Development of a Generalized, Quantitative Physicochemical Model of CYP3A4 Inhibition for Use in Early Drug Discovery

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Purpose. To examine the structure-activity relationships for the inhibition of the activity of recombinant human CYP3A4 and to establish a generalized, quantitative physicochemical model for use in early drug discovery.

Methods. Inhibition of the activity of recombinant human CYP3A4 (erythromycin N-demethylase) by 30 diverse chemicals was studied using enhanced throughput methodology.

Results. There was a general, strong correlation between the IC_{50} value determined against erythromycin N-demethylase activity and lipophilicity ($LogD_{7.4}$) ($r^2 = 0.68$, $p < 0.0001$). This relationship was strengthened further by subdividing the structures studied into two distinct subpopulations of chemistry within the dataset. These could be identified by the absence ($r^2 = 0.80$, $p < 0.0001$) or presence ($r^2 = 0.69$, $p < 0.0001$) of a sterically uninhindered N-containing heterocycle, more specifically a pyridine, imidazole, or triazole function. The presence of these structural motifs increased the potency of CYP3A4 inhibition by approximately 10-fold for a given lipophilicity ($LogD_{7.4}$.value). More detailed analyses of AstraZeneca compounds demonstrated that the inhibitory potency of the pyridine structure can be attenuated through direct steric effects or electronic substitution resulting in a modulation of the pKa of the pyridine nitrogen, thereby influencing its ability to interact with the CYP heme.

Conclusions. A generalized, quantitative model is proposed for the inhibition of the major drug metabolizing enzyme, CYP3A4. This model indicates the importance of lipophilicity and rationalizes increased potency arising through additional interactions with the heme iron. These general relationships were shown to be applicable to a selection of compounds of interest to several early research projects.

KEY WORDS: CYP3A4; IC_{50}; lipophilicity; quantitative physicochemical model.

INTRODUCTION

Cytochrome P450 (CYP) enzymes are a class of heme-thiolate proteins that catalyse the regio- and stereo-selective oxidation of a wide variety of xenobiotics and pharmaceutical agents. Cytochrome P450 3A4 (CYP3A4) is quantitatively the most important human hepatic CYP isoform and catalytically the most promiscuous. This enzyme constitutes up to 50% total hepatic P450 content (1) and contributes to the metabolism of perhaps as much as 60% of major marketed therapies (2). Although CYP3A4 tends to catalyse preferentially the oxidation of lipophilic neutral or basic compounds, its active site appears capable of accommodating a wide range of structures from simple, rigid steroids to macromolecules such as cyclosporins. Noteworthy pharmaceutical substrates include anaesthetics, antibiotics, steroids, and cancer chemotherapeutics (2).

Since the majority of marketed drugs are metabolised by CYP enzymes, it has become commonplace to characterize the interactions of potential new therapies with members of this enzyme. In turn, this should facilitate the discovery and development of safer drugs with fewer side effects, predictable pharmacokinetic properties, and minimized drug-drug interactions and discussions with regulatory authorities. It is generally accepted that drug interactions arising through enzyme (CYP) inhibition are more likely to be clinically significant (3). Recent experiences with drugs such as terfenadine (4), cisapride (5), and mebefradil (6) have supported this basic tenet and have highlighted the importance of elucidating the underlying molecular basis (7).

With the advent of combinatorial chemistry, increased emphasis has been placed on enhanced throughput metabolic studies to generate data with which to eradicate such pharmacokinetic defects. In theory, this may arise either through serendipity, perhaps as a direct consequence of the ability to measure appropriate parameters in sufficient volume, or design *via* the establishment of predictive databases. In the absence of a crystal structure for human CYPs, most predictive (*in silico*) efforts to date have focused on basic pharmacophores and homology models, perhaps coupled with site-directed mutagenesis and NMR studies of ligand-protein interactions (8). It is interesting to note that specific hydrophobes are key, common components of the CYP3A4 molecular models described to date (9,10), which have applied a number of sophisticated computational chemistry and QSAR approaches. This report describes the development of a generalized, quantitative physicochemical model for CYP3A4 inhibition compiled from an investigation of the relationships between physicochemical properties of a series of structurally diverse compounds and their ability to inhibit CYP3A4 *in vitro*.

