DMD Fast Forward. Published on May 4, 2012 as doi:10.1124/dmd.112.045799

In Vivo Information-Guided Prediction Approach for Assessing the Risks of Drug-Drug Interactions Associated with Circulating Inhibitory Metabolites

Zhe-Yi Hu, Robert B. Parker, and S. Casey Laizure

University of Tennessee Health Science Center, College of Pharmacy, Department of Clinical Pharmacy, Memphis, Tennessee

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: *In vivo* information approach for predicting drug interaction

Corresponding author:

Zhe-Yi Hu, PhD

Department of Clinical Pharmacy

University of Tennessee

Room 328, 881 Madison Ave., Memphis, TN 38163

Phone: 9014487240

E-mail: zhu13@uthsc.edu.

Number of:

Text pages: 22

Tables: 4

Figures: 2

References: 37

Words in Abstract: 244

Words in Introduction: 561

Words in Discussion: 1431

Abbreviations:

DDI, drug-drug interaction; CYP, Cytochrome P450; IVIP, *in vivo* information-guided prediction; AUC, area under the plasma concentration-time curve; [I], inhibitor concentration; K_i, inhibition constant; K_I, concentration of inhibitor required to achieve half-maximal inactivation; k_{inact}, maximal rate constant of enzyme inactivation; f_{mCYP}, the fraction of substrate clearance mediated by the inhibited enzyme; E_g, intestinal extraction; CR, the contribution ratio; IR, the apparent inhibition ratio of the inhibiting drug; UHI, unbound hepatic inlet concentration; k_a, absorption rate constant; F_a, the fraction absorbed; D, dose

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

of the inhibitor; Q_h, liver blood flow; f_u, unbound fraction of drug in plasma; k_{deg}, degradation rate constant; Q_g, enterocytic blood flow; PBPK, physiologically based pharmacokinetics.

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT:

The *in vivo* drug-drug interaction (DDI) risks associated with Cytochrome P450 (CYP) inhibitors that have circulating inhibitory metabolites cannot be accurately predicted by conventional *in vitro*-based methods. A novel approach, *in vivo* information-guided prediction (IVIP), was recently introduced for CYP3A- and CYP2D6-mediated DDIs. This technique should be applicable to the prediction of DDIs involving other important CYP metabolic pathways. Therefore, the aims of this study are to extend the IVIP approach to CYP2C9-mediated DDIs and evaluate the IVIP approach for predicting DDIs associated with inhibitory metabolites. The analysis was based on data from reported DDIs in the literature. The IVIP approach was modified and extended to CYP2C9-mediated DDIs. Thereafter, the IVIP approach was evaluated for predicting the DDI risks of various inhibitors with inhibitory metabolites. Although the data on CYP2C9-mediated DDIs were limited compared to CYP3A and CYP2D6-mediated DDIs, the modified IVIP approach successfully predicted CYP2C9-mediated DDIs. For the external validation set, the prediction accuracy for area under the plasma concentration-time curve (AUC) ratios ranged from 70 to 125%. The accuracy (75 to 128%) of the IVIP approach in predicting DDI risks of inhibitors with circulating inhibitory metabolites was more accurate than *in vitro*-based methods (27 to 452%). The IVIP model accommodates important confounding factors in the prediction of DDIs, which are difficult to handle using *in vitro*-based methods. In conclusion, the IVIP approach could be used to predict CYP2C9-mediated DDIs, and is easily modified to incorporate the additive effect of circulating inhibitory metabolites.

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Drug-drug interactions (DDIs) can result when one drug alters the pharmacokinetics of another drug or its metabolites. According to the new FDA Draft Guidance (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf), the pharmacokinetic interactions between an investigational new drug and other drugs should be defined during drug development, as part of an adequate assessment of the drug's safety and effectiveness. Therefore, predicting clinically significant drug interactions during drug development is essential for the pharmaceutical industry and regulatory agencies. The large number of clinically significant DDIs due to the inhibition of cytochrome P450 (CYP) substrate metabolism and the availability of *in vitro*, *in vivo*, and clinical methods for assessing CYP DDIs have made this a logical starting point for the development and validation of techniques to predict clinically significant DDIs.

There exists a broad consensus as to the common principles underlying prediction of the magnitude of an *in vivo* DDI from *in vitro* data. The increase in the area under the plasma concentration-time curve (AUC) of a substrate when co-administered in the presence of a reversible inhibitor of the substrate's elimination pathway is a function of the ratio of inhibitor concentration ([I]) to inhibition constant (K_i) (Ito et al., 1998; Shou, 2005; Brown et al., 2006; Obach et al., 2006; Einolf, 2007). A similar model involving K_I (concentration of inhibitor required to achieve half-maximal inactivation) and k_{inact} (maximal rate constant of enzyme inactivation) for DDIs associated with irreversible (mechanism-based) inhibitors has also been proposed (Obach et al., 2007). In addition, researchers have incorporated the fraction of substrate clearance mediated by the inhibited enzyme (f_{mCYP}), the plasma protein binding of the inhibitor

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

(Shardlow et al., 2011), and fraction of absorbed substrate dose escaping gut metabolism by CYP3A (F_G) (Galetin et al., 2008) to improve predictions for certain drug classes.

Although *in vitro*-based models can quantitatively predict many *in vivo* DDIs with acceptable accuracy, the application of this model to the prediction of DDIs associated with CYP inhibitors that have inhibitory metabolites has not been successful (Yeung et al., 2011; McDonald et al., 2012). The prediction accuracy of *in vitro* models may be improved to some extent when data pertaining to metabolites were included in the model; however, prediction accuracy (35-188%) was still unsatisfactory for DDIs associated with ten typical inhibitors that have inhibitory metabolites (Yeung et al., 2011). Another recent study found that prediction accuracy decreased when more inhibitory metabolites of amiodarone were taken into account (McDonald et al., 2012). A novel approach, *in vivo* information-guided prediction (IVIP), was recently introduced for CYP3A- and CYP2D6-mediated drug interactions (Ohno et al., 2007; Tod et al., 2011). This model relies primarily on *in vivo* data and uses two characteristic parameters: one for the substrate, and the other for the inhibitor. This model has the potential to take into account inhibitory metabolites, different mechanisms of inhibition, and intestinal inhibition. Although information on the inhibitory metabolites can also be incorporated into an *in vitro*-based model, the IVIP approach has certain advantages when compared with *in vitro*-based methods. Validation of the IVIP approach for the prediction of DDIs mediated by other CYP450 enzymes or the effects of inhibitory metabolites on DDIs is still lacking, due to the paucity of available data. Therefore, the aims of this study are to extend the IVIP approach to CYP2C9-mediated interactions, and to validate the modified IVIP approach for prediction of DDIs associated with inhibitors that have inhibitory metabolites.

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

Materials and Methods

Extending the IVIP approach to CYP2C9-mediated interactions. Medline, PubMed, and Embase databases (from 197 5 until December 31, 2011) were searched using the terms "CYP2C9," "inhibition," and "pharmacokinetics". Citations within the retrieved articles were used to search for additional relevant studies. Studies were included if: (1) they were conducted in humans, (2) they provided the ratio between the AUC of the substrate when administered alone and when co-administered at the same dose with the inhibitor, (3) the dose of the inhibitor was within the therapeutic dose range, and (4) the inhibitor and substrate drugs were orally or intravenously administered to the subjects. Drug-drug interaction studies associated with herbal products, combination therapies, and oral contraceptives were excluded. Both reversible and irreversible inhibitors were included in the analysis.

