SUPPORTING INFORMATION

TABLE 1: Sequences of Oligonucleotides Used for Mutagenesis

mutant	sense oligonucletide	complementary oligonucletide
WT[a]	AGGAGGGACTGAAGGAGGAGTCGGGCTTTCTGCG	CGCAGAAAGCCCGACGCCTCCTTCAGTCCCTCCTG
E216A	AGGAGGGACT**T**AAGGAGGCGTCGGGCTTTCTGCG	CGCAGAAAGCCCGACGCCTCCTTAAGTCCCTCCTG
E216L	AGGAGGGACT**T**AAGGAGTTGTCGGGCTTTCTGCG	CGCAGAAAGCCCGACAACTCCTTAAGTCCCTCCTG
E216H	AGGAGGGACT**T**AAGGAGCATTCGGGCTTTCTGCG	CGCAGAAAGCCCGAATGCTCCTTAAGTCCCTCCTG
E216N	AGGAGGGACT**T**AAGGAGAACTCGGGCTTTCTGCG	CGCAGAAAGCCCGAGTTCTCCTTAAGTCCCTCCTG
E216Q	AGGAGGGACT**T**AAGGAGCAATCGGGCTTTCTGCG	CGCAGAAAGCCCGATTGCTCCTTAAGTCCCTCCTG
E216D	AGGAGGGACT**T**AAGGAGGACTCGGGCTTTCTGCG	CGCAGAAAGCCCGAGTCCTCCTTAAGTCCCTCCTG
E216K	AGGAGGGACT**T**AAGGAGAAGTCGGGCTTTCTGCG	CGCAGAAAGCCCGACTTCTCCTTAAGTCCCTCCTG

[a]The corresponding wild type sequence (WT) is shown on the first row for comparison.

Table 2: Alignment of P450 2C5 (P_1TD6) and P450 2D6 (CYP2D6) sequences used to generate the homology model developed in this work. P450 2D6 residues in upper case represent regions designated as structurally conserved whereas those in lower case represent loops built *de novo*.

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                    50
P_1DT6:                                        PPGPTPFPIIGNILQID
CYP2D6: mglealvplavivaifllllvdlmhrrqrwaaryPPGPLPLPGLGNLLHVD

         51                                                   100
P_1DT6: AKDISKSLTKFSECYGPVFTVYLGMKPTVVLHGYEAVKEALVDLGEEFAG
CYP2D6: FQNTPYCFDQLRRRFGDVFSLQLAWTPVVVLNGLAAVREALVTHGEDTAD

        101                                                  150
P_1DT6: RGSVPILEK---VSKGLGIAFS-NAKTWKEMRRFSLMTLRNFGMGKRSIE
CYP2D6: RPPVPITQIlgfGPRSQGVFLArYGPAWREQRRFSVSTLRNLGLGKKSLE

        151                                                  200
P_1DT6: DRIQEEARCLVEELRKTNASPCDPTFILGCAPCNVICSVIFHNRFDYKDE
CYP2D6: QWVTEEAACLCAAFANHSGRPFRPNGLLDKAVSNVIASLTCGRRFEYDDP

        201                                                  250
P_1DT6: EFLKLMESLHENVELLGTP|---------LDYFPGIHKTLLKNADYIKNF
CYP2D6: RFLRLLDLAQEGLKEESGFlrevlnavpvllhIPALAGKVLRFQKAFLTQ

        251                                                  300
P_1DT6: IMEKVKEHQKLLDVNN-PRDFIDCFLIKMEQENN---LEFTLESLVIAVS
CYP2D6: LDELLTEHRMTWDPAQpPRDLTEAFLAEMEKAKGnpeSSFNDENLRIVVA

        301                                                  350
P_1DT6: DLFGAGTETTSTTLRYSLLLLLKHPEVAARVQEEIERVIGRHRSPCMQDR
CYP2D6: DLFSAGMVTTSTTLAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQ

        351                                                  400
P_1DT6: SRMPYTDAVIHEIQRFIDLLPTNLPHAVTRDVRFRNYFIPKGTDIITSLT
CYP2D6: AHMPYTTAVIHEVQRFGDIVPLGVTHMTSRDIEVQGFRIPKGTTLITNLS

        401                                                  450
P_1DT6: SVLHDEKAFPNPKVFDPGHFLDESGNFKKSDYFMPFSAGKRMCVGEGLAR
CYP2D6: SVLKDEAVWEKPFRFHPEHFLDAQGHFVKPEAFLPFSAGRRACLGEPLAR

        451                                                  500
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P_1DT6: MELFLFLTSILQNFKLQSLV-EPKDLDITAVVNGFVSVPPSYQLCFIPIHH
CYP2D6: MELFLFFTSLLQHFSFSVPTgQPRP-SHHGVF-AFLVSPSPYELCAVPR

