

DMD #13888

Three Dimensional-Quantitative Structure Activity Relationship (3D-QSAR) Analysis Of Human CYP51 Inhibitors

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DMD #13888

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Abbreviations: CYP, cytochrome P450; 3D-QSAR, 3-dimensional-quantitative structure activity relationship; DTT, dithiothreitol; EDTA, ethylenediamine tetraacetic acid.

DMD #13888

ABSTRACT

CYP51 fulfills an essential requirement for all cells, by catalyzing three sequential monooxidations within the cholesterol biosynthesis cascade. Inhibition of fungal CYP51 is used as a therapy for treating fungal infections, while inhibition of human CYP51 has been considered as a pharmacological approach to treat dyslipidemia and some forms of cancer. In order to predict the interaction of inhibitors with the active site of human CYP51, a 3-dimensional quantitative structure activity relationship (3D-QSAR) model was constructed. This pharmacophore model of the common structural features of CYP51 inhibitors was built using the program CatalystTM from multiple inhibitors (n = 26) of recombinant human CYP51-mediated lanosterol 14 α -demethylation. The pharmacophore, which consisted of 1 hydrophobe, 1 hydrogen bond acceptor and 2 ring aromatic features, demonstrated a high correlation between observed and predicted IC₅₀ values (r = 0.92). Validation of this pharmacophore was performed by predicting the IC₅₀ of a test set of commercially available (n = 19) and CP-320626-related (n = 48) CYP51 inhibitors. Using predictions below 10 μ M as a cutoff indicative of active inhibitors, 16 of 19 commercially available inhibitors (84 %) and 38 of 48 CP-320626-related inhibitors (79.2 %) were predicted correctly. In order to better understand how inhibitors fit into the enzyme, potent CYP51 inhibitors were used to build a Cerius² TM receptor surface model representing the volume of the active site. This study has demonstrated the potential for ligand-based computational pharmacophore modeling of human CYP51 and enables a high-throughput screening system for drug discovery and database mining.

DMD #13888

Cytochrome P450 enzymes (CYPs) are a widely studied, large superfamily of heme-thiolate proteins involved in the metabolism of endobiotics and xenobiotics across eukaryotes and prokaryotes (Nelson et al., 1996). The clinical relevance of CYPs is their central role in drug metabolism, which can occur in all human tissues and may be inhibited by the co-administration of competing xenobiotics for the same enzyme (Wrighton et al., 1995). Much less is known about the endogenous functions of P450, although their role in steroid metabolism is an exception. It has been postulated that other endogenous roles might be in neurotransmitter metabolism (Hiroi et al., 1998) and signaling pathways (Chan et al., 1998). One of these enzymes is the ubiquitously expressed CYP51, also known as lanosterol 14 α -demethylase (P450-14DM) (Nelson et al., 1996). This enzyme fulfills an essential requirement for all cells by catalyzing three sequential monooxidations within the cholesterol biosynthesis cascade (Trzaskos et al., 1986; Waterman and Lapesheva, 2005).

CYP51 is one of the most conserved CYPs across phyla (Aoyama et al., 1996; Yoshida et al., 1997), with a 93 % amino acid sequence identity between rat and human and 39-42% identity between mammalian and fungal enzymes (Aoyama et al., 1996; Yoshida et al., 1997). In humans there are three CYP51 genes, including two pseudogenes and a functional gene on chromosome 7 (Rozman et al., 1996). Substrates for CYP51 from mammals, plants and fungi are lanosterol, obtusifoliol and 24-methylene-dihydroxylanosterol, respectively (Lamb et al., 1998). Such substrate specificities can be explained by a limited number of amino acid substitutions in substrate recognition sites 1, 2 and 5 between mammalian and fungal CYP51, and it is likely that these locations are responsible for conferring selectivity for sterol metabolism (Yoshida et al., 1997). Before the sequences of fungal and human CYP51 were known, this enzyme was identified as an antifungal therapeutic target that could be inhibited by many

DMD #13888

imidazole, triazole (Vanden Bossche et al., 1987; Vanden Bossche et al., 1995; Hartman and Sanglard, 1997; Kelly et al., 1997) and non-azole compounds (Aoyama et al., 1983; Yoshida and Aoyama, 1985; Aoyama et al., 1987; Gebhardt et al., 1994; Hartman and Sanglard, 1997). Many of these marketed compounds are specific towards a single fungal species while others have widespread application (Hartman and Sanglard, 1997). The selectivity of these antifungals is also variable, with some exhibiting no interaction with the mammalian enzyme. Many of these azole antifungals are also potent inhibitors of other mammalian P450s involved in drug metabolism and there is thus the potential for clinically significant drug-drug interactions when co-administered with substrates for the same enzymes (Strolin Benedetti and Bani, 1998).

There have been many studies utilizing azole, oxysterol and mechanism-based inactivator-type inhibitors of fungal or mammalian CYP51 (Trzaskos et al., 1986; Frye et al., 1993; Trzaskos et al., 1993; Frye et al., 1994; Trzaskos et al., 1995). In addition, the purification (Sonoda et al., 1993) and expression of human CYP51 has allowed the determination of its regulation by oxysterols (Stromstedt et al., 1996). However, due to the membrane-bound nature of mammalian P450s, there is as yet no crystal structure for human CYP51. Therefore, our understanding of the structural requirements of the human CYP51 active site are limited to homology models constructed using various bacterial P450s (Ishida et al., 1988; Tuck et al., 1991; Tuck et al., 1992; Tafi et al., 1996; Talele et al., 1997; Holtje and Fattorusso, 1998; Lewis et al., 1999) including *M. tuberculosis* CYP51 (Matsuura et al., 2005; Rupp et al., 2005), and the crystal structure of *M. tuberculosis* CYP51 (Podust et al., 2001).

There have also been few quantitative structure activity relationship (QSAR) models of CYP51 to date and they have dealt exclusively with the fungal CYP51s (Tafi et al., 1996; Fujita, 1997). The utility of more classical QSAR has also been reviewed for the production of azole-

DMD #13888

type agricultural fungicides (Fujita, 1997). However, approaches towards modeling the common features of inhibitors of other human P450s have been widely shown using a CatalystTM pharmacophore approach (Ekins et al., 2001), comparative molecular field analysis (CoMFA, (Jones et al., 1996)), and other QSAR methods (Ekins et al., 2003). These techniques have also been used to predict a test-set of molecules excluded from the training set (Ekins et al., 1999).

