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ORIGINAL PAPER

Analysis of clinically relevant substrates of CYP2B6 enzyme by computational methods

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Abstract Mounting evidence thus far indicates that human cytochrome P450 2B6 (CYP2B6), an enzyme expressed at a relatively low level functionally, is primarily responsible for the metabolism of several clinically relevant drugs, including propofol, efavirenz, bupropion, mephobarbital, and the propofol analog 2,6-di-sec-butyl phenol. We used molecular dynamics and molecular docking methods to predict such interactions and to compare with experimentally measured metabolisms. Insight II and Discover Studio 2.5 were used to carry out the docking of these substrates into CYP2B6 to explore the critical residues and interaction energies of the complexes. Phe297, Glu301, Thr302 and Val367 were identified as major drug-binding residues, which is consistent with previous data on site-directed mutagenesis, crystallography structure, and from modeling and docking studies. In addition, our docking results suggest that nonpolar amino acid clusters and heme also participate in binding to mediate drug oxidative metabolism. The binding modes of the five clinically relevant substrates mentioned above for metabolism on CYP2B6 are presented.

Keywords CYP2B6 · Clinically relevant · Substrates · Molecular dynamics · Molecular docking

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Introduction

Cytochrome P450s (CYPs) constitute a superfamily of heme-containing biotransformation enzymes involved in the oxidative metabolism of a wide variety of endogenous and exogenous compounds [1, 2]. P450 2B6 (CYP2B6) is the only functional member of the human CYP2B family, and it was initially regarded as a minor enzyme in the overall xenobiotic metabolism [3, 4]. More recent publications recognize rising numbers of clinically relevant CYP2B6 substrates, and cite an estimate of the contribution of CYP2B6 to drug metabolism of about 8% [5]. CYP2B6 participates in the oxidative metabolism of several clinically important drugs, including the anesthetic propofol [6], the antiretroviral agent efavirenz [7], the antidepressant bupropion [8], the antiepileptic agent mephobarbital [9], and the propofol analog 2,6-di-sec-butyl phenol.

Propofol is a short-acting anesthetic commonly used in clinical practice. The ~40% oxidation of propofol via ring hydroxylation is catalyzed by cytochrome P450 [10]. Several lines of evidence suggest that CYP2B6 is the principal CYP isoform involved in the metabolism of propofol by human liver [11]. Efavirenz, a first generation non-nucleoside reverse transcriptase inhibitor of HIV-1, is one of the preferred components of the first-line treatment regimen for HIV infection worldwide [12, 13]. Efavirenz is used as a probe compound when studying induction or inhibition. The antidepressant and antismoking agent bupropion is extensively metabolized to three principal metabolites: hydroxybupropion, erythrohydrobupropion, and threobupropion [8]. Hydroxybupropion is pharmacologically active, and its formation is thought to be a selective and useful model reaction for CYP2B6 in vitro [14, 15]. Mephobarbital (5-ethyl-1-methyl-5-phenylbarbituric acid) has been used in the treatment of epilepsy since the 1930s,

and it undergoes extensive hepatic metabolism in humans [9]. Mephobarbital is a chiral compound that is commercially available as a racemate of *R*- and *S*-mephobarbital [16]. In addition, the *S*-enantiomer is principally *N*-demethylated [17]. CYP2B6 is the sole enzyme responsible for the *N*-demethylation of *S*-mephobarbital in human liver microsomes [9].

Computational approaches, molecular dynamics (MD) simulations and automatic docking provide a group of methods that can be used to illustrate the functions of proteins and show the binding modes of substrates. These methods are successfully used in CYP systems, and provide useful information for further studies [1]. In the present study, we used semiflexible molecular docking and molecular dynamics simulations to investigate the substrate specificity of CYP2B6 when catalyzing the metabolic activations of propofol, efavirenz, bupropion, mephobarbital and 2,6-di-sec-butyl phenol, and to try to identify the docking region and the critical residues, thus further illuminating the reaction mechanism of the ligand.

