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The Effects of Nitrogen-Heme-Iron Coordination on Substrate Affinities for Cytochrome P450 2E1.

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Abstract

A descriptor based computational model was developed for cytochrome P450 2E1 (CYP2E1) based on inhibition constants determined for inhibition of chlorzoxazone, or 4-nitrophenol, metabolism. An empirical descriptor for type II binding was developed and tested for a series of CYP2E1 inhibitors. Inhibition constants were measured for 51 different compounds. A fast 2-dimensional predictive model was developed based on 40 compounds, and tested on 8 compounds of diverse structure. The trained model ($n=40$) had an r value of 0.76 and an RMSE of 0.48. The correlation between the predicted and actual pK_i values of the test set of compounds not included in the model gives an r^2 value of 0.78. The features that described binding include heme coordination (type II binding), molecular volume, octanol/water partition coefficient, solvent accessible surface area, and the sum of the atomic polarizabilities. The heme coordination parameter assigns an integer between 0 and 6 depending on structure, and is a new descriptor, based on simple quantum chemical calculations with correction for steric effects. The type II binding parameter was found to be important in obtaining a good correlation between predicted and experimental inhibition constants increasing the r^2 value from 0.38 to 0.77.

Keywords

P450; CYP; 2E1; type II binding; nitrogen-iron coordination; QSAR; inhibition

1.0 Introduction

Xenobiotics interacting with the hepatic microsomal cytochrome P450 (CYP) enzymes are divided into two groups according to their effect on the spectral properties of microsomal suspensions [1]. One group of compounds are termed type I binding compounds, and are characterized by the appearance in the UV spectrum of an absorption peak at 388 nm and the disappearance of an absorption band at 420–422 nm. These types of compounds displace water as the sixth ligand increasing the amount of high-spin heme, making the heme easier to reduce, and initiating the catalytic cycle. The other group of compounds gives an

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absorbance spectrum that is characterized by the appearance of an absorption peak around 424 nm and the disappearance of absorption at 390 nm. The interaction between the type II ligands and heme stabilizes the low spin iron state and slows or prevents initiation of the catalytic cycle.

Thus type II ligands can play an important role in enhancing drug stability. If the strength of the interaction can be controlled the introduction of a type II interaction can increase the half-life of drug candidates that are rapidly cleared by CYP enzymes. Another benefit of type II binding is that it can increase the affinity of compounds for CYP drug target enzymes. A number of CYP enzymes are therapeutic targets, such as aromatase for breast cancer [2], CYP2A6 for smoking cessation [3,4] and CYP51 for high cholesterol [5,6], yeast infections, and fungal infections (*tinea pedis*, *tinea cruris*, and *candida* in HIV-AIDS) [7]. If specific inhibitors could be made, CYP51 is also a target for parasitic diseases including *American trypanosomiasis* (Chagas disease) and *African trypanosomiasis* (sleeping sickness) [8-10], which affect tens of millions of people around the world. The CYP 51 enzyme is also a target for antifungal agents such as those presented by Wu and coworkers [11]. The currently available therapeutic agents used to treat these diseases are designed to inhibit CYP target enzymes by coordination of nitrogen to the heme iron (type II binding). It is the coordinating nitrogen from a heteroaromatic azole or pyridine moiety within the therapeutic agent that increases binding affinity [7].

While type II binding enhances affinities for CYP enzymes relative to other non-CYP targets (most likely reducing some forms of toxicity), the non-specific inhibition of CYP enzymes leads to toxicity. For example, one major form of toxicity is related to drug/drug interactions by inhibition of drug metabolizing enzymes [7,12]. A second form of toxicity, seen with antifungal agents that inhibit CYP51, is related to altered steroid metabolism [12]. It has also been shown that azole antifungal agents cause birth defects similar to those observed with a phenotypic variation in the CYP reductase [12]. To date, relatively little is known about how to design specific type II binding compounds for individual CYP enzymes, and the difference in type II binding relative to type I binding is not well accounted for in most models for predicting affinity.