Recently a series of dual-acting hypoglycemic and hypocholesterolemic agents was found to inhibit glycogen phosphorylase and human CYP51 (Harwood et al., 2005). The prototype of this series, CP-320626, inhibited glycogen phosphorylase and human CYP51 with IC₅₀'s of 155 nM and 4 μM respectively, reduced glycogenolysis and cholesterolgenesis in cultured cells, and lowered plasma glucose and plasma cholesterol in experimental animals (Harwood et al., 2005). In order to understand the characteristics of these CP-320626 related CYP51 inhibitors and other inhibitors of this human CYP51 enzyme, we have used a computational approach to produce a predictive CatalystTM pharmacophore. This model was constructed using a training set of structurally diverse commercially available molecules and CP-320626-related CYP51 inhibitors with varying IC₅₀ values. The validity of the predictions made by this model was then evaluated using test sets of molecules not in the training set. These test sets contained both commercially available molecules and structurally diverse CP-320626-related CYP51 inhibitors for which *in vitro* data were subsequently generated.

DMD #13888

Materials and Methods

Materials. Human cytochrome P450 reductase was obtained from Panvera (now Invitrogen, Madison, WI). Rat microsomal lipid was prepared as described by Sundin *et al.* (Sundin *et al.*, 1987). Triarimol, CP-320626 and all other related CYP51 inhibitors denoted as A-P and 1-48 (see Supplemental Table 1 for structures) in the tables were synthesized as described previously (Hoover *et al.*, 1998; Martin *et al.*, 1998; Rath *et al.*, 2000; Treadway *et al.*, 2001; Harwood *et al.*, 2005; Yu *et al.*, 2006) and in the patents cited in these publications. Lovastatin was purchased from Calbiochem (La Jolla, CA). Itraconazole and terconazole were obtained from Research Diagnostics Inc. (Flanders, NJ). Sulphaphenazole was purchased from Gentest Corp. (now BD Gentest, Woburn, MA). Dioleoyl phosphatidyl choline (DOPC), lanosterol (97 % pure), ergosterol and all other chemicals were obtained from Sigma (St. Louis, MO) or Steraloids Inc, (Newport, RI).

CYP51 Lanosterol Demethylase Assay. Human CYP51 expressed in Topp 3 cells was partially purified using the method of Stromstedt (Stromstedt *et al.*, 1996). Protein concentrations were measured using a BCA Protein Assay kit (Pierce Chemical, Rockford, Ill.) with bovine serum albumin as a standard as described by the manufacturer. Cytochrome P450 content was measured by the method of Omura and Sato (Omura and Sato, 1964). Initial rate conditions for the formation of 4,4-dimethylcholesta-8,14,24-trien-3 β -ol (triene) were determined in preliminary studies with the CYP51 reconstitution system. Briefly, 25 μ L of lanosterol (1 mM, suspended in a mixture of 50/50 tyloxapol / acetone, DOPC micelles (5 mg/ml), methanol, and 100 mM potassium phosphate buffer, pH 7.4) was added to tubes to provide a final lanosterol concentration of 50 μ M (approximate K_m). Inhibitors (ranging from

DMD #13888

0.01 to 200 μ M in methanol) or methanol (control) were then added to tubes, and the lanosterol / inhibitor mixture was allowed to dry under nitrogen for 10 minutes. To dried substrate and inhibitor tubes, 20 pmol CYP51, 125 pmol human reductase, and 50 μ l rat lipid were added and allowed to sit at room temperature for 10-15 minutes for enzyme reconstitution. Buffer (100 mM potassium phosphate, pH 7.4, with 20% glycerol, 0.1 mM DTT, 0.1 mM EDTA, and 0.5 mM potassium cyanide) was added to tubes after the reconstitution period for a final incubation volume of 0.5 ml. Tubes were preincubated for 2 minutes at 37⁰ C and the reaction was initiated by the addition of 50 μ l NADPH regenerating system (final incubation concentration; 10 mM MgCl₂, 0.54 mM NADP, 6.2 mM DL-Isocitric Acid, 0.5 U/ml Isocitric Dehydrogenase). Reactions were terminated at 60 min with the addition of 25 μ l ergosterol (1 mg/ml in ethyl acetate), followed by extraction with 5 ml ethyl acetate. Tubes were then vortexed before centrifugation followed by removal of 3-4 ml of supernatant and transfer to fresh tubes and evaporation under nitrogen at 50 °C. The evaporated samples were reconstituted in 150 μ l mobile phase, and 25-50 μ l was injected onto the HPLC system (see below).

Triene formation was determined using a modification of the method of Strömstedt (Stromstedt et al., 1996). Briefly, triene metabolite and ergosterol were separated on a Waters Novapak C18 column (4.0 μ M; 150 mm X 3.9 mm, Bedford, MA) with a mobile phase consisting of methanol:acetonitrile:HPLC grade water (45:45:10), at a flow rate of 1.5 ml/min. The triene and ergosterol were monitored by UV detection at 248 nm using a SpectroMonitor variable wavelength detector (LDC Analytical, Riviera Beach, FL) and the Multichrom™ data acquisition system (Version. 2.11, Fisons Instruments, Beverly, MA) was used for data

DMD #13888

collection, analysis and reporting. Approximate retention times for triene metabolite and ergosterol were 25 and 30 minutes, respectively.

Interpretation of CYP51 lanosterol demethylase assay results. The percent inhibition of triene formation with methanol controls was determined with each inhibitor at 3-6 concentrations in triplicate and the average was used as described previously (Harwood et al., 2005). The IC_{50} was calculated from a graph of percent CYP51 activity remaining vs. inhibitor concentration using Deltagraph (Version 4.0, Monterey, CA).

Molecular Modeling. The computational molecular modeling studies were carried out using a Silicon Graphics Octane workstation and based on a pharmacophore methodology previously described for other CYPs (Ekins et al., 1999; Ekins et al., 2001). The pharmacophore method described below represents the features on the ligands used and cannot be related easily to features in the protein binding site. This method has also been applied to other proteins of relevance to absorption, distribution, metabolism, excretion and toxicity (Ekins and Swaan, 2004). The receptor surface modeling method also described below attempts to provide information relating to the volume requirements of the enzyme active site for the heme and non-heme binding compounds with highest affinity.

