The Reliability of Estimating $K_i$ Values for Direct, Reversible Inhibition of Cytochrome P450 Enzymes from Corresponding IC$_{50}$ Values: A Retrospective Analysis of 343 Experiments

Lois J. Haupt, Faraz Kazmi, Brian W. Ogilvie, David B. Buckley, Brian D. Smith, Sarah Leatherman, Brandy Paris, Oliver Parkinson, and Andrew Parkinson


Received July 28, 2015; accepted September 8, 2015

ABSTRACT

In the present study, we conducted a retrospective analysis of 343 in vitro experiments to ascertain whether observed (experimentally determined) values of $K_i$ for reversible cytochrome P450 (P450) inhibition could be reliably predicted by dividing the corresponding IC$_{50}$ values by two, based on the relationship (for competitive inhibition) in which $K_i = IC_{50}/2$ when [S] (substrate concentration) = $K_m$ (Michaelis-Menten constant). Values of $K_i$ and IC$_{50}$ were determined under the following conditions: 1) the concentration of P450 marker substrate, [S], was equal to $K_m$ (for IC$_{50}$ determinations) and spanned $K_m$ (for $K_i$ determinations); 2) the substrate incubation time was short (5 minutes) to minimize metabolism-dependent inhibition and inhibitor depletion; and 3) the concentration of human liver microsomes was low (0.1 mg/ml or less) to maximize the unbound fraction of inhibitor. Under these conditions, predicted $K_i$ values, based on IC$_{50}$/2, correlated strongly with experimentally observed $K_i$ determinations ($r = 0.940$; average fold error (AFE) = 1.10). Of the 343 predicted $K_i$ values, 316 (92%) were within a factor of 2 of the experimentally determined $K_i$ values, and only one value fell outside a 3-fold range. In the case of noncompetitive inhibitors, $K_i$ values predicted from IC$_{50}$/2 values were overestimated by a factor of nearly 2 (AFE = 1.85; $n = 13$), which is to be expected because, for noncompetitive inhibition, $K_i = IC_{50}$ (not IC$_{50}$/2). The results suggest that, under appropriate experimental conditions with the substrate concentration equal to $K_m$, values of $K_i$ for direct, reversible inhibition can be reliably estimated from values of IC$_{50}$/2.

ABBREVIATIONS: AFE, average fold error; AUC, area under the plasma concentration-time curve; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; NRMSE, normalized root mean square error; P450, cytochrome P450; RMSE, root mean square error.
exceed values of $[I]$ (total $C_{max}$); accordingly, the FDA’s cutoff value for inhibition of intestinal P450 enzymes is 11 (based on $1 + [I]_{gut}/K_{unbound}$). The corresponding EMA cutoff is 10 (based on $[I]_{gut}/K_{unbound}$).

Typically, inhibition of P450 enzymes is first evaluated in vitro by determining the concentration of investigational drug (or significant circulating metabolite) that causes 50% inhibition of P450 enzyme activity ($IC_{50}$) with a selective P450 probe at a substrate concentration approximately equal to $K_m$ (Michaelis-Menten constant). Determining the mechanism of reversible inhibition (competitive, noncompetitive, mixed, or uncompetitive) and measuring the value of $K_i$ requires an in vitro evaluation of the effects of multiple inhibitor concentrations versus multiple substrate concentrations, ideally with the former spanning $K_i$ and the latter spanning $K_m$. Determining values of $IC_{50}$ and $K_i$ based on the unbound concentration of inhibitor in the test system (typically human liver microsomes) requires knowledge of $f_{un}$ (the fraction of unbound drug in the microsomal incubation), which can be determined experimentally or estimated theoretically from the inhibitor’s logP or logD value and the concentration of microsomal protein (Austin et al., 2002; Hallifax and Houston, 2006).

