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A COMBINED MODEL FOR PREDICTING CYP3A4 CLINICAL NET DRUG-DRUG INTERACTION BASED ON CYP3A4 INHIBITION, INACTIVATION, AND INDUCTION DETERMINED IN VITRO

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A COMBINED MODEL FOR PREDICTING CYP3A4 CLINICAL NET DDI

Abbreviations

AUC, area under the concentration-time curve; TDI, Time-dependent Inactivation; DDI, DDIs.

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Abstract

Although approaches to the prediction of DDIs arising via time-dependent inactivation have recently been developed, such approaches do not account for simple competitive inhibition or induction. Accordingly, these approaches do not provide accurate predictions of DDIs arising from simple competitive inhibition (e.g. ketoconazole) or induction of P450s (e.g. phenytoin). In addition, methods which focus upon a single interaction mechanism are likely to yield misleading predictions in the face of mixed mechanisms (e.g. ritonavir). As such, we have developed a more comprehensive mathematical model that accounts for the simultaneous influences of competitive inhibition, time-dependent inactivation and induction of CYP3A in both the liver and intestine in order to provide a net drug-drug interaction prediction in terms of AUC ratio. This model provides a framework by which readily obtained in vitro values for competitive inhibition, time-dependent inactivation and induction for the precipitant compound as well as literature values for f_m and F_G for the object drug can be used to provide quantitative predictions of DDIs. Using this model, DDIs arising via inactivation (e.g. erythromycin) continue to be well predicted, while those arising via competitive inhibition (e.g. ketoconazole); induction (e.g. phenytoin) and mixed mechanisms (e.g. ritonavir) are also predicted within the ranges reported in the clinic. This comprehensive model quantitatively predicts clinical observations with reasonable accuracy and can be a valuable tool to evaluate candidate drugs and rationalize clinical DDIs.

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Introduction

The cytochrome CYP450 superfamily of enzymes, are the most important enzymes in the metabolism of a wide variety of compounds. Many drug-drug interactions (DDIs) result from altering the activities of these enzymes. A considerable effort in the area of drug metabolism is dedicated toward predicting clinical pharmacokinetics and DDIs from in vitro data (Ito et al., 1998; Ito et al., 2004; Mayhew et al., 2000; Wang et al., 2004; Bjornsson et al., 2003; Venkatakrishnan and Obach 2007). The prediction of DDIs based on an assumption of reversible CYP450 inhibition has been a standard part of preclinical programs in the pharmaceutical industry for many years. It is now well-appreciated that this common practice will under predict the magnitude of DDI when the underlying inhibitory mechanism includes time-dependent inactivation (e.g., erythromycin and verapamil). As such, recent efforts have yielded methods whereby in vitro data can be used to provide accurate predictions of DDI arising via a time-dependent inactivation mechanism (Mayhew et al., 2000; Wang et al., 2004; Obach et al 2007). Although the magnitude of DDI predicted via these newer approaches is often close to that observed in the clinic for drugs known to inhibit CYP3A via time-dependent inactivation, it is important to acknowledge discrepancies that have been observed with some of the drugs tested, e.g., cyclosporin, erythromycin, ethinyl estradiol, indinavir, mibefradil, oleandomycin, rifampicin and verapamil (Lamberg et al., 1998). Interestingly, some of these inactivators are also potent competitive inhibitors and inducers of CYP3A. As such, these discrepancies may represent the mixed nature of the underlying mechanisms by which the precipitants interact with CYP3A. In this work, we attempt to improve previously published mathematical models by simultaneously considering the relative contribution of competitive inhibition, inactivation and induction in both the liver and intestine on the net DDI observed in the clinic. The validity of the derived approach was examined through a comparison of observed DDI's to those predicted using the mathematical model populated with competitive

inhibition, inactivation and induction parameters derived from in vitro systems. For this study, we have chosen the term “precipitant” for the drug causing the effect and “object” for the drug whose AUC is being affected. These terms are consistent with those used in the University of Washington database (http://www.druginteractioninfo.org/).

Methods

Clinical Data Source

Literature compound clinical data were collected from the University of Washington Metabolism and Transport drug interaction database (http://www.druginteractioninfo.org/). The database contains in vitro and in vivo information on drug interactions in human from 5979 publications referenced in PubMed, 19 New Drug Applications and 223 excerpts of product labels. Thirty two drugs were chosen for this study, based on available data from clinical studies with midazolam, triazolam, simvastatin and nifedipine and commercial availability. Also, efforts were made to choose precipitants that have exhibited net clinical inhibition as well as drugs that have exhibited net clinical induction as determined by the object (e.g. midazolam) AUC in the presence and absence of the precipitant. For rifampin, only the 15 mg midazolam dose studies were included to capture the maximum effect, along with all other object probes. For phenytoin and nifedipine, in vivo data were obtained from Backman et al., 1996 and Horsmans et al., 1991, respectively.