Modeling with Catalyst™. The 3-D structures of CYP51 inhibitors from the present study or literature data sets were built interactively using Catalyst™ version 4.0 (Accelrys, San Diego, CA). The number of conformers generated for each inhibitor was limited to a maximum of 255 with an energy range of 0 to 20 kcal/mol. A training set of 26 molecules was selected and used to build the pharmacophore (Table 1). Ten hypotheses were generated using these conformers for each of the molecules and IC_{50} values generated after selection of the following features for the inhibitors: hydrogen bond donor, hydrogen bond acceptor, hydrophobic, positive

DMD #13888

ionizable and ring aromatic. After assessing all 10 hypotheses generated, the lowest energy cost hypothesis was considered the best.

The goodness of the structure activity correlation was estimated by means of the correlation coefficient r-value. CatalystTM also calculates the total energy cost of the generated pharmacophores from the deviation between the estimated activity and the observed activity, combined with the complexity of the hypothesis (i.e. the number of pharmacophore features). A null hypothesis is additionally calculated which presumes that there is no relationship in the data and that experimental activities are normally distributed about their mean. Hence, the greater the difference between the energy cost of the generated hypothesis and the energy cost of the null hypothesis, the less likely it is that the hypothesis reflects a chance correlation. This criteria is then used as an assessment of the pharmacophore model selected.

CatalystTM CYP51 pharmacophore validation using test sets of IC₅₀ values. Two test sets of 19 commercially available molecules and 48 proprietary compounds, respectively were subjected to the fast-fit algorithm for the CatalystTM hypothesis in order to predict a IC₅₀ value. None of these molecules was included in the initial training set for the CYP51 IC₅₀ pharmacophore. The fast fit algorithm refers to the method of finding the optimum fit of the substrate to the hypothesis among all the conformers of the molecule without performing an energy minimization on the conformers of the molecule (CatalystTM tutorials release 3.0, Accelrys, San Diego, CA.). The computational predictions derived using the fit to the CYP51 CatalystTM pharmacophore were compared to the observed *in vitro* values. We selected an acceptable arbitrary cut-off for potency of inhibition as accepted less than or equal to 10 μ M.

CatalystTM CYP51 pharmacophore validation using permuting of activity data. The statistical significance of the pharmacophore hypothesis utilized was tested by permuting

DMD #13888

(randomizing) the structures and the activities and then repeating the CatalystTM hypothesis generation procedure ten times.

Modeling with Cerius²™. The 3-D structures of CYP51 inhibitors generated using CatalystTM described previously were imported into Cerius²™ (Accelrys, San Diego, CA). Inhibitors with an IC₅₀ less than or equal to 10 μM were selected and a receptor surface model (Hahn and Rogers, 1995) was generated. Additional molecules were then fitted to this model in order to discover which features were outside of the volume occupied by the active molecules.

DMD #13888

RESULTS

CYP51 in vitro inhibition data. Partially purified CYP51 was characterized for protein and carbon monoxide-difference spectra (data not shown). Total P450 was determined as 0.22 nmol/mg protein. The assay conditions were also developed to provide optimal lanosterol turnover using a final ratio of P450:reductase of 1:6. The K_m determined for lanosterol with the reconstituted human CYP51 was approximately 30 μM (data not shown), in agreement with previously determined values (Lamb et al., 1998). Using a standard concentration of 50 μM lanosterol, 93 commercially available molecules including the competitive inhibitor CP-320626 (Harwood et al., 2005) and related CYP51 inhibitors were screened in the IC_{50} assay (Tables 1-3).

CatalystTM CYP51 pharmacophore model. CatalystTM utilizes a collection of molecules with inhibitory activity over multiple orders of magnitude for the enzyme of interest, in order to construct a model of the structural features (pharmacophore) necessary for interaction of the molecules with the active site of the enzyme. The resultant pharmacophore hypotheses explain the variability of the potency of inhibition with respect to the geometric localization of these features of the molecules. The IC_{50} values for structurally diverse inhibitors of CYP51 generated in this study cover over 3 orders of magnitude (0.13 μM to >200 μM , Table 1). These values were used to build a pharmacophore, with the lowest energy model yielding 4 structural features (Figure 1) necessary for inhibitor binding to the active site of CYP51: 1 hydrophobe, 1 hydrogen bond acceptor and 2 ring aromatic features (additional hydrophobes). The CatalystTM pharmacophore demonstrated a good correlation of observed versus estimated IC_{50} values ($r = 0.92$, Figure 2).

DMD #13888

The total energy cost of the hypothesis (117.3) and the null hypothesis (154.1) suggest that the model was significant as the difference was sizeable. Permuting the training set molecule structures with activities can be used to provide further evidence that the model is statistically reliable (Ekins et al., 1999). After permuting the hypothesis 10 times the mean r value decreased considerably to 0.32 and mean energy cost difference increased to 148.7, almost identical to the null hypothesis value.

CatalystTM CYP51 pharmacophore validation using test sets of IC₅₀ values. After constructing the CatalystTM 3D-QSAR model for a training set of 26 IC₅₀ values generated by this laboratory *in vitro* (Table 1), the model was used to predict IC₅₀ values of a test set of 19 commercially available molecules for which *in vitro* IC₅₀ values were also generated (Table 2) and not included in the initial CatalystTM model. In 16 cases the IC₅₀ values were correctly predicted to be either active (IC₅₀ < 10 μ M) or inactive molecules (IC₅₀ >10 μ M). The poorly predicted molecules were made up of 5.3 % false negatives (n = 1, bifonazole) and 10.5 % false positives (n = 2, Triarimol and Azacozazole).

A second test set of IC₅₀ values was composed of 48 CP-320626 related CYP51 inhibitors of which 38 were correctly predicted using the CatalystTM 3D-QSAR model following the criteria as described above (Table 3). The poorly predicted molecules were made up of 4.2 % false negatives (n = 2, molecules 1, 37) and 16.6 % false positives (n = 8, molecules 7, 12, 38, 39, 44, 45, 46, 47).

Modeling with Cerius² TM. Fifteen molecules with *in vitro* IC₅₀ values < 10 μ M produced a volume using the receptor surface model software present in Cerius² TM (Figure 3A). When fluconazole, an inhibitor with a low affinity for human CYP51 was fitted to this model, part of the molecule lies outside of the volume (Figure 3B).