The relationship between $K_i$ and values of $IC_{50}$ determined when $[S]$ (substrate concentration) = $K_m$ depends on the mechanism of inhibition, as summarized in Table 1 (Cheng and Prusoff, 1973; Brandt et al., 1987; Cer et al., 2009). In the case of noncompetitive inhibition, $K_i = IC_{50}$. In the case of competitive and uncompetitive inhibition, $K_i = IC_{50}/2$. In the case of mixed inhibition, $K_i$ values range from $IC_{50}$ to $IC_{50}/2$. The FDA’s 2012 Guidance for Industry on drug interactions acknowledges that $K_i$ values are often estimated from values of $IC_{50}/2$, based on the conservative assumption that the mechanism of reversible inhibition is competitive in nature. In the present study, we conducted a retrospective analysis of 343 in vitro $K_i$ determinations to investigate whether experimentally determined $K_i$ values can, in fact, be reliably estimated from values of $IC_{50}/2$ when $IC_{50}$ values are determined under conditions of $[S] \approx K_m$.

### Materials and Methods

#### Chemicals and Reagents

The commercial sources of most substrates, metabolites, internal standards, and reagents have been described previously (Pearce et al., 1996; Paris et al., 2009; Parkinson et al., 2011). Efavirenz was purchased from U.S. Pharmacopeia (Rockville, MD). 8-Hydroxyefavirenz, 8-hydroxyefavirenz-d$_1$ (internal standard), and 6-hydroxyefavirenz-d$_2$ (internal standard) were purchased from Toronto Research Chemicals, Inc. (North York, Ontario, Canada). The compounds represent a set of structurally diverse, small drug molecules under development for several different therapeutic indications. The design of the experiments and the interpretation of the results of this study (a comparison of two endpoints of P450 inhibition) required neither knowledge of chemical structures nor physicochemical properties.

#### Test System

Pooled human liver microsomes ($n = 16$ or 200; mixed gender) were prepared from nontransplantable livers and characterized at Xenotech, LLC (Lexena, KS) as described previously (Pearce et al., 1996; Parkinson et al., 2004).

#### Incubation Conditions

$K_i$ and $IC_{50}$ values were determined in accordance with recommendations in the FDA and EMA guidance documents and consensus papers (Tucker et al., 2001; Bjornsson et al., 2003). All experiments were performed under the following conditions: 1) the concentration of P450 marker substrate was approximately equal to $K_m$ for $IC_{50}$ determinations and spanned $K_m$ for $K_i$ determinations (i.e., $[S]$ ranged from 0.25 times $K_m$ to 10 times $K_m$; solubility permitting); 2) the substrate incubation time was 5 minutes to minimize metabolism-dependent inhibition and inhibitor depletion; and 3) the concentration of human liver microsomes was 0.1 mg/ml or less to maximize the unbound inhibitor concentration.

In general, incubations were conducted at 37°C in 200- or 400-μl incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), MgCl$_2$ (3 mM), EDTA (1 mM), NADPH-generating system, and human liver microsomes. Most of the P450 reactions examined have been described in detail elsewhere (Paris et al., 2009; Parkinson et al., 2011). The P450 substrates, analytes (metabolites measured), internal standards, and microsomal protein concentration for all of the P450 reactions examined are summarized in Supplemental Table 1.

#### Analytical Methods

All metabolites and their internal standards (usually isotopically labeled metabolites) were measured with validated liquid chromatography–tandem mass spectrometry methods on AB Sciex (Framingham, MA) API 2000, 3000, or 4000 mass spectrometers with Shimadzu (Kyoto, Japan) high-performance liquid chromatography pumps and autosampler systems according to methods described previously (Paris et al., 2009; Parkinson et al., 2011). Peak areas for all metabolites were integrated with reference to a standard calibration curve based on back-calculation of a weighted (1/x), linear, least-squares regression.

