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**Supporting Information**

# Hydroxylation and dechlorination of 3,3′,4,4′-tetrachlorobiphenyl (CB77) by rat and human CYP1A1s and critical roles of amino acids composing their substrate-binding cavity

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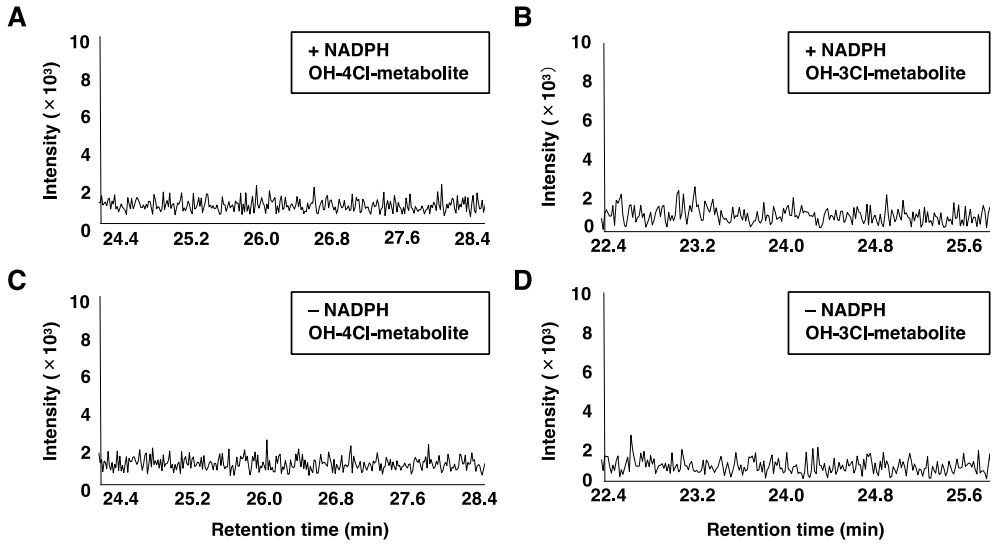


**Fig. S1** Measurement of 3,3′,4,4′-tetrachlorobiphenyl (CB77) metabolites produced by control microsomes. Vector control microsomes were incubated in the reaction mixture with 6.9 μM CB77 for 2 h. The extracted metabolites were quantified by performing high-resolution gas chromatography/high-resolution mass spectrometry after methylation. (A, B) Vector control with NADPH. (C, D) Vector control without NADPH.