An IVIP approach that has been previously described was modified and applied to the quantitative prediction of CYP2C9-mediated DDIs (Ohno et al., 2007; Tod et al., 2011). This modeling framework utilizes two characteristic parameters: the contribution ratio (CR) defined as the contribution of the specific enzyme to the oral clearance or total clearance (intravenous administration) of the drug whose metabolism is inhibited (victim), and the apparent inhibition ratio (IR) of the inhibiting drug (perpetrator). If reasonable estimates of CR ($0 \leq CR \leq 1$) and IR ($0 \leq IR \leq 1$) can be determined, then the ratio of the AUC in the presence (AUC_I) and absence (AUC) of the inhibitor can be estimated using the following equation (Ohno et al., 2007; Hisaka et al., 2010; Tod et al., 2011):

$$\frac{AUC_I}{AUC} = \frac{1}{1 - CR \cdot IR} \quad (1)$$

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

In contrast to the complex derivation by previous studies (Ohno et al., 2007; Tod et al., 2011), equation 1 is readily derived from the well-known *in vitro*-based model (reversible inhibition, intestinal metabolism ignored) below (equation 2), where f_{mCYP} is the contribution of the specific enzyme to the overall clearance.

$$\frac{AUC_I}{AUC} = \frac{1}{\left(\frac{f_{mCYP}}{1+\frac{[I]}{K_i}}\right) + (1 - f_{mCYP})} \quad (2)$$

After defining f_{mCYP} = CR and IR = [I]/([I]+K_i), equation 2 is transformed to equation 1. Since the IR in this model relies on *in vivo* data, this avoids the confounding issues due to extrapolation from K_i (or k_{inact}/K_I) and does not require distinction between reversible and irreversible (mechanism based) inhibitors.

For the CYP2D6- and CYP3A-mediated DDIs, the CR values of most victim drugs can be estimated directly from *in vivo* data using the pharmacogenetic or drug interaction methods (Ohno et al., 2007; Tod et al., 2011). The pharmacogenetic method (Tod et al., 2011) allows determining CR from equation 3, where AUC_{PM} is the AUC in poor metabolizers and AUC_{EM} is the AUC in extensive metabolizers.

$$CR = \frac{AUC_{PM} / AUC_{EM} - 1}{AUC_{PM} / AUC_{EM}} \quad (3)$$

Estimating CR using the interaction method (equation 4) is based on transformation of equation 1 using a known IR value of the inhibitor (IR was assumed to be 1.0 for very strong inhibitor), where AUC_I is the AUC of the drug when the inhibitor is coadministered.

$$CR = \frac{AUC_I / AUC - 1}{AUC_I / AUC \cdot IR} \quad (4)$$

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

However, the IVIP approach developed for CYP2D6- and CYP3A-mediated DDIs cannot be directly extended to CYP2C9-mediated DDIs without modification. The CR values of CYP2C9 substrates cannot be reliably estimated by the pharmacogenetic method because relevant studies in CYP2C9 poor metabolizers are limited. Furthermore, the CR of most CYP2C9 substrates cannot be calculated by equation 4 due to the absence of IR data (e.g. The IR value can be assumed to be 1 for a very strong CYP inhibitor, but no strong inhibitor of CYP2C9 has been found according to the new FDA Draft Guidance and a recent study (Polasek et al., 2011)). Therefore, the CR of CYP2C9 substrates was estimated using equation 5; where f_m is the contribution of CYP2C9 to hepatic clearance (estimated *in vitro* by CYP2C9-specific inhibitor or functional neutralizing antibody), and f_h is the contribution of the hepatic clearance to the total clearance of the drug (estimated by the recovery of excreted CYP2C9 metabolites in urine, bile and feces).

$$CR = f_m \cdot f_h \quad (5)$$

The CR values of most CYP2C9 substrates were estimated by the extrapolation method ($CR = f_m \cdot f_h$) using literature data. A learning set (learning set 1) was selected to calculate the IR of CYP2C9 inhibitors. Only the DDIs associated with typical CYP2C9 substrates (S-warfarin, tolbutamide, diclofenac, and phenytoin) were included in this learning set. For the inhibitors with different levels of doses, IR values were estimated for each dose because IR should be dose dependent. For the remaining few substrates whose CR could not be calculated by the extrapolation method (learning set 2), CR values were estimated by equation 4 using the known IR of the inhibitors. An external validation of these estimates was carried out by comparing the AUC ratios predicted by equation 1 with the observed values from data not included in the preceding steps. An algebraic mean of the AUC increase was used in the calculation whenever

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

results from multiple studies were available for a single combination of substrate and inhibitor (same dose of inhibitor was used in these studies).

Evaluation of the IVIP approach in predicting the DDI risks of various inhibitors with circulating inhibitory metabolites. The relevant data of clinical DDIs associated with CYP inhibitors that have inhibitory metabolites were mainly retrieved from a single recent report (Yeung et al., 2011), which is based on the University of Washington Metabolism and Transport Drug Interaction Database (MTDI database: http://www.druginteractioninfo.org). Whenever available, additional data from the literature were included. *In vivo* DDI studies were included in our analysis only if they had been conducted with a reliable CYP probe. For CYP3A- and CYP2D6-mediated DDIs, only data from oral administration were included in the analysis. An algebraic mean of the AUC increase was used in the calculation when multiple studies were available for a single combination of victim drug and inhibitor (same dose of inhibitor was used in these studies).

Using the above retrieved data, *in vivo* DDIs associated with inhibitory metabolites were predicted by the fully validated IVIP approach. In order to avoid "self-prediction", the data in the learning set were not included in the validation set, and vice versa. The learning sets for CYP2D6- and CYP3A-mediated DDIs were selected according to the following criteria: (1) the dose of the inhibitor in the learning set is the same as in the validation set, (2) the regimens of the inhibitor in the learning set and the validation set are both multiple-dose or single-dose regimen, and (3) the victim drug is a known probe, or a substrate with a relatively high CR value.

These retrieved data were also used to predict *in vivo* DDIs by each of the *in vitro*-based methods. The steady-state concentrations [I] of the inhibitors were estimated in two ways; (1) total systemic C_{max}, and 2) unbound hepatic inlet concentration (UHI) defined by equation 6,

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

where k_a is the absorption rate constant (0.03/min, an assumed average value (Obach et al., 2006)), F_a is the fraction absorbed (assumed to be 1), D is the dose of the inhibitor, Q_h is the liver blood flow (1498 mL/min), and f_u is the unbound fraction of drug in plasma. This equation assumes that metabolism of the inhibitor in the gut is negligible.

$$[\mathrm{I}]_{\mathrm{hepatic,inlet}} = f_u \cdot \left(C_{\max} + \frac{k_a \cdot F_a \cdot D}{Q_h} \right) \tag{6}$$

For reversible inhibitors of CYP2C9 and CYP2D6, equation 7 was used to predict the clinical DDI. The effect of multiple inhibitors was accounted for by summing the [I]/K_i ratios (Yeung et al., 2011). It should be pointed out that both f_{mCYP} (*in vitro*-based model) and CR (IVIP model) indicate the contribution ratio of the target metabolizing enzyme to the clearance of a substrate drug after oral absorption or intravenous administration, so the same value is used in our analysis.

$$\frac{\mathrm{AUC_I}}{\mathrm{AUC}} = \frac{1}{\left(\dfrac{f_{mCYP}}{1 + \sum_j^n \left(\dfrac{[\mathrm{I}]_j}{K_{ij}} \right)} \right) + (1 - f_{mCYP})} \tag{7}$$