#### Data Processing

$IC_{50}$ data were processed with one of two validated software packages, Galileo- Laboratory Information Management System (Galileo version 3.3; Thermo Fisher Scientific Inc., Grand Island, NY) or XLfit (version 3.0.5; ID Business Solutions Ltd., Guildford, Surrey, UK), which is

### Table 1

<table>
<thead>
<tr>
<th>Type of Inhibition</th>
<th>Modified Michaelis-Menten Equations</th>
<th>Relationship between $K_i$ and $IC_{50}$ when $[S] = K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive</td>
<td>$v = \frac{V_{max} [S]}{K_m + [S]}$</td>
<td>$K_i = \frac{IC_{50}}{2}$</td>
</tr>
<tr>
<td>Uncompetitive</td>
<td>$v = \frac{V_{max} [S]}{K_m + [S]}$</td>
<td>$K_i = IC_{50}$</td>
</tr>
<tr>
<td>Mixed</td>
<td>$v = \frac{V_{max} [S]}{K_m + [S]}$</td>
<td>$K_i = IC_{50}$ to $IC_{50}$ depending on the ratio of $K_i$ to $K_m$</td>
</tr>
<tr>
<td>Noncompetitive</td>
<td>Same equation as above but this is a special case of mixed inhibition where $K_i = K_m$ and $K_m$ remains unchanged.</td>
<td>$K_i = IC_{50}$</td>
</tr>
</tbody>
</table>

* $K_m = \frac{[S]}{C_{avg}}$ (binding of inhibitor, I, to the enzyme, E); $K_p = \frac{[S]}{C_{avg}}$ (binding of the inhibitor, I, to the enzyme-substrate complex, ES).
used within a customized software program (DI IC50 LCMS Template version 2.0.3) for Microsoft Excel Office 2000 (version 9.0; Microsoft Inc., Redmond, WA). Both software programs use a Levenberg-Marquardt algorithm (Levenberg, 1944; Marquardt, 1963), also known as a damped least-squares algorithm, to fit a nonlinear regression (sigmoidal) curve to IC50 data based on the following equation:

$$\text{Fit} = \min + \frac{\max - \min}{1 + \left(\frac{\text{Inhibitor}}{\text{IC50}}\right)^{\text{slope}}}$$

where Min = zero (no inhibition) and Max = 100 (complete inhibition). In XLfit, the terms Min and Max are called background and range, respectively. Both software programs have been validated for their ability to calculate IC50 values only when they lie within the actual range of inhibitor concentrations tested. In other words, none of the IC50 values reported here was extrapolated from data that fell above or below the highest or lowest concentration of inhibitor, respectively.

The data for Ki determinations were processed with one of two comparable methods. The first method used Microsoft Excel to calculate rates of metabolite formation, which were imported into GraFit (Erithacus Software Ltd., Horley, Surrey, UK) to perform nonlinear regression according to the Michaelis-Menten equations associated with each type of direct inhibition. The second method used a Galileo Laboratory Information Management System (Thermo Scientific, Waltham, MA) with Crystal Reports-SAP Business Objects (SAP, Newtown Square, PA). The data (i.e., reaction rates at all concentrations of inhibitor at all concentrations of P450 marker substrates) were fitted to the Michaelis-Menten equations for competitive, noncompetitive, uncompetitive, and mixed (competitive-noncompetitive) inhibition (see Table 1) by nonlinear regression analysis. The goodness of fit to each of the four inhibition equations was determined by $\chi^2$ analysis (with lower values indicating better fit) or by comparison of Akaike information criterion values (with higher values indicating better fit), which provided an initial basis for identifying the mechanism of inhibition. Eadie-Hofstee plots (rate versus rate/$[S]$) were inspected visually. At times, the nonlinear regression lines did not correlate well with the data points depicted on the Eadie-Hofstee plot, and visual inspection of the kinetic plots was necessary to deduce the mechanism of inhibition. Both methods of data processing are validated to calculate $K_i$ values only when they lie within the range of inhibitor concentrations tested.