CYP2E1 is a major CYP isoform expressed in human liver, which is known to play a major role in the metabolic activation and detoxification of many low molecular weight carcinogens such as dimethyl- and diethylnitrosamine and other toxic agents. The substrates of human CYP2E1 include some hydrophilic substrates such as EtOH and acetone, and also small molecules such as chloroform and benzene. Drugs metabolized by CYP2E1 include acetaminophen [13], theophylline [14], the halogenated inhalation anesthetics such as enflurane and halothane [15], and chlorzoxazone [16]. Since this enzyme appears to be rather selective it provides a logical starting place for understanding the important features involved in type II binding, and inhibition. In this study, we develop structure activity relationships for binding to CYP2E1 for 51 type II binding inhibitors to investigate the physicochemical properties involved in the interaction between ligands and receptor. Our goal is to develop a simple parameter related to coordination to the heme that corrects for this interaction relative to compounds that only interact with the protein backbone with no heme coordination.

2.0 Material and methods

2.1 Materials

Chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and Lancaster Synthesis (Windham, NH). Solvents were from Fisher Scientific Co. (Pittsburgh, PA).

2.2 Microsomal and Purified CYP2E1 Preparations

CYP2E1 rat liver microsomes were obtained from Fisher 344 rats induced with pyridine as described previously [17]. Rabbit CYP2E1 was prepared as described in Koop and Coon [18].

2.3 Inhibition Methods

The production of 6-hydroxychlorzoxazone from chlorzoxazone appears to be predominantly mediated by CYP2E1 so this process was used to assess the activity of CYP2E1. Briefly, 160 μ L 10 mM potassium phosphate buffer (pH 7.4), 50 μ L 1 mM NADP (Nicotinamide adenine dinucleotide phosphate), 50 μ L 0.2 units G6PD (Glucose-6-phosphate dehydrogenase), 50 μ L 10 mM G6P (Glucose-6-phosphate), 50 μ L 5 mM $MgCl_2$, 10 μ L 100 μ M chlorzoxazone, 20 μ L inhibitor and 10 μ L CYP2E1 human liver microsomes were added to each reaction vial and incubated at 37°C with shaking for 10 minutes. Acetonitrile (200 μ L) containing phenacetin (300 ng) as internal standard was added to stop the reaction. The solution was centrifuged at 4°C at 3000 rpm for 10 minutes to remove the proteins. The supernatant was extracted with 2 mL ethyl ether, the top layer was evaporated under nitrogen and then dissolved in buffer (30:70 acetonitrile: 0.5% $H_2PO_4 \cdot H_2O$). HPLC (HPLC system was a Agilent 1100 Series with an Agilent C_8 3.5 μ m 4.6 $\times$ 150 mm column) was run using the method of Tracy and coworkers [19]. Incubations were carried out in triplicate. This method was used for all compounds except the substituted pyrazoles, which were tested with purified rabbit CYP2E1, for the ability to inhibit p-nitrophenol formation using the methods described in Koop [20]. (Note: While it would be ideal to have the K_i measurements made in the same system, the pyrazole compounds are no longer available for testing, and it is not uncommon for models to be built from data taken from the literature using different systems.)

2.4 Kinetic Analysis

Inhibition constants were obtained by keeping the chlorzoxazone concentrations constant and using three different concentrations of each inhibitor around the IC_{50} value. Rough fits of preliminary data were used to estimate the IC_{50} value. The K_i values were obtained from IC_{50} values by fitting the data to nonlinear regression plots on GraphPad Prism software using the sigmoidal dose response equation. The K_i values are the average of at least two separate experiments on different days.

3.0 Theory/calculation

3.1 Partial Least Squares Predictive Models

Regression models made by partial least squares regression of the negative log of the $1/K_i$ versus the descriptor Log P (octanol water partition coefficient), volume, apol (sum of the atomic polarizabilities with polarizabilities), ASA (water accessible surface area), and rank type II (a descriptor developed from DFT calculation on nitrogen-heme interaction presented in the results section). All descriptor calculations besides the rank type II descriptor were calculated using the Chemical Computing Group (2009) Molecular operating environment (moe) <http://www.chemcomp.com>.