Predictions for reversible inhibitors of CYP3A used equation 8 and incorporated the contribution of gut metabolism, where F_G is the fraction of absorbed substrate escaping gut metabolism by CYP3A. The concentration in the gut, $[\mathrm{I}]_{gut}$, was defined by equation 9, where Q_g is the enterocytic blood flow (248 mL/min).

$$\frac{\mathrm{AUC_I}}{\mathrm{AUC}} = \frac{1}{\left(\dfrac{f_{mCYP}}{1 + \sum_j^n \left(\dfrac{[\mathrm{I}]_j}{K_{ij}} \right)} \right) + (1 - f_{mCYP})} \cdot \frac{1}{F_G + \left(\dfrac{1 - F_G}{1 + \dfrac{[\mathrm{I}]_{gut}}{K_i}} \right)} \tag{8}$$

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

$$[\mathrm{I}]_{\mathrm{gut}} = \frac{k_{\mathrm{a}} \cdot F_{\mathrm{a}} \cdot D}{Q_{\mathrm{g}}} \tag{9}$$

Predictions for irreversible inhibitors of CYP3A used equation 10 with a k_{deg} (hepatic) of 0.000321/min (Obach et al., 2007), F_{G} of the substrate and the degradation rate constant for CYP3A in the enterocyte [k_{deg}(gut) = 0.000481/min (Obach et al., 2007)].

$$\frac{\mathrm{AUC_I}}{\mathrm{AUC}} = \frac{1}{\left(\dfrac{f_{\mathrm{mCYP}}}{1 + \sum_j^n \dfrac{[\mathrm{I}]_j \cdot k_{\mathrm{inact}\,j}}{k_{\mathrm{deg}} \cdot \left([\mathrm{I}]_j + K_{\mathrm{I}\,j} \right)}} + (1 - f_{\mathrm{mCYP}}) \right)} \cdot \frac{1}{F_{\mathrm{G}} + \left(\dfrac{1 - F_{\mathrm{G}}}{1 + \dfrac{[\mathrm{I}]_{\mathrm{gut}} \cdot k_{\mathrm{inact}}}{k_{\mathrm{deg(gut)}} \cdot \left([\mathrm{I}]_{\mathrm{gut}} + K_{\mathrm{I}} \right)}} \right)} \tag{10}$$

Assessment of predictive performance. To assess the quantitative accuracy of each model, a prediction error was calculated from the difference between each predicted AUC ratio and the observed ratio. The prediction bias of each assumption was calculated as an average deviation (AD) of the predicted versus observed AUC ratios. The precision of each assumption was calculated as the root-mean-square error (RMSE). The observed and predicted DDIs were assigned as "positive" if AUC ratio was ≥ 1.25 or otherwise were termed as "negative." This threshold was selected to maximize our ability to make conservative decisions on the necessity for a clinical DDI study, and approximates the FDA standards for bioequivalence and weak inhibition (FDA Draft Guidance for Industry, 2012). The sensitivity and specificity of each prediction model was determined. Sensitivity is a measure of the ability of the prediction approach to successfully identify a positive DDI. The specificity of a prediction approach is defined as its ability to successfully identify a negative (AUC ratio < 1.25) or weak DDI (1.25 ≤ AUC ratio < 2.00).

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

Results

Extending the IVIP approach to CYP2C9-mediated interactions. A total of 44 different combinations (substrate and inhibitor) of *in vivo* DDI studies were identified by the literature search. The estimated values of CR for CYP2C9 substrates determined by the extrapolation method are listed in Table 1. Calculated IR values of all CYP2C9 inhibitors and CR of the remaining four substrates (whose CR cannot be estimated by the extrapolation method) are shown in Table 2, which comprises learning sets 1 and 2.

References for the DDI studies involving CYP2C9 that were used for the external validation are shown in Table 3. A total of 19 AUC ratios were available. The relationship between the observed and predicted AUC ratios is plotted in Fig. 1A. All the points are inside the range of acceptable predictions (50% to 200%). The prediction accuracy of AUC_I/AUC ranged from 70 to 125% (Fig. 1B). The predictive sensitivity and specificity were both 93%. The predictive error and precision were −0.09 and 0.29, respectively.

The AUC_I/AUC ratios of 180 possible interactions between the 12 substrates and the 15 inhibitors listed in Table 2 and 3 were calculated (Fig. 1C). Only a small proportion (21%) of all possible combinations between substrates and inhibitors had been studied *in vivo*.

Evaluation of the IVIP approach in predicting the DDI risks of various inhibitors with circulating inhibitory metabolites. The details of the data and calculation are provided as Supplemental Material 1. A total of 14 different combinations of *in vivo* DDI studies (including 12 inhibitors with inhibitory metabolites) were identified (Table 4). Two inhibitors (diltiazem and erythromycin) and their metabolites have been shown to possess both reversible and irreversible inhibitory effects on CYP3A (Zhang et al., 2009a). However, both the reversible and irreversible *in vitro* models could not accurately predict the AUC ratios (Table 4). In general, the

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

predictive performance of the model incorporating unbound hepatic inlet concentration (UHI) was not superior to the model that used total systemic C_{max} (Fig. 2). The predictive error of the three *in vitro*-based models was 5.04 (parent drug, [I] = C_{max}), 7.34 (parent drug and metabolites, [I] = C_{max}), and −0.18 (parent drug and metabolites, [I] = UHI), respectively. The precision of these *in vitro*-based models was 13.2, 19.0, and 6.31, respectively. In contrast, the predictive error (0.10), precision (0.57), and absolute accuracy (75% to 128%) of the IVIP approach were significantly better. The predictive sensitivity of the *in vitro* model could be improved to some extent, when data pertaining to metabolites were included in the model (64% vs. 43%). The IVIP approach could successfully identify 12 out of 14 positive DDIs (86%). For the two failures, the observed vs predicted AUC ratios were 1.28 vs 1.17, and 1.59 vs 1.24, respectively. The predictive specificities of all three *in vitro*-based models and the IVIP approach were 100%, 86%, 100%, and 100%, respectively.

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

There were two primary findings of this study. The first finding is that the modified IVIP approach can be extended to the prediction of CYP2C9-mediated DDIs. For the external validation set, the prediction accuracy for AUC ratios ranged from 70 to 125%. The second finding is that the accuracy of the IVIP approach in predicting DDI risks of 12 inhibitors with circulating inhibitory metabolites was more accurate than *in vitro*-based methods.

To our knowledge, this is the first proof-of-concept study demonstrating that the IVIP approach is a useful tool for the prediction of drug interaction risks associated with CYP inhibitors that have circulating inhibitory metabolites. The IR in IVIP model relies on *in vivo* data, thereby avoiding the confounding issues due to extrapolation from *in vitro* K_i (or k_{inact}/K_I). The IVIP approach accommodates important confounding factors (inhibitory metabolites, different mechanisms of inhibition, and intestinal inhibition) in the prediction of DDIs, which are difficult to handle by *in vitro*-based methods. The theoretical basis for the versatility of the IVIP approach is provided in Supplemental Material 2. In brief, the versatility of the IVIP approach is dependent on the apparent inhibition ratio (IR) of the inhibitors. The underlying meaning of the IR is different for different confounding factors. For instance, the IR reflects the total extent of inhibition occurring in the liver and intestines, if intestinal inhibition exists. In cases where there are multiple inhibitors that act via independent inhibition mechanisms, the IR reflects the combined inhibitory effects of all inhibitors present. Another advantage of the IVIP approach is that it is easy to use and does not require statistical or pharmacokinetic simulation software to perform the analysis.

