Can K_i values for direct inhibition of CYP enzymes be reliably estimated from IC_{50} values?

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INTRODUCTION

Regulatory agencies recommend that the potential for a drug candidate to cause clinically relevant, direct inhibition of cytochrome P450 (CYP) enzymes be estimated based on the ratio of [I]/K_i (or 1+[I]/K_i) where [I] is the *in vivo* concentration of drug candidate and K_i is the dissociation constant for the enzyme-inhibitor complex for direct inhibition (US FDA, 2006). Typically, inhibition of CYP enzymes by a drug candidate is first evaluated *in vitro* by determining the concentration of drug candidate that causes 50% inhibition of a specific CYP enzyme activity (IC_{50}) using a marker substrate concentration approximately equal to K_m for the marker substrate reaction. Determining the mechanism of direct inhibition (competitive, uncompetitive, noncompetitive and mixed) and measuring the K_i value requires an *in vitro* evaluation of the effects of multiple concentrations of the drug candidate versus multiple concentrations of CYP probe substrate (the former spanning K_i and the latter spanning K_m). The Cheng-Prusoff equation (Cheng and Prusoff, 1973) allows the estimation of the K_i value from an experimentally-determined IC_{50} value. The most conservative version of this calculation (*i.e.*, that for competitive inhibition) is shown below:

$$K_i = \frac{IC_{50}}{\left(\frac{S}{K_m} + 1\right)}$$

This equation simplifies to $K_i = IC_{50}/2$ when the substrate concentration used to measure IC_{50} is equal to the K_m for the marker substrate metabolism.

In the present study we conducted a retrospective analysis of 251 *in vitro* K_i determinations to ascertain whether the experimentally-determined K_i values could be reliably estimated by dividing the corresponding experimentally-determined IC_{50} values by two, based on the relationship that, for competitive inhibition, $K_i = IC_{50}/2$ (when [S] = K_m).

MATERIALS & METHODS

Chemicals

The CYP2B6 marker substrate efavirenz was purchased from US Pharmacopeia (Rockville, Maryland). Its metabolite, 8-hydroxyefavirenz, was purchased from Toronto Research Chemicals, Inc (Ontario, Canada). All other reagents were purchased from commercial sources described previously (Pearce *et al*, 1996, Parkinson *et al*, 2011).

Test system

Pooled human liver microsomes (n = 16, mixed gender) were prepared from nontransplantable livers and characterized at XenoTech, LLC (Lenexa, KS) as described previously (Parkinson *et al*, 2004, Pearce *et al*, 1996).

Incubation conditions and analytical methods

The K_i and IC_{50} values were determined using methods consistent with guidance documents and consensus papers (US FDA, 2006, Bjornsson *et al*, 2004). These experiments were performed under the following conditions: (1) the concentration of CYP marker substrate was approximately equal to K_m for IC_{50} determinations and spanned K_m for K_i determinations (*i.e.*, ranging from 0.25 times K_m to 10 times K_m, solubility permitting); (2) the substrate incubation time was 5 min or less to minimize metabolism-dependent inhibition and inhibitor depletion, and (3) the concentration of human liver microsomes was 0.1 mg/mL or less to minimize nonspecific binding and depletion of the inhibitor.

In general, incubations were conducted at 37 °C in 200- or 400-µL incubation mixtures (pH 7.4) containing potassium phosphate buffer (50 mM), $MgCl_2$ (3 mM), EDTA (1 mM), NADPH-generating system and human liver microsomes. Experimental conditions for assays except CYP2B6 (as measured by efavirenz 8-hydroxylation) are described in previous publications (Pearce *et al*, 1996, Parkinson *et al*, 2011). For CYP2B6 (as measured by efavirenz 8-hydroxylation), experiments were performed by incubating efavirenz with human liver microsomes (0.1 mg/mL) at concentrations equal to or bracketing the experimentally-determined K_m (3 µM) for the marker substrate reaction. All reactions were initiated by the addition of NADPH-generating system and terminated by the addition of a nearly equal volume of organic solvent (*i.e.*, acetonitrile) containing the appropriate internal standard. Precipitated protein was removed by centrifugation prior to analyzing the samples by LC/MS/MS.

All analyses were performed using validated LC/MS/MS methods on an AB Sciex API 2000, 3000 or 4000 instrument (AB Sciex) with Shimadzu HPLC pumps and autosampler systems according to methods described previously (Parkinson *et al*, 2011). For CYP2B6 (as measured by efavirenz 8-hydroxylation), the rate of 8-hydroxyefavirenz formation was quantified by LC/MS/MS using a negative electrospray ionization method monitoring mass transitions from 330 to 286 amu. The internal standard used was 8-hydroxyefavirenz-d_4. Peak areas for all metabolites were integrated with an AB Sciex Analyst data system, and metabolites were quantified by reference to a standard calibration curve based on back calculation of a weighted (1/x), linear, least-squares regression.

IC_{50} values were calculated using the Levenberg-Marquardt algorithm to perform non-linear regression fitting of the data. The entire data set for K_i determinations (*i.e.*, reaction rates at all concentrations of drug candidate, at all marker substrate concentrations) were fitted to the Michaelis-Menten equations for competitive, noncompetitive, uncompetitive and mixed (competitive-noncompetitive) inhibition by nonlinear regression analysis.

RESULTS

Figure 1 shows the distribution of the types of direct inhibition observed for 251 K_i determinations for nine different CYP enzymes as determined using pooled human liver microsomes. Nearly 96% of all compounds reviewed were found to be either mixed (competitive-noncompetitive) or competitive inhibitors of the CYP enzymes evaluated.