**Statistical Analysis.** The accuracy of the prediction of observed $K_i$ values from values of IC50/2 was assessed by determining the average fold error (AFE) according to eq. 4 (Obach et al., 1997). This method is based on absolute values of the logarithm of the ratio of predicted-to-observed values, meaning that all negative values are converted to positive values so that, for example, values 50% less and 100% more than the observed value both represent a 2-fold error. An AFE value of 1 represents a perfect prediction.
Correlation of Estimated $K_i$ and Experimental $K_i$ Values

The precision of the prediction was assessed by calculating the root mean square error (RMSE) according to eq. 5 (Sheiner and Beal, 1981):

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum (\text{predicted } K_i - \text{observed } K_i)^2}$$  \hspace{1cm} (5)

The normalized RMSE (NRMSE; expressed as a percentage) was calculated as follows:

$$\text{NRMSE} = \frac{\text{RMSE}}{\text{Max observed } K_i - \text{Min observed } K_i} \times 100$$  \hspace{1cm} (6)

In all cases, the observed $K_i$ is the experimentally determined value, and the predicted $K_i$ corresponds to $IC_{50}/2$.

### Results

The nine P450 enzymes and 14 marker substrates examined in the current study, and the distribution of the types of reversible inhibition (i.e., competitive, noncompetitive, mixed, and uncompetitive inhibition) observed in vitro with human liver microsomes are summarized in Fig. 1 and Table 2. The inhibitors were 132 structurally diverse investigational drugs, some of which were examined as reversible inhibitors of more than one P450 enzyme (data not shown). The correlations between the 343 observed (experimentally determined) $K_i$ values and those predicted from the corresponding values of $IC_{50}/2$ (when $[S] \approx K_m$) are shown in Fig. 2 and Table 3. The correlations between observed and predicted $K_i$ values as a function of the mechanism of P450 inhibition are shown in Fig. 3. The ratios of the observed-to-predicted $K_i$ values as a function of the mechanism of P450 inhibition are shown in Figs. 4 and Table 3. Measures of the accuracy (AFE) and precision (RMSE and NRMSE) of predicting $K_i$ values from $IC_{50}/2$ when $[S] \approx K_m$ are shown in Figs. 2 and 3, and summarized in Table 3.

### Discussion

A total of 343 $K_i$ values for reversible inhibition of various P450 enzymes was determined experimentally (from studies with multiple inhibitor concentrations versus multiple substrate concentrations) with 132 structurally diverse investigational drugs (some of which were examined with more than one P450 enzyme) after determining $IC_{50}$ values in human liver microsomes under conditions where $[S] \approx K_m$. Table 2 shows the distribution of the four mechanisms of reversible inhibition for the 343 $K_i$ determinations. The compounds were predominantly (~95%) mixed ($n = 217$) or competitive ($n = 108$) P450 inhibitors; however, noncompetitive inhibitors ($n = 13$) and

### Table 3

Summary of the accuracy (AFE) and precision (RMSE and NRMSE) of predicting $K_i$ values for reversible cytochrome P450 inhibition from values of $IC_{50}/2$ when $[S] \approx K_m$

<table>
<thead>
<tr>
<th>Inhibition Type</th>
<th>$n$</th>
<th>Ratio of Observed versus Predicted $K_i$</th>
<th>Correlation coefficient ($r$)</th>
<th>AFE$^a$</th>
<th>RMSE$^b$</th>
<th>NRMSE$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>$n &lt; 0.5$</td>
<td>$n &gt; 2.0$</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>217</td>
<td>1.13</td>
<td>0.481–2.95</td>
<td>1</td>
<td>9</td>
<td>0.926</td>
</tr>
<tr>
<td>Competitive</td>
<td>108</td>
<td>1.19</td>
<td>0.335–3.41</td>
<td>1</td>
<td>9</td>
<td>0.985</td>
</tr>
<tr>
<td>Noncompetitive</td>
<td>13</td>
<td>1.90</td>
<td>1.26–2.79</td>
<td>0</td>
<td>6</td>
<td>0.994</td>
</tr>
<tr>
<td>Uncompetitive</td>
<td>5</td>
<td>1.35</td>
<td>0.725–2.55</td>
<td>0</td>
<td>1</td>
<td>0.951</td>
</tr>
<tr>
<td>Mixed, competitive, and uncompetitive</td>
<td>330</td>
<td>1.15</td>
<td>0.335–3.41</td>
<td>2</td>
<td>19</td>
<td>0.941</td>
</tr>
<tr>
<td>Total</td>
<td>343</td>
<td>1.18</td>
<td>0.335–3.41</td>
<td>2</td>
<td>25</td>
<td>0.940</td>
</tr>
</tbody>
</table>

$^a$See eq. 4.  
$^b$See eq. 5.  
$^c$See eq. 6.
uncompetitive inhibitors \((n = 5)\) were also represented in the data set.

