Articles

Validation of Model of Cytochrome P450 2D6: An in Silico Tool for Predicting Metabolism and Inhibition

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There has been much interest in the development of a predictive model of cytochrome P450 2D6 particularly because this enzyme is involved in the oxidation of at least 50 drugs. Previously we have described the combined use of homology modeling and molecular docking to correctly position a range of substrates in the CYP2D6 active site with the known sites of metabolism above the heme. Here, our approach identifies correctly the site of metabolism of the atypical (no basic nitrogen) cytochrome P450 2D6 substrate, spirosulfonamide. The same method is used to screen a small compound database for cytochrome P450 2D6 inhibition. A database containing 33 compounds from the National Cancer Institute database was docked into our cytochrome P450 2D6 homology model using the program GOLDv2.0. Experimental IC_{50} values for the 33 compounds were determined; comparison with the corresponding docked scores revealed a correlation with a regression coefficient of $r^2 = 0.61$ ($q^2 = 0.59$). The method was able to discriminate between tight and weak binding compounds and correctly identified several novel inhibitors. The results therefore suggest that our approach, which combines homology modeling with molecular docking, has produced a useful predictive in silico tool for cytochrome P450 2D6 inhibition, which is best used as one filter in a multifilter database screen.

Introduction

Cytochromes P450 are a large superfamily of heme-containing monooxygenases responsible for the oxidative metabolism of a wide variety of substrates. Human cytochrome P450 2D6 (CYP2D6) has been the subject of much research. CYP2D6 plays a key role in the metabolism of many drugs in current clinical use including antiarrhythmics, antidepressants, antipsychotics, β-blockers, and analgesics.[1] CYP2D6 is also susceptible to inhibition by other drugs, for example, quinidine,[2] fluoxetine,[3] and ritonavir,[4] which can give rise to potentially significant drug−drug interactions.[5]

Developing new drugs is a lengthy and very expensive process, with no guarantee that the product will be completely free of harmful side effects or drug−drug interactions. A major advance in drug development would therefore be the prediction of drug−drug interactions at an early stage of development. This would shorten the time required to bring a new drug to the market and reduce its failure rate in clinical trials. A breakthrough in this direction would be the availability of a reliable in silico model of CYP2D6 inhibition.

There are several reports of in silico methods for predicting binding affinity against CYP2D6, for example, recursive partitioning[6−8] and pharmacophore modeling[9,10] using two-dimensional properties and chemical structures of CYP2D6 inhibitors. Two-dimensional methods are very fast, but they do not provide information about binding modes. Here, we describe an in silico method that utilizes molecular docking and a three-dimensional homology model of CYP2D6. The advantage of an in silico method over high-throughput screening (HTS) is that it can be used to screen virtual libraries and eliminate compounds prior to synthesis. In a recent review article[11] the results from several molecular docking applications are compared very favorably with high-throughput screening. In one example, molecular docking and HTS were used to screen commercially available compounds against protein tyrosine phosphate-1B.[12] Molecular docking gave an enriched hit rate over a random screen, and the hits were judged to be more druglike than the HTS hits.

In our method the molecular docking program GOLDv2.0,[13] with the fitness function ChemScore,[14,15] is used to dock compounds into our CYP2D6 model. The ChemScore function was chosen because unlike the Goldscore function it has been parametrized against binding affinities.[14] We have shown previously that the CYP2D6 homology model can yield results consistent with known patterns of oxidation of basic CYP2D6 substrates, codeine and 1-methyl-4-phenyl-1,2,3,6-tetra-

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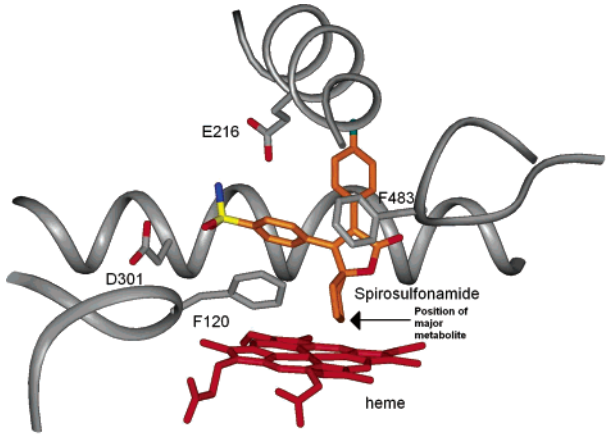