Although the IVIP approach was developed based on previous methods (Ohno et al., 2007; Tod et al., 2011), some differences merit discussion. In the current study, only the well-known

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

CYP2C9 probes (S-warfarin, tolbutamide, diclofenac, and phenytoin) were eligible for the learning set used to calculate the IR values of various inhibitors. This criterion is likely to minimize the possibility of introducing misleading CR information. In addition, the IR values of the inhibitors that are calculated based on reliable CYP2C9 probes will only reflect the inhibition of CYP2C9 rather than other inhibitory mechanisms (such as transporter-mediated inhibition). In the current study, the CR values of most CYP2C9 substrates cannot be directly estimated from *in vivo* information because relevant data for CYP2C9 are limited. In order to overcome this difficulty, estimation of CR values was mainly based on the extrapolation method, using *in vitro* data. This modified approach was demonstrated to be accurate and reliable, suggesting the extrapolation method is a reasonable alternative for estimation of CR values. Both the data from oral and intravenous administration were included in our prediction of CYP2C9-mediated DDIs because the activity of CYP2C9 in the intestine is only 4% of the activity in the liver (Galetin and Houston, 2006).

The IVIP model for CYP2C9-mediated DDIs was also used to forecast the magnitude of a large number of drug interactions that have not been studied. The most potent CYP2C9 inhibitors are predicted to be bucolome and miconazole. Respectively they caused 6.25-, and 6.25-fold increases in the plasma AUC values of the CYP2C9 probe tolbutamide (Fig. 1C). According to the FDA classifications of strong, moderate or weak inhibitors (FDA Draft Guidance for Industry, 2012), these perpetrators may be strong inhibitors of CYP2C9. However, no strong inhibitor of CYP2C9 was listed in the new FDA draft guidance and a recent criteria-based assessment of perpetrators (Polasek et al., 2011). Although these three perpetrators have limited clinical application, they may serve as useful tools for drug metabolism studies. With regard to the moderate (amiodarone, benzbromarone, sulphaphenazole, and fluconazole) and

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

weak (voriconazole, sulfinpyrazone, and fluvoxamine) inhibitors of CYP2C9, our predictions are consistent with the FDA DDI guidance.

In the present study, the predictive performances of three *in vitro*-based models were compared with the IVIP approach. These *in vitro*-data based mathematical models require an assumption of the perpetrator concentration available to the enzyme ([I]) for the prediction of DDIs. This elusive value is not easy to determine for human CYP drug metabolism. Therefore, alternative assumptions have been explored to optimize a static value for [I], including systemic C_{max}, C_{avg}, and estimated hepatic inlet concentrations. However, at present there is no consensus on the *in vivo* inhibitor concentration that should be used. For instance, the FDA DDI guidance advocates that a clinical DDI study be conducted if the $[I]/K_i$ ratio > 0.1, where [I] = total systemic C_{max}. It has also been reported that different estimates work better for different classes of inhibitors; for example, unbound systemic concentration has been used for predicting DDIs caused by irreversible inhibition, whereas the estimated unbound hepatic inlet concentration has been the preferred value for reversible inhibition (Obach, 2009). Recently, Shardlow et al. (Shardlow et al., 2011) observed unbound hepatic inlet concentration allowed the accurate prediction of DDIs for the drugs in their dataset (including different types of inhibitors). Therefore, both total systemic C_{max} and estimates of unbound hepatic inlet concentrations were used as surrogates for the inhibitor concentration in our analysis. Furthermore, *in vitro*-based models differentiated reversible and irreversible inhibition, and also considered the contribution of intestinal metabolism for CYP3A substrates.

Physiologically based pharmacokinetic (PBPK) approaches to define dynamic perpetrator and victim concentrations are becoming more widespread (Zhao et al., 2011). However, accurate prediction by this complex model relies on the accurate estimation of comprehensive *in vivo* and

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

in vitro pharmacokinetic (PK) parameters of the substrates and inhibitors, as well as all of the inhibitory metabolites (Zhang et al., 2009b). These data are often not available for compounds early in drug discovery.

In order to improve decision making in drug development and discovery, we suggest different models should be used at the different stages of new drug development. Although the *in vitro*-based model is less accurate, it is useful in the earlier stages of drug development, before any clinical data are available (clinical [I] can be estimated based on preclinical data). If a new drug is a potential victim drug, one clinical DDI study for this victim drug (co-administered with a strong inhibitor) is needed to investigate the potential DDI risk (FDA Draft Guidance for Industry, 2012). The *in vivo* contribution ratio of this victim drug can be accurately estimated based on the result of this clinical study. Then, the IVIP model can be used for predicting the DDI risks of this victim drug if co-administered with other inhibitors. According to the new draft guidance, when a strong inhibitor alters this victim drug, subsequent clinical studies or modeling are advised to define interactions with other less potent specific inhibitors. However, if no significant DDI is predicted for this victim drug and other weaker inhibitors based on IVIP model, we suggest a secondary clinical study may not be necessary. If a new drug is a potential perpetrator, most of the DDIs involving this drug can also be predicted using the IVIP model from a single clinical DDI study of the perpetrator and a sensitive substrate. The PBPK model may be used in the later stages of drug development after more comprehensive clinical PK data and DDI data are available.

The limitations of the present study need to be considered. It is noteworthy that the value of IR is dose dependent (as shown in Table 2). Because it is estimated from specific in vivo study, and the exposure of the perpetrator has already been considered with the certain relationship with

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

K_i or k_{inact}/K_I. Therefore, in order to accurately predict a certain clinical DDI, the dose and regimen of the perpetrator in the learning set should not be significantly different from that of the same perpetrator in this clinical DDI study. In addition, in its present form, the IVIP is not applicable to inhibitors that can inhibit both CYP enzymes and transporters such as P-glycoprotein. Failing to account for the interaction with P-glycoprotein may result in under prediction of the AUC ratio. However, our study included an inhibitor (quinidine) that inhibits both CYP2D6 and P-glycoprotein, but no significant under prediction of the AUC ratio was observed. This can be explained by the fact that both victim drugs (desipramine and metoprolol) in the validation set and learning set are not P-glycoprotein substrates. Further studies are underway in our laboratory to apply the model to transporter-mediated DDIs.

The IVIP approach is validated to be accurate in the prediction of CYP2C9-mediated DDIs and is a useful tool for the prediction of drug interaction risks associated with CYP inhibitors that have circulating inhibitory metabolites. This approach can be used in new drug development after the result of the first clinical DDI study is available.

Acknowledgements

The authors wish to thank Vanessa L. Herring for her help in the writing of the manuscript. The authors also thank Dr. Yuan-Sheng Zhao from Mount Sinai School of Medicine for his fruitful discussions during the preparation of this paper.

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

Authorship Contributions

Participated in research design: Zhe-Yi Hu, Robert B. Parker, S. Casey Laizure

Conducted experiments: Zhe-Yi Hu

Contributed new reagents or analytic tools: none

Performed data analysis: Zhe-Yi Hu

Wrote or contributed to the writing of the manuscript: Zhe-Yi Hu, Robert B. Parker, S. Casey Laizure

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

References

Albers LJ, Reist C, Vu RL, Fujimoto K, Ozdemir V, Helmeste D, Poland R, and Tang SW (2000) Effect of venlafaxine on imipramine metabolism. *Psychiatry research* **96:**235-243.