The distribution of P450 enzymes analyzed as well as the marker substrates used are shown in Fig. 1 and Table 2. For the seven drug-metabolizing P450 enzymes listed in the FDA and EMA guidance documents on drug interactions, the number of \(K_i\) determinations ranged from 21 (CYP1A2) to 94 (CYP3A4/5). Relatively few \(K_i\) values were determined with CYP2A6 \((n = 6)\) and CYP2E1 \((n = 2)\). Four P450 enzymes (CYP2B6, 2C8, 2D6, and 3A4/5) were assayed with more than one substrate.

The correlation between the observed (experimentally determined) \(K_i\) values and the predicted \(K_i\) values (from IC50/2) for the entire data set is shown in Fig. 2. The experimentally determined \(K_i\) values ranged 200,000-fold from 9 nM to 1.9 mM. Overall, predicted values of \(K_i\) (based on IC50/2) correlated well \((r = 0.940)\) with observed values of \(K_i\), and the prediction was both accurate \((AFE = 1.10)\) and precise \((RMSE = 64.2; NRMSE = 3.38\%)).

When the data were segregated by inhibition type, the observed and predicted \(K_i\) values were highly correlated for all four types of inhibition \((r values ranged from 0.926 to 0.994)\), as shown in Table 3 and Fig. 3. Values of IC50/2 served as accurate predictors of observed \(K_i\) values for competitive inhibition \((AFE = 1.10)\) and uncompetitive inhibition \((AFE = 1.24)\). This was as expected because, in theory, \(K_i\) should equal IC50/2 for these types of inhibition (Table 1). Values of IC50/2 also accurately predicted \(K_i\) values for mixed inhibition \((AFE = 1.07)\), where, in theory, \(K_i\) values can range from IC50 to IC50/2 (Table 1). However, IC50/2 was a less accurate predictor of \(K_i\) values for noncompetitive inhibition. In this case, the AFE value of 1.85 indicates that the prediction was off by a factor of nearly 2, which is consistent with the equation in Table 1, indicating that, for noncompetitive inhibition, \(K_i = IC_{50}\) and not IC50/2. Omitting the 13 \(K_i\) values for noncompetitive inhibition (where theoretically \(K_i = IC_{50}\) and not IC50/2) resulted in a negligible improvement in overall AFE; the value decreased from 1.10 to 1.08, and NRMSE decreased slightly from 3.38 to 3.34%, as shown in Table 3.

The ratios of observed-to-predicted \(K_i\) values are shown in Fig. 4 and summarized in Table 3. Of the 343 predicted \(K_i\) values, 316 (92%) were within a factor of 2 of the experimentally determined \(K_i\) value. Two predicted values were less than 0.5 (but not less than 0.33) and 25 values were greater than 2, only one of which was greater than 3. In other words, of the 343 predicted \(K_i\) values, 316 were within a factor of 2 and 342 were within a factor of 3, meaning only one value fell outside a 3-fold range (the actual value was 3.41). A preliminary account of this work included a second case in which the predicted \(K_i\) value was more than 3-fold greater than the experimentally determined \(K_i\) value (Haupt et al., 2011). However, in this case, the initial IC50 value was below the

---

**Fig. 3.** Comparison of observed (experimentally determined) \(K_i\) values with \(K_i\) values estimated from values of IC50/2 (when \([S] \approx K_m\)) as a function of the mechanism of reversible P450 inhibition. The line shown is a line of identity.