Figure 1. Docked structure of the atypical substrate spirosulfonamide in the CYP2D6 homology model. The heme moiety and key residues are also shown.

hydropyridine (MPTP).[16] Here, we show that the method can be extended to a substrate devoid of a basic nitrogen, spirosulfonamide. We also apply the method to dock several small databases of druglike compounds into our CYP2D6 homology model.[16] The first two databases, the Ekins[9] training set and the Strobl[10] training set contain 21 and 30 CYP2D6 inhibitors with known $K_{i(apparent)}$ values, respectively. The third database contains 33 druglike compounds randomly selected from the National Cancer Institute (NCI) database (NCI set).

There are two main objectives of this study. First, we aim to develop an in silico method capable of screening out CYP2D6 inhibitors from a database of potential drug candidates and additionally to investigate if a three-dimensional method can give improved results over faster two-dimensional approaches and provide additional useful structural information. Second, these results can then be used to assess the quality and predictive accuracy of our CYP2D6 model. Molecular docking optimizes favorable contacts between ligands and receptors to estimate binding modes; therefore, good results rely on an accurate three-dimensional representation of the binding site. Most docking applications utilize X-ray crystal structures, but here, a homology model of CYP2D6 is used. Therefore, a comparison between the docking results and experiment provides an assessment of the accuracy of our model.

Results and Discussion

As a first validation exercise of our CYP2D6 homology model,[16] we docked two typical basic nitrogen-containing substrates, codeine and MPTP, into the model. The results have been described elsewhere.[16] In brief, for both substrates the docked solutions positioned known sites of metabolism above the heme. A recent article[17] questioned the ability of CYP2D6 homology models to predict the binding modes of substrates devoid of a basic nitrogen. We therefore docked the substrate spirosulfonamide into our CYP2D6 model using the protocol described in the Experimental Section. The best ranked docked solution of spirosulfonamide is shown in Figure 1. The docking positioned the cyclopentyl moiety above the heme and hence correctly identified a major metabolite.[17] We can therefore conclude that our CYP2D6 model can yield results consistent with known patterns of oxidation of a range of substrates.

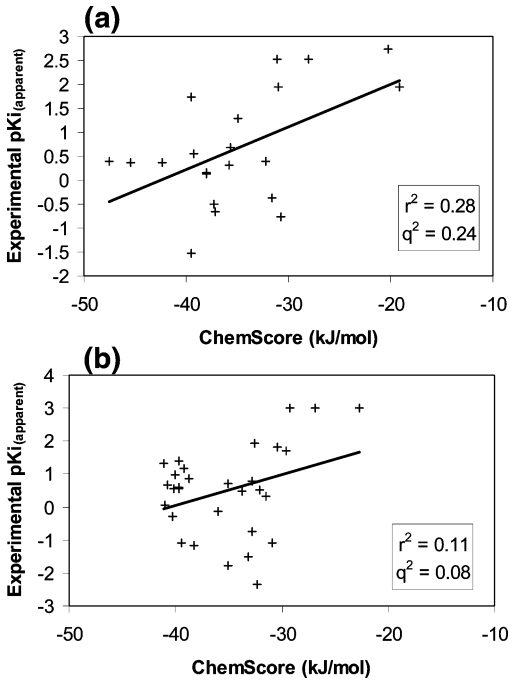


Figure 2. For each inhibitor in (a) the Ekins training set and (b) the Strobl training set, the value of the ChemScore (kJ/mol) fitness function for the best ranked docked solution is plotted against the experimental $pK_{i(apparent)}$. There is no significant correlation between the ChemScore values and the experimental $pK_{i(apparent)}$.