Alderman J, Preskorn SH, Greenblatt DJ, Harrison W, Penenberg D, Allison J, and Chung M (1997) Desipramine pharmacokinetics when coadministered with paroxetine or sertraline in extensive metabolizers. *Journal of clinical psychopharmacology* **17:**284-291.

Ayesh R, Dawling S, Hayler A, Oates NS, Cholerton S, Widdop B, Idle JR, and Smith RL (1991) Comparative effects of the diastereoisomers, quinine and quinidine in producing phenocopy debrisoquine poor metabolisers (PMs) in healthy volunteers. *Chirality* **3:**14-18.

Backman JT, Kivisto KT, Olkkola KT, and Neuvonen PJ (1998) The area under the plasma concentration-time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. *European journal of clinical pharmacology* **54:**53-58.

Backman JT, Olkkola KT, Aranko K, Himberg JJ, and Neuvonen PJ (1994) Dose of Midazolam Should Be Reduced during Diltiazem and Verapamil Treatments. *Brit J Clin Pharmaco* **37:**221-225.

Brown HS, Galetin A, Hallifax D, and Houston JB (2006) Prediction of in vivo drug-drug interactions from in vitro data : factors affecting prototypic drug-drug interactions involving CYP2C9, CYP2D6 and CYP3A4. *Clinical pharmacokinetics* **45:**1035-1050.

Einolf HJ (2007) Comparison of different approaches to predict metabolic drug-drug interactions. *Xenobiotica; the fate of foreign compounds in biological systems* **37:**1257-1294.

Galetin A, Gertz M, and Houston JB (2008) Potential role of intestinal first-pass metabolism in the prediction of drug-drug interactions. *Expert opinion on drug metabolism & toxicology* **4:**909-922.

Galetin A and Houston JB (2006) Intestinal and hepatic metabolic activity of five cytochrome P450 enzymes: impact on prediction of first-pass metabolism. *The Journal of pharmacology and experimental therapeutics* **318:**1220-1229.

Hisaka A, Ohno Y, Yamamoto T, and Suzuki H (2010) Prediction of pharmacokinetic drug-drug interaction caused by changes in cytochrome P450 activity using in vivo information. *Pharmacol Ther* **125:**230-248.

Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H, and Sugiyama Y (1998) Prediction of pharmacokinetic alterations caused by drug-drug interactions: Metabolic interaction in the liver. *Pharmacol Rev* **50:**387-411.

Jerling M, Huan BL, Leung K, Chu N, Abdallah H, and Hussein Z (2005) Studies to investigate the pharmacokinetic interactions between ranolazine and ketoconazole, diltiazem, or simvastatin during combined administration in healthy subjects. *Journal of clinical pharmacology* **45:**422-433.

Kurtz DL, Bergstrom RF, Goldberg MJ, and Cerimele BJ (1997) The effect of sertraline on the pharmacokinetics of desipramine and imipramine. *Clin Pharmacol Ther* **62:**145-156.

McDonald MG, Au NT, Wittkowsky AK, and Rettie AE (2012) Warfarin-Amiodarone Drug-Drug Interactions: Determination of [I](u)/K(I,u) for Amiodarone and Its Plasma Metabolites. *Clin Pharmacol Ther* **91:**709-717.

Neuvonen PJ, Kantola T, and Kivisto KT (1998) Simvastatin but not pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole. *Clin Pharmacol Ther* **63:**332-341.

Nolan PE, Marcus FI, Hoyer GL, Bliss M, and Gear K (1989) Pharmacokinetic Interaction between Intravenous Phenytoin and Amiodarone in Healthy-Volunteers. *Clin Pharmacol Ther* **46:**43-50.

O'Reilly RA (1982) Stereoselective interaction of sulfinpyrazone with racemic warfarin and its separated enantiomorphs in man. *Circulation* **65:**202-207.

Obach RS (2009) Predicting drug-drug interactions from in vitro drug metabolism data: challenges and recent advances. *Current opinion in drug discovery & development* **12:**81-89.

Obach RS, Walsky RL, and Venkatakrishnan K (2007) Mechanism-based inactivation of human cytochrome p450 enzymes and the prediction of drug-drug interactions. *Drug metabolism and disposition: the biological fate of chemicals* **35:**246-255.

Obach RS, Walsky RL, Venkatakrishnan K, Gaman EA, Houston JB, and Tremaine LM (2006) The utility of in vitro cytochrome P450 inhibition data in the prediction of drug-drug interactions. *The Journal of pharmacology and experimental therapeutics* **316:**336-348.

Ohno Y, Hisaka A, and Suzuki H (2007) General framework for the quantitative prediction of CYP3A4-mediated oral drug interactions based on the AUC increase by coadministration of standard drugs. *Clinical pharmacokinetics* **46:**681-696.

Olkkola KT, Ahonen J, and Neuvonen PJ (1996) The effects of the systemic antimycotics, itraconazole and fluconazole, on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. *Anesthesia and analgesia* **82:**511-516.

Olkkola KT, Aranko K, Luurila H, Hiller A, Saarnivaara L, Himberg JJ, and Neuvonen PJ (1993) A Potentially Hazardous Interaction between Erythromycin and Midazolam. *Clin Pharmacol Ther* **53:**298-305.

Olkkola KT, Backman JT, and Neuvonen PJ (1994) Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther* **55:**481-485.

Polasek TM, Lin FP, Miners JO, and Doogue MP (2011) Perpetrators of pharmacokinetic drug-drug interactions arising from altered cytochrome P450 activity: a criteria-based assessment. *Br J Clin Pharmacol* **71:**727-736.

Preskorn SH, Alderman J, Chung M, Harrison W, Messig M, and Harris S (1994) Pharmacokinetics of desipramine coadministered with sertraline or fluoxetine. *Journal of clinical psychopharmacology* **14:**90-98.

Reese MJ, Wurm RM, Muir KT, Generaux GT, St John-Williams L, and McConn DJ (2008) An in vitro mechanistic study to elucidate the desipramine/bupropion clinical drug-drug interaction. *Drug metabolism and disposition: the biological fate of chemicals* **36:**1198-1201.

Shardlow CE, Generaux GT, MacLauchlin CC, Pons N, Skordos KW, and Bloomer JC (2011) Utilizing drug-drug interaction prediction tools during drug development: enhanced decision making based on clinical risk. *Drug metabolism and disposition: the biological fate of chemicals* **39:**2076-2084.

Shou M (2005) Prediction of pharmacokinetics and drug-drug interactions from in vitro metabolism data. *Current opinion in drug discovery & development* **8:**66-77.

Tod M, Goutelle S, Clavel-Grabit F, Nicolas G, and Charpiat B (2011) Quantitative prediction of cytochrome P450 (CYP) 2D6-mediated drug interactions. *Clinical pharmacokinetics* **50:**519-530.

Toon S, Low LK, Gibaldi M, Trager WF, O'Reilly RA, Motley CH, and Goulart DA (1986) The warfarin-sulfinpyrazone interaction: stereochemical considerations. *Clin Pharmacol Ther* **39:**15-24.

Yeung CK, Fujioka Y, Hachad H, Levy RH, and Isoherranen N (2011) Are circulating metabolites important in drug-drug interactions?: Quantitative analysis of risk prediction and inhibitory potency. *Clin Pharmacol Ther* **89:**105-113.

Zamuner S, Johnson BM, Pagliarusco S, Fina P, Peroni M, Fiore M, Adams LM, and Fernandes SA (2010) Effect of single and repeat doses of casopitant on the pharmacokinetics of CYP450 3A4 substrates midazolam and nifedipine. *Br J Clin Pharmacol* **70:**537-546.