**Fig. 4.** Ratio of observed (experimentally determined) \(K_i\) values with \(K_i\) values estimated from values of IC50/2 (when \([S] \approx K_m\)) as a function of the mechanism of reversible P450 inhibition. The line shown is a line of identity.
lowest concentration of inhibitor tested, and an interpolated IC_{50} value was used in the analysis. This observation underscores the importance of predicting K_i values only when the IC_{50} value falls within the range of inhibitor concentrations tested.

It is important to note that other experimental conditions can affect the measured values of IC_{50} and the kinetic constants K_{m} and K_i (Obach, 1996, 1997, and 1999; McLeur et al., 2000; Austin et al., 2002; Di Marco et al., 2003; Margolis and Obach, 2003; Hallifax and Houston, 2006; Howgate et al., 2006; Brown et al., 2007a,b; Ogilvie et al., 2011; Parkinson et al., 2011). In this study, all K_{m} and IC_{50} values were determined at 0.1 mg microsomal protein/ml or less with a short substrate incubation time (5 minutes) to minimize metabolic loss of the inhibitory drug, to reduce the possibility of metabolism-dependent inhibition, and to maximize the concentration of unbound inhibitor. Membrane partitioning (commonly but erroneously called nonspecific binding (Nagar and Korzekwa, 2012)) decreases the unbound concentration of inhibitor, but this effect can be corrected by measuring or calculating f_{unb} (Austin et al., 2002; Hallifax and Houston, 2006) and expressing the inhibition constant as K_{i,unbound} now recommended by both the FDA and EMA. This same approach can be applied to measurements of K_i based on IC_{50}/2. It should be noted that, in the current study, IC_{50} and K_i values for a given P450 enzyme were determined under identical experimental conditions (i.e., the same concentration of microsomal protein), such that values of f_{unb} were the same for predicted and observed K_i determinations. Accordingly, correcting for f_{unb} would have had no effect on estimates of the ratio of the prediction-to-observed K_i values or the accuracy and precision of predicting K_i values from IC_{50}/2.

Although the experimentally determined K_i values varied approximately 200,000-fold, most of them (240 of 343) fell between 1 and 25 µM (with 61 greater than 25 µM and 42 less than 1 µM). In the majority of cases (287 of 343 determinations), the initial IC_{50} value was 50 µM or less (hence, the predicted K_i value was 25 µM or less), suggesting that the decision to measure K_i values was biased toward those cases in which the initial IC_{50} determination suggested relatively strong P450 inhibition. There were 37 cases in which the initial IC_{50} was greater than 100 µM (i.e., the inhibition was relatively weak). It might be expected that, in these cases, the decision to perform a K_i determination was biased toward CYP3A4/5 because of its intestinal location, where the relevant inhibitor concentration is molar dose/250 ml, not plasma C_{max,ss}. However, of the 37 cases of weak inhibition, K_i values were determined for a wide variety of P450 enzymes: 13 for CYP3A4/5, 6 for CYP2C9, 6 for CYP2C19, 5 for CYP2D6, 3 for CYP2B6, 2 for CYP1A2, 1 for CYP2A6, and 1 for CYP2C8. Therefore, CYP3A4/5 represented only 35% of the cases in which K_i was determined when the initial IC_{50} was greater than 100 µM.

Interestingly, uncompetitive inhibition, characterized by a decrease in both V_{max} and K_{m}, was observed only when nifedipine was the substrate. Of the 10 assays performed with nifedipine, 5 exhibited uncompetitive inhibition. The CYP3A substrates used in the current study, midazolam, testosterone, and nifedipine, are thought to bind to three distinct regions within the substrate-binding site and exhibit distinct enzyme kinetics characterized by hyperbolic (typical Michaelis-Menten) kinetics in the case of midazolam, substrate activation (sigmoidal kinetics due to homotropic cooperativity) in the case of testosterone, and substrate inhibition in the case of nifedipine. The high prevalence of uncompetitive inhibition observed with nifedipine, which suggests some inhibitors bind to the CYP3A-nifedipine (enzyme-substrate) complex, is consistent with previous studies documenting the so-called stand-alone properties of nifedipine as a CYP3A4/5 substrate (Kennworthy et al., 1999; Wang et al., 2000; Galetin et al., 2002, 2003; Foti et al., 2010).