MATERIALS AND METHODS

Materials

All chemicals (see Table I) and reagents used were of the highest available commercial grade. [*N-methyl-* ^{14}C] erythromycin (specific radioactivity 55°mCi/mmole, radiochemical purity >97%) was purchased from DuPont NEN (Stevenage, UK). Other chemicals were synthesised at AstraZeneca R&D, Charnwood (Loughborough, UK) or obtained from sources documented previously (11). Pooled human liver microsomes (HLM) were purchased from IIAM (Leics, UK).

CYP3A4 Inhibition

The main body of the study used N-demethylation of [^{14}C]-erythromycin (59 μM) as probe reaction for CYP3A4 catalysed by HLM (12,13). All incubations and sample extractions were fully automated using a robotic sample processor (RSP) (Genesis RSP 150, Tecan, Reading, UK) and conducted as detailed previously (11). For some compounds, the 6β-hydroxylation of testosterone (50 μM) was also used as the CYP3A4 activity (13)and data were compared with literature values for the inhibition of cyclosporin A (CsA) oxi-

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Table I. Physicochemical Properties of Compounds Investigated as Initial Training Set and Their CYP3A4 Inhibition Potency (IC_{50})

Compound	Log P	$LogD_{7.4}$	IC_{50} (μM)	Log IC_{50}
Erythromycin	2.54	1.26	132	2.12
Chloroquine	4.63	1.23	350	2.54
Diltiazem	2.8	2.1	217	2.34
Verapamil	3.79	2.44	76	1.88
Bromocriptine	6.69[a]	6.21	3	0.48
Dihydroergotamine	4.94[a]	4.94	3	0.48
Troleandomycin	3.2[a]	3.2	8	0.90
Sulfamethizole	0.54	–1.11	836	2.92
Timoprazole	1.33	1.33	309	2.49
Piroxicam	1.98	0.26	1000	3.00
Tazanolast	2.03[a]	–0.54	290	2.46
Cimetidine	0.4	0.33	1000	3.00
Nifedipine	3.27	3.27	47	1.67
Omeprazole	2.23	2.23	78	1.89
1-Me imidazole	–0.06	–0.06	2700	3.43
2-Me imidazole	–0.4[a]	–0.99	1252	3.10
3-OH pyridine	0.52	0.52	768	2.89
2-anilino pyridine	2.75	2.75	431	2.63
AZ-2	3.8	3.8	43	1.63
Thioperamide	3[a]	2.59	6.1	0.79
Triadimefon	2.77	2.77	9.3	0.97
Propiconazole	3.5	3.5	1.04	0.02
Ketoconazole	4.34	4.33	0.2	–0.70
Metyrapone	1.5	1.5	4.93	0.69
Glipizide	1.91	1.91	7.44	0.87
Econazole	5.25[a]	5.25	0.43	–0.37
Miconazole	5.96[a]	5.96	0.85	–0.07
Clotrimazole	5.05[a]	5.05	0.05	–1.30
Fluconazole	0.5	0.5	25	1.40
AZ-1	4.0	4.0	3.5	0.54

[a] cLog P quoted.

dation in human hepatocytes (ketoconazole, troleandomycin, nifedipine, verapamil, erythromycin, and diltiazem). Inter-assay variation was previously determined using ketoconazole as the standard inhibitor (IC_{50} = 0.26 ± 0.02 μM, mean ± SD, N = 5)(11).