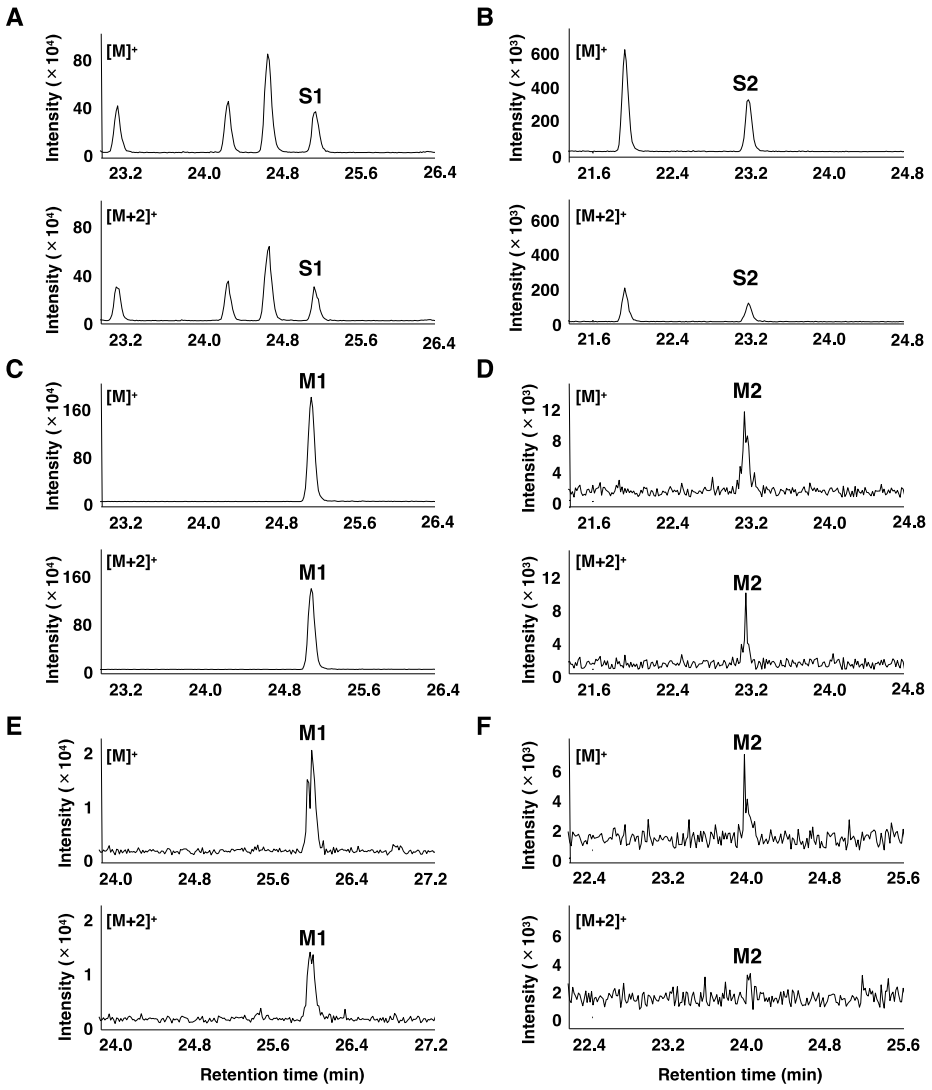


**Fig. S2** Isotope ratios of methylated 3,3′,4,4′-tetrachlorobiphenyl (CB77) metabolites produced by rat (C, D) and human (E, F) CYP1A1s. (A, B) Authentic standards: peak S1, 5-MeO-2,3′,4,4′- or 4′-MeO-3,3′,4,5′-tetrachlorobiphenyl; peak S2, 4′-MeO-3,3′,4-trichlorobiphenyl. (C, D) Rat CYP1A1 with NADPH. (E, F) Human CYP1A1 with NADPH.