In Vitro Data

Data reflecting competitive inhibition, time-dependent inactivation and induction of CYP3A were collected from the in vitro systems described below. Thirty two drugs were included in this study. Of these, 16 drugs exhibited competitive inhibition, 18 drugs exhibited time-dependent inactivation and 21 compounds exhibited induction. Thirteen drugs exhibited all three interaction mechanisms in vitro (troleandomycin, ethinyl estradiol, fluoxetine, mibefradil,

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pioglitazone, rosiglitazone, troglitazone, saquinavir, nelfinavir, ritonavir, ethinyl estradiol, mibefradil and verapamil).

Competitive Inhibition Data

Competitive inhibition data were determined using previously described validated methods (Walsky and Obach, 2004) for CYP3A (midazolam 1'-hydroxylase). Human liver microsomes pooled from 53 individual donors were used as the source of enzyme activity. IC_{50} values were measured using the substrates at a concentration equal to previously determined K_M values. Inhibitors were examined up to a maximum concentration of 300 μM.

Time-Dependent Inactivation Data

Data for the kinetic parameters determinations, K_i and k_{inact} were run in our laboratory using previously described methods (Walsky and Obach, 2004; Obach et al., 2006; Obach et al., 2007).

Induction Assay Data

CYP3A4 induction data were carried out as described by the manufacturer, utilizing human cryopreserved hepatocytes (lot Hu4026, CellzDirect, Pittsboro, NC). Total RNA was extracted from cells using the RNeasy Mini kit according to instructions provided by the manufacturer (Qiagen, Germantown MD). Quantification of cytochrome CYP3A4 mRNA was performed using the TaqMan two-step RT-PCR method by using the ABI 7500 Fast Real Time PCR (Applied Biosystems, Foster City, CA). The relative quantity of the target CYP3A4 gene (Hs00604506_m1) compared with the endogenous control (GAPDH) was determined by the ΔΔCT method. EC_{50} and E_{max} (ψ) parameters were determined based on CYP3A4 mRNA, using a sigmoid 3-parameter curve fitting (Sigma Plot 9.0, Systat Software, Inc. Chicago, IL).

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Mathematical Model

As reported previously, the ratio of area under the exposure – time curve in the presence (AUC'_{po}) and absence (AUC_{po}) of a pharmacokinetic drug-drug interaction can be defined as stated in equation 1 (Wang et al., 2004).

$$\frac{AUC'_{po}}{AUC_{po}} = \frac{F'_G}{F_G} \times \frac{CL_{int,H}}{CL'_{int,H}} \qquad \text{Equation 1}$$

F'_G and F_G, represent the fraction of drug escaping intestinal metabolism in the pharmacokinetically altered and unaltered state, respectively. $CL'_{int,H}$ and $CL_{int,H}$ represent the intrinsic hepatic clearance in the pharmacokinetically altered and unaltered state, respectively. The expanded forms of these ratios of intrinsic clearance and fraction escaping gut metabolism are derived separately in detail below.

Alterations in hepatic intrinsic clearance

The intrinsic hepatic clearance of a theoretical drug can be defined as in Equation 2, where $V_{max,H}$, K_m, $k_{cat,H}$, and $E_{ss,H}$ represent the maximum velocity, drug concentration associated with half-maximum velocity, catalysis rate and the steady-state amount of clearing enzyme, respectively.

$$CL_{int,H} = \frac{V_{max,H}}{K_m} = \frac{k_{cat,H} \times E_{ss,H}}{K_m} \qquad \text{Equation 2}$$

Since the steady state amount of enzyme is determined by the ratio of the zero-order rate of synthesis ($K_{syn,H}$) and first-order rate of degradation ($k_{deg,H}$), equation 2 can be written as follows:

$$CL_{int,H} = \frac{k_{cat,H} \times \left(K_{syn,H} \Big/ k_{deg,H} \right)}{K_m} \qquad \text{Equation 3}$$

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In the presence of competitive inhibition, the apparent K_m (K'_m) will also be determined by the concentration of inhibitor in the liver ($[I]_H$) and its associated equilibrium dissociation constant (K_I) as follows:

$$K'_m = K_m\left(1+\frac{[I]_H}{K_I}\right) \qquad \text{Equation 4}$$

In the presence of inactivation, the overall rate of loss of the enzyme will also be determined by the pseudo-first-order apparent inactivation rate ($k_{inact,app}$). This apparent inactivation rate is dependent upon $[I]_H$, K_I and the true first-order inactivation rate constant (k_{inact}).

$$k'_{deg,H} = k_{deg,H} + k_{inact,app} = k_{deg,H} + \frac{[I]_H \times k_{inact}}{[I]_H + K_I} \qquad \text{Equation 5}$$