Methods

Refinement of CYP2B6

The atomic coordinates of CYP2B6 were retrieved from the Protein Data Bank [PDB ID: 3IBD] [18]. The structure of CYP2B6 in complex with the inhibitor 4-(4-chlorophenyl) imidazole (4-CPI) was determined by X-ray crystallography to a resolution of 2.0 Å [19]. The inhibitor 4-CPI, the ligand Cymal-5 and thiocyanate ions were removed from the PDB file. The molecular dynamics simulations were conducted by Discovery Studio (DS) 2.5. First, the system was relaxed by a 2000-step energy minimization using the conjugated gradient (CG) method with the heme constraint fixed until the root-mean-square (RMS) gradient energy was lower than 0.1 kcal mol^{-1} $Å^{-1}$). It was then solvated in a truncated octahedron of explicit boundary solvent water molecules with a spacing distance of 8.5 Å to account for solvent effects. A 3000-step steepest decent (SD) energy minimization followed by a 5000-step CG were carried out to improve the structure. After that, the system was gradually heated from 50 to 300 K over 60 ps, and then equilibration dynamics were performed at 300 K for 100 ps. Finally, 500 ps production dynamics simulations were conducted in the NPT ensemble at 1 atm and 300 K. A time step of 1 fs and a nonbonding interaction cutoff distance of 10 Å were used. The CHARMm force field [20] was assigned for the protein. After all of these, the Analyze Trajectory protocol was used for the clustering analysis to get the total energy and the root-mean-square deviation (RMSD) for the last 300 ps of the 500 ps production dynamics simulations, as shown in Fig. 1. The structure was checked using Profile-3D and PROCHECK [21]. All of the figures were created with Pymol [22].

Docking study

The initial structures of four substrates were obtained from the DrugBank database [23], and then optimized in the Ampac/Mopac module with the Consistent Valence Force-Field to convergence. These drugs were propofol (DrugBank: DB00818), efavirenz (DrugBank: DB00625), bupropion (DrugBank: DB01156) and mephobarbital (DrugBank: DB00849). The substrate 2,6-di-sec-butyl phenol was built by the Builder module of Insight II [24]. The two-dimensional structures of all of these drugs are shown in Fig. 2.

The Affinity [24] module of Insight II was employed to dock five substrates into CYP2B6. In Affinity, a combination of Monte Carlo (MC) and Simulated Annealing (SA) methods was used to search for the optimal orientation of substrate binding. To include solvent effects, the active sites of the substrate–enzyme complexes were solvated in a layer of TIP3P water molecules of radius 8 Å. The final substrate–enzyme complex structures were chosen based on the interaction energy and the geometrical match quality.

Results and discussion

Refinement of CYP2B6

Initial structures were refined by the energy minimization and MD simulations mentioned in the "Methods" section above. The RMSD vs. time profile collected during the last 300 ps of the 500 ps production dynamics is displayed in Fig. 1. Obviously, the RMSD of the system remained in equilibrium during the last 300 ps, and this indicates that