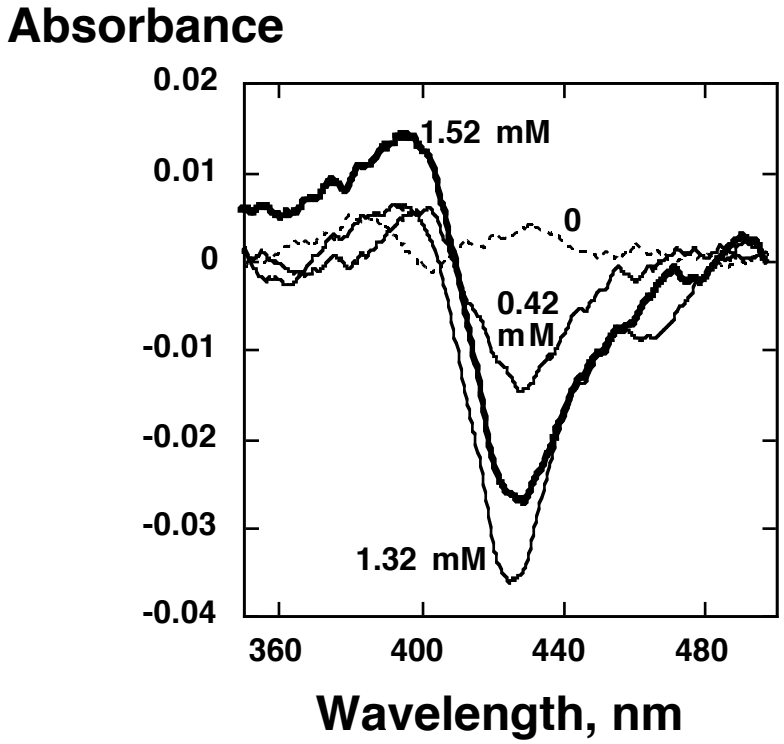

FIGURE 1: Binding of bufuralol to P450 2D6 E216H. The general procedure described in Figure 1 of the main text and the Experimental Procedures was used with P450 2D6 E216H (3 μM P450 in 0.10 M potassium phosphate buffer, pH 7.0, containing 45 μM di-12:0 GPC) except that a reference cuvette was utilized with the same concentration of P450. Additions shown are for 0, 0.42, 1.31, and 1.52 mM bufuralol.

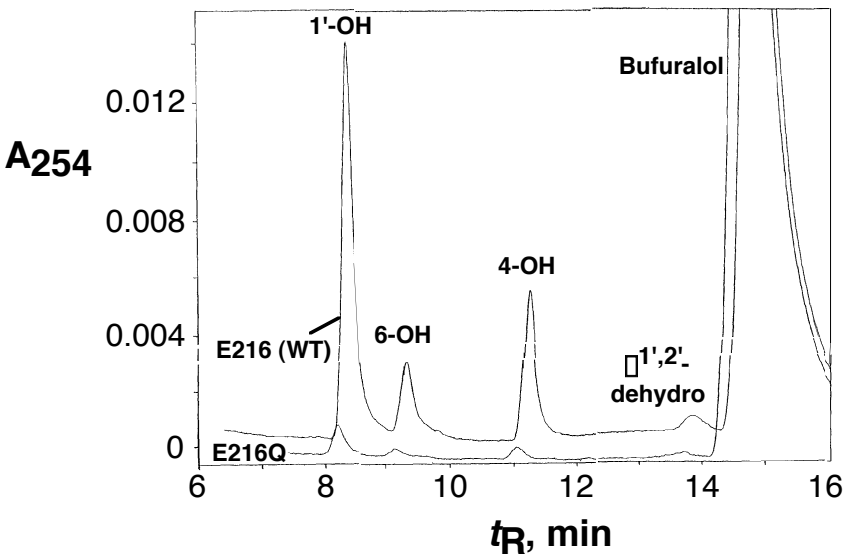

FIGURE 2: Effect of substitution of Gln for Glu at position 216 of P450 2D6 on bufuralol oxidation activity. Assays were done with 0.4 μM P450 2D6 and 100 μM bufuralol for 5 min at 37 °C and, following deproteinization, 50 μL aliquots were analyzed by HPLC. Traces are shown for wild type P450 2D6 (WT) (upper trace) and P450 2D6 E216Q (lower trace), with the four products identified (1´-hydroxybufuralol, 6-hydroxybufuralol, 4-hydroxybufuralol, and $\Delta^{1',2'}$-dehydrobufuralol). t_R = retention time.

