-5,6-diol							38 ± 3.7	0.044 ± 0.003	0.0012
B[*c*]Phe	1.1 ± 0.44	0.049 ± 0.005	0.045	>20			2.5 ± 0.31	0.039 ± 0.001	0.016
B[*c*]Phe-3,4-diol	>20						>20		
fluoranthene	2.4 ± 1.5	0.044 ± 0.011	0.018	>20			1.6 ± 0.32	0.066 ± 0.003	0.042
FA-2,3-diol	0.86 ± 0.16	0.052 ± 0.003	0.060				7.4 ± 1.3	0.043 ± 0.003	0.0058
chrysene	>20						>20 μM		
chrysene-1,2-diol	>20						>20μM		
5-methylchrysene	>20						6.9 ± 2.4	0.044 ± 0.008	0.0064
5MeCh-1,2-diol	>20						>20 μM		
1-nitropyrene	0.23 ± 0.08	0.055 ± 0.004	0.24	>20			1.4 ± 0.001	0.037 ± 0.001	0.026
B[*a*]P	>20			>20			>20		
B[*a*]P-4,5-diol							23 ± 10	0.032 ± 0.010	0.0014
(±)B[*a*]P-7,8-diol	>20						6.8 ± 1.2	0.024 ± 0.002	0.0035
2-AA	>20			>20			33 ± 36	0.085 ± 0.076	0.0026
2-AF	>20			>20			4.6 ± 1.3	0.032 ± 0.003	0.0070
2-AAF							20 ± 7.8	0.035 ± 0.009	0.0018
Trp-P-1							>20		
PhIP							>20		
AFB_1	>20			>20					

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The results for K_S and ΔA_{max} values indicate SE, obtained from fitting of hyperbolic plots in GraphPad Prism, and values for $\Delta A_{max}/K_S$ inculde further analysis of the SE of the quotients. Not all chemicals were used for spectral analysis; the assays were restricted to chemicals that were of the most interest.

Table 2

Metabolic activation of procarcinogens by human P450 2A13, 2A6, and 1B1 in *S. typhimurium* strain NM2009

	activation of procarcinogens *umu* gene expression (units/min/nmol P450)		
procarcinogen	**P450 2A13**	**P450 2A6**	**P450 1B1**
PAHs			
7,12-DMBA	<10	<10	190 ± 21
B[*a*]P	<10	<10	92 ± 18
B[*c*]Phe	55 ± 11	22 ± 8	25 ± 10
FA	<10	<10	<10
1-NP	<10	<10	<10
B[*a*]A-3,4-diol	<10	<10	<10
acenaphthene	<10	<10	<10
acenaphthylene	<10	<10	<10
PAH diols			
7,12-DMBA-3,4-diol	280 ± 33	55 ± 13	2700 ± 110
(±)B[*a*]P-7,8-diol	136 ± 22	45 ± 11	2600 ± 110
5MeCh-1,2-diol	86 ± 15	52 ± 11	2600 ± 180
B[*c*]Phe-3,4-diol	45 ± 8	33 ± 10	140 ± 21
FA-2,3-diol	88 ± 13	36 ± 8	30 ± 11
chrysene-1,2-diol	<10	<10	220 ± 19
B[*g*]C-11,12-diol	<10	<10	207 ± 25
B[*a*]P-4,5-diol	<10	<10	<10
B[*a*]A-3,4-diol	<10	<10	<10
aryl and heterocyclic amines			
2-AF	1400 ± 77	450 ± 31	160 ± 21
2-AA	560 ± 40	1200 ± 89	1900 ± 210
2-AAF	<10	<10	<10
MeIQ	260 ± 30	350 ± 44	601 ± 59
IQ	430 ± 40	75 ± 11	220 ± 29
MeIQx	110 ± 15	45 ± 15	87 ± 11
Trp-P-1	40 ± 9	61 ± 11	910 ± 90
PhIP	<10	<10	<10

Incubation mixtures contained 10 nM P450 and 2.5 μM procarcinogen, and other details for the assay of *umu* gene expression are described in Materials and methods. Results are expressed as means ± range of duplicate experiments, using two significant digits.