3.2 Nitrogen-Heme Iron Interactions

Density functional calculations were performed using Gaussian 03 [21]. The B3LYP functional was used with the LANL2DZ basis set with effective core potential on iron and the 3-21G basis set on all other elements. The heme model was the abbreviated heme with an S-H fifth ligand that has been used by Shaik and coworkers [22,23]. The nitrogen atom was positioned over the heme and optimized using the DIIS algorithm. When convergence

problems were observed we switched to quadratic convergence. The small basis set was used in an effort to keep the calculation approachable without large computing resources.

4.0 Results

The inhibition constants for chlorzoxazone metabolism by the 51 compounds tested are listed in Table 1. Overall, the dataset covers a broad range of inhibition constants that range from 60 nM to over 5 mM. Imidazoles and pyrazoles comprise the top 6 binding compounds, with inhibition constants less than 2.3 μ M. The quinolines in general are poor binding compounds with the parent compound quinoline having the highest affinity of the quinolines with a K_i of 60 μ M. Isoquinolines bound much tighter than quinolines of similar structure with the parent compound, isoquinoline, binding with K_i values of 2.5 μ M. Pyridines were also relatively poor binding compounds. Beyond these simple generalizations it is difficult to rationalize the difference in affinity, with rather large changes occurring with relatively small structural changes, e.g., 6-methoxyquinoline has a K_i of 1,100 μ M while the 6-hydroxyquinoline has a K_i of 218 μ M, and the 6-methyquinoline has a K_i of 125 μ M.

Since all the compounds have the potential to coordinate to the heme-iron we used theoretical calculations to assess the electronic features associated with binding to a naked heme. Density functional theory (DFT) calculations were performed on the different type II binding substructures quinoline, isoquinoline, pyridine, pyrazole, and imidazole to determine the electronic affinity of each group of compounds for coordination to the heme. The difference in the gas phase SCF energies were determined by taking the difference between the energies of the inhibitor plus the sextet spin state of the heme and the bound inhibitor-heme complex (doublet). The tightest binding substructure was found to be *N*-alkylated imidazole which is predicted to have an almost two kcal/mol higher affinity (lower energy, see Table 2) than imidazole. Pyrazole bound less tightly than imidazole, followed by pyridines with electron donating methyl groups, pyridine, and isoquinoline. The quinoline substructure caused significant distortion of the heme ring in the optimized structures, and these structures had much lower affinity. These energies were used as part of a rank-order descriptor as described below.

In an effort to determine what features are important in binding besides heme coordination we attempted to correlate the pK_i values with different descriptors including heme affinity. Predictive models for CYP2E1 inhibition were constructed using the compounds in Table 1. The inhibition constants K_i 's were predicted using the five descriptors; vol (molecular volume), Log P (octanol/water partition coefficient), ASA (solvent accessible surface area), apol (sum of the atomic polarizabilities), and RTII a rank order type II binding descriptor made from the data shown in Table 2 for nitrogen-heme affinities plus empirical steric corrections. These descriptors were chosen because they are relatively easy to interpret with respect to different features associated with binding, and we have used similar descriptors to describe other CYP mediated binding events [24,25]. The Log P descriptor accounts for the entropic contributions to binding resulting from the ordering of water around the substrate, and ASA and volume account for too large and too small size molecules respectively as well the general shape of the molecule, while the polarizability descriptor addresses any potential electrostatic interactions.