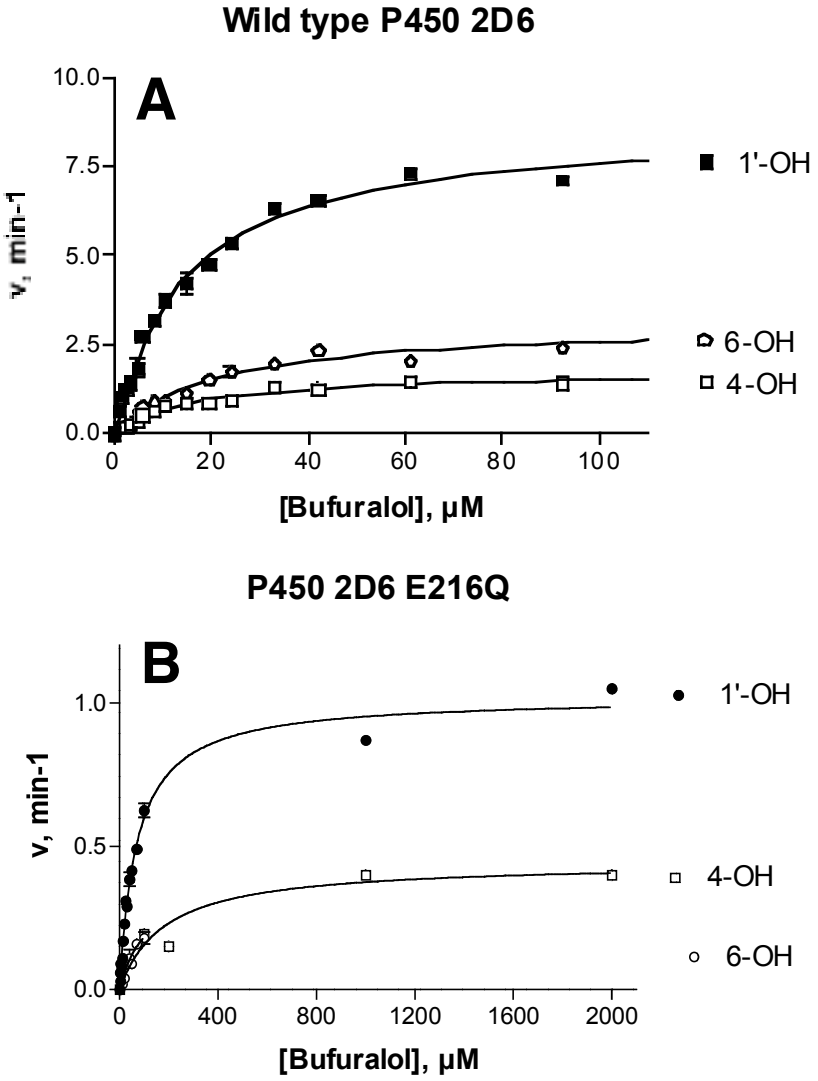

FIGURE 3: Bufuralol hydroxylation by wild type P450 2D6 (Asp301) (A) and the E216Q mutant (B). The rates of formation of the three major products are indicated.

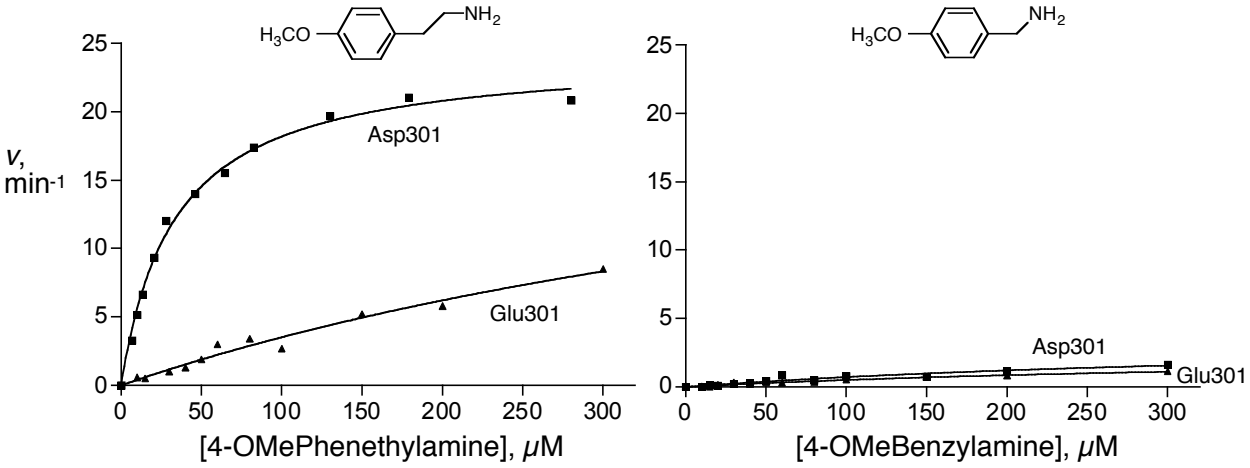

FIGURE 4: *O*-Demethylation of 4-methyoxyphenethylamine (*A*) and 4-methoxybenzylamine (*B*) by wild type P450 2D6 (Asp301) (■) and the D301E mutant (Glu301) (▲).