In the presence of induction, the rate of enzyme synthesis will also be determined by inducer concentrations in the liver $[I]_H$, the maximum fold induction (ψ) and the concentration of inducer associated with half-maximum induction ($EC_{50,I}$). The d parameter in Equation 6 represents an empirical calibration factor for the purposes of in vitro to in vivo induction scaling. As such, its value was estimated through correlation of predicted and observed AUC ratios.

$$K'_{syn,H} = K_{syn,H} \times \left(1+\frac{d \bullet \psi \bullet [I]_H}{[I]_H + EC_{50,I}}\right) \qquad \text{Equation 6}$$

The expected net effect of simultaneous competitive inhibition, induction and inactivation, can be illustrated by substituting Equations 4, 5 and 6 into Equation 3.

$$CL'_{int,H} = \frac{k_{cat,H} \times K_{syn,H} \times \left(1+\frac{d \bullet \psi \bullet [I]_H}{[I]_H + EC_{50,I}}\right)}{K_m \times \left(1+\frac{[I]_H}{K_I}\right) \times \left(k_{deg,H} + \frac{[I]_H \times k_{inact}}{[I]_H + K_I}\right)} \qquad \text{Equation 7}$$

Assuming that the object drug is only fractionally metabolized by the altered pathway, the f_m parameter represents the fraction of object drug cleared via the altered metabolic pathway (Equation 8).

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$$CL'_{int,H} = \left[\frac{k_{cat,H} \times K_{syn,H} \times \left(1 + \frac{d \bullet \psi \bullet [I]_H}{[I]_H + EC_{50,I}}\right)}{K_m \times \left(1 + \frac{[I]_H}{K_I}\right) \times \left(k_{deg,H} + \frac{[I]_H \times k_{inact}}{[I]_H + K_I}\right)} \right] \times f_m + CL_{int,H} \times (1 - f_m) \qquad \text{Equation 8}$$

If one lets

Time-Dependent Inactivation Term or $A = \frac{k_{deg}}{k_{deg,H} + \frac{[I]_H \times k_{inact}}{[I]_H + K_I}}$

Induction Term or $B = 1 + \frac{d \bullet \psi \bullet [I]_H}{[I]_H + EC_{50,I}}$

and Reversible Inhibition Term or $C = \frac{1}{1 + \frac{[I]_H}{K_I}}$,

then the ratio of Equations 3 and 8 will yield the expected ratio of object drug AUC_{po} due to the hepatic component of the drug-drug interaction arising from simultaneous inactivation (A), induction (B) and competitive inhibition (C).

$$\frac{AUC'_{po}}{AUC_{po}} = \frac{CL_{int,H}}{CL'_{int,H}} = \frac{1}{[A \times B \times C] \times f_m + (1 - f_m)} \qquad \text{Equation 9}$$

Alterations in intestinal intrinsic clearance

Assuming that absorption rate is unaffected, the ratio of the fraction of drug escaping intestinal extraction in the altered and unaltered state will equal the ratio of intrinsic clearance in the unaltered and altered state (Equation 10).

$$\frac{F'_G}{F_G} = \frac{CL_{int,G}}{CL'_{int,G}} \qquad \text{Equation 10}$$

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The intrinsic intestinal clearance of a theoretical drug in the altered and unaltered state can be derived in an identical manner to that depicted above for hepatic intrinsic clearance to provide Equations 11 and 12.

$$CL_{int,G} = \frac{k_{cat,G} \times \left(K_{syn,G} / k_{deg,G} \right)}{K_m} \qquad \text{Equation 11}$$

$$CL'_{int,G} = \left[\frac{k_{cat,G} \times K_{syn,G} \times \left(1 + \frac{d \bullet \psi \bullet [I]_G}{[I]_G + EC_{50,I}} \right)}{K_m \times \left(1 + \frac{[I]_G}{K_I} \right) \times \left(k_{deg,G} + \frac{[I]_G \times k_{inact}}{[I]_G + K_I} \right)} \right] \times (1 - F_G) + CL_{int,G} \times F_G \qquad \text{Equation 12}$$

If one lets

Time-dependent Inhibition term for the intestinal portion or $X = \frac{k_{deg}}{k_{deg,G} + \frac{[I]_G \times k_{inact}}{[I]_G + K_I}}$

Induction Term for the intestinal portion or $Y = 1 + \frac{d \bullet \psi \bullet [I]_G}{[I]_G + EC_{50,I}}$

and Reversible Inhibition term for the intestinal portion or $Z = \frac{1}{1 + \frac{[I]_G}{K_I}}$. Equation 13

then the ratio of Equation 11 and 12 will yield the expected change in AUC_{po} due to the intestinal component of the DDIs arising from simultaneous inactivation (A), induction (B) and competitive inhibition (C).

$$\frac{F'_G}{F_G} = \frac{CL_{int,G}}{CL'_{int,G}} = \frac{1}{[X \times Y \times Z] \times (1 - F_G) + F_G} \qquad \text{Equation 14}$$

As previously reported (Obach et al., 2007), Equation 15 was used to estimate intestinal drug concentrations.

$$[I]_G = \frac{Dose \times k_a \times f_a}{Q_g \times freq} \qquad \text{Equation 15}$$

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Dose, k_a, f_a, Q_g and *freq* represent, total daily dose of inhibitor given orally, first order absorption rate constant, fraction of dose absorbed, enterocytic blood flow and frequency of daily dose respectively.