Zhang X, Jones DR, and Hall SD (2009a) Prediction of the Effect of Erythromycin, Diltiazem, and Their Metabolites, Alone and in Combination, on CYP3A4 Inhibition. *Drug Metabolism and Disposition* **37:**150-160.

Zhang X, Quinney SK, Gorski JC, Jones DR, and Hall SD (2009b) Semiphysiologically based pharmacokinetic models for the inhibition of midazolam clearance by diltiazem and its major metabolite. *Drug metabolism and disposition: the biological fate of chemicals* **37:**1587-1597.

Zhao P, Zhang L, Grillo JA, Liu Q, Bullock JM, Moon YJ, Song P, Brar SS, Madabushi R, Wu TC, Booth BP, Rahman NA, Reynolds KS, Gil Berglund E, Lesko LJ, and Huang SM (2011) Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. *Clin Pharmacol Ther* **89:**259-267.

Zimmermann T, Yeates RA, Laufen H, Scharpf F, Leitold M, and Wildfeuer A (1996) Influence of the antibiotics erythromycin and azithromycin on the pharmacokinetics and pharmacodynamics of midazolam. *Arzneimittel-Forschung* **46:**213-217.

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

Footnotes

This study was supported by grant [R15GM096074] from National Institute of General Medical Sciences. Address correspondence to: Dr. Zheyi Hu, Department of Clinical Pharmacy, University of Tennessee, Room 328, 881 Madison Ave., Memphis, TN 38163. E-mail: zhu13@uthsc.edu

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

Legends for Figures

FIG. 1. Predicted versus observed AUC_I/AUC ratios (CYP2C9) in the external validation set (panel A, the dotted red lines represent 50–200% ranges of the prediction accuracy), prediction accuracy for the external validation set (panel B), and predicted AUC_I/AUC of substrates in the presence of various inhibitors (panel C). In panel C, the magnitude of the DDI increased from yellow to red; the doses for inhibitors amiodarone, fluconazole and fluvoxamine are 400, 400, and 150 mg/day, respectively. The doses for other inhibitors are shown in Table 2. DIC, diclofenac; LOS, losartan; IBU, S-ibuprofen; MEL, meloxicam; ZAF, zafirlukast; FLT, fluvastatin; FLP, flurbiprofen; ETR, etravirine; PHE, phenytoin; WAR, S-warfarin; GLI, glimepiride; TOL, tolbutamide; PAR, paroxetine; DIL, diltiazem; SER, sertraline; FLX, fluvoxamine; CIM, cimetidine; SUR, sulphinpyrazone; KET, ketoconazole; AMI, amiodarone; VOR, voriconazole; FLN, fluconazole; BEN, benzbromarone; SUN, sulphaphenazole; BUC, bucolome; MIC, miconazole.

FIG. 2. Predicted versus observed AUC_I/AUC ratios for *in vivo* drug-drug interaction studies associated with inhibitors that have inhibitory metabolites. The prediction was based on four different models. The dotted red lines represent 50–200% ranges of the prediction accuracy. For the inhibitors diltiazem and erythromycin, only the predicted AUC ratios based on the irreversible inhibition model were shown. *In vitro* (P), only the data of the parent drugs were included in the *in vitro*-based prediction approach and total systemic C_{max} was used to estimate perpetrator concentration available to the enzyme; *In vitro* (P+M), total systemic C_{max} for the parent drug and metabolites were used in the *in vitro*-based prediction approach; *In vitro* UHI (P+M), unbound hepatic inlet concentrations of parent drug and metabolites were used to estimate perpetrator concentration available to the enzyme; IVIP, *in vivo* information-guided prediction approach.

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

TABLE 1

The estimated values of the contribution ratio of CYP2C9 substrates

Substrates	CR[a]	f_m[b]	References for f_m[e]	f_h[c]	References for f_h[e]
Diclofenac	0.48	0.95	(Yamazaki et al., 1998a; Tang et al., 1999)	0.50	(Stierlin and Faigle, 1979; Kumar et al., 2002)
Flurbiprofen	0.68	0.99	(Tracy et al., 1996; Yamazaki et al., 1998a)	0.69	(Zgheib et al., 2007)
Fluvastatin	0.60	0.65	(Andersson et al., 2004)	0.92	(Tse et al., 1992)
S-Ibuprofen	0.50	0.70	(Hamman et al., 1997)	0.70	(Rudy et al., 1991; Davies, 1998)
Meloxicam	0.50	0.60–0.80[d]	(Chesne et al., 1998)	0.70	(Schmid et al., 1995)
Phenytoin	0.75	0.95	(Miners and Birkett, 1998; Komatsu et al., 2000b)	0.79	(Dickinson et al., 1985)
Tolbutamide	0.84	0.99	(Miners and Birkett, 1998; Komatsu et al., 2000a)	0.85	(Madsen et al., 2001)
S-Warfarin	0.69	0.96	(Yamazaki et al., 1998a; Yamazaki et al., 1998b)	0.72	(Toon et al., 1986; Heimark et al., 1992)

[a] Calculated by the extrapolation method (equation 5).
[b] The contribution of CYP2C9 to hepatic clearance.
[c] The contribution of the hepatic clearance to the total clearance of the drug.
[d] Value varies depending on the CYP3A4-to-CYP2C9 concentration ratio in different livers. A mean value was used in our analysis.
[e] The bibliography are only shown in Supplemental Material 3.

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

TABLE 2

The data of learning set 1 (data selected to calculate the IR of all CYP2C9 inhibitors) and learning set 2 (data selected to calculate the CR of remaining CYP2C9 substrates)

Substrates	Inhibitors	Dose and regimen (mg/day, days)[a]	IR	CR	AUC_I/AUC	References[g]
Learning set 1[b]						
S-Warfarin	Amiodarone	300, 3d	0.31	0.69	1.27	(Heimark et al., 1992)
		400, 3d	0.76	0.69	2.11	(O'Reilly et al., 1987)
S-Warfarin	Benzbromarone	50, unknown	0.78	0.69	2.15	(Takahashi et al., 1999b)
S-Warfarin	Bucolome	300, unknown	1.00	0.69	3.29	(Takahashi et al., 1999a)
S-Warfarin	Cimetidine	1200, 3d/15d	0.33	0.69	1.30[f]	(O'Reilly, 1984; Sax et al., 1987)
Tolbutamide	Diltiazem	60, 0d	0.11	0.84	1.10	(Dixit and Rao, 1999)
S-Warfarin	Fluconazole	100, 7d	0.38	0.69	1.35	(Black et al., 1996)
		200, 7d	0.67	0.69	1.86	(Neal et al., 2003)
		300, 7d	0.72	0.69	2.00	(Neal et al., 2003)
		400, 7d	0.94	0.69	2.84	(Neal et al., 2003)
Diclofenac	Fluvastatin	40, 7d	0.42[e]	0.48	1.25	(Transon et al., 1995)
Tolbutamide	Fluvastatin	40, 15d	0.00[e]	0.84	1.00	(Appel et al., 1995)
Tolbutamide	Fluvoxamine	75, 3d	0.22	0.84	1.23	(Madsen et al., 2001)
		150, 3d	0.40	0.84	1.50	(Madsen et al., 2001)
Tolbutamide	Ketoconazole	200, 7d	0.52	0.84	1.77	(Krishnaiah et al., 1994)
S-Warfarin	Miconazole	125, 3d	1.00	0.69	4.72	(O'Reilly et al., 1992)
S-Warfarin	Paroxetine	30, 14d	0.09	0.69	1.07	(Bannister et al., 1989)
Phenytoin	Sertraline	200, 17d	0.21	0.75	1.19	(Rapeport et al., 1996)
Tolbutamide	Sulphaphenazole	1000, 3d/1d	0.91	0.84	4.19[f]	(Back et al., 1988; Veronese et al., 1990)
Tolbutamide	Sulphinpyrazone	800, 7d	0.48	0.84	1.67	(Miners et al., 1982)
Phenytoin	Voriconazole	800, 9d	0.60	0.75	1.81	(Purkins et al., 2003)
Learning set 2[c]						
Losartan	Fluconazole	200, 9d/3d	0.67	0.47	1.47[f]	(Kazierad et al., 1997; Kaukonen et al., 1998)
Zafirlukast	Fluconazole	200, 2d[d]	0.67	0.56	1.60	(Karonen et al., 2011)
Glimepiride	Fluconazole	200, 3d[d]	0.67	0.87	2.38	(Niemi et al., 2001)
Etravirine	Fluconazole	200, 7d	0.67	0.69	1.86	(Kakuda et al., 2011)