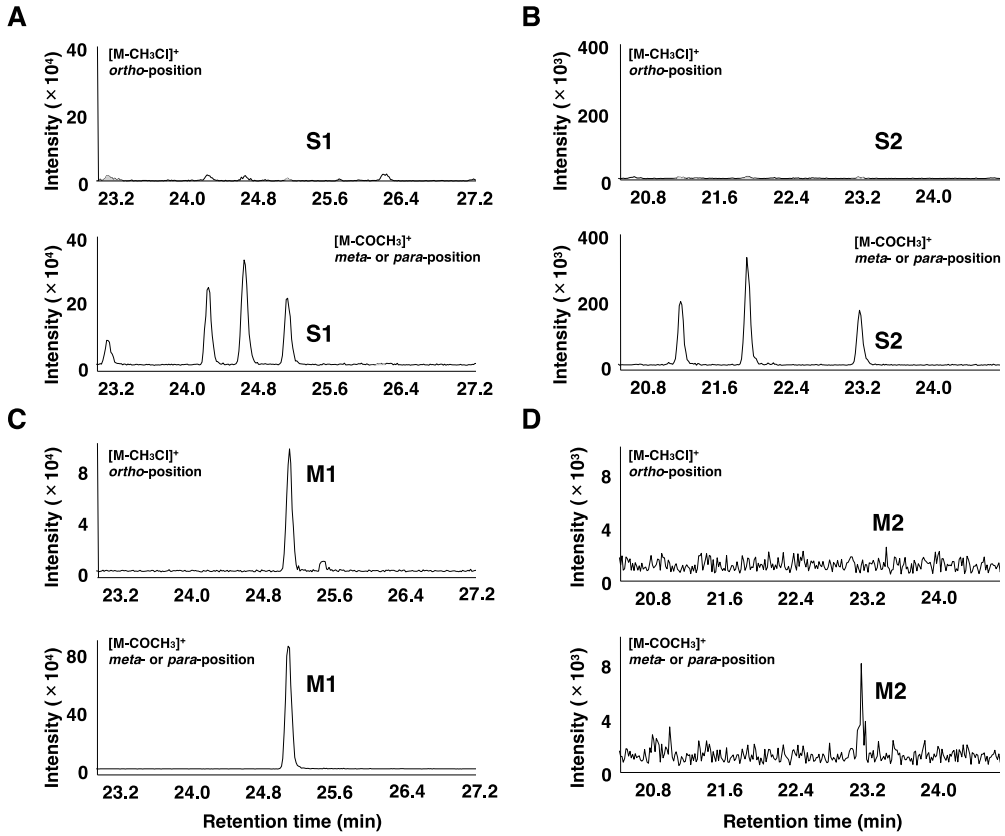


**Fig. S3** Chromatograms of fragment ions from 3,3′,4,4′-tetrachlorobiphenyl (CB77) metabolites. (A, B) Authentic standards: peak S1, 5-MeO-2,3′,4,4′- or 4′-MeO-3,3′,4,5′-tetrachlorobiphenyl; peak S2, 4′-MeO-3,3′,4-trichlorobiphenyl. (C, D) Rat CYP1A1 with NADPH.