The RTII parameter assigns an integer between 0 and 6 depending on the structure. Steric effects and the effects of alternate tautomeric states are combined with the rank order electronic effects from Table 2 to produce a simple empirical descriptor. The bin values based on the DFT calculations are given in Figure 1A. A bin value of 0 is given to all compounds with a substituent ortho to the nitrogen that would coordinate with the heme iron

(See Figure 1B). These steric restrictions associated with an ortho substituent decrease or obviate type II binding [26,27], and we have seen the effect of ortho substitution in the amounts of N-oxide formed from quinoline versus isoquinoline [28,29]. A value of 1 is assigned to 4-hydroxypyridine since a high population of the ground state will be a tautomer that cannot coordinate to the heme (See Figure 1C). A value of 3 is given to all isoquinolines and other pyridines without ortho substituents based on the electronic effects that show weaker coordination of these substructures relative to the 5-membered azaheterocycles (See Figure 1A). A value of 4 is given to all pyrazoles that do not have an alkyl group on one nitrogen of the pyrazole ring. Pyrazoles that have an N-alkyl group and imidazoles without an N-alkyl group are assigned a 5, and finally all imidazoles with an N-alkyl group are assigned a 6. We believe that the effect of the N-alkyl group is a result of electronic and the tautomeric configuration that results in a lone-pair that is always available for interaction with the heme iron (See Figure 1C). The lack of the N-alkyl group means that only a fraction of the coordinating nitrogen is in the correct tautomeric state to interact with the iron. The values assigned each compound are given in Table 1.

To validate the model and assess the effectiveness of the RTII descriptor, 8 compounds (Table 3) selected for diversity using moe fingerprint analysis were removed from the set of 51. This test set spanned the binding affinities and structural space, and the remaining 43 compounds were used to train a predictive model. The trained model had an r^2 value of 0.55, with an RMSE of 0.7. The model was able to predict the diverse test set better with an r^2 value of 0.71. The ability to predict the test set better than the training set indicates that some compounds in the training set were not covered well by the descriptors. Analysis of residuals and refitting the data indicates that the main source of error in the fit arises from three compounds, 1-(3-aminopropyl)-imidazole, acetylamino pyrazole, and 3,5-lutidine. The first compound 1-(3-aminopropyl)-imidazole, is the only charged molecule in the training set, the second has a K_i value over two orders of magnitude less than the next tightest binding pyrazole, and 3,5-lutidine is the only meta di-substituted pyridine. We hypothesize that having two group meta to the nitrogen results in a steric clash with the I-helix over the heme disrupting the iron-nitrogen bond. When these compounds were removed the complete model ($n=48$) had an r^2 value of 0.77 and an RMSE of 0.51. The predictive equation is shown below (Eq. 1). The predicted pK_i values are given in Table 1 and a plot of the predicted versus actual pK_i for a model including all test and training compounds is given in Figure 2.

$$pK_i = 0.43 * RTII + 0.51 * apol + 0.049 * ASA + 0.59 * \log P - 0.17 * vol - 1.15 \quad \text{Eq. 1}$$

The equation using all 51 compounds is given in Eq. 2, and shows that removal of the 3 outliers does not drastically alter the fit, with the major change being in the polarizability parameter and $\log P$ as might be expected after removal of a charged compound from the training set.

$$pK_i = 0.41 * RTII + 0.39 * apol + 0.052 * ASA + 0.85 * \log P - 0.16 * vol - 1.05 \quad \text{Eq. 2}$$

When the test set is removed from the model and the resulting $n=40$ regression model is used to predict the 8 compound test-set the correlation between the predicted and actual pK_i values give an r^2 value of 0.78. The predicted values are given in Table 3.

To determine if the model was overly influenced by a few compounds in the training set we used leave-one-out cross-validation to generate 48 different models with $n = 47$. The cross-validated correlation coefficient (q^2) was 0.69 and the plot of the predicted versus actual pK_i

values is given in Figure 3. Comparison of Figure 3 and Figure 2 showed very similar predictions when each compound is left out of the training set indicating that the model is not overly dependent on specific molecules in the training set.

The model indicates in addition to the nitrogen-iron coordination enhancing binding, large volume decreases binding affinity, more solvent accessible surface area enhances binding, lipophilic compounds bind better, and polarizable molecules bind better. The enhanced binding for polarizable molecules may indicate some type of electrostatic interaction with the protein, or the ability to donate electrons to the nitrogen enhancing the type II interaction.