DMD #13888

DISCUSSION

In humans, beyond its obvious role in cholesterol and steroid hormone biosynthesis (Trzaskos et al., 1986), the physiological roles of CYP51 in, for example spermatogenesis and oogenesis (Stromstedt et al., 1998), are poorly defined. In addition, inhibition of cholesterol biosynthesis through inhibition of mammalian CYP51 results in an increase in cellular lanosterol concentrations (Miettinen, 1988), the consequences of which remain poorly defined. Further, the poor specificity of most current mammalian CYP51 inhibitors for CYP51 versus steroidogenic or drug-metabolizing P450s (Hartman and Sanglard, 1997; Strolin Benedetti and Bani, 1998) implies that molecules aimed at other therapeutic targets should preferably avoid interaction with this enzyme.

Since there is no crystal structure for the human membrane-bound CYP51, alternative techniques must be considered to understand whether a novel molecule will bind to it. It is possible, however, to begin to understand the specificity of an enzyme without possessing information regarding its crystal structure. The most widely used computational method for modeling in the cytochrome P450 field is homology modeling based on crystallized bacterial CYPs. The initial models of the active site for CYP51 from *S. cerevisiae* and an inactive mutant (Ishida et al., 1988) were constructed using alignment with P450cam. Using these, novel substrate analogs were developed as competitive inhibitors for this enzyme (Tuck et al., 1991). The formation of a fungal CYP51 biphenyl-iron complex also yielded information as to the approximate height of the cavity (10Å) above the porphyrin ring in the active site (Tuck et al., 1992). Further work studying the I-helix of the fungal CYP51 with lanosterol has shown that Gly-310 is an important residue, although interactions at His-317 and Met-313 may also occur (Vanden Bossche et al., 1995). It has been suggested that whereas ketoconazole and itraconazole

DMD #13888

have multiple interactions with the CYP51 I-helix, fluconazole has fewer and may result in its lower antifungal activity (Vanden Bossche and Koymans, 1998). Comparative homology models have identified differences in the active sites of C. albicans and S. cerevisiae (Boscott and Grant, 1994) in agreement with earlier work (Yoshida and Aoyama, 1987). When the human CYP51 I-helix sequence is aligned with those from C. albicans and S. cerevisiae (Vanden Bossche and Koymans, 1998) there are 6 amino acids which are different between the fungal and the human P450s. It is therefore likely that the surface volume of inhibitors for fungi and human CYP51 could be markedly different due to the alteration in the active site caused by these amino acid substitutions. Numerous studies have used homology modeling and docking for CYP51 to show: lanosterol hydrogen bonding interactions (Holtje and Fattorusso, 1998), triazole andazole inhibitors interacting with the hydrophobic active site and the heme iron (Talele et al., 1997) and functional group modifications that alter enzyme-inhibitor interactions (Tsukuda et al., 1998). CYP51 homology models have been used in de novo design (Ji et al., 2000) and in understandingazole binding to human and crystallized M. tuberculosis CYP51 enzymes (Matsuura et al., 2005; Rupp et al., 2005). The latter study indicated that the human enzyme may have a larger and differently shaped active site cavity due to the replacement of Phe-255 with Leu-310 (Matsuura et al., 2005; Rupp et al., 2005).

Although there are data sets in the literature describing the fungicidal activity of CYP51 inhibitors (Stehmann and de Waard, 1995), there is little information relating to human disease. The present study has described to our knowledge the first 3D-QSAR relationship using *in vitro* data for inhibitors of human CYP51. By using 26 structurally diverse molecules with a range of inhibitory activity, we constructed a predictive pharmacophore model that was used to score the activity of test molecules and predict whether new chemical entities might interact with this

DMD #13888

enzyme. The model for the data set generated in this study suggested a pharmacophore with four features, indicating the predominance of hydrophobic features (Figure 1) in agreement with previous reports (Matsuura et al., 2005; Rupp et al., 2005), and a high correlation ($r = 0.92$, Figure 2) between predicted and observed IC_{50} values.

One of the best ways to test the validity of a 3D-QSAR is to predict the activity of molecules excluded from the training set and then compare these to observed values. In addition to prospectively assessing many proprietary compounds, several commercially available molecules were evaluated *in vitro* as they had either important endogenous roles (cholesterol, arachidonic acid, progesterone, pregnenolone and anandamide), were known CYP substrates (erythromycin, tolbutamide, lovastatin, chlorpropamide, propafenone) or were known CYP inhibitors (sulphaphenazole, fluconazole, azaconazole, econazole, bifonazole, terconazole, itraconazole, triarimol and sulfinpyrazone). It was thought that these molecules would provide a structurally diverse test set in order to validate the pharmacophore constructed from a similarly structurally diverse training set including heme-binding azoles and non heme-binding CP-320626-related compounds (Harwood et al., 2005). *In vitro* IC_{50} values were generated for CYP51 inhibition for 19 commercially available molecules. Sixteen of the 19 (84 %) molecules fulfilled the criterion (Table 2). Non heme-binding CP-320626-related CYP51 inhibitors were also successfully predicted using the same pharmacophore model with an approximate 80 % prediction rate (Table 3). These results suggest that the CYP51 pharmacophore has utility for both classification of novel molecules that are either known heme binders or non-heme binders with an acceptable level of accuracy for early screening.

By aligning the most potent CYP51 inhibitors (azoles and CP320626-related compounds) with the pharmacophore it was possible to generate a receptor surface model of

DMD #13888

their volume. The receptor surface model of these active inhibitors forms an elongated shape (Figure 3A), not too dissimilar to that of lanosterol and the volume generated by Rupp et al., (Rupp et al., 2005). This receptor surface model can also be used to explain the apparent lack of inhibitory activity towards human CYP51 by inactive molecules, by first mapping them to the pharmacophore and then placing them inside this receptor surface model. Features outside of the volume could suggest poor steric interactions within the active site of the enzyme that would be unfavorable for binding and therefore inhibition. As an example, we have fitted fluconazole to the pharmacophore and shown that a triazole ring is outside of the surface volume generated by the active compounds (Figure 3B). This may explain the lack of potency of fluconazole towards the human CYP51 but subsequent activity towards the fungal P450. Indeed this is in agreement with a recent study that has also shown that fluconazole is considerably less active than ketoconazole with human CYP51 with K_d values of 120 μ M and 8 μ M, respectively. The differential potency between these two compounds may also be in part because fluconazole possesses a hydrophilic triazole and hydroxyl groups that would decrease affinity for the hydrophobic binding site relative to the more hydrophobic ketoconazole (Matsuura et al., 2005).