Data Analysis

Incubations were conducted using an erythromycin concentration equivalent to the Km in HLM, such that the IC_{50} estimated would be within two-fold of the Ki, regardless of the mechanism of inhibition, since under these conditions,

$$\mathrm{Ki} = \frac{\mathrm{IC_{50}}}{1 + (\mathrm{S/Km})}$$

for competitive inhibition and Ki = IC_{50} for noncompetitive or uncompetitive inhibition (14). IC_{50} values were estimated in Win-NonLin (Scientific Consulting Inc., Cary NC) using the formula below:

$$v = \frac{Vo}{1 + (I/IC_{50})^s} - b$$

where v = velocity or % control activity, Vo = control activity, I = inhibitor concentration, s = slope factor (usually = 1), and b = background (uninhibited) activity.

RESULTS AND DISCUSSION

Figure 1 shows the relationship between the IC_{50} values obtained for 11 compounds (some of which are listed in Table I) screened in this laboratory against erythromycin N-demethylase activity and by other groups using either cyclosporin A (CsA) (15) or testosterone as the probe substrate. The IC_{50} values obtained span three orders of magnitude. The data show clearly that, under the experimental conditions chosen, the interactions of these inhibitors or co-substrates with CYP3A4 are quite similar. These observations are consistent with data generated by other labs (16). Erythromycin N-demethylase would therefore appear useful as a probe reaction for the early assessment of inhibition of human hepatic CYP3A4 activity within drug discovery. However, reports of atypical observations for some substrate-inhibitor combinations may indicate the value of occasional confirmation with a secondary substrate. These include nifedipine-testosterone (17) and haloperidol-benzyloxyresorufin (16). The data presented in this report would benefit from confirmation with further probe substrates.

Using erythromycin N-demethylase as the CYP3A4 activity, preliminary analysis of the data obtained for all the compounds studied (Table I) demonstrated a strong relationship between lipophilicity ($logD_{7.4}$) and the potency of CYP3A4 inhibition (Fig. 2). The regression equation is shown below:

$$\mathrm{Log\ IC_{50}} = -0.524\ \mathrm{LogD_{7.4}} + 2.693 \qquad \text{(all compounds)}$$

$$(\mathrm{N} = 30 \quad r^2 = 0.69 \quad p < 0.0001)$$

More detailed analysis suggested the existence of two distinct subpopulations of chemistry within the dataset. These could be identified by the presence or absence of a sterically uninhindered N-containing heterocycle, more specifically a pyri-

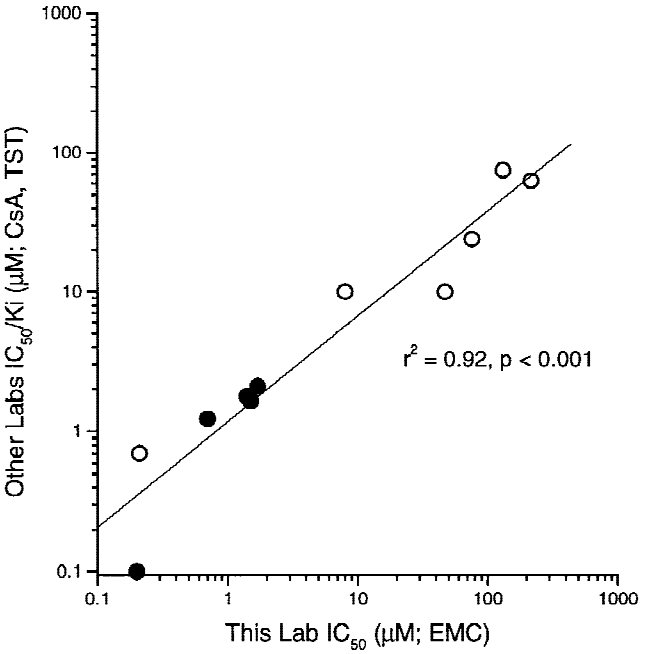


Fig. 1. Comparison between inhibition of CYP3A4 activity observed with several prototypic substrates: erythromycin (EMC), cyclosporin A (5 μM CsA, ○), and testosterone (TST, ●) using human liver microsomes or human hepatocytes (CsA).