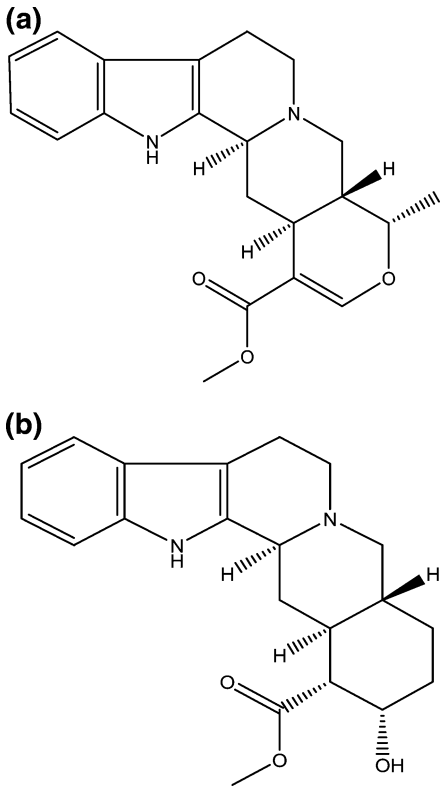
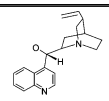
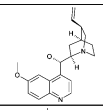
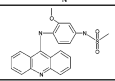
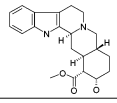


Figure 3. Two-dimensional structures of (a) ajmalicine and (b) yohimbine, members of the indole alkaloid family of compounds.

To test whether our model can be used to screen compounds for CYP2D6 inhibitors, two small databases of druglike molecules were assembled. The databases

Table 1. IC_{50}, K_i, and ChemScore Values[a]

Compound	Chemical Structure	IC_{50} (μM)	K_i (μM)	ChemScore (kJ/mol)
Cinchonine		1.8 ± 0.5	21	-39.6
Quinidine		0.015 ± 0.006	0.030	-39.5
Cinchonidine		10.5 ± 1	7.5	-38.8
Yohimbine		0.0084 ± 0.0007	0.18	-32.8

[a] Experimental IC_{50} (mM) values were obtained for a selection of Strobl[9,10] inhibitors. The table lists the compounds, their chemical structures, experimental IC_{50} (μM) values, published K_i values,[9,10] and ChemScore (kJ/mol) values of the best ranked docked solutions. The compounds are ranked from the highest to lowest ChemScore values.

Table 2. Individual Contributions to the ChemScore Value for Ajmalicine and Quinidine[a]

Compound	$\Delta G'_{binding}$ (kJ/mol)	S_{hbond}	S_{metal}	S_{lipo}	E_{clash}
Ajmalicine	32.3	0.0	0.0	335.4	9.3
Quinidine	39.5	1.0	0.0	262.9	2.2

[a] $\Delta G'_{binding}$ is the ChemScore function used in GOLD. S_{hbond}, S_{metal}, and S_{lipo} are scores for hydrogen-bonding, acceptor−metal, and lipophilic interactions resepectively. E_{clash} is the protein−ligand clash penalty term.

contained 21 and 30 compounds with published inhibition data for CYP2D6 (Ekins[9] training set and the Strobl[10] training set).

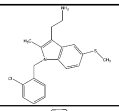
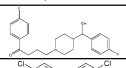
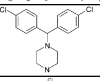
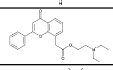
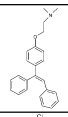
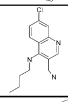
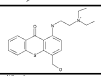
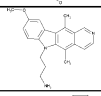
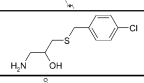
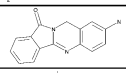
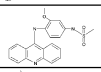
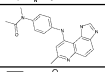
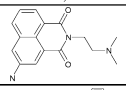
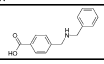
Each compound in the database was docked into the CYP2D6 model, and the value of the ChemScore fitness function for the best ranked solution is plotted against the published $pK_{i(apparent)}$ in Figure 2. There is no significant correlation for either the Ekins training set (a) $r^2 = 0.28$ ($q^2 = 0.24$) or the Strobl training set (b) $r^2 = 0.11$ ($q^2 = 0.08$).[18]