The computational techniques described and applied in this paper may allow elucidation of areas within the human CYP51 active site that can be targeted by *directed* synthesis to enable production of more selective antifungal agents. Similarly, if human CYP51 is pursued as a therapeutic target, for example in cancer (Downie et al., 2005; Kumarakulasingham et al., 2005), this pharmacophore model enables mining of compound databases for inhibitors and may help to understand the affinity of inhibitors for the human CYP51 active site. Specific inhibitors of human CYP51 (Harwood et al., 2005) might also be applicable, for example, as agents for lipid lowering, either alone or in combination with other agents (e.g. HMG CoA reductase inhibitors)

DMD #13888

currently marketed and / or in development (Harwood and Hamanaka, 1998) for use as therapeutic intervention in areas with substantial medical need such as coronary heart disease, obesity and diabetes.

While the pharmacophore technique could represent an approach for identifying modulators of human CYP51, possibly by database screening, we have taken the same approach with *in vitro* data for antifungal activity (Tafi et al., 1996), to create a second pharmacophore for fungal CYP51 (data not shown). Therefore it is possible to differentiate between the human and fungal CYP51 enzymes *in silico* to develop selective modulators of this enzyme across phyla. The publication of X-ray structures of CYP51 in *M. tuberculosis* with bound inhibitors (Podust et al., 2001) suggests we will require selective inhibitors if we are to capitalize on CYP51 as a therapeutic target; the human CYP51 pharmacophores may allow this in combination with the protein models (de Groot and Ekins, 2002).

In conclusion, this study has described a large number of molecules tested as potential CYP51 inhibitors and has enabled the identification of non-azoles with low μM potency. These molecules have been used to build and test the first 3D-QSAR for human CYP51, which can be used to predict the inhibitory potential of other molecules towards this enzyme. We suggest that this CYP51 pharmacophore may be used to garner information regarding the interactions with the active site of this P450 and visually describe the selectivity of antifungal agents. This model could also enable rapid discovery of further modulators of this enzyme for therapeutic use.

DMD #13888

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DMD #13888

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DMD #13888

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DMD #13888

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DMD #13888

FOOTNOTES PAGE

a) Send reprint requests to:

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Figure Legends

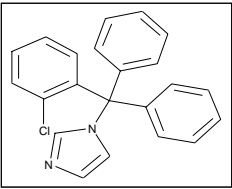
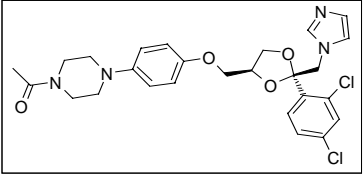
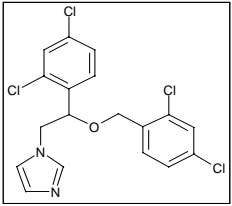
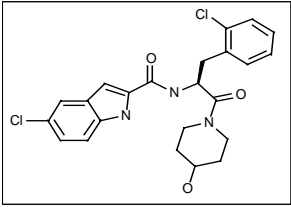
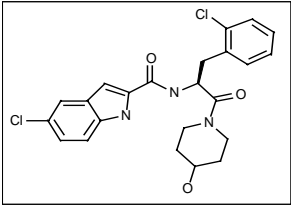
Figure 1. The human CYP51 pharmacophore for $n = 26$ inhibitors generated using CatalystTM (see Materials and Methods) illustrating 1 hydrophobe (cyan), 1 hydrogen bond acceptor (green) and 2 ring aromatic features (orange). The inter bond angles and the distances between features are also annotated.

Figure 2. Correlation of observed and estimated inhibition IC_{50} (log units) for the CYP51 CatalystTM model. The central line corresponds to the regression of the data; the dashed lines represent the 95% confidence interval for the regression while the outer lines are the 95% confidence interval for the population.

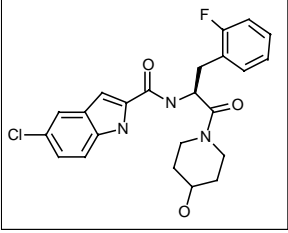
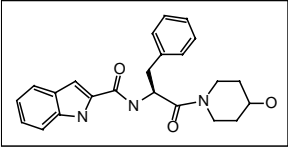
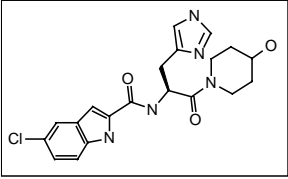
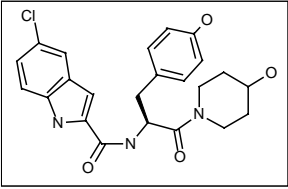
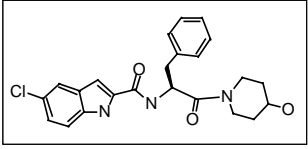
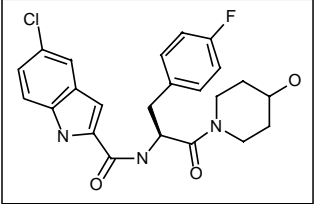
Figure 3. A. Opaque Cerius2TM receptor surface model of active CYP51 inhibitors showing hydrogen bond acceptor regions (blue coloring) - front view. This model was generated with training set molecules with IC_{50} values less than or equal to $10 \mu\text{M}$ (see Materials and Methods). B. Fluconazole fitted to Cerius2TM receptor surface model (side view) for human CYP51, showing an area of the molecule outside of the surface boundary for this volume (see Materials and Methods).