Figure 1

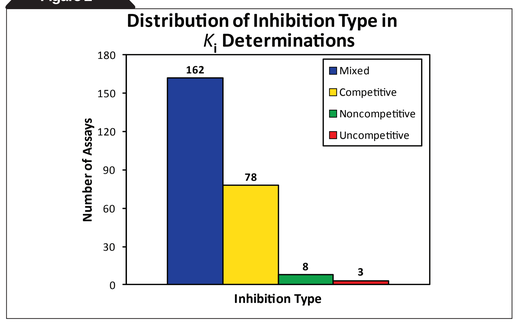


Figure 2 shows the distribution of assays related to CYP enzyme activity studied. CYP3A4/5 (as measured by three different marker substrate activities) was the most commonly evaluated CYP enzyme with 26% of the experiments performed.

Figure 2

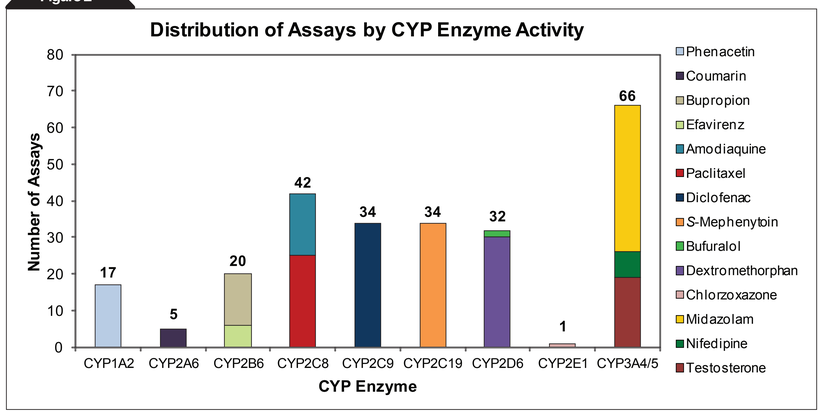


Figure 3 illustrates the comparison of experimentally-determined K_i values versus estimated K_i values (*i.e.*, calculated by $IC_{50}/2$). Estimated and actual K_i values correlated well (Pearson product coefficient (r) = 0.944) regardless of the type of inhibition observed.

Figure 3

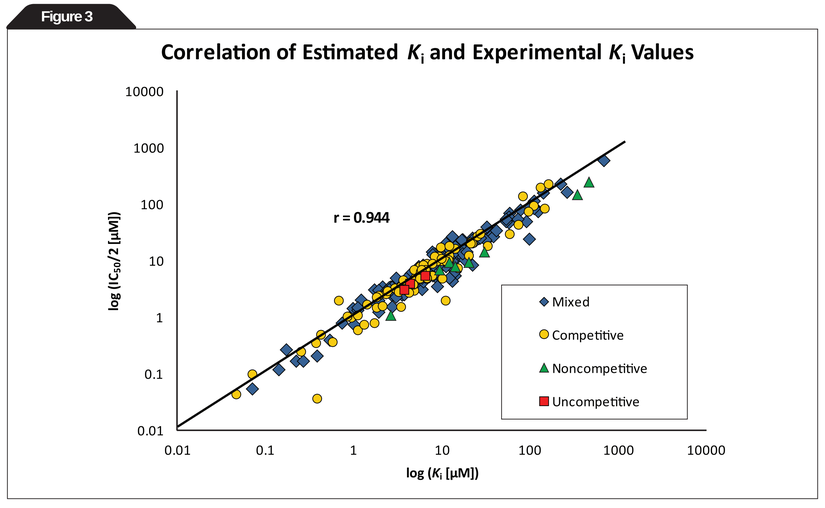
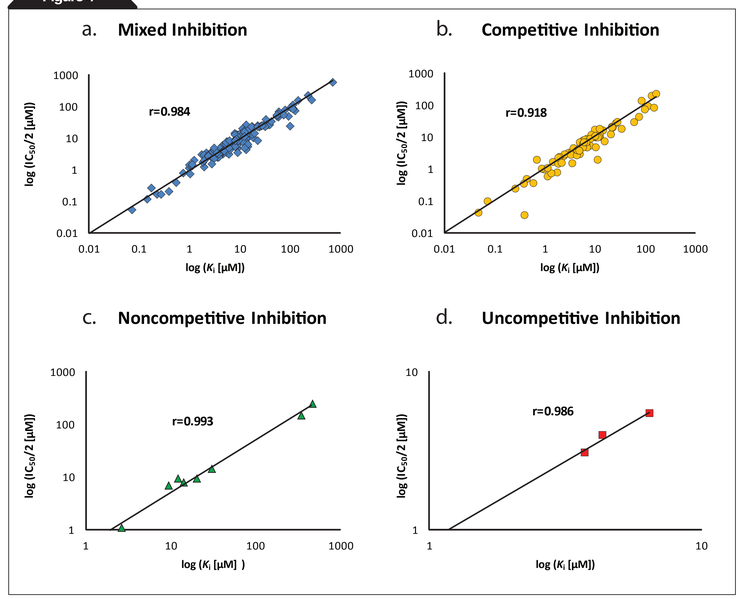


Figure 4 shows the correlation of estimated K_i values and experimentally-determined K_i values with respect to the mechanism of direct inhibition

Figure 4



CONCLUSIONS

- K_i values estimated from experimentally-determined IC_{50} values for 251 compounds correlated well with experimentally-determined K_i values for those same compounds regardless of the type of direct inhibition observed (*i.e.*, competitive, noncompetitive, uncompetitive or mixed) yielding a Pearson product coefficient (r) of 0.944.
- The results of this retrospective analysis suggest that the K_i value for direct inhibition of CYP enzyme activity can reliably, albeit somewhat conservatively, be estimated by the formula $IC_{50}/2$.

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