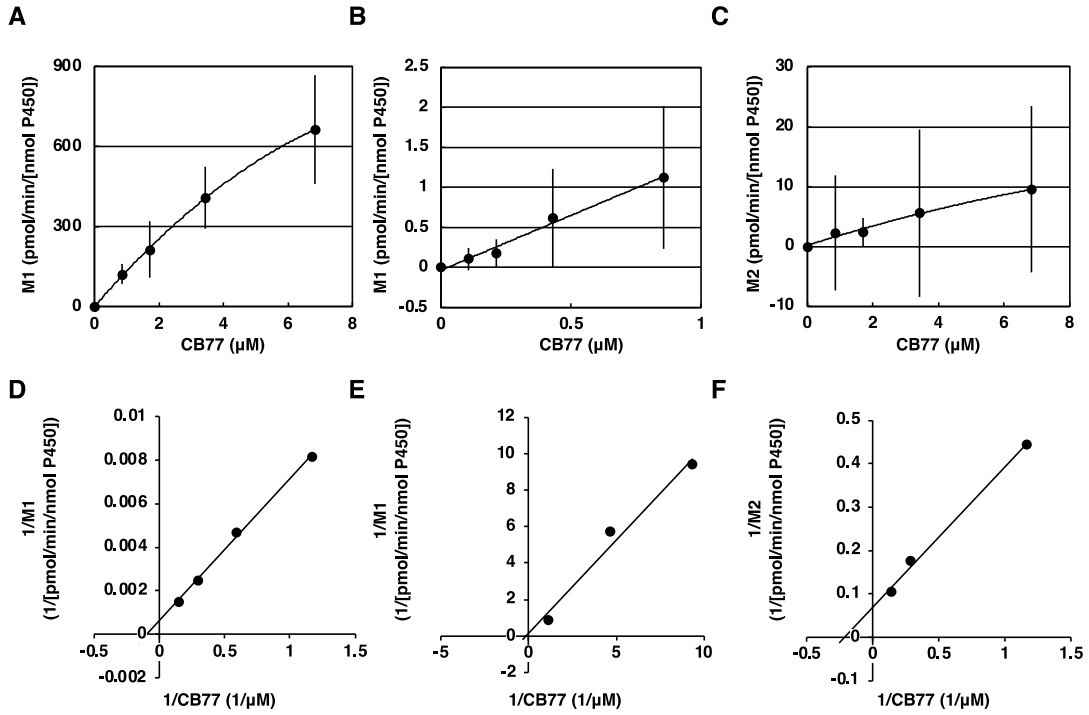


**Fig. S4** Michaelis–Menten (A–C) and Lineweaver–Burk (D–F) plots of kinetics of CYP1A1s for hydroxylation of 3,3′,4,4′-tetrachlorobiphenyl (CB77). Microsomes containing 80 nM of rat and human CYP1A1s were reacted with 0–6.9 and 0–0.86 μM CB77, respectively. The extracted metabolites were quantified by performing high-resolution gas chromatography/high-resolution mass spectrometry after methylation. All values are shown as the mean ± standard deviation of five independent experiments. (A, D) M1 predicted as 4′-OH-3,3′,4,5′-tetrachlorobiphenyl produced by rat CYP1A1. (B, E) M1 produced by human CYP1A1. (C, F) M2 identified as 4′-OH-3,3′,4-trichlorobiphenyl produced by rat CYP1A1.

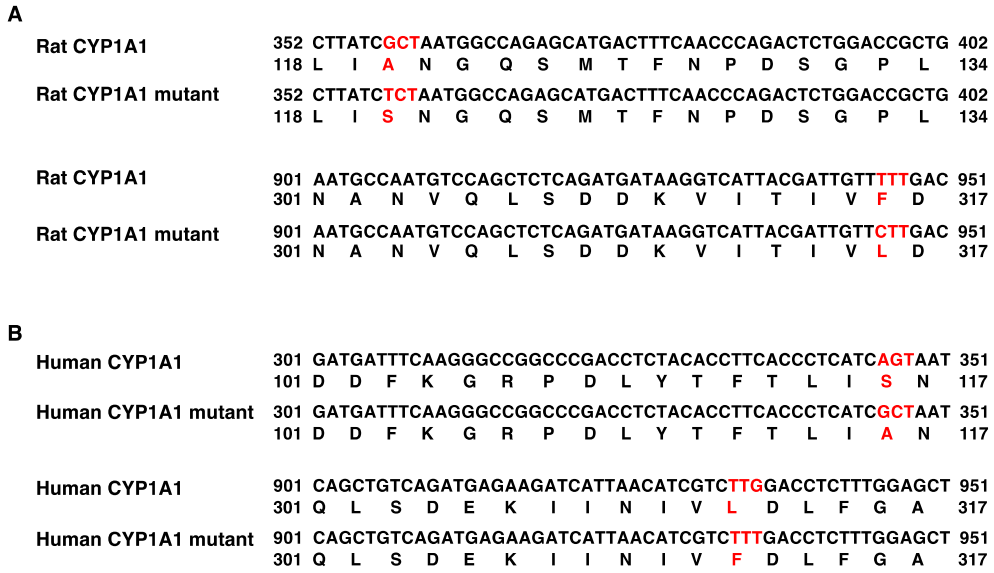


**Fig. S5** Mutation of DNA sequences of rat (A) and human (B) *CYP1A1*s and deduced amino acid sequences. Red codons and amino acids are mutated sequences.