From Figure 2b it is noticeable that ChemScore underpredicts the strength of binding of several tight binding compounds. From the Strobl training set many of the ajmalicine and yohimbine derivatives are the underpredicted.[10] They are bulky molecules with five fused rings, and the two-dimensional structures of ajmalicine and yohimbine are shown in Figure 3. If the 12 yohimbine and ajmalicine derivatives in the Strobl training set are removed from the regression, the r^2 improves significantly from $r^2 = 0.11$ to $r^2 = 0.56$. Although GOLDv2.0 performs flexible ligand docking, it treats ring systems and the protein as rigid. It is therefore difficult to find the correct binding modes of the large and inflexible compounds without suffering heavy protein−ligand clashes. The breakdown of the ChemScore energy components for the yohimbine and ajmalicine derivatives in the Strobl training set show the clash term has added large penalties to the final score; the average clash penalty for these compounds is 7.0 kJ/mol. In comparison, the average clash penalty for the quinidine derivates in the Strobl training set is 2.9 kJ/mol. Table 2 lists the hydrogen bond energies, acceptor−metal and lipophilic interactions, and the clash penalty contributions to the final ChemScore values for the best ranked ajmalicine and quinidine solutions. The results show that the predicted binding mode of quinidine clashes less with the heavy atoms of the CYP2D6 model than the predicted binding mode of ajmalicine.

The Ekins training set only contains one compound with a large ring system, LY333531; the docking positioned it on the surface of the protein instead of trying to fit it into the rigid active site. The ChemScore function underestimated the binding affinity of LY333531 and removing it from the regression improved the r^2 from $r^2 = 0.28$ to $r^2 = 0.36$. Considering the nature of the training sets and the inability of the method to treat ring systems as flexible, it is perhaps not surprising that the regression is disappointing.

We decided to assemble a third database consisting of compounds from the NCI database[19] (NCI set). The NCI database was chosen because it lists compounds

Table 3. Experimental IC_{50} (μM) and ChemScore Values for 33 NCI Compounds[a]

NCI Number	Chemical Structure	IC_{50} (μM)	ChemScore (kJ/mol)
17383		3.5 ± 0.3	-47.7
169453		2.6 ± 0.1	-43.5
343513		1.6 ± 0.2	-41.9
142496		7.3 ± 0.7	-40.7
10777		2.1 ± 0.5	-39.7
293015		8.7 ± 1.6	-39.1
180973		7.2 ± 1	-37.0
13239		2.2 ± 0.4	-36.7
142982		1.5 ± 0.1	-35.5
176327		2.4 ± 0.2	-34.7
24915		8 ± 1	-34.5
320846		48 ± 5.2	-33.9
249992		10.2 ± 2.8	-33.3
305884		18.1 ± 1.8	-33.2
308847		39.6 ± 3.5	-33.0
122451		570 ± 175	-32.5
163376		554 ± 120	-30.8
320000		8.5 ± 1	-30.5
321803		196 ± 65	-30.5
278214		11 ± 1.6	-30.3
3088		65.1 ± 14.2	-29.7
172112		310 ± 73	-29.4
372939		30 ± 1.8	-29.3
303812		71 ± 7	-29.4
100857		234 ± 40	-29.3
193457		265 ± 58	-28.5
165563		514 ± 59	-26.4
267213		288 ± 21	-23.0
301739		76.3 ± 10	-22.3
56410		94.3 ± 7	-18.5
314055		1017 ± 114	-15.0
329680		699 ± 87	-14.3
178248		2021 ± 411	-13.6

[a] The table lists the 33 NCI compounds, their chemical structures, experimental IC_{50} values, and ChemScore (kJ/mol) values of the best ranked docked solutions.

that have been tested for the treatment of cancer. Therefore, we hope the compounds are representative of the types of molecules in current pharmaceutical research. Initially 111 compounds were chosen from the NCI database. The compounds were chosen to be diverse and within the same weight range as the compounds in the Ekins and Strobl training sets; compounds with more than four fused rings were removed. Consistent with the majority of known 2D6 substrates, several of the compounds were included because they contained a basic nitrogen and an aromatic group. IC_{50} values for 33 of the NCI compounds were calculated by measuring the inhibition of AMMC demethylase activity of recombinant CYP2D6. This smaller subset of compounds represents those molecules that were available from NCI and ammenable for experimental study.