Net effect

Substituting Equations 9 and 13 back into Equation 1, one obtains the following mathematical model for the net effect of competitive inhibition, inactivation and induction in both the intestine and liver.

$$\frac{AUC'_{po}}{AUC_{po}} = \left(\frac{1}{[A \times B \times C] \times f_m + (1 - f_m)}\right) \times \left(\frac{1}{[X \times Y \times Z] \times (1 - F_G) + F_G}\right) \qquad \text{Equation 16}$$

Prediction of AUC Ratio

Equation 16 was used to make AUC ratio predictions against which comparisons were made to clinical AUC ratios obtained from the University of Washington Metabolism and Transport drug interaction database (http://www.druginteractioninfo.org/). For purposes of prediction, fraction of precipitant drug absorbed (f_a) and absorption rate (k_a) were assumed to be 1.0 and 0.03 min^{-1}. These values were combined with an assumed enterocytic blood flow (Q_g) of 248 ml/min to calculate intestinal precipitant drug concentration using Equation 15. Average plasma concentrations and unbound fractions associated with the precipitant were also obtained from the literature as indicated in the Results in order to calculate drug concentration available to interact at the level of the liver. Competitive inhibition, inactivation and induction parameters used in the predictions were estimated in vitro as described above (i.e. K_I, k_{inact}, EC_{50} and ψ). Consistent with previous reports, the degradation rate for CYP3A4 (k_{deg}) was assumed to be 0.019 hr^{-1} ($t_{1/2}$ = 36 hr) and 0.029 hr^{-1} ($t_{1/2}$ = 24 hr) in the liver and intestine, respectively. The fraction of object drug metabolized by CYP3A4 was assumed to be the same as that previously

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reported for midazolam, nifedipine, simvastatin and triazolam (f_m=0.93). The fraction of object drugs metabolized by CYP3A4 in the intestines, were assumed to be 0.57, 0.66, 0.75 and 0.78 for midazolam, simvastatin, triazolam and nifedipine, respectively and as previously reported (Obach et al., 2007).

AUC ratio predictions were made under the following 4 conditions: (1) assuming a liver effect only and employing total precipitant concentrations in the plasma, (2) assuming a gut and liver effect and employing total precipitant concentrations in the plasma, (3) assuming a liver effect only and employing unbound precipitant concentrations in the plasma and (4) assuming a gut and liver effect and employing unbound precipitant concentrations in the plasma.

The scaling parameter for induction (i.e. d) in each of the four sets of predictions was estimated through linear regression to a value which minimized the geometric mean fold error (GMFE) of the prediction via linear weighted least squares regression. Briefly, GMFE of the prediction set of interest was calculated as depicted in Equation 17. All parameters, with the exception of d, were fixed to those estimated in vitro. The value of d was then estimated via weighted least squares regression to a value that provided the lowest GMFE. The derived GMFE was also subsequently used to assess the relative accuracy of the examined approaches.

$$GMFE = 10^{\frac{\sum \left| \log \frac{\text{Predicted AUC ratio}}{\text{Observed AUC ratio}} \right|}{N}} \qquad \text{Equation 17}$$

Results

The in vivo drug-drug interaction data gathered from the University of Washington database utilized in this study are summarized in Table 1. Clinical CYP3A4 probe substrate data were used from midazolam, triazolam, nifedipine or simvastatin in vivo studies, where f_m by CYP3A4 is hypothesized to be 0.93 (Obach et al., 2007).

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Reversible inhibition

The reversible inhibition of CYP3A4 for the test compounds was assessed and the K_i data are shown in Table 2. About 16 compounds showed a K_i value less than 10 μM. The predicted clinical DDI predicted assuming a purely competitive mechanism is also shown, along with observed clinical DDI, expressed as AUC ratios in the presence and absence of inhibitor. Predictions depicted in Table 2 were made assuming competitive inhibition via total precipitant concentrations at the level of the liver via the "C" component of Equation 9. The results demonstrate that using the reversible inhibition data alone fails to predict the extent of drug-drug interaction within 2-fold for over 50% of the examined cases. As one might expect, several compounds for which CYP3A4 interaction is known to be limited to a competitive inhibition mechanism are well predicted by this approach (e.g. cimetidine, fluonazole, itraconazole and ketoconazole). As has been reported previously, this approach produced significant under predictions of AUC ratio for compounds known to produce time-dependent inactivation of CYP3A4 (e.g. cyclosporin, diltazem, eryhtromycin, mibefradil, nelfanivir, ritonavir, saquinavir, troleandomycin and verapamil).