5.0 Discussion

Given the prevalence of nitrogen containing heteroaromatic compounds used in drug scaffolds, nitrogen coordination to the heme-iron of CYP enzymes can play a major role in the binding affinity, the potential for drug/drug interactions, the half-life of a potential drug, and the ability of a drug to alter the metabolism of toxins. Tracy and coworkers, have provided evidence that type II interactions with CYP2C9 reveal minor binding modes, indicative of only weak interactions [30]. Wienkers and coworkers [31] have shown that PH-302, while giving a type II spectra also bound to the CYP3A4 in an alternate orientation that led to metabolism at a site distant from the imidazole that coordinated to the iron. Interestingly, this compound inhibits inducible nitric oxide synthase (iNOS) by coordinating to the heme, and preventing dimerization. Again this indicates that the type II interaction with CYP3A4 is relatively weak for this compound, while coordination to iNOS is stronger. In contrast, azole antifungal agents have been found to have a very slow off-rate [32] consistent with tight binding, and Chiba et al., [26,27] have found that type II binding can decrease turnover by up to two orders of magnitude for 3A4. Based on our work with nicotine and a closely related analog we estimated that type II binding to the pyridine ring enhanced binding by over 250 fold to CYP101A1 [33]. More recently we have reported that type II binding quinoline carboxamides bind very tightly to both cytochrome CYP2C9 and 3A4, but that these compounds are also good substrates for CYP3A4 [34,35]. Obviously, the structure of the molecule, and the type of nitrogen coordinating has a profound effect on how affinity is altered by type II interactions. Our goal in these studies is to begin to differentiate between heme-nitrogen and apoprotein effects in type II binding to CYP enzymes. It is reasonable to assume that heme interaction will be universal, while each individual CYP enzyme may show different apoprotein profiles in type II binding.

To determine the strength of nitrogen-heme interaction in the absence of apoprotein we used density functional theoretical methods (DFT) to look at the different nitrogen containing heterocycles in Table 2. Consistent with the overall trends in the inhibition constants we measured for CYP2E1, N-alkylimidazoles, imidazoles and pyrazoles have the highest affinity, followed by pyridines, isoquinolines and quinolines in that order. Quinoline still appeared to have favorable interactions with the heme, but the interaction was almost 10 kcal/mol weaker than the less hindered type II binding compounds. This is also consistent with our earlier finding that the quinoline ring hindered approach towards the heme decreasing N-oxide formation, while isoquinoline gave large amounts of the N-oxide [28]. Placing two electron donating methyl groups in the 3 and 5 position of the pyridine ring (3,5-lutidine) pushed electron density towards the nitrogen lone pair, and slightly increased the affinity. This is in contrast to the experimental results, and indicates that the apoprotein must play a role in the decrease in affinity seen for this molecule. Placing an alkyl group on the nitrogen of the imidazole significantly increased the strength of the nitrogen-iron interaction. This would also be augmented by the fact that N-alkylimidazoles and N-alkylpyrazoles would always have a single nitrogen lone pair available for coordination.

Other imidazole and pyrazole isomers would only have a fraction of the compound readily able to bind to the heme iron (Figure 1).

The optimized DFT ground states for each complex proved to have the plane of the heterocyclic ring orthogonal to the plane of the heme. The heme itself was only significantly distorted for quinoline, and for other ortho substituents. For example 2,6-lutidine could not be optimized with the nitrogen coordinating to the ring (data not shown). The optimized bond distance between the sixth coordinate nitrogen and the iron ranged from 2.05 to 2.10 Å, except for quinoline which was 2.30 Å. Docking and molecular dynamics studies such as those done for 1A1 and 2B1 [36] could be used to assess the proteins role in this interaction. However, we chose to use a relatively simple QSAR approach that can be easily applied without the need to correct the docking models for type II binding interactions, which require constraints to be placed on the nitrogen-iron bond to give the correct binding geometry.