Overall, our analysis of 343 determinations supports theoretical considerations (Table 1) that, when determined under in vitro conditions of low protein concentration, short substrate incubation time, and [S] ≈ K_{m}, values of IC_{50}/2 provide an accurate prediction (generally within a factor of 2) of experimentally determined K_i values for all types of reversible, direct inhibition of P450 enzymes. In the case of noncompetitive inhibitors, values of K_i estimated from IC_{50}/2 were off by a factor of ~ 2 (AFE = 1.85), which is consistent with theoretical considerations (Table 1). However, noncompetitive inhibitors accounted for a relatively low percentage (~4%) of the types of inhibition observed (13 of 343 determinations). In conclusion, the results of our analysis suggest that, under appropriate experimental conditions, K_i values for direct, reversible inhibition can be reliably, albeit somewhat conservatively, estimated from values of IC_{50}/2.

The following excerpt is from the FDA’s Guidance for Industry on drug interactions (from footnote 2 on page 21): “For a drug that is a reversible inhibitor, R = 1 + [I]/K_i. K_i is the unbinding inhibition constant determined in vitro. Sometimes inhibitor concentration causing 50% inhibition (IC_{50}) is determined, and K_i can be calculated as IC_{50}/2 by assuming competitive inhibition.” The results of our analysis suggest that measuring IC_{50} is sufficient to estimate K_i for the purpose of evaluating the potential of an investigational drug to cause clinically relevant P450 inhibition, with two stipulations. First, IC_{50} is determined under appropriate experimental conditions, as described in the previous paragraph. Second, the K_i value estimated from IC_{50}/2 is corrected for the fraction of unbound drug in the test system, such that K_{i,unbound} is estimated from IC_{50}/2/f_{unb}, where f_{unb} is determined experimentally or calculated from log P (in the case of nonionic and basic drugs) or log D_{7.4} (in the case of acidic and zwitterionic drugs), as described by Austin et al. (2002) and Hallifax and Houston (2006).

Acknowledgments

The authors thank the Analytical Sciences and Enzyme Inhibition departments at XenoTech LLC for technical assistance.

Authorship Contributions

Participated in research design: Kazmi, Ogilvie, Buckley, Paris, A. Parkinson.

Conducted experiments: Haupt, Smith, Leatherman.

Performed data analysis: Haupt, Kazmi, Ogilvie, Buckley, Paris, O. Parkinson, A. Parkinson.

Wrote or contributed to the writing of the manuscript: Haupt, Kazmi, Ogilvie, Buckley, O. Parkinson, Paris, A. Parkinson.

References


Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (E50) of an enzymatic reaction. Biochem Pharmacol 22:3099-3108.

Drugs Metab Dispos 27:1350–1359.


Drugs Metab Dispos 37:2045–2054.


Drugs Metab Dispos 39:1370–1387.


Address correspondence to: Dr. Andrew Parkinson, XPD Consulting, 18000 West 68th Street, Shawnee, KS 66217. E-mail: aparkinson@xpd.us.com
Supplemental Table

The reliability of estimating $K_i$ values for direct, reversible inhibition of cytochrome P450 enzymes from corresponding IC$_{50}$ values: A retrospective analysis of 343 experiments

Lois J. Haupt, Faraz Kazmi, Brian W. Ogilvie, David B. Buckley, Brian D. Smith, Sarah Leatherman, Brandy Paris, Oliver Parkinson, and Andrew Parkinson

XPD Consulting, Shawnee, KS, USA (B.P., O.P., A.P.)