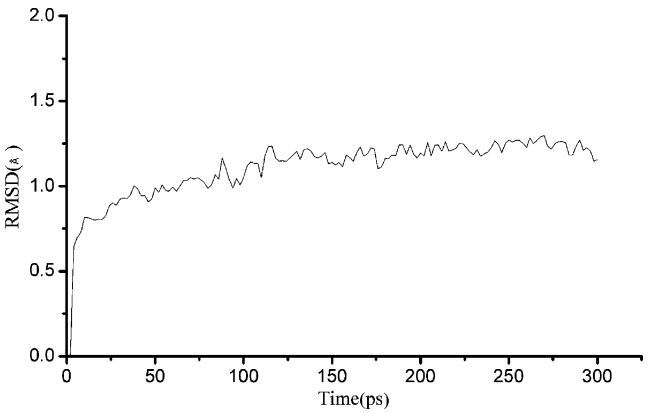

Fig. 1 The RMSD of CYP2B6 in the last 300 ps of the production simulations

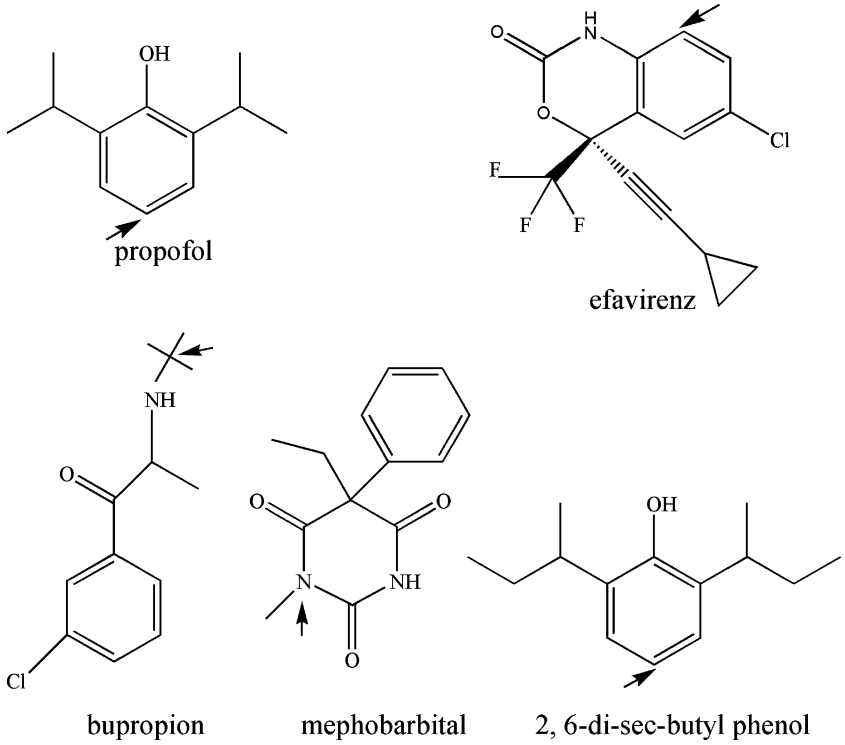

Fig. 2 Two-dimensional structures of the substrates used in this study. *Arrows* point to the known major site of reaction for each substrate

the three-dimensional (3D) model is stable and can be used in further docking studies. The final structure was checked by Profile 3D, and the self-compatibility score for this protein was 202.1, which was higher than the lowest score 108.6 and was close to the top score 210.5. The structure of CYP2B6 was then evaluated using PROCHECK. Among the 476 residues, no residue was found in the disallowed regions of the Ramachandran plot. The statistical score for the Ramachandran plot shows that 86.6% are in the most favored regions, 11.1% in the additional allowed regions, and 2.3% in the generously allowed regions. The above results indicate that the structure is reliable.

Docking propofol and 2,6-di-sec-butyl phenol into CYP2B6

The binding 3D conformations of the propofol–CYP2B6 and 2,6-di-sec-butyl phenol–CYP2B6 complexes are displayed in Fig. 3 and Fig. 4, respectively. Interaction energies were calculated between each amino acid at the active site and the substrate in order to evaluate the docking result in general and identify the significant binding-site residues in the models. A residue was considered a key residue in the substrate-binding complex if it bound the substrate with an interaction energy of less than −1 kcal mol^{-1} [25, 26]. The interaction energies of propofol and 2,6-di-sec-butyl phenol with each residue at the active site of CYP2B6 were calculated and are listed in Tables 1 and 2, respectively.

Propofol is reported to undergo ring hydroxylation by CYP to form 4-hydroxypropofol [6, 10, 11]. In the propofol–CYP2B6 complex, propofol is positioned firmly over the heme group due to hydrogen bond and hydrophobic interactions. The total interaction energy calculated for propofol with heme is −9.804 kcal mol^{-1}, which is the second largest interaction energy. The site of metabolism