In an attempt to determine what other features play a role in binding we used regression analysis (Eq. 1) with descriptors for size (vol), polarizability (apol), solvent accessible surface area (ASA), water octanol partitioning (Log P), and a rank order type II interaction terms (RTII). Each of these descriptors was chosen by itself or in combination with another descriptor to probe the driving forces for binding to CYP2E1. For example, Log P should reflect to some extent the entropic driving force resulting from desolvation of the inhibitor. Volume obviously probes how large a molecule can be, while when combined with solvent accessible surface area gives an idea of the best shape of a molecule for binding, and allows for an optimum size to be accommodated. Polarizability gives an indication of the potential for electrostatic interactions, and can modify the type II interaction term. Overall, Eq. 1 can be interpreted to indicate that active site prefers small lipophilic molecules that are long versus globular, and that are polarizable. Aliphatic nonpolar chains drive binding by increasing entropy upon binding, and by increasing the enthalpic lipophilic interactions in the binding site.

The utility of the RTII descriptor is illustrated by comparing Figures 2 and 4. A significantly better description of the data is given when the RTII descriptor is included (r^2 of 0.77 versus 0.38).

However, the RTII descriptor is not the most important descriptor in equation 1 and each descriptor plays a role in fitting the data. Eq. 3 shows the normalized fit, with the coefficients magnitude reflecting the relative importance of each descriptor. Thus the most important descriptor is volume, and the least important is Log P.

$$\begin{aligned} \text{pKi} / \text{SD}(\text{pKi}) = & 0.88757 * \text{RTII} \\ & / \text{SD}(\text{RTII}) \\ & + 2.28598 * \text{apol} \\ & / \text{SD}(\text{apol}) \\ & + 2.13252 * \text{ASA} \\ & / \text{SD}(\text{ASA}) \\ & + 0.55078 * \log P \\ & / \text{SD}(\log P) \quad -4.50646 * \text{vol} / \text{SD}(\text{vol}) \quad -1.77874 \end{aligned} \quad \text{eq. 3}$$

These simple models appear to do a good job of describing binding affinity, and are easy to use. While the descriptor based model was constructed mainly to elucidate binding forces, the ability of the model to predict the affinities of a test set of diverse compounds provide

some degree of confidence that within this structural space binding affinities can be estimated. The outliers in the model are 1-(3-aminopropyl)-imidazole, acetylaminopyrazole, and 3,5-lutidine. Excluding these compounds increases the correlation coefficient, but does not significantly alter the regression constants. However, analysis of why these compounds are not well predicted can lead to interesting predictions. The amine group in the aminopropyl side chain of 1-(3-aminopropyl)-imidazole has a pKa above the pH of the buffer and is most likely protonated. This indicates that 1) positive charge is not well tolerated by the active site, or 2) desolvation is not well predicted for these kinds of compounds. The disubstituted 3,5-lutidine (Figure 2) has essentially the same binding affinity as 2,6-lutidine (524 versus 360 μM), which is sterically hindered and should not coordinate to the heme-iron. While other compounds such as methyl-nicotinate with only a single substituent meta to the nitrogen bind an order of magnitude tighter (22 μM) and are accurately predicted by the QSAR model (Eq. 1). The most likely explanation is that the meta disubstituted compound has steric interactions with the apoprotein that prevent the nitrogen from coordinating with the heme. It is not clear why acetylaminopyrazole is not a much tighter binding compound. To probe if electronic effects may be responsible we calculated the interaction enthalpy using the same DFT methods we used in Table 2. The results provide some insight in that, unlike pyrazoles, which have the strongest interaction energies of 25 kcal/mol, acetylaminopyrazole has a 3 kcal/mol weaker interaction energy. This can be rationalized by the electron withdrawing nature of the acetyl group decreasing electron density on the pyrazole nitrogen coordinated to the heme iron.

The RTII parameter should be of use for other series of compounds for both CYP2E1 and other CYP enzymes. As more structurally diverse type II binding substrate are tested more bins might need to be added over the 7 used in this study. One possible use is to correct docking scores, which fail to account for the energetics of nitrogen-iron coordination.