Induction

As shown in Table 3, 21 compounds showed CYP3A4 induction in cryopreserved human hepatocytes. The scaling parameters for induction (i.e. d) in each of the two sets of predictions were estimated through linear least squares regression to a value which minimized the geometric mean fold error (GMFE). Using a composite of known strong inducers as described in Materials and Methods, the calibration constant, d, was set to 0.8. This gave a balance of slightly over predicting to slightly under predicting AUC effects for the high inducers. Predictions made assuming induction via total precipitant concentrations at the level of the liver using the "B" component of Equation 9, provided AUC ratio predictions associated with greater than 2-fold error for the more than 50% of the examined cases. As one might expect, large under predictions (exceeding an order of magnitude) of AUC ratio were associated with compounds

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possessing mixed mechanisms of CYP3A4 interaction (e.g. mibefradil, nelfinavir, ritonavir, saquinavir, troleandomycin, verapamil).

Time-dependent inactivation

As shown in Table 4, 18 compounds showed time-dependent CYP3A4 inactivation. Predictions made assuming inactivation via total precipitant concentrations at the level of the liver using the A component of Equation 9 provided AUC ratio predictions associated with greater than 2-fold error for about 40% of the examined cases. As one might expect, many of the over-predictions of DDI were associated with drug known to also possess the ability to induce CYP3A4 (e.g. fluoxetine, nelfinavir, pioglitazone, rosiglitazone, saquinavir, troglitazone and verapamil). When these values are compared to estimates of AUC ratio changes obtained from clinical study reports, some compounds that show positive time-dependent inactivation ($k_{inact} > 0$) are reasonably predicted.

Integrated approach

To account for all known mechanisms affecting CYP3A4 activity, data from the three possible mechanisms; induction, inactivation and competitive inhibition were used simultaneously to make a prediction on the AUC ratio change, as shown in Table 5, Table 6 and Figures 1A-1D. Table 5, Figures 1A &1C show the AUC ratio predictions made by employing total precipitant concentrations in the plasma, assuming a liver effect only and gut plus liver effect, by applying Equations 9 and 16, respectively.

The scaling parameter for induction (i.e. d) in each of the two sets of predictions was estimated through linear least squares regression to a value which minimized the geometric mean fold error (GMFE). The values for d were 0.8 and 0.3, and GMFE values were calculated as 1.8 and 2.5, for the liver and the gut plus liver analyses, respectively. Table 6, Figure 1B and Figure 1D

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show the DDI predictions made by employing unbound precipitant concentrations in the plasma, assuming a liver effect only and gut plus liver effect, by applying Equations 9 and 16, respectively. The values for d were 1.0 and 0.4, and GMFE values were calculated as 1.9 and 2.0, for the liver and gut plus liver analyses, respectively.

Discussion

Pharmacokinetic DDI's via CYP3A pose a serious safety risk and attrition factor in drug development. As such, significant efforts are made in the pharmaceutical industry to design compounds that are devoid of this risk. These design efforts almost exclusively rely upon in vitro data. Frequently, mathematical models are also employed which enable scaling of these and other pertinent information into a quantitative prediction of the expected magnitude of DDI (typically expressed as an AUC ratio of the object in the presence and absence of the precipitant). Prior work has demonstrated that this approach can indeed yield accurate DDI predictions (Ito et al., 1998; Ito et al., 2004; Mayhew et al., 2000; Wang et al., 2004; Bjornsson et al., 2003; Venkatakrishnan and Obach 2007). However, to date, such quantitative approaches have not been comprehensive in that they assume a singular mechanism of action. In cases where the mechanism of CYP3A interaction is mixed (e.g. competitive inhibition, time-dependent inactivation, induction) such approaches may yield inaccurate predictions. A classic example of this is the under prediction of DDI for time-dependent inactivators when only a competitive mechanism is presumed (illustrated here in Table 2). Conversely, the presence of an induction mechanism may yield over predictions of DDI in the event that only inhibition mechanisms are considered.

As such, we have developed and tested a comprehensive mathematical model which simultaneously accounts for competitive inhibition, time-dependent inhibition and induction to yield a net DDI prediction from in vitro data. This model was combined with data from in vitro

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systems to provide 59 DDI predictions on 32 drugs. Of the 32 drugs examined in this study, 16 exhibited in vitro reversible inhibition, 18 drugs exhibited time-dependent inactivation and 21 drugs exhibited in vitro induction of CYP3A4. Thirteen drugs namely; troleandomycin, ethinyl estradiol, fluoxetine, mibefradil, pioglitazone, rosiglitazone, troglitazone, saquinavir, nelfinavir, ritonavir, ethinyl estradiol, mibefradil and verapamil were positives in all three in vitro assays.