[a] mg/day, the daily doses of the inhibitors; days, the duration of the multiple dosing before administration of substrates. Single dose is shown as 0d.

[b] Only the DDIs associated with typical CYP2C9 substrates (S-warfarin, tolbutamide, diclofenac, and phenytoin) were included in the learning set 1. In learning set 1, IR of all CYP2C9 inhibitors were calculated by equation 1 using known CR and AUC_I/AUC values.

[c] In learning set 2, CR of the remaining CYP2C9 substrates (not included in Table 1) were calculated by equation 3 using known IR and AUC_I/AUC values.

[d] 400 mg on the first day.

[e] Two IR values of fluvastatin were calculated and the mean value was used in the further analysis.

[f] An algebraic mean of the AUC_I/AUC was used if multiple studies are reported for a single combination of substrate and inhibitor (same dose of inhibitor was used in these studies).

DMD Fast Forward. Published on May 4, 2012 as DOI: 10.1124/dmd.112.045799
This article has not been copyedited and formatted. The final version may differ from this version.

[g] The bibliography are only shown in Supplemental Material 3.

TABLE 3

The CYP2C9-mediated drug-drug interaction studies that were used for external validation

Substrates	Inhibitor	Dose and regimen (mg/day, days)[a]	IR	CR	Observed AUC_I/AUC	Predicted AUC_I/AUC[b]	Accuracy (%)	References[f]
Phenytoin	Amiodarone	200, 21d	0.31	0.75	1.40	1.30	93	(Nolan et al., 1989)
Losartan	Bucolome	300, 7d	1.00	0.47	1.67	1.90	114	(Kobayashi et al., 2008)
Tolbutamide	Cimetidine	1200, 3d	0.33	0.84	1.20	1.39	116	(Cate et al., 1986)
Tolbutamide	Fluconazole	100, 6d	0.38	0.84	2.09	1.47	70	(Lazar and Wilner, 1990)
Phenytoin	Fluconazole	200, 13d	0.67	0.75	1.75	2.01	115	(Blum et al., 1991)
Fluvastatin	Fluconazole	200, 3d[c]	0.67	0.60	1.84	1.67	91	(Kantola et al., 2000)
Flurbiprofen	Fluconazole	200, 1d/6d	0.67	0.68	1.92[e]	1.84	96	(Greenblatt et al., 2006; Kumar et al., 2008)
		400, 6d	0.94	0.68	3.03	2.77	91	(Kumar et al., 2008)
S-Ibuprofen	Fluconazole	200, 2d[c]	0.67	0.50	1.83	1.50	82	(Hynninen et al., 2006)
Losartan	Fluvastatin	40, 13d	0.21	0.47	1.01	1.11	110	(Meadowcroft et al., 1999)
Glimepiride	Fluvoxamine	100, 3d	0.22	0.87	1.33	1.24	93	(Niemi et al., 2001)
Etravirine	Paroxetine	20, 7d	0.09	0.69	1.00	1.07	107	(Brown et al., 2009)
Tolbutamide	Sertraline	200, 21d	0.21	0.84	1.19	1.22	102	(Tremaine et al., 1997)
Phenytoin	Sulphaphenazole	2000, 7d	0.91	0.75	3.06	3.12	102	(Hansen et al., 1979)
S-Warfarin	Sulphinpyrazone	400, 3d	0.48	0.69	1.82[e]	1.49	82	(O'Reilly, 1982; Toon et al., 1986)
Diclofenac	Voriconazole	400, 2d[d]	0.60	0.48	1.78	1.40	79	(Hynninen et al., 2007)
Meloxicam	Voriconazole	400, 2d[d]	0.60	0.50	1.46	1.43	98	(Hynninen et al., 2009)
Etravirine	Voriconazole	400, 7d	0.60	0.69	1.36	1.70	125	(Kakuda et al., 2011)
S-Ibuprofen	Voriconazole	400, 2d[d]	0.60	0.50	2.01	1.43	71	(Hynninen et al., 2006)

[a] mg/day, the daily doses of the inhibitors; days, the duration of the multiple dosing before administration of substrates.
[b] The AUC_I/AUC was predicted by equation 1.
[c] 400 mg on the first day.
[d] 800 mg on the first day.
[e] An algebraic mean of the observed AUC_I/AUC was used if multiple studies are reported for a single combination of substrate and inhibitor (same dose of inhibitor was used in these studies).
[f] The bibliography are only shown in Supplemental Material 3.

TABLE 4

Predicted versus observed AUC ratios (AUC_I/AUC) calculated with the in vitro-based model and in vivo information-guided prediction approach

Inhibitors (parent drug)	Inhibitors (metabolites)	Inhibitor dose (mg/day)	Victim drugs	CYP	Observed AUC_I/AUC	Predicted AUC_I/AUC				References for observed AUC_I/AUC
						In vitro (P)	*In vitro* (P+M)	*In vitro* UHI (P+M)	IVIP	
Casopitant	Hydroxycasopitant	30	Midazolam	3A	1.76	1.04	1.08	1.00	1.74	(Zamuner et al., 2010)
Diltiazem	N-desmethyldiltiazem	240	Midazolam	3A	3.88[a]	1.01/13.69[c]	1.14/17.74[c]	1.06/15.02[c]	3.91	(Backman et al., 1994; Zhang et al., 2009b)
Erythromycin	N-desmethyl erythromycin	1500	Midazolam	3A	4.12[a]	1.05/20.80[c]	1.05/21.90[c]	1.01/18.68[c]	4.72	(Olkkola et al., 1993; Zimmermann et al., 1996)
Itraconazole	Hydroxyitraconazole	200	Midazolam	3A	7.86[a]	15.79	17.25	3.52	8.93	(Olkkola et al., 1994; Olkkola et al., 1996; Backman et al., 1998)
Itraconazole	Hydroxyitraconazole	200	Simvastatin	3A	18.61	62.62	84.78	5.35	18.26	(Neuvonen et al., 1998)
Ranolazine	O-Desmethylranolazine	2000	Simvastatin	3A	1.59	1.06	1.09	1.04	1.24	(Jerling et al., 2005)
Bupropion	Erythrohydrobupropion Hydroxybupropion Threohydrobupropion	150	Desipramine	2D6	5.21	1.03	1.76	1.39	6.67	(Reese et al., 2008)
Fluoxetine	Norfluoxetine	20	Desipramine	2D6	4.42	2.09	3.29	1.19	4.67	(Preskorn et al., 1994)
Quinidine	Quinidine N-oxide 3-Hydroxyquinidine	50[b]	Desipramine	2D6	3.14	5.27	5.29	3.38	2.64	(Ayesh et al., 1991)
Sertraline	N-Desmethylsertraline	50	Desipramine	2D6	1.30[a]	1.00	1.01	1.00	1.54	(Preskorn et al., 1994; Alderman et al., 1997)
Sertraline	N-Desmethylsertraline	150	Imipramine	2D6	1.68	1.00	1.01	1.00	1.26	(Kurtz et al., 1997)
Venlafaxine	N-Desmethylvenlafaxine	150	Imipramine	2D6	1.28	1.00	1.00	1.00	1.17	(Albers et al., 2000)
Amiodarone	N-Desethylamiodarone	200	Phenytoin	2C9	1.40	1.01	1.36	1.01	1.30	(Nolan et al., 1989)
Sulfinpyrazone	Sulfinpyrazone sulfide Sulfinpyrazone sulfone	400	S-Warfarin	2C9	1.82[a]	1.16	2.24	1.01	1.49	(O'Reilly, 1982; Toon et al., 1986)