In conclusion, size, polarizability, solvation, and the strength of the nitrogen iron bond are the major determinants of binding to cytochrome CYP2E1 by compounds containing an sp^2 nitrogen. The tightest binding compounds all can have strong nitrogen-iron coordination interactions, and have relatively small volumes.

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**A. Bin number based on DFT energetics,
the arrow indicates the site of iron coordination**

B. Bin number adjusted to zero based on steric (ortho) effects

C. Effect of tautomers on bin number

Figure 1. Illustration of the assignment of RTII, the type II binding parameter

1A) The initial bin value is a result of the data given in Table 1 and reflects electronic effects. 1B) Any substituent ortho to the nitrogen disrupts coordination to iron resulting in a bin value of zero. 1C) Tautomers can lead to decreased ability to coordinate as for 4-hydroxypyridine decrease bin value, or the bin value increases if it limits the compound to a single tautomer as for the 1-substituted imidazoles.

Figure 2.
Plot of predicted pKi using eq. 1 versus measured pKi values.

Figure 3.
Plot of cross-validated pKi versus measured pKi values.

Figure 4.
Plot of predicted values without the RTII descriptor versus measured pKi values.

Table 1

Names, inhibition constants, pK_i^a , predicted pK_i^b , and descriptors^c.

Name	Ki (μM)	pKi	Pred pKi	RTII	vol	logP	ASA	apol
4-pentylpyrazole	0.06	7.2	7.4	4	158	2.60	371	25.6
4-propylpyrazole	0.16	6.8	6.3	4	123	1.72	307	19.4
N-(n-butyl)imidazole	0.63	6.2	5.9	6	145	0.47	336	22.5
4-O-propylpyrazole	0.79	6.1	6	4	131	1.41	322	20.2
4-iodopyrazole	1.0	6	5.5	4	100	1.69	264	14.8
4-methylpyrazole	1.3	5.9	5.7	4	86	0.80	246	13.2
isoquinoline	2.5	5.6	4.5	3	138	1.90	307	21.6
methyl isonicotinate	2.5	5.6	4.5	3	132	0.62	318	19.7
4-bromo-3-methylpyrazole	3.0	5.5	5.2	4	114	1.56	286	15.6
3-methylpyrazole	5.4	5.3	5.4	4	88	0.76	245	13.2
1-methylimidazole	6.3	5.2	4.9	6	90	-0.93	246	13.2
4-(2-methoxyethyl)-imidazole	8.4	5.1	5.5	5	134	-0.22	332	20.2
pyridine	11.8	4.9	4.4	3	90	0.67	244	13.2
4-nitroimidazole	12.6	4.9	5.3	5	88	-0.02	255	12.2
4-bromoimidazole	15.1	4.8	4.5	5	98	0.84	259	12.5
6,7-dimethoxyisoquinoline	17.0	4.8	4.2	3	188	1.63	391	29.4
5-hydroxyisoquinoline	20.0	4.7	4.7	3	142	1.59	318	22.4
4-nitropyrzole	21.0	4.7	4.7	4	88	0.44	247	12.2
methyl nicotinate	22.4	4.7	4.3	3	133	0.61	320	19.7
4-acetylpyridine	29.0	4.5	4.4	3	70	0.50	214	10.1
pyrazole	35.5	4.4	5.1	4	96	0.37	255	14.0
3-hydroxypyridine	36.0	4.4	4.2	3	135	0.61	321	19.7
3-acetoxypyridine	50.1	4.3	4.1	3	138	2.11	317	21.6
quinoline	60.0	4.2	3.8	0	72	-0.36	217	10.1
imidazole	70.0	4.2	4.7	5	143	1.80	320	22.4
8-hydroxyquinoline	87.0	4.1	3.4	0	154	2.61	340	24.7
3-methylquinoline	106.0	4	4.2	0	124	0.71	308	19.4
2-ethyl-4-methylimidazole	107.2	4	3.9	0	131	2.13	299	20.3