Application of this model to the data indicates that reasonably accurate predictions can be made with this approach (Figures 1A-1D, Tables 5 and 6). In this particular case, the greatest predictive accuracy and least bias was observed when total plasma precipitant concentrations were employed and the interaction at the level of the gut was excluded (Figure 1A). Under this set of conditions, the geometric mean fold error of the prediction was 1.8-fold with 66% of the predictions being within 2 fold (Table 5). Considering unbound precipitant concentrations (rather than total) yielded comparable accuracy with a GMFE of 1.9, and 66% of predictions within 2 fold of actual (Figure 1B and Table 5).

Assuming total precipitant concentrations and inclusion of the intestinal component (Figure 1C) provided the least accuracy (GMFE = 2.5, 46% within 2-fold) and a clear bias at the extremes of induction and inhibition. Assuming unbound precipitant concentrations (Figure 1D) and inclusion of the intestinal component reduced bias, but provided less accuracy than those approaches utilizing total precipitant concentrations (GMFE = 2.0, 64 % within 2-fold).

Of particular interest in the examination of this approach are the subsets of precipitants which are CYP3A inducers. For this subset analysis, total drug concentrations and a hepatic only effect was assumed and the relative predictive accuracy was assessed using in vitro data on competitive interaction only, TDI only or by including all data on competitive interaction, TDI and induction (Table 7). Overall, the least fold error was observed using in vitro data on all the

relevant mechanisms (GMFE = 3.2) for this subset of compounds. Importantly, drugs with a net induction effect in the clinic (e.g. avasimibe, carbamazepine, modafinil, nafcillin, phenytoin and rifampin) were predicted with reasonable accuracy via this approach. This was achieved while maintaining reasonable predictive accuracy for compounds with a net inhibition in the clinic (e.g. mibefradil, nelfinavir, ritonavir saquinavir, troleandomycin, and verapamil) and for which the clinical DDI is negligible (e.g. ethinyl estradiol, nifedipine, pioglitazone and rosiglitazone).

Nevertheless, it is important to note several important limitations of this work which represent areas for future improvement. Most of the limitations of this work relate to the mathematical model which is derived to address the extent of DDI under the equilibrium, steady-state condition. For example, this aspect of the mathematical model requires a singular value of precipitant concentrations in the intestine and liver for the prediction of DDI. Candidate measures of this exposure include maximum, minimum or average systemic blood concentrations at steady-state. Calculated concentrations in the portal venous blood and intestine are also candidates for this singular exposure value. Lastly, each of these candidate exposure measures can be entered into the mathematical model as total or unbound exposure. As such, it is common practice with such approaches to empirically select surrogate measures of exposure in the liver and intestine which provide the best correlation of predicted and observed DDIs reported in the literature. Similar exercises have also been employed herein to guide the incorporation or exclusion of the intestinal effect and the consideration of unbound fraction.

While practically useful, such empirical oversimplifications surely limit predictive accuracy within the dataset and may translate to lower accuracy when the method is applied outside the dataset for which it was developed. In addition, caution should be used not to over interpret such

empirical validation as mechanistic validation. For example, it would be inappropriate to conclude from this analysis that CYP3A4 interactions are fundamentally driven by total precipitant concentration or that the intestine is not a source of DDI's arising from alterations in CYP3A4 activity (only that, for whatever reason, the best correlation was observed when total steady-state average blood concentrations were employed and intestinal interaction ignored). Likewise, this empirical aspect makes it difficult to reasonably speculate as to the mechanistic source of observed prediction errors (e.g. troglitazone, pleconaril, bosentan). Another empirical aspect of this work which imposes similar limitations relates to the estimation of the induction scaling parameter d. Because the value of d is estimated through correlation, its absolute value is shown to be dependent upon the assumptions regarding affected tissue (i.e. intestine and/or liver) and exposure surrogate (total or unbound blood concentrations). Likewise, the value of this parameter is expected to depend upon the in vitro system employed. For example, the authors have noted similar predictive accuracy associated with the need for a much higher value of d when in vitro induction data from immortalized hepatocytes, rather than cryopreserved hepatocytes, are employed (data not shown).

All of these limitations indicate the need for more sophisticated approaches to the prediction of DDI's arising from interaction at the level of CYP3A4 metabolism. In this regard, approaches which link the time course of predicted precipitant and object liver and intestine exposure to the known CYP3A4 interaction mechanism will likely be required to address the aforementioned limitations. Ideally, such approaches should also provide the framework for mechanistic, physiologically-based scaling of in vitro CYP3A4 interaction data such that interindividual variability in the interaction can also be predicted. Such approaches would also be much more appropriate for drawing fundamental mechanistic inferences through the comparison of predicted and observed DDI's.