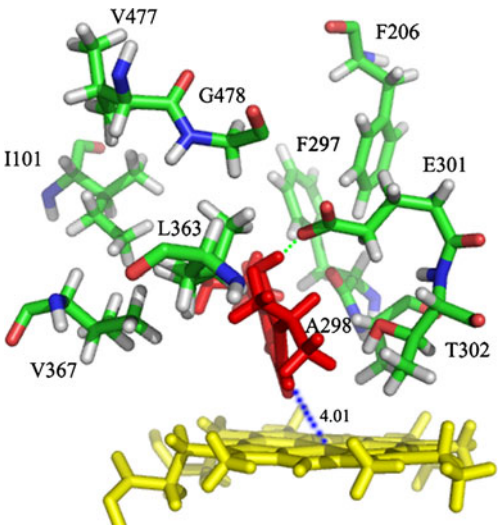

Fig. 3 The binding mode of the CYP2B6–propofol complex. The heme group and substrates are represented by *yellow* and *red sticks*, respectively. The hydrogen bonds are shown as *green dotted lines*. The distance between the heme iron and the site of metabolization is displayed as a *blue dotted line*

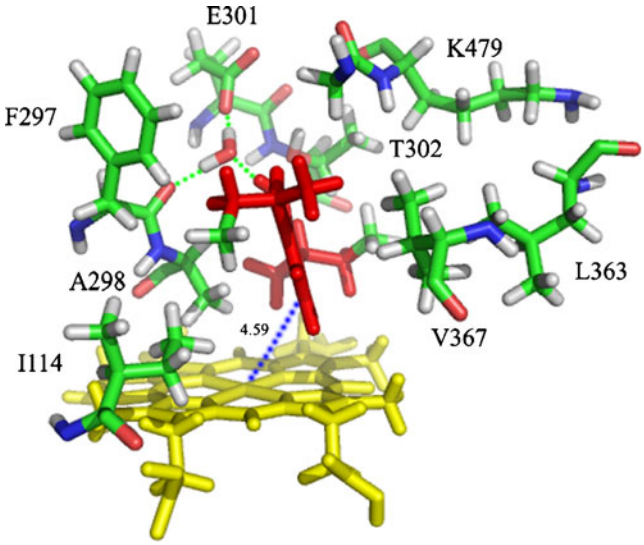

Fig. 4 The binding mode of the CYP2B6-(2,6-di-sec-butyl phenol) complex. The heme group and substrates are represented by *yellow* and *red sticks*, respectively. The hydrogen bonds are shown by *green dotted lines*. The distance between the heme iron and the site of metabolization is displayed as a *blue dotted line*

for propofol was 4.01 Å from the heme iron atom. Through the interaction analysis described above, we know that Ile101, Phe297, Ala298, Glu301, Thr302, and Val367 have interaction energies that are less than −1 kcal mol^{-1}. Glu301 has the highest electrostatic and total interaction energies, and the carboxyl O of Glu301 forms a hydrogen bond with the H of propofol (showed in Fig. 3). On the other hand, of the four nonpolar amino acids (Phe297, Ala298, Leu363 and Val477) around the benzyl group of propofol, Phe297 and Ala298 show large hydrophobic interactions with the whole system.

2,6-Di-sec-butyl phenol is an analog of propofol; the only differences between them are the substituents at the 2 and 6 positions. We therefore thought that the site of metabolism for 2,6-di-sec-butyl phenol would be the same as for propofol. In the 2,6-di-sec-butyl phenol–CYP2B6 complex, the distance between the oxidation site and the iron atom of the heme is 4.59 Å. Te heme has the largest interaction energy (−12.814 kcal mol^{-1}) with 2,6-di-sec-butyl phenol. The O atom of the phenolic group in 2,6-di-sec-butyl phenol forms two pairs of hydrogen bonds with Glu301 and Phe297 via water bridges. Phe297, Ala298, Glu301, Thr302, Val367, and Gly478 contribute large interaction energies with 2,6-di-sec-butyl phenol.