To test the AMMC system, a small selection of known inhibitors from the Ekins and Strobl sets were analyzed and compared with the published K_i values calculated from bufuralol 1′-hydroxylation by human liver microsomes (Table 1). When compared with the published K_i values, the AMMC system produced similar values for quinidine and cinchonidine. However, there were some differences in the rank order, which may be a reflection of differences in the assay systems and/or substrate/inhibitor interactions within the active site. The results are summarized in Table 3.

The experimental log IC_{50} values for the 33 NCI set have been plotted against the ChemScores of the best ranked docked solution in each case (Figure 4). The ChemScores correlate with the experimental log IC_{50} values with $r^2 = 0.61$ ($q^2 = 0.59$), significantly better

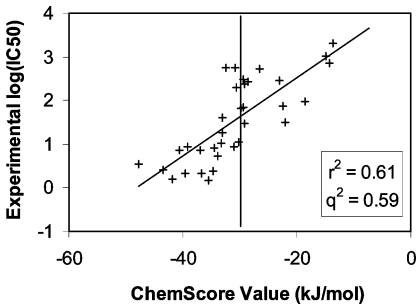


Figure 4. For each NCI compound listed in Table 3, the value of the ChemScore (kJ/mol) fitness function for the best ranked docked solution is plotted against the experimental log IC_{50}. There is good correlation between the ChemScore value and the experimental log IC_{50}.

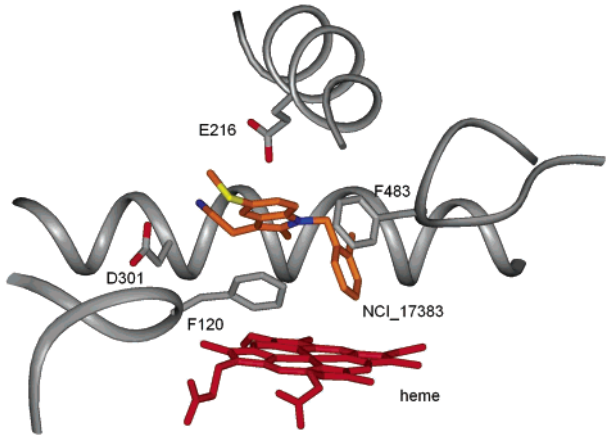


Figure 5. Docked structure of compound NCI_17383 in the CYP2D6 homology model. The heme moiety and key residues are also shown.

than random and a big improvement on the Ekins and Strobl training set predictions. It is also worth noting that the regression coefficient is better than the result obtained for the training set of 60 crystal structures in GOLDv2.0[15] where $r^2 = 0.53$ was found.

With a ChemScore of −47.7 kJ/mol, the NCI compound predicted to be the tightest binder is NCI_17383. Figure 5 shows the predicted binding mode of NCI_17383 in the CYP2D6 homology model. The method has docked NCI_17383 in the CYP2D6 active site close to the heme. Similar to many CYP2D6 inhibitors, NCI_17383 contains a basic nitrogen atom; Figure 5 shows this atom is positioned between residues E216 and D301, both of which have been identified as key to substrate binding.[16,20] The aromatic groups of NCI_17383 are packed between two important phenylalanine residues F120[21] and F483.[22] The experimental IC_{50} value for NCI_17383 is 3.5 μM (Table 3), and therefore, the method correctly predicts this compound to inhibit CYP2D6.

It is difficult to determine the number of compounds correctly predicted as inhibitors by the method because cutoff values for both the IC_{50} and the ChemScore must chosen. One option is to use an $IC_{50} < 10$ μM to define an inhibitor. When a ChemScore of −30 kJ/mol is selected as a cutoff, 20 compounds are predicted to be inhibitors. Of these, 13 have IC_{50} less than 10 μM and 7 are false positives. There are 14 compounds with ChemScore values greater than −30 kJ/mol, of which there are no false negatives; all are correctly predicted to not inhibit CYP2D6. Obviously the choice of these