dine, imidazole, or triazole function. Separating the data into these two series resulted in stronger correlations for both resultant trends:

$$\text{Log } IC_{50} = -0.384 \text{ Log}D_{7.4} + 1.564 \quad \text{(pyridines, imidazoles, triazoles)}$$

$$(N = 11 \quad r^2 = 0.69 \quad p < 0.0001)$$

$$\text{Log } IC_{50} = -0.389 \text{ Log}D_{7.4} + 2.883 \quad \text{(other compounds)}$$

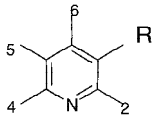
$$(N = 19 \quad r^2 = 0.80 \quad p < 0.0001)$$

The data demonstrate that much of the variance in the inhibition of CYP3A4 by this diverse range of compounds could be described from a consideration of their lipophilicity. This is consistent with previous observation with CYP3A4 pharmacophore models and the apparent lack of functionality in the amino acid residues comprising the CYP3A4 active site (8,10). The gradients for both datasets show a similar dependence upon $\text{logD}_{7.4}$. Moreover, the presence of a sterically uninhindered N-containing heterocycle in the structure appeared to increase the potency of CYP3A4 inhibition by an order of magnitude for a given value of $\text{LogD}_{7.4}$. It is likely that this reflects the increased binding energy afforded by the lone pair on the nitrogen atoms ligating to the CYP heme iron (some 6 kcal/mol), as suggested for the prototypic CYP inhibitors ketoconazole, sulfaphenazole, quinidine, and pyridine (18).

The importance of the position of the substitution on the pyridine nucleus was shown with two AstraZeneca congeners (AZ1 and AZ2 in Table I): the 3′- and 2′-pyridyl analogues conferred an IC_{50} of 3 or 43 μM at CYP3A4. These observations prompted a synthetic effort to explore the structure-activity of pyridine substitution in more detail. Table II shows that the inhibitory potency of the pyridine structure can be attenuated through direct steric effects (eg., NMe_2 or CH_2OH at the 2'-position) or electronic substitution (halogens at the 2'-, 4'-, or 6'-position) resulting in a modulation of the pKa of the pyridine nitrogen thereby influencing its ability to interact with the CYP heme.

The data presented here support the concept that basic guidelines may be established for CYP3A4 inhibition akin to those proposed by Lipinski and colleagues for passive drug absorption (19):

Table II. Modifications of CYP3A4 Inhibition Within a Series of Pyridine-Containing Analogues (R = side chain; "—", not tested)



Substituent	IC_{50} (μM) with substituent at position: 2	4	5	6
H	3	3	3	3
F	71	>200	5	—
OCH_3	>200	>200	1	7
CH_2OH	74	—	—	—
NMe_2	13	—	1	5
OH	—	—	10	—
Cl	—	—	—	15
CH_3	—	—	—	5
OCH_2Ph	—	—	—	2