DMD #13888

Table 1. Commercial and CP-320626 analog CYP51 inhibitors used as a CatalystTM training set

Inhibitor	Structure	Observed CYP51 IC ₅₀ (μ M)*	Estimated CYP51 IC ₅₀ (μ M)*
Clotrimazole		0.13	1.3
Ketoconazole		0.19	0.082
Miconazole		0.2	1.7
Analog A		0.3	1.2
Analog B		0.8	2.2

DMD #13888

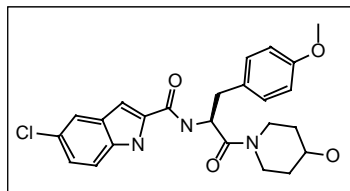
			
Analog C		0.91	2
			
Analog D		1.3	6.9
			
Analog E		2.5	2.9
			
Analog F		3.1	1.5
			
CP-320626		4.0	2.8
			

DMD #13888

Analog G

4.5

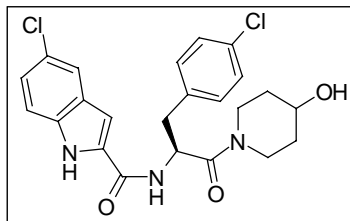
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Analog H

9.7

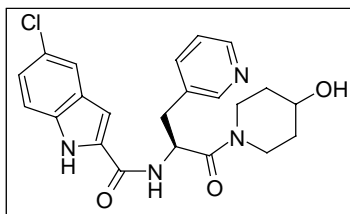
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Analog I

26

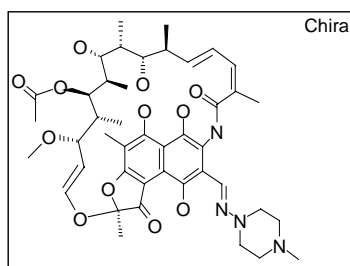
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Rifampicin

30

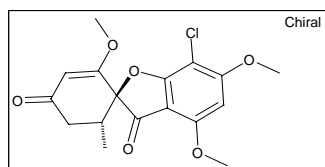
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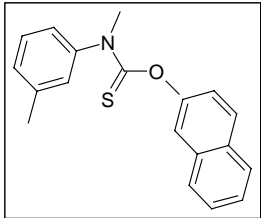
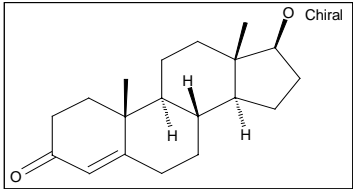
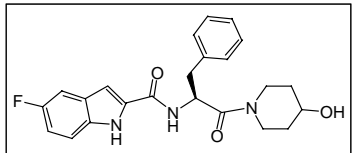
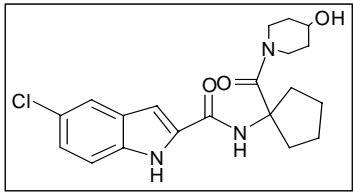
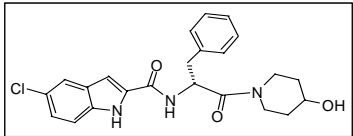
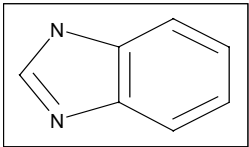
Griseofulvin

50

120



DMD #13888

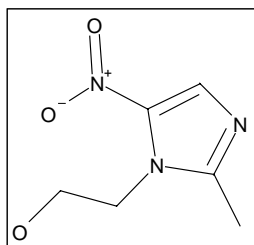
Tolnaftate	50	25
		
Testosterone	50	160
		
Analog J	75	44
		
Analog K	100	60
		
Analog L	100	14
		
Benzimidazole	>200	150
		

DMD #13888

Metronidazole

>200

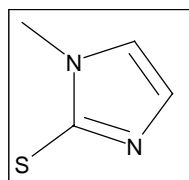
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Methimazole

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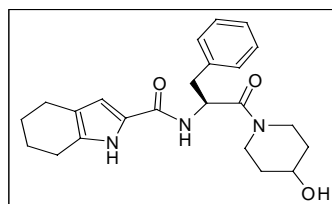
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Analog M

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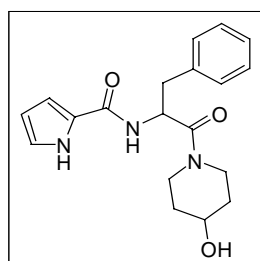
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Analog N

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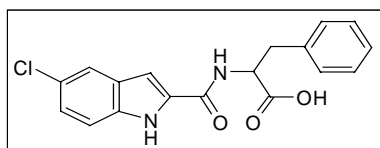
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Analog O

>200

89



* Experimental data is the average of triplicate determinations.

DMD #13888

Table 2. Observed and predicted IC₅₀ values for diverse molecules tested as inhibitors of CYP51 that were fast fit to the Catalyst™ CYP51 pharmacophore

Inhibitor	Observed In vitro	Predicted Catalyst™
	IC ₅₀ (μM)*	fast fit IC ₅₀ (μM)*
Cholesterol	>200	96
Erythromycin	>200	89
Sulphaphenazole	>200	16
Tolbutamide	>200	120
Fluconazole	>200	96
Propafenone	>200	17
Progesterone	>200	92
Arachidonic acid	>200	92
Chlopropamide	>200	92
Anandamide	>200	90
Sulfinpyrazone	>200	93
Triarimol	>10	0.85
Bifonazole	0.342	140
Azaconazole	>10	0.88
Econazole	0.05	0.51

DMD #13888

Lovastatin	>200	89
Pregnenolone	>200	98
Terconazole	3	0.43
Itraconazole	3.6	0.82

* Experimental data is the average of triplicate determinations.

DMD #13888

Table 3. Observed and predicted IC₅₀ values for CP-320626-related analogs tested as inhibitors of CYP51 and fast fit to the CatalystTM CYP51 pharmacophore (Structures for molecules provided in supplemental Table 1).

CP-320626-related molecules	Observed <i>in vitro</i> IC ₅₀ (μM)*	Predicted Catalyst TM fast fit IC ₅₀ (μM)*
1	6	43
2	22	91
3	>30	72
4	>30	79
5	3.5	0.91
6	>10	91
7	>10	6
8	>10	90
9	>10	90
10	>10	97
11	>10	52
12	>10	5.8
13	>10	92
14	>10	30
15	>10	91

DMD #13888

16	20	91
17	50	95
18	>30	95
19	>30	90
20	14	92
21	5	5.2
22	>30	79
23	>30	42
24	>30	90
25	>10	95
26	>10	93
27	>10	60
28	>10	93
29	>10	91
30	>10	91
31	>10	63
32	>10	39
33	>10	14
34	>10	92
35	1.2	4.8
36	5.7	8.1
37	6.0	43

DMD #13888

38	28	1.9
39	>200	1.9
40	>10	26
41	>10	120
42	>10	170
43	>10	29
44	>10	0.46
45	>10	0.51
46	>30	0.39
47	>30	0.41
48	0.7	0.43

* Experimental data is the average of triplicate determinations.