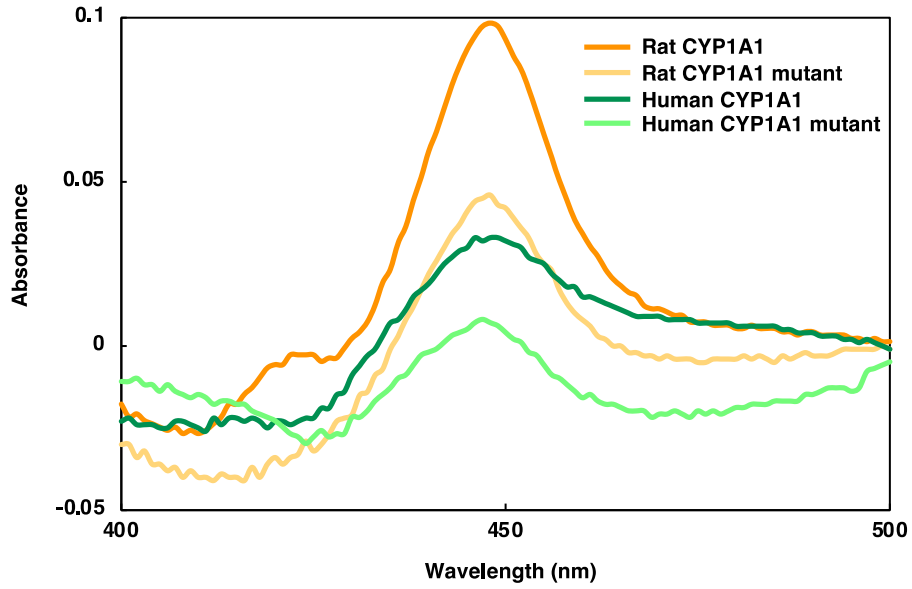


**Fig. S6** Reduced difference CO spectra of rat and human CYP1A1s and their mutants.

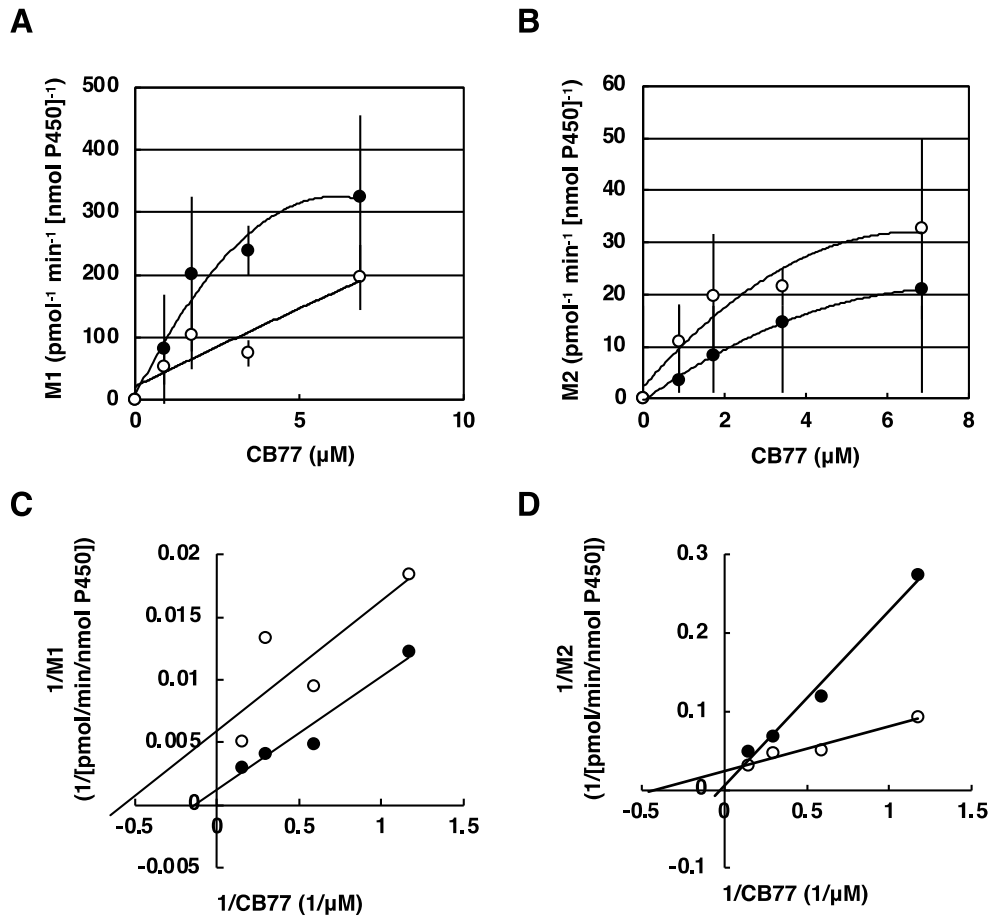


**Fig. S7** Michaelis–Menten (A, B) and Lineweaver–Burk (C, D) plots of kinetics of rat CYP1A1 and its mutant for hydroxylation of 3,3′,4,4′-tetrachlorobiphenyl (CB77). Microsomes containing 80 nM of rat CYP1A1s and its mutant were reacted with 0–6.9 μM CB77. The extracted metabolites were quantified by performing high-resolution gas chromatography/high-resolution mass spectrometry after methylation. All values are shown as the mean ± standard deviation of five independent experiments. (A, C) M1 predicted as 4′-OH-3,3′,4,5′-tetrachlorobiphenyl produced by rat CYP1A1 and its mutant indicated by closed and open circles, respectively; (B, D) M2 identified as 4′-OH-3,3′,4-trichlorobiphenyl produced by rat CYP1A1 and its mutant indicated by closed and open circles, respectively.