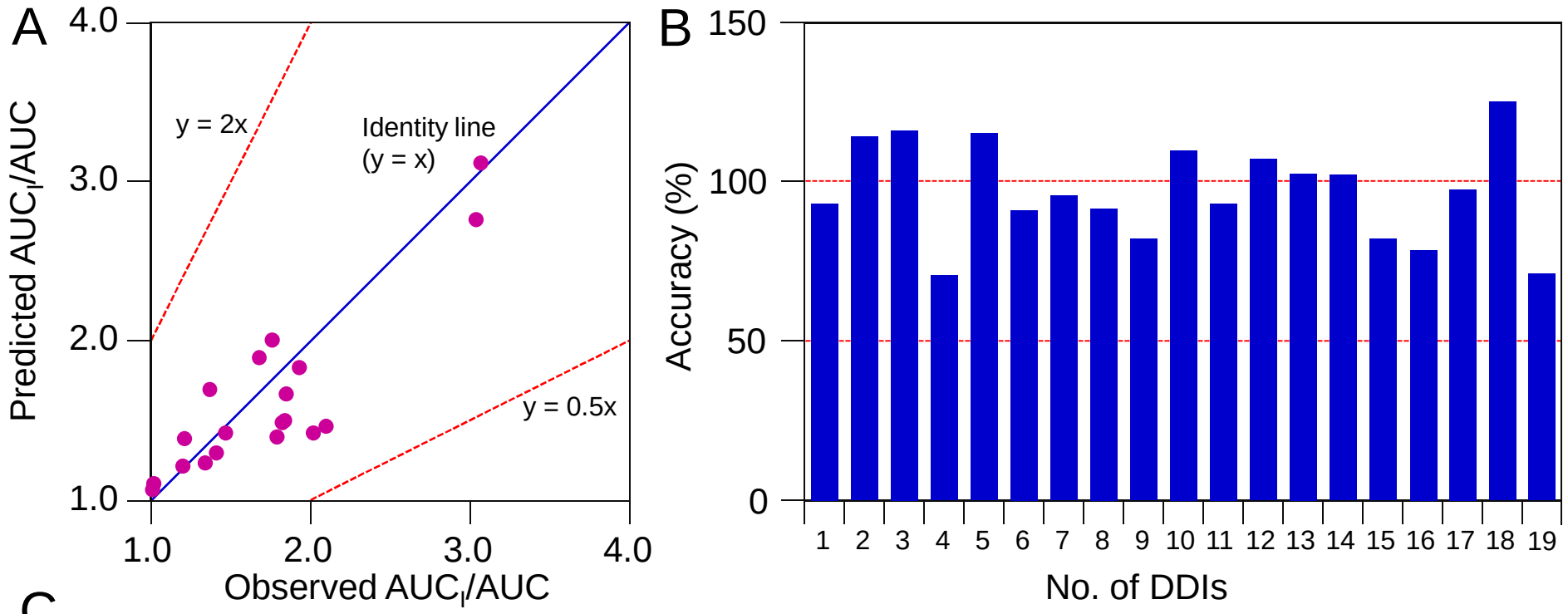
[a] An algebraic mean of the observed AUC_I/AUC was used if multiple studies are reported for a single combination of substrate and inhibitor (same dose of inhibitor was used in these studies).

[b] Single dose of quinidine was administrated to the subjects in this study.

[c] the left data were calculated based on the reversible inhibition model while the right data were based on the irreversible inhibition model.

In vitro (P), only the data of the parent drugs were included in the *in vitro*-based prediction approach and total systemic C_{max} was used to estimate perpetrator concentration available to the enzyme; *In vitro* (P+M), both the data of the parent drug and metabolites were included in the *in vitro*-based prediction approach; *In vitro* UHI (P+M), unbound hepatic inlet concentration was used to estimate perpetrator concentration available to the enzyme; IVIP, *in vivo* information-guided prediction approach.

Figure 1



C

	Substrates	LOS	DIC	IBU	MEL	ZAF	FLT	FLP	ETR	WAR	PHE	TOL	GLI
Inhibitors	IR/CR	0.47	0.48	0.50	0.50	0.56	0.60	0.68	0.69	0.69	0.75	0.84	0.87
PAR	0.09	1.04	1.05	1.05	1.05	1.05	1.06	1.07	1.07	1.07	1.07	1.08	1.08
DIL	0.11	1.05	1.06	1.06	1.06	1.07	1.07	1.08	1.08	1.08	1.09	1.10	1.11
FLT	0.21	1.08	1.08	1.09	1.09	1.10	1.11	1.12	1.12	1.12	1.14	1.16	1.16
SER	0.21	1.11	1.11	1.12	1.12	1.13	1.14	1.17	1.17	1.17	1.19	1.21	1.22
CIM	0.33	1.18	1.19	1.20	1.20	1.23	1.25	1.29	1.29	1.29	1.33	1.38	1.40
FLX	0.40	1.23	1.24	1.25	1.25	1.29	1.32	1.37	1.38	1.38	1.43	1.51	1.53
SUR	0.48	1.29	1.30	1.32	1.32	1.37	1.40	1.48	1.50	1.50	1.56	1.68	1.72
KET	0.52	1.32	1.33	1.35	1.35	1.41	1.45	1.55	1.56	1.56	1.64	1.78	1.83
VOR	0.60	1.39	1.40	1.43	1.43	1.51	1.56	1.69	1.71	1.71	1.82	2.02	2.09
AMI	0.76	1.56	1.57	1.61	1.61	1.74	1.84	2.07	2.10	2.10	2.33	2.77	2.95
BEN	0.78	1.58	1.60	1.64	1.64	1.78	1.88	2.13	2.17	2.17	2.41	2.90	3.11
SUN	0.91	1.75	1.78	1.83	1.83	2.04	2.20	2.62	2.69	2.69	3.15	4.24	4.80
FLN	0.94	1.79	1.82	1.89	1.89	2.11	2.29	2.77	2.85	2.85	3.39	4.75	5.49
BUC	1.00	1.89	1.92	2.00	2.00	2.27	2.50	3.13	3.23	3.23	4.00	6.25	7.69
MIC	1.00	1.89	1.92	2.00	2.00	2.27	2.50	3.13	3.23	3.23	4.00	6.25	7.69

Figure 2