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Overall, this approach provides an accuracy comparable to previous methods designed to predict DDI arising from a singular CYP3A4 interaction mechanism (i.e. within 2-fold). The advantage of this approach is that it allows investigators to generate a DDI prediction which reflects the net influence of competitive inhibition, time-dependent inactivation and induction. Use of this model requires 6 kinetic constants which are readily estimatable from in vitro systems, namely: K_i and k_{inact} for inhibitor binding and inactivation, d, ψ and EC_{50} for P450 induction, and f_m for fraction of metabolism by the pathway being observed. As such, this method provides an attractive means to support early efforts to identify and mitigate DDI risk during drug design and development.

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Footnotes

[ξ]This work was presented in part at the 2005 & 2006 Institute for Scientific Exchange Conferences on Drug-drug interactions, and at the 2006 International Symposium on Microsomes and Drug Oxidations (MDO) meeting.

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Fig. Legends

Figure 1. Predicted versus observed AUC ratios and associated geometric fold error assuming total plasma concentration and hepatic interaction only (A), unbound plasma concentration and hepatic interaction only (B), total plasma concentration and interaction in the liver and intestine (C) and unbound plasma concentration and interaction in the liver and intestine (D). Solid black line represents unity. Dashed red lines represent 2 – fold boundary around the line of unity.

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Table 1: Literature compounds dosing regimen information

Precipitant	Precipitant Dose	Precipitant Interval	Object	Object Dose	AUC Ratios Change	Range of AUC Ratios Change
avasimibe	50 mg/day (7 d)	NA	midazolam	2 mg	0.27	
avasimibe	750 mg/day (7 d)	NA	midazolam	2 mg	0.06	
azithromycin (n=3)	500 mg (3 d)	q.d.	midazolam	15 mg	1.1	0.87-1.3
azithromycin	250 mg (2 d)	q.d.	triazolam	0.125 mg	1	
bosentan	125 mg (5.5 d)	b.i.d.	simvastatin	40 mg	0.66	
carbamazepine	300 mg (14 d)	b.i.d.	simvastatin	80 mg	0.26	
cimetidine (n-3)	400 mg (1.5 d)	b.i.d.	midazolam	15 mg	1.7	1.3-2.0
clarithromycin	250 mg (5 d)	b.i.d.	midazolam	15 mg	3.6	
clarithromycin (n=2)	500 mg (7 d)	b.i.d.	midazolam	8 mg	7	5.5-8.4
clarithromycin	500 mg (9 d)	b.i.d.	simvastatin	40 mg	10	
clarithromycin	500 mg (2 d)	b.i.d.	triazolam	0.125 mg	5.3	
cyclosporine	1.1-3.8 mg/kg/day	NA	simvastatin	20 mg	2.6	
cyclosporine	236 mg/day (7 M)	NA	simvastatin	5-10 mg	8	
diltiazem	60 mg (2 d)	t.i.d.	midazolam	15 mg	3.8	
diltiazem	60 mg (6 d)	t.i.d.	nifedipine	20 mg	3	
diltiazem	120 mg (2 W)	b.i.d.	simvastatin	20 mg	4.8	
diltiazem (n=2)	60 mg	t.i.d.	triazolam	0.25 mg	2.8	2.3-3.4
erythromycin	200 mg (7 d)	qid	midazolam	5 mg	3.3	
erythromycin (n=2)	500 mg (7 d)	t.i.d.	midazolam	15 mg	4.1	3.8-4.4
erythromycin	500 mg (2 d)	b.i.d.	triazolam	0.125 mg	3.8	
erythromycin	500 mg (2 d)	t.i.d.	simvastatin	40 mg	6.2	
erythromycin	333 mg (3 d)	t.i.d.	triazolam	0.5 mg	2.1	
Ethinyl estradiol	30 ug (10 d)	daily	midazolam	7.5 mg	1.2	
fluconazole	200 mg (5 d)	q.d.	midazolam	7.5 mg	3.6	
fluconazole (n=2)	400 mg	single dose	midazolam	3 mg	4.3	3.7-4.9
fluconazole	200 mg/day	NA	nifedipine	40 mg	2.7	
fluconazole (n=3)	100 mg (4 d)	q.d.	triazolam	0.25 mg	3.2	2.1-4.4
fluoxetine	60 mg (12 d)	q.d.	midazolam	10 mg	0.87	
fluoxetine	60 mg (8 d)	q.d.	triazolam	0.25 mg	1	
itraconazole (n=3)	200 mg (4 d)	q.d.	midazolam	7.5 mg	7.9	6.2-11
itraconazole	200 mg (4 d)	q.d.	simvastatin	40 mg	10	
itraconazole (n=4)	200 mg	single dose	triazolam	0.25 mg	3.9	3.1-4.5
ketoconazole	200 mg (1.5 d)	b.i.d.	midazolam	6 mg	13.6	13.6
ketoconazole (n=3)	200 mg	single dose	midazolam	2 mg	6.8	5.2-8.7
ketoconazole (n=2)	400 mg	q.d.	midazolam	5.5 mg	12.6	9.5-16
ketoconazole (n=3)	200 mg	b.i.d.	triazolam	0.125 mg	15.1	9.2-22
mibefradil	100 mg	single dose	midazolam	2 mg	8.9	
mibefradil	50 mg (3 d)	q.d.	triazolam	0.25 mg	8.4	