Drug Metabolism and Disposition
**Supplemental Table 1:** Summary of experimental conditions for measuring cytochrome P450 inhibition in human liver microsomes (HLM)

<table>
<thead>
<tr>
<th>P450 Enzyme</th>
<th>P450 Enzyme Activity¹</th>
<th>[Substrate]² (K_m)</th>
<th>[Protein] (mg/mL)</th>
<th>Analyte measured by LC-MS/MS (internal standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Phenacetin O-dealkylation</td>
<td>40 – 60 or 90 µM</td>
<td>0.1</td>
<td>Acetaminophen (Acetaminophen-d4)</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Coumarin 7-hydroxylation</td>
<td>0.6 - 0.75 µM</td>
<td>0.0125 or 0.1 ³</td>
<td>7-Hydroxycoumarin (7-Hydroxycoumarin-d6)</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Bupropion hydroxylation</td>
<td>50 µM</td>
<td>0.1</td>
<td>Hydroxybupropion (Hydroxybupropion-d6)</td>
</tr>
<tr>
<td></td>
<td>Efavirenz 8-hydroxylation</td>
<td>3 or 5 µM</td>
<td>0.1</td>
<td>8-Hydroxyefavirenz (8-Hydroxyefavirenz-d4)</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Amodiaquine N-dealkylation</td>
<td>1.5 – 7 or 2 µM</td>
<td>0.0125 or 0.1 ³,⁴</td>
<td>N-Desethylamodiaquine (N-Desethylamodiaquine-d5)</td>
</tr>
<tr>
<td></td>
<td>Paclitaxel 6α-hydroxylation</td>
<td>10 µM</td>
<td>0.05</td>
<td>6α-Hydroxypaclitaxel (6α-Hydroxypaclitaxel-d5 or deacetyltaxol)</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Diclofenac 4′-hydroxylation</td>
<td>6 – 7.5 or 12 µM</td>
<td>0.1</td>
<td>4′-Hydroxydiclofenac (4′-Hydroxydiclofenac-d4)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-Mephenytoin 4′-hydroxylation</td>
<td>40 or 60 µM</td>
<td>0.1</td>
<td>4′-Hydroxymephenytoin (4′-Hydroxymephenytoin-d3)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Dextromethorphan O-demethylation</td>
<td>7.5 or 10 µM</td>
<td>0.1</td>
<td>Dextrorphan (Dextrorphan-d3)</td>
</tr>
<tr>
<td></td>
<td>Bufurolol 1′-hydroxylation</td>
<td>12 µM</td>
<td>0.1</td>
<td>1′-Hydroxybufurolol (Dextrorphan-d3)</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Chlorzoxazone 6-hydroxylation</td>
<td>30 µM</td>
<td>0.1</td>
<td>6-Hydroxychlorzoxazone (6-Hydroxychlorzoxazone-d2)</td>
</tr>
<tr>
<td>CYP3A4/5</td>
<td>Testosterone 6β-hydroxylation</td>
<td>70-100 or 60 µM⁵</td>
<td>0.1</td>
<td>6β-Hydroxytestosterone (6β-Hydroxytestosterone-d3)</td>
</tr>
<tr>
<td></td>
<td>Midazolam 1′-hydroxylation</td>
<td>2 - 5 or 3 µM</td>
<td>0.05 or 0.1 ³</td>
<td>1′-Hydroxymidazolam (1′-Hydroxymidazolam-d4)</td>
</tr>
<tr>
<td></td>
<td>Nifedipine dehydrogenation</td>
<td>10 µM</td>
<td>0.1</td>
<td>Dehydronifedipine (Dehydronifedipine-d6)</td>
</tr>
</tbody>
</table>

¹ All substrates were incubated for 5 min.
² Substrate concentrations in plain text are for the HLM pool of 16 whereas values in italics are for the HLM pool of 200.
³ Active microsomal protein concentration was normalized to 0.1 mg/mL with an individual preparation of HLM with low enzyme activity.
⁴ A single assay was performed with 0.1 mg/mL of active HLM and a marker substrate concentration of 7 µM.
⁵ In the case of testosterone, which exhibits homotropic cooperativity (substrate activation), the value represents S_50 (with a Hill coefficient of n = 1.3).