Table 1 The total energy (E_{total}), van der Waals energy (E_{vdw}) and electrostatic energy (E_{ele}) between propofol and individual residues in CYP2B6

Residue	E_{vdw} (kcal/mol)	E_{ele} (kcal/mol)	E_{total} (kcal/mol)
Total	−36.293	0.806	−35.481
Glu301	3.539	−15.235	−11.696
Heme	−8.921	−0.883	−9.804
Ala298	−3.577	0.067	−3.510
Thr302	−1.702	−0.430	−2.132
Phe297	−1.986	0.568	−1.418
Val367	−1.056	−0.235	−1.291
Ile101	−1.078	−0.107	−1.185

Table 2 The total energy (E_{total}), van der Waals energy (E_{vdw}) and electrostatic energy (E_{ele}) between 2,6-di-sec-butyl phenol and individual residues in CYP2B6

Residue	E_{vdw} (kcal/mol)	E_{ele} (kcal/mol)	E_{total} (kcal/mol)
Total	−35.508	−2.162	−37.670
Heme	−12.992	0.178	−12.814
Ala298	−3.801	−0.943	−4.744
Phe297	−2.634	−1.284	−3.918
Glu301	−0.978	−2.551	−3.529
Gly478	−1.319	−0.088	−1.407
Val367	−1.263	0.044	−1.219
Lys479	−0.822	−0.184	−1.006

The total interaction energy of 2,6-di-sec-butyl phenol with CYP2B6 is a little more than that of propofol with CYP2B6 (−37.67 vs. −35.48), which is due to the increased hydrophobicity. The results indicate that the nature and the sizes of the substituents at the 2 and 6 positions of propofol may be critical determinants of potency. This is also consistent with experimental results, which indicate that most of the residues at the binding site of CYP2B6 are hydrophobic [19]. We also found that both Leu363 and Val477 have weak interaction energies with the two substrates.

Docking efavirenz into CYP2B6

Efavirenz is extensively metabolized in humans by the P450 system to hydroxylated metabolites that include 8-hydroxyefavirenz [7, 12, 13]. The binding 3D conformation of efavirenz–CYP2B6 is displayed in Fig. 5. The Fe–C interatomic distance in the complex is 5.67 Å. The total interaction energy between efavirenz and heme is −6.608 kcal mol^{-1}, while the vdW energy and the electrostatic energy were −7.315 kcal mol^{-1} and 0.707 kcal mol^{-1}. Docking simulations of efavirenz result in the formation of two hydrogen bonds via water molecules with Glu301 and Thr302 in the efavirenz–CYP2B6 complex. Table 3 gives the interaction energies of efavirenz with each residue at the active site of CYP2B6. From Table 3, we can see that Ile101, Phe297, Glu301, Thr302, Val367, and Gly478 were the six residues with total interaction energies that were less than −1 kcal mol^{-1}. Glu301 contributes the

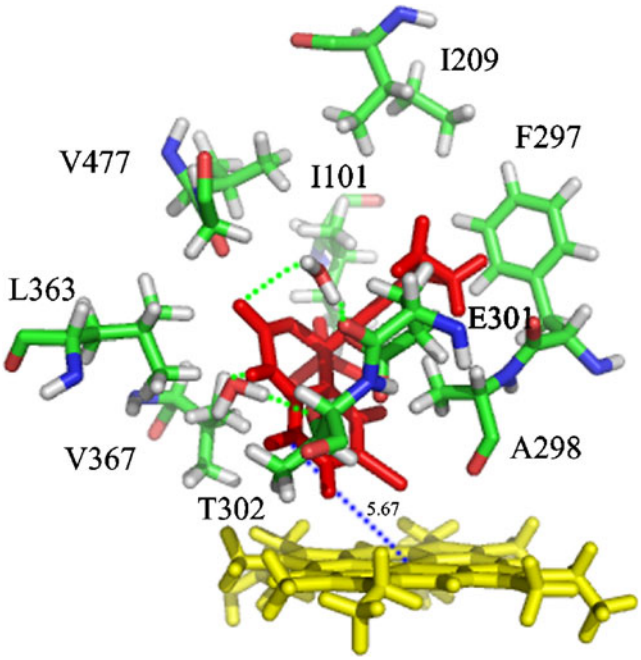

Fig. 5 The binding mode of the CYP2B6–efavirenz complex. The heme group and substrates are represented by a *yellow* and *red sticks*, respectively. The hydrogen bonds are shown as *green dotted lines*. The distance between the heme iron and the site of metabolization is displayed as a *blue dotted line*

largest interaction energy with efavirenz, just as in the propofol–CYP2B6 complex. The total energy between Leu363 and CYP2B6 is 0.516 kcal mol^{-1}, and that between Val477 and CYP2B6 is −0.297 kcal mol^{-1}.