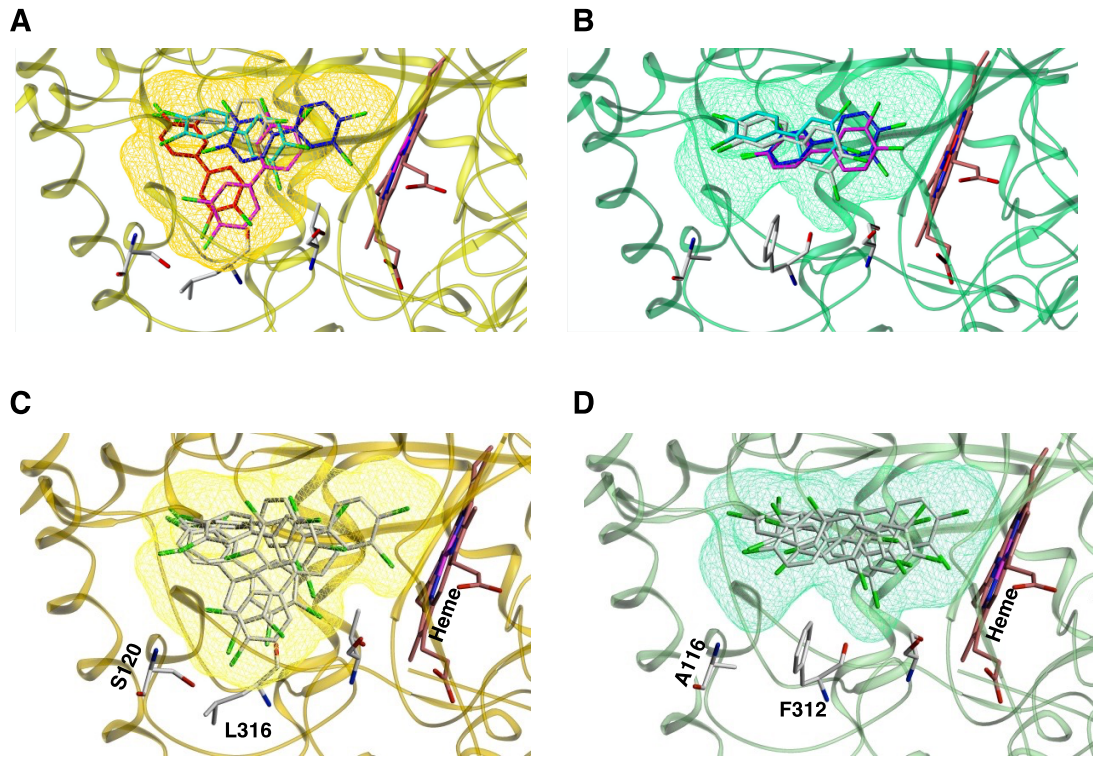


**Fig. S8** Docking models of rat (A) and human (B) CYP1A1 mutants with 3,3′,4,4′-tetrachlorobiphenyl (CB77). Connolly channel surfaces of the cavities are displayed using a colored mesh. (A, B) Representative orientations of CB77 in the substrate-binding cavity of rat (five orientations) and human (four orientations) CYP1A1 mutants; (C, D) Conformations of CB77 close to the heme iron in rat (six conformations) and human (five conformations) CYP1A1 mutants.

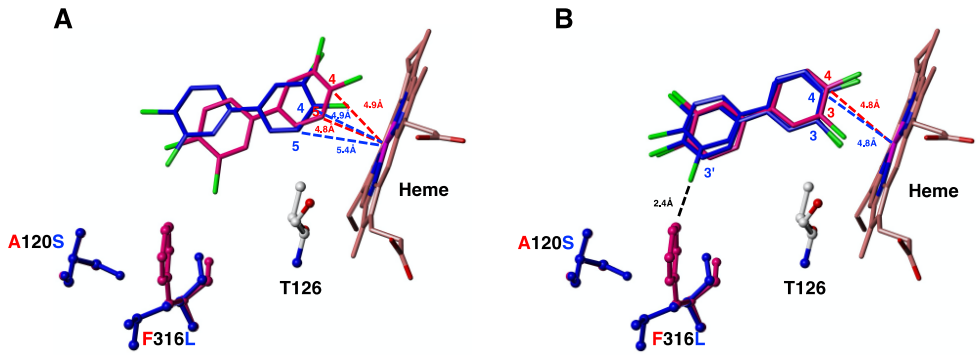


**Fig. S9** Conformations of 3,3′,4,4′-tetrachlorobiphenyl (CB77) to produce the metabolites M1 and M2 by rat CYP1A1 and its mutant. (A) Difference in conformations to produce M1, predicted as 4′-OH-3,3′,4,5′-tetrachlorobiphenyl, between rat CYP1A1 and its mutant. (B) Difference in conformations to produce M2, identified as 4′-OH-3,3′,4-trichlorobiphenyl, between rat CYP1A1 and its mutant. CB77 structures shown in red and blue represent CB77 docked to rat CYP1A1 and its mutant, respectively. The amino acids in red and blue represent rat CYP1A1 and its mutant, respectively.