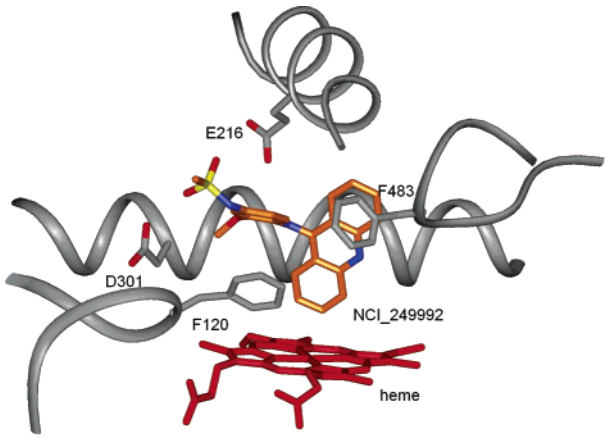


Figure 6. Docked structure of compound NCI_249992 in the CYP2D6 homology model. The heme moiety and key residues are also shown.

cutoffs could be optimized to fit the data; however, it could be argued that this is subjective and would not apply generically to different data sets.

Several NCI compounds predicted to be tight binders do not contain a basic nitrogen group. For example, NCI_249992 was docked into the CYP2D6 model with a ChemScore of −33.3 kJ/mol. The experimental IC_{50} value of NCI_249992 is 10.2 μM. The predicted binding mode of NCI_249992 is shown in Figure 6. The docking has positioned the compound in the active site close to the heme with the aromatic rings π-stacking with the two phenylalanine residues. In the absence of a basic nitrogen atom, no hydrogen bonds are formed to the two negatively charged residues E216 and D301. Hence, the method has correctly predicted novel CYP2D6 inhibitors from the NCI compounds.

From Figure 4, we can conclude that the method works well at predicting the weak binders in the NCI set; there are no false negatives (i.e., all the compounds with ChemScores greater than −30 kJ/mol are weak binders and have IC_{50} greater than 10 μM). If ChemScore less than −30 kJ/mol is taken as defining a tight binder, then the method is not as successful at predicting tight binders as weak binders; there are several false positives in the set of compounds with ChemScores less than or equal to −30 kJ/mol. The results suggest that for database screening, one can have confidence that compounds with ChemScore greater than −30 kJ/mol will not inhibit CYP2D6. However, some noninhibitors may be overpredicted and therefore missed.

The time taken to dock the 33 NCI compounds on one processor of a Silicon Graphics R14 000[23] took an average of 6.9 CPU minutes per compound. This is very slow compared to two-dimensional screening methods and even some high-throughput techniques. However, one advantage of in silico screening is that it can be applied to virtual compounds and even molecular fragments. An increase of CPU power would make the method practical for a larger database, but a more realistic use of the method would be as one filter in a multiple-filter screen. Simple property and two-dimensional filters could be applied to a very large database to reduce the numbers prior to docking. It would be an interesting study to perform such a screening exercise on a very large database. However, as we have shown, without consistent kinetic data for all the compounds

the results would be difficult to interpret. Another valuable exercise would be to apply the same techniques to a database containing CYP2D6 substrates. Simply predicting the strength of binding does not differentiate between inhibitors and substrates, but hopefully, an examination of the predicted binding modes would provide more insight into this problem, and we have shown above that we can yield results consistent with known oxidation patterns of a range of CYP2D6 substrates.

We are continuing this study by examining in more detail the differences between the compounds in the Ekins and Strobl training sets and the NCI compounds. Hopefully this analysis will help us to modify the method and improve the results, particularly for bulky hydrophobic molecules with large ring systems.

Conclusions

In summary, we have used a structure-based approach that combines homology modeling and molecular docking to correctly position codeine, MPTP, and spirosulfonamide (an atypical CYP2D6 substrate devoid of a basic nitrogen) in the CYP2D6 active site. The molecular docking program GOLDv2.0 was used with the ChemScore fitness function option. The best ranked docked solution of spirosulfonamide positioned the cyclopentyl moiety above the heme correctly identifying a major metabolite.

By use of the same method, compounds with known inhibition and 33 druglike compounds randomly chosen from the NCI database were docked into a model of CYP2D6. The molecular docking program GOLDv2.0 was used with the ChemScore fitness function option. Experimental IC_{50} values were obtained for 33 druglike (NCI) compounds, and the method predicted inhibition with a regression coefficient $r^2 = 0.61$ and $q^2 = 0.59$. The method correctly identified several NCI compounds to be CYP2D6 inhibitors, including some compounds that do not contain a basic nitrogen atom.