Docking bupropion into CYP2B6

The cytochrome P450 (CYP) enzyme system, especially CYP2B6, plays an important role in bupropion hydroxylation [8, 14, 15]. The binding 3D conformation of bupropion–CYP2B6 is displayed in Fig. 6. The interaction energies of the bupropion with each residue at the active site of CYP2B6 were calculated and are listed in Table 4. In

Table 3 The total energy (E_{total}), van der Waals energy (E_{vdw}) and electrostatic energy (E_{ele}) between efavirenz and individual residues in CYP2B6

Residue	*Evdw* (kcal/mol)	E_{ele} (kcal/mol)	E_{total} (kcal/mol)
Total	−27.422	−2.030	−29.452
Glu301	−5.338	−5.102	−10.440
Heme	−7.315	0.707	−6.608
Val367	−2.162	−0.268	−2.430
Thr302	−1.235	−0.647	−1.882
Ile101	−1.103	−0.285	−1.388
Phe297	−0.933	−0.367	−1.300
Gly478	0.490	−1.555	−1.065

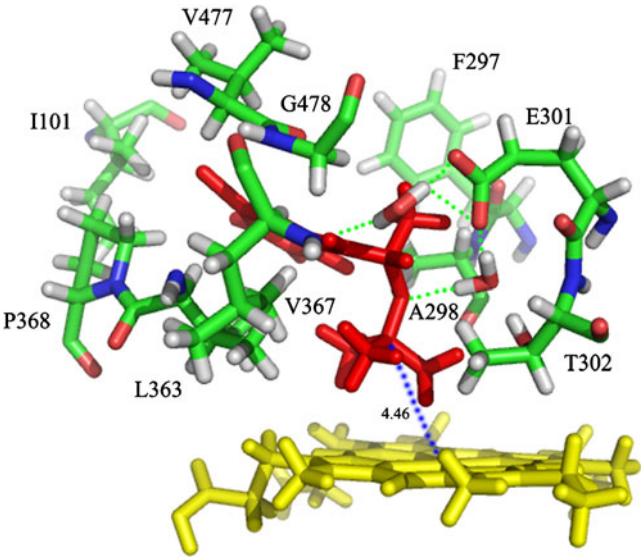

Fig. 6 The binding mode of the CYP2B6–bupropion complex. The heme group and substrates are represented by *yellow* and *red sticks*, respectively. The hydrogen bonds are shown as *green dotted lines*. The distance between the heme iron and the site of metabolization is displayed as a *blue dotted line*

examining the position and orientation of the bupropion at the active site of CYP2B6, as predicted by our docking procedure, it is observed that two residues Glu301 and Thr302 form three hydrogen bonds with the oxygen and hydrogen atoms of the amino group of bupropion via two water molecules. The distance between the oxidation site and the iron of heme is 4.46 Å. The heme exhibits the largest interaction energy with bupropion. The interaction energies of the two water molecules with the substrate bupropion and CYP2B6 were calculated. The energies between the water molecules and bupropion are −1.904 and −3.499 kcal mol^{-1}, and between the water molecules and CYP2B6 they are −6.286 and −21.606 kcal mol^{-1}. The

Table 4 The total energy (E_{total}), van der Waals energy (E_{vdw}) and electrostatic energy (E_{ele}) between bupropion and individual residues in CYP2B6