**Table S1** Sequences of the primers used in this study.

| Primer | Sequence |
|---|---|
| t946c as | 5′-CTCCAAAGAGGTCAAGAACAATCGTAATGACCTTATCATCTGAG-3′ |
| g358t as | 5′-CATGCTCTGGCCATTAGAGATAAGTGTGAAGCTGT-3′ |
| g936t as | 5′-GCTCCAAAGAGGTCAAAGACGATGTTAATGATCTTCTCATC-3′ |
| a346g g347c as | 5′-GGACATGCTCTGACCATTAGCGATGAGGGTGAAGGTGTAC-3′ |

**Table S2** 7-Ethoxycoumarin *O*-deethylation activity of CYP1A1s and its mutants.

| P450 | P450 content[1] | 7-Ethoxycoumarin *O*-deethylation[2,3] |
|---|---|---|
| Rat CYP1A1 | 110 | 31.5 ± 1.46 |
| Rat CYP1A1 mutant | 24.2 | 27.8 ± 1.33** |
| Human CYP1A1 | 15.8 | 0.544 ± 0.0470 |
| Human CYP1A1 mutant | 7.61 | 1.2 ± 0.164** |

[1] pmol/mg; [2] nmol/min/(nmol P450); [3] All values are mean ± standard deviation of five independent experiments. **, $p < 0.01$ by Student's *t*-test (wild-type)