Name	Ki (μM)	pKi	Pred pKi	RTII	vol	logP	ASA	apol
phthalazine	114.8	3.9	3.4	0	126	0.53	296	18.9
3-acetylpyridine	117.5	3.9	3.9	3	155	2.61	341	24.7
6-methylquinoline	125.0	3.9	4.1	0	155	2.61	345	24.7
7-methylquinoline	151.4	3.8	4.3	0	141	1.84	322	22.4
3-hydroxyquinoline	155.0	3.8	3.9	0	142	1.80	321	22.4
5-hydroxyquinoline	158.0	3.8	3.7	0	142	1.84	319	22.4
6-hydroxyquinoline	218.0	3.7	3.5	0	116	0.11	288	15.5
1-(trifluoroacetyl)-imidazole	239.9	3.6	4.4	6	149	2.52	325	23.1
7-chloroisoquinoline	251.2	3.6	4.8	3	83	0.91	235	11.9
pyridazine	257.0	3.6	3.2	0	124	1.53	306	19.4
2,6-lutidine	363.1	3.4	4.2	0	98	0.37	258	14.0
4-hydroxypyridine	470.0	3.3	3.2	1	95	-0.92	256	14.0
4-hydroxymethylimidazole	508.0	3.3	4.6	5	145	2.03	322	22.4
3-hydroxyisoquinoline	569.0	3.2	3.3	0	143	1.80	320	22.4
4-hydroxyquinoline	592.0	3.2	3.4	0	125	0.49	300	18.9
2-acetylpyridine	631.0	3.2	2.9	0	146	2.20	331	22.4
2-hydroxyquinoline	930.9	3	3.6	0	165	2.10	357	25.5
6-methoxyquinoline	1150	2.9	3.3	0	162	4.12	344	23.3
1,4-dichlorophthalazine	1349	2.9	3.2	0	156	2.36	339	24.7
3-methylisoquinoline	1399	2.9	3.7	0	124	0.53	300	18.9
1-(3-aminopropyl)-imidazole	5012	2.3	ND	6	137	-1.41	331	21.9
4-acetylaminopyrazole	5750	2.2	ND	4	116	-0.21	289	17.6
3,5-lutidine	525	3.3	ND	3	129	1.65	312	19.4

^aLog (1/Ki).

^bPredicted pKi based on the descriptors used in Eq 1 training with compounds 1-48.

^cEach descriptor is explained in the text, and have been rounded from the numbers used in the calculations. All quinolines and isoquinolines were dissolved in acetonitrile, all other compounds were dissolved in potassium phosphate buffer.

Table 2

DFT binding energies for nitrogen coordination to free heme.

Compound	Heat of Reaction
quinoline	-15.8
isoquinoline	-23.4
pyridine	-23.7
3,5-lutidine	-23.8
pyrazole	-25.0
imidazole	-26.4
1-methylimidazole	-28.3

DFT from G03 using the B3LYP functional, LANLDZ basis set and ECP on Fe, and the 3-21G basis set on C, N, S, and H. Energies are reported in kcal/mol and are the difference between the five-coordinate sextet ground state plus the free ligand energy and the energy of the complex.

Table 3

Test set of molecules selected for diversity.

Name	$pK_i \pm \text{stdev}$	Type II Rank	Predicted pK_i
4-propylpyrazole	6.8 ± 0.6	4	6.3
methyl isonicotinate	5.6 ± 0.1	3	4.4
4-nitroimidazole	4.9 ± 0.2	5	5.1
5-hydroxyisoquinoline	4.7 ± 0.1	3	4.8
Quinoline	4.2 ± 0.1	0	3.9
6-methylquinoline	3.9 ± 0.1	0	4.2
4-hydroxypyridine	3.3 ± 0.2	1	3.2
2-hydroxyquinoline	3.0 ± 0.1	0	3.7
3-methylisoquinoline	2.9 ± 0.1	0	3.8

The predicted pK_i is a result of prediction from the regression analysis excluding the compounds listed in the table.