Residue	E_{vdw} (kcal/mol)	E_{ele} (kcal/mol)	E_{total} (kcal/mol)
Total	−36.968	3.099	−33.879
Heme	−9.976	−0.653	−10.629
Val367	−5.029	0.341	−4.687
Gly478	−2.015	−1.860	−3.874
Thr302	−0.957	−0.952	−1.909
Leu363	−1.805	0.012	−1.794
Val477	−1.105	−0.529	−1.634
Ala298	−2.167	0.553	−1.614
Ile101	−0.845	−0.184	−1.029
Phe297	−1.108	0.158	−0.950
Pro368	−0.500	−0.439	−0.939

water molecules have strong interaction energies with both CYP2B6 and bupropion. In addition, Glu301 moves to the edge of the active site, which differs from the behavior seen for the substrates propofol and efavirenz. This is why there is a lower interaction energy between Glu301 and bupropion. Phe297, Ala298, Glu301, Thr302, Leu363, Val367, Val477, and Gly478 are the eight amino acid residues that contribute the largest van der Waals interaction energies. The total energy between Leu363 and efavirenz is −1.794 kcal mol^{-1}, and that between Val477 and efavirenz is −1.634 kcal mol^{-1}, which differ from the corresponding energies seen for propofol and 2,6-di-sec-butyl phenol.

Docking mephobarbital into CYP2B6

Mephobarbital is metabolized by *N*-demethylation to phenobarbital [9, 16, 17]. The binding 3D conformation of mephobarbital–CYP2B6 is displayed in Fig. 7. The interaction energies of the mephobarbital with each residue at the active site of CYP2B6 were calculated and are listed in Table 5. The distance between the site of metabolism for mephobarbital and the heme iron atom is 5.81 Å. The total interaction energy with the heme is −2.439 kcal mol^{-1}, which is next to Glu301 and Phe297. A hydrogen bond is formed between mephobarbital and the residue Glu301. Two pairs of hydrogen bonds are formed between mephobarbital and Lys479 via a water molecule. Mephobarbital interacts through another hydrogen bond with Thr302 via a water molecule. In the mephobarbital–CYP2B6 complex, Glu301 contributes the largest interaction energy −7.666 kcal mol^{-1} with mephobarbital. From Table 5, we can see that Leu363 and Val 477 contribute the largest van der Waals interaction energies and total interaction energies with mephobarbital.

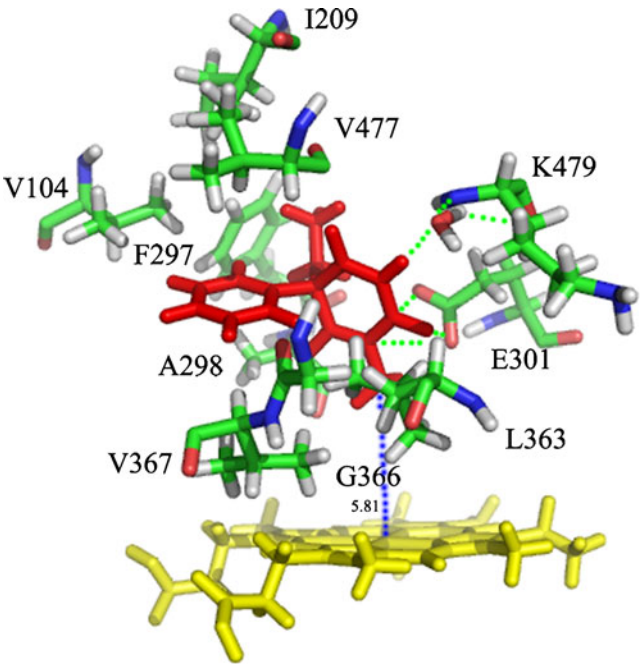

Fig. 7 The binding mode of the CYP2B6–mephobarbital complex. The heme group and substrates are represented by *yellow* and *red sticks*, respectively. The hydrogen bonds are shown as *green dotted lines*. The distance between the heme iron and the site of metabolization is displayed as a *blue dotted line*

Table 5 The total energy (E_{total}), van der Waals energy (E_{vdw}) and electrostatic energy (E_{ele}) between mephobarbital and individual residues in CYP2B6