Fig 1

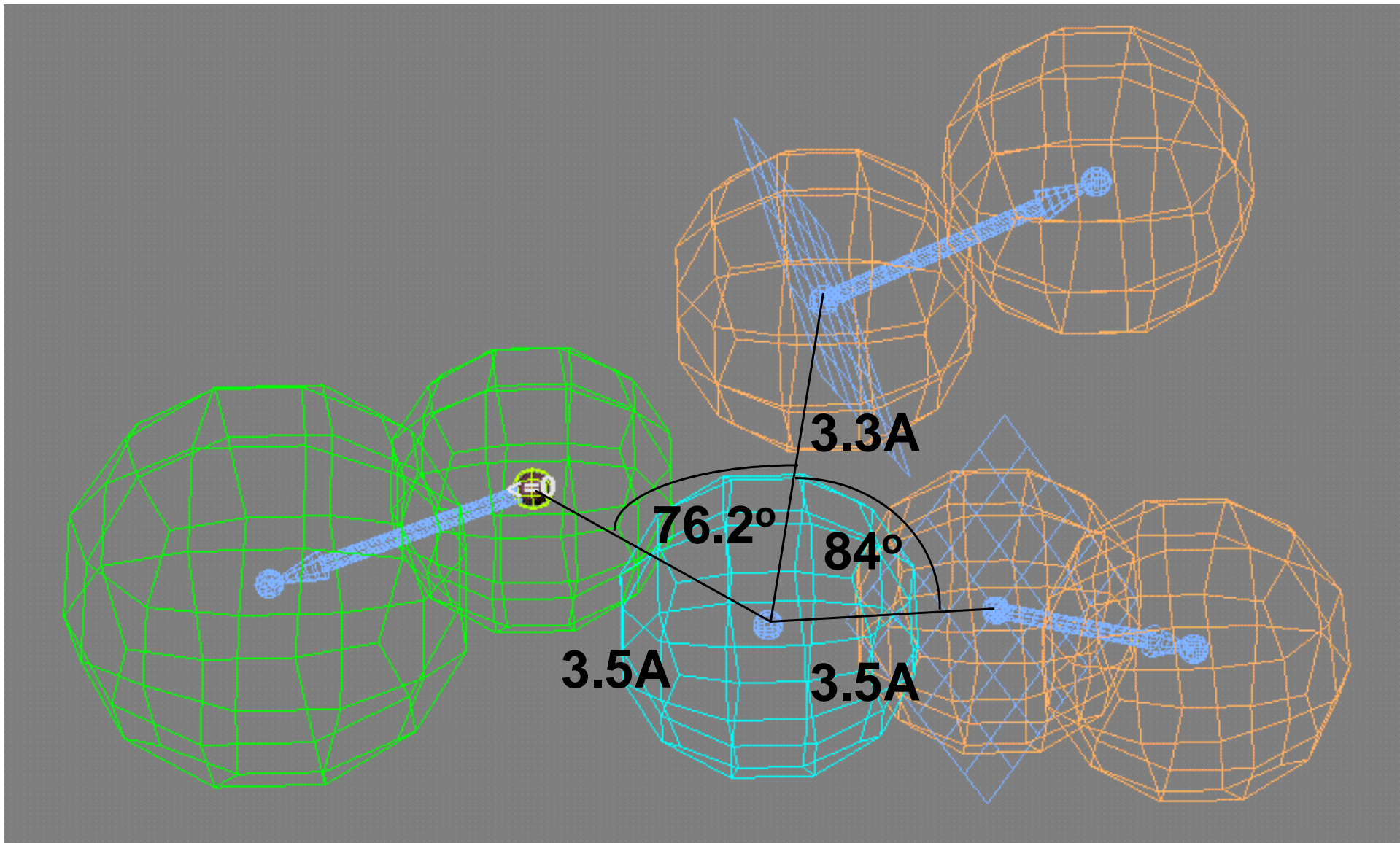


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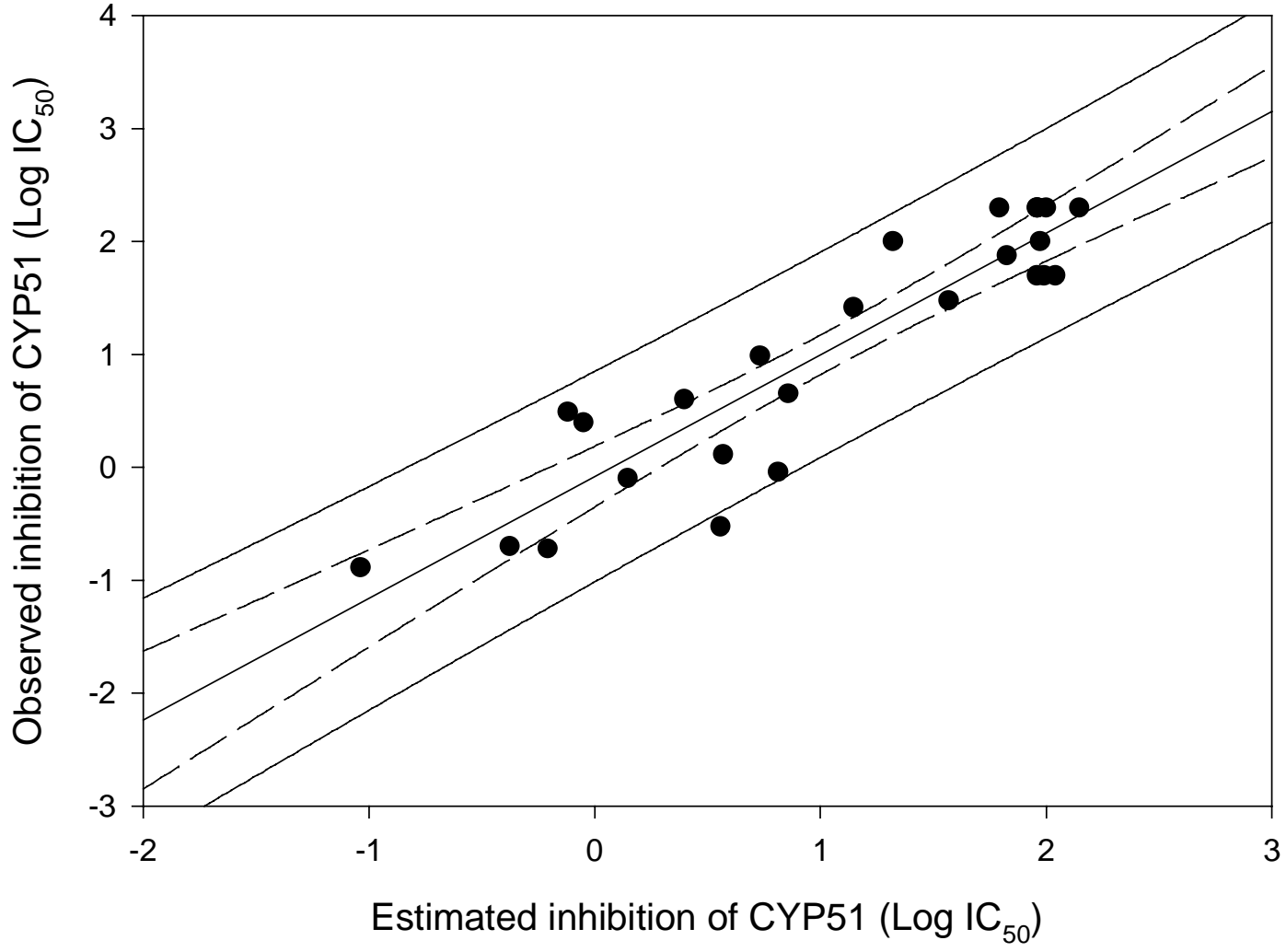
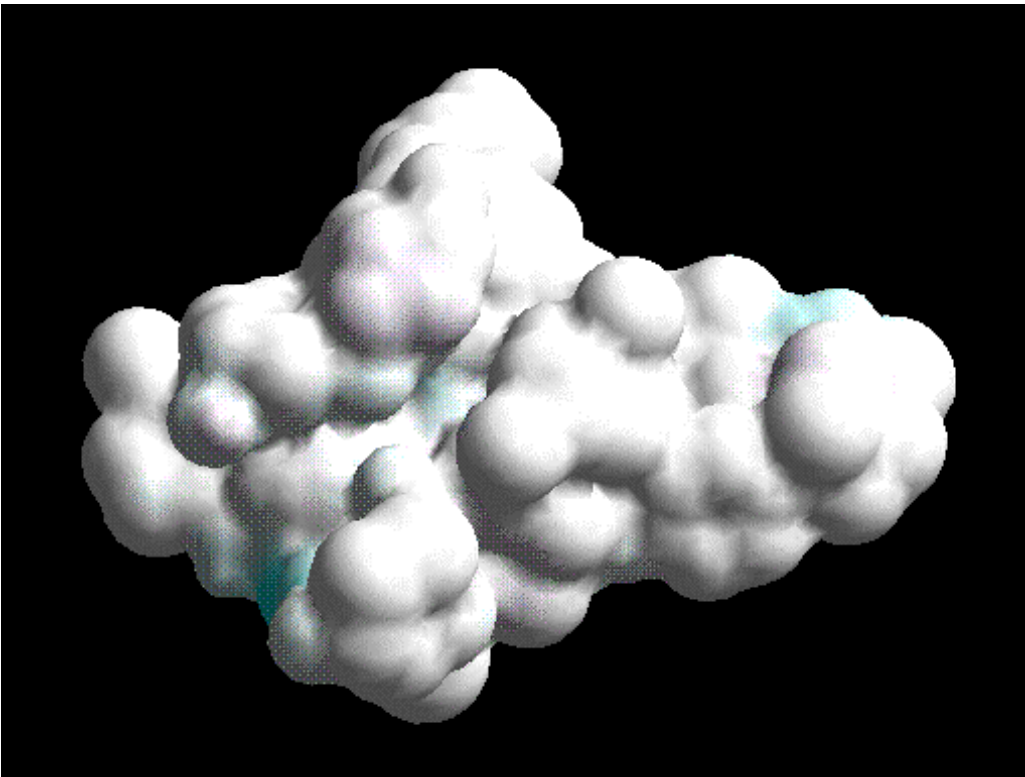
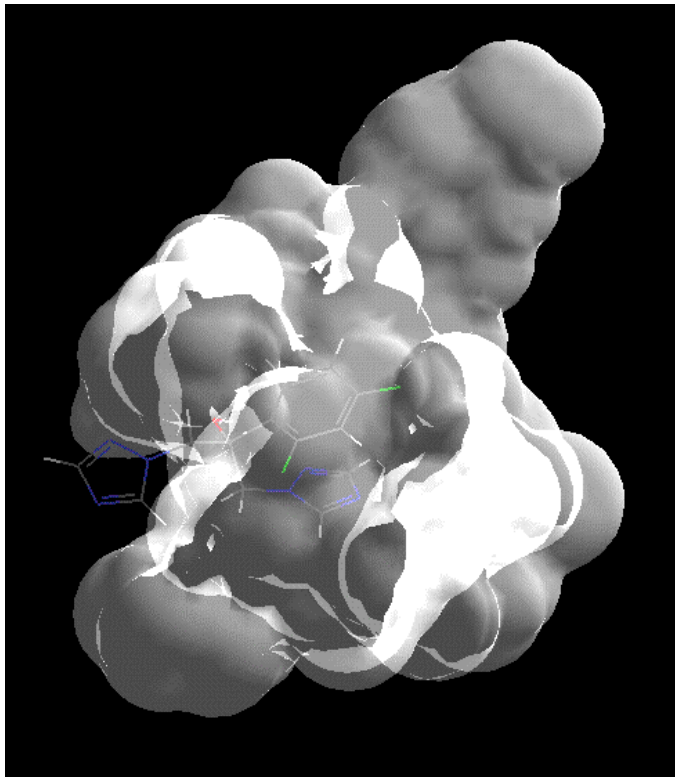


Fig 3

A



B



CORRECTION TO “THREE-DIMENSIONAL QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS OF HUMAN CYP51 INHIBITORS”

In Table 1 on page 496 of the article above [Ekins S, Mankowski DC, Hoover DJ, Lawton MP, Treadway JL, and Harwood HJ Jr (2007) *Drug Metab Dispos* **35**:493–500], the chemical structure for analog L was inadvertently dropped and replaced with a duplicate of the structure for benzimidazole. The correct structure for analog L appears below.

The online version of this article will be corrected in departure from the print version.

The printer regrets this error and apologizes for any confusion or inconvenience it may have caused.