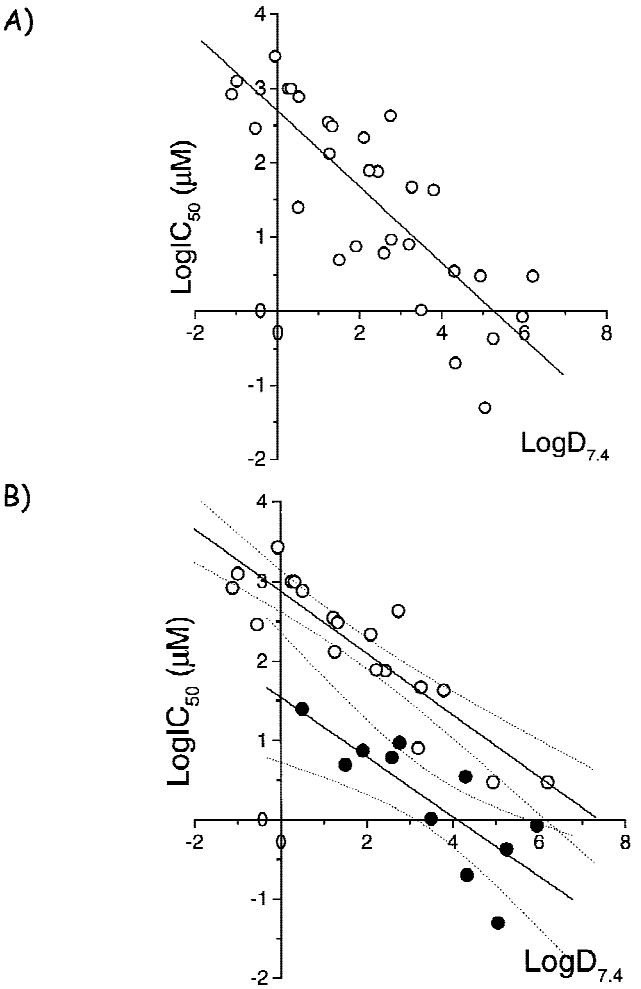


Fig. 2. Relationships between lipophilicity ($\text{LogD}_{7.4}$) and CYP3A4 inhibition for (A) initial dataset and (B) two distinct sub-populations of chemistry within the dataset identified by the presence (●) or absence (○) of a sterically uninhindered pyridine, imidazole, or triazole function. The broken lines in (B) indicate the 95% confidence intervals.

- The IC_{50} for CYP3A4 inhibition is significantly correlated with lipophilicity ($\text{logD}_{7.4}$);
- The IC_{50} values for structures possessing a nitrogen-containing heterocycles (triazoles, pyridines, imidazoles, quinolines, thiazoles) may be an order of magnitude lower for a given $\text{LogD}_{7.4}$;
- The proposed interaction between the nitrogen lone pair and the CYP heme can be modified specifically by steric hindrance or perturbation of the pKa;

Figure 3 shows the application of the relationships established for the different chemicals studied to several Research projects at AstraZeneca. This figure shows the CYP3A4 IC_{50} predicted from the relationships shown in Fig. 2 following a visual "by eye" inspection of the structures plotted against the measured data.

It is interesting to speculate that a similar relationship may exist for the metabolism of CYP3A4 substrates. Indeed, several of the compounds studied here are actually CYP3A4 substrates, for which IC_{50} would be related to Km (or Kd). In this case, the intrinsic clearance, Clint ($=V_{max}/Km$), may be

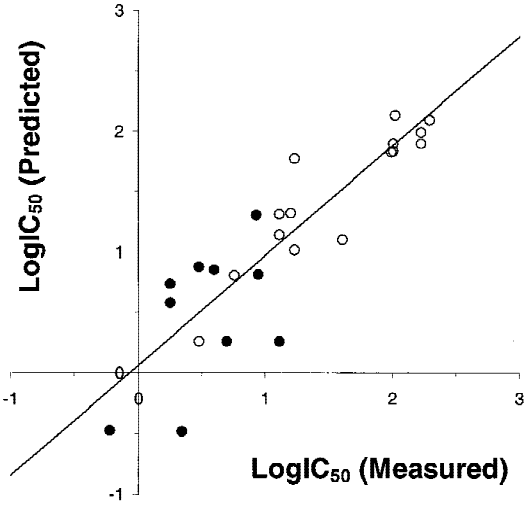


Fig. 3. Application of model from initial training set to ongoing Discovery projects at AstraZeneca Charnwood (external validation). *Closed symbols* represent compounds containing a sterically uninhindered pyridine, imidazole, or triazole function. The relationship between the predicted and measured $\log IC_{50}$ values is given by $y = 0.910x + 0.056$ ($r^2 = 0.76$, $p < 0.001$).

expected to vary with $LogD_{7.4}$ for a range of CYP3A4 substrates exhibiting similar biotransformations where Vmax for the metabolic route may be quite similar (20).

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