Residue	E_{vdw} (kcal/mol)	E_{ele} (kcal/mol)	E_{total} (kcal/mol)
Total	−30.615	3.817	−26.798
Glu301	−1.986	−5.080	−7.666
Phe297	−3.086	−0.780	−3.866
Heme	−1.783	−0.656	−2.439
Val367	−2.164	0.079	−2.085
Ala298	−0.781	−1.049	−1.830
Val477	−2.277	0.645	−1.632
Leu363	−1.901	0.371	−1.530
Gly366	−1.238	−0.176	−1.414
Val104	−1.324	−0.029	−1.354
Ile209	−1.017	−0.004	−1.021
Thr302	−1.006	0.331	−0.675

In summary, the analysis of interactions between all substrates and CYP2B6 reveals that the van der Waals energy makes a larger contribution to ligand binding than the electrostatic energy. This is in line with the fact that the binding pocket of CYP2B6 is mainly composed of hydrophobic residues. The distances between the substrate site of metabolism and the heme iron atom were never greater than 6.0 Å, which is the distance used to check whether a docking pose is correct or not [1]. The heme is held into place by hydrogen bonds and strong interactions with Arg98, Trp121, Arg125, Ser430 and Arg434, which are highly conserved across family 2 enzymes. It also exhibits large interaction energies with the substrates. The docked structures of CYP2B6 exhibited a relatively large number of residues that were involved in drug interactions. The heme of CYP2B6 was also observed to play a role in each clinically relevant drug interaction.

Key residue analysis

In light of the crystal structure of CYP2B6 and the docking results, we found that the substrates occupied the same binding pocket. The positions of the active site residues are consistent with the crystal structure study of CYP2B6. Glu301 and Thr302 are two residues with polar side chains, which form hydrogen bonds with substrates directly or via water molecules. They may be the key amino acid residues,

as they show strong hydrogen bonding interactions with the substrates. In addition to Glu301 and Thr302, several other residues have been identified as the key residues responsible for substrate binding based on the docking results. Phe297 and Val367 are two critical residues for the binding of substrates to CYP2B6 according to our calculations. They exhibit large interaction energies with all of the drugs, and act as anchoring residues for substrate binding. Val477 and Leu363 show stronger interactions with bupropion and mephobarbital, but they contribute little to the interactions with propofol, efavirenz and 2,6-di-sec-butyl phenol. Among the five substrates, propofol and 2,6-di-sec-butyl phenol is a member of the phenol family, efavirenz is an oxazine, and bupropion and mephobarbital are amino-amides. The different sizes and shapes of the substrate structures are thought to be responsible for the inclusion of residues located outside of the substrate binding site.

In general, the smaller the apparent K_m (for substrates) or IC_{50} (for inhibitors), the greater the affinity an enzyme has for its ligand [25, 27]. Based on the energy analysis, we can also see that the total interaction energies between the substrates and CYP2B6 can be ordered as follows: 2,6-di-sec-butyl phenol (-37.67 kcal mol^{-1})>propofol (-35.48 kcal mol^{-1})>bupropion (-33.88 kcal mol^{-1})> efavirenz>(-29.45 kcal mol^{-1})>mephobarbital (-26.80 kcal mol^{-1}). These results are consistent with the available experimental pK_m (M) values: propofol (5.000), efavirenz (4.907) and mephobarbital (3.578) [27].

Conclusions

In this work, the X-ray crystal structure of CYP2B6 was refined by energy minimization and MD simulations. Molecular docking studies were performed to explore possible binding modes of clinically relevant drugs to CYP2B6. Using a semiflexible docking approach, five substrate–enzyme complexes were obtained. The docked results show that the five substrates present a similar binding mode for metabolism at the CYP2B6 enzyme. Glu301 and Thr302, two residues with polar side chains, make important contributions to hydrogen bond formation. Phe297 and Val367 may also be important residues according to the computational results. The results also identified residues Leu363 and Val 477 as critical residues that take part in substrate recognition. The identified binding mode of the drugs to CYP2B6 will be useful in the development of new drugs, and it also provides valuable insights into the metabolism of drugs.

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