Thus, our approach of combining homology modeling with molecular docking provides a useful tool for predicting inhibition. We propose the method could be one filter in a database screen to eliminate compounds that may inhibit CYP2D6 and potentially give rise to drug−drug interactions.

Experimental Section

Modeling. The homology model of CYP2D6 was produced as described previously.[16] In brief, the model was produced using the comparative modeling program Modeler[24] with five structural templates: P450s cam,[25] terp,[26] eryF,[27] BM3,[28] and 2C5.[29]

Compound Selection. Ekins and Strobl Sets. Sixty-five compounds with known inhibition data for CYP2D6[9,10] were built and minimized within SYBYL.[30]

NCI Set. One-hundred-eleven druglike compounds, within the same molecular weight range as the known inhibitors (<600), were selected (and their structures downloaded) from the National Cancer Institute (NCI) database (NCI set).

Molecular Docking. Docking studies have been carried out using the program GOLDv2.0[13] with the ChemScore[9,15] fitness function. The docking was performed with a standard Genetic Algorithm (GA) protocol and an active site cavity defined as a sphere with a 20 Å radius centered on the heme Fe atom. There were 10 solutions of each ligand generated and ranked according to the value of the ChemScore fitness function. Only the best ranked solution of each ligand was included in further analysis. Inspection of the results was performed using the molecular visualization package InsightII.[31]

IC_{50} Determinations. A fluorescence-based screening approach was used to determine IC_{50} values, which was adapted from a standard Gentest protocol.[32] The probe substrate 3-[2-(*N*,*N*-diethyl-*N*-methylamino)ethyl]-7-methoxy-4-methylcoumarin (AMMC) was used, which produces the fluorescent metabolite 3-[2-(*N*,*N*-diethylamino)ethyl-7-hydroxy-4-methylcoumarin (AHMC) (λ_{Em} = 460 nm; λ_{Ex} = 390 nm) when demethylated by CYP2D6.

For the assay, reactions were performed in a 96-well microtiter plate using a final volume of 200 μL. Compounds were serially diluted 3-fold across eight lanes in 100 μL of a 2× enzyme/substrate stock solution (0.01 pmol/μL P450 and 2 μM AMMC in 100 mM potassium phosphate buffer, pH 7.4). A solvent control was included to correct for any solvent effects across the dilution range.

Plates were preincubated for 5 min at 37 °C, and the enzyme reaction was initiated by the addition of a 100 μL aliquot of prewarmed 2× NADPH generating system (16.4 μM $NADP^+$, 0.82 mM glucose 6-phosphate, 0.82 mM $MgCl_2 \cdot 6H_2O$, 1.6 U/mL glucose 6-phosphate dehydrogenase). The reaction mixture was maintained at 37 °C, and the change in emission at 460 nm was followed for 30 min using a Fluoroskan Ascent FL microtiter plate reader (Labsystems). Activity was determined from the initial linear phase of the reaction plot using Prism 3.0[33] and expressed as a percentage of the activity determined for the corresponding solvent-only control. Data were plotted and IC_{50} determinations were done using GraFit 5.0.4.[34]

Only small quantities of compounds were available from NCI (10 mg), and there was limited solubility data. Thus, extensive physicochemical analysis was not feasible and empirical determinations were carried out to optimize solubility conditions. To test for solubility, compounds were dissolved to a final concentration of 50 mM in water, 100% methanol, or 100% DMSO. Solubility was defined by the presence (insoluble) or absence (soluble) of a visible pellet following Eppendorf microfugation at 10000*g* for 1 min at room temperature. The NCI compounds 3088, 1077, 13239, 17383, 24915, 56410, 100857, 122451, 142496, 163376, 172112, 178248, 180973, 193457, 249992, 267213, 293015, 320000, 329680, and 343513 were dissolved in 100% methanol. Compounds 142982, 165563, 169453, 176327, 301739, 303812, 305884, and 314055 were dissolved in water, and compounds 269148, 278214, 308847, 320846, 321803, and 372939 were dissolved in 100% DMSO.

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Supporting Information Available: All compounds studied are listed together with ChemScore values. This material is available free of charge via the Internet at http://pubs.acs.org.

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