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Table 1 (Continued): Literature compounds dosing regimen information

Precipitant	Precipitant Dose	Precipitant Interval	Object	Object Dose	AUC Ratios Change	Range of AUC Ratios Change
modafinil	200-400 mg (28 d)	q.d.	triazolam	0.125 mg	0.42	
nafcillin	500 mg (5 d)	q.i.d.	nifedipine	10 mg	0.37	
nelfinavir	1250 mg (14 d)	b.i.d.	simvastatin	20 mg	6.1	
nifedipine	60 mg	single dose	cerivastatin	0.3 mg	1	
phenobarbital	100 mg/day (8 d)	q.d.	nifedipine	20 mg	0.39	
phenytoin	150-300 mg	b.i.d.	midazolam	15 mg	0.06	
pioglitazone	45 mg (24 d)	q.d.	simvastatin	80 mg	0.98	
pleconaril	400 mg (6 d)	t.i.d.	midazolam	5 mg	0.65	
rifampin (n=2)	600 mg (5 d)	q.d.	midazolam	15 mg	0.03	0.02-0.04
rifampin	600 mg (7 d)	q.d.	nifedipine	20 mg	0.08	
rifampin (n=2)	600 mg (9 d)	q.d.	simvastatin	40 mg	0.12	0.09-0.14
rifampin	600 mg (5 d)	q.d.	triazolam	0.5 mg	0.05	
ritonavir (n=3)	200 mg	b.i.d.	triazolam	0.125 mg	27	20-41
rosiglitazone	8 mg (14 d)	q.d.	nifedipine	20 mg	0.88	
roxithromycin	300 mg (6 d)	q.d.	midazolam	15 mg	1.5	
saquinavir	1200 mg (5 d)	t.i.d.	midazolam	7.5 mg	5.2	
troglitazone	400 mg (24 d)	q.d.	simvastatin	40 mg	0.62	
troleandomycin	1 g (7.5 d)	b.i.d.	triazolam	0.25 mg	3.8	
verapamil	80 mg (2 d)	t.i.d.	midazolam	15 mg	2.9	
verapamil	80 mg (2 d)	t.i.d.	simvastatin	40 mg	4.7	

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Table 2: K_i values obtained from reversible inhibition experiment and DDI predicted AUC Ratio. (Inhibition Term used from Equation 9). The values used for [I] in vivo were estimated total systemic plasma concentration. GFE is the geometric fold error in predicting DDI (predicted/observed DDI)

Precipitant	DDI (Observed)	K_i (μM)	Predicted AUC'_{PO}/AUC_{PO} (Reversible Inhibition)	**GFE**
Avasimibe	0.27	0.80	1.6	**5.8**
Avasimibe	0.06	0.80	2.5	**42**
Bosentan	0.66	10	1.2	1.9
Cyclosporine	8.0	8.9	1.2	**6.6**
Cyclosporine	2.6	8.9	1.1	**2.4**
Erythromycin	3.3	13	1.0	**3.2**
Erythromycin	4.1	13	1.1	**3.8**
Erythromycin	3.8	13	1.1	**3.6**
Erythromycin	6.2	13	1.1	**5.8**
Erythromycin	2.1	13	1.2	1.7
Fluconazole	3.6	6.8	1.9	1.9
Fluconazole	4.3	6.8	4.1	1.1
Fluconazole	2.7	6.8	3.5	1.3
Fluconazole	3.2	6.8	1.9	1.7
Fluoxetine	0.87	5.3	1.1	1.2
Fluoxetine	1.0	5.3	1.1	1.1
Itraconazole	7.9	0.01	9.7	1.2
Itraconazole	10	0.01	9.7	1.0
Itraconazole	3.9	0.01	9.7	**2.5**
ketoconazole	14	0.012	14	1.0
ketoconazole	6.8	0.012	13	1.9
ketoconazole	13	0.012	14	1.1
ketoconazole	15	0.012	14	1.1
Mibefradil	8.9	2.3	1.5	**6.0**
Mibefradil	8.4	2.3	1.5	**5.6**
Nelfinavir	6.1	4.0	2.5	**2.5**
Pioglitazone	1.0	10	1.2	1.3
Raloxifene	1.0	9.9	1.0	1.0
Ritonavir	27	0.04	14	**2.0**
Rosiglitazone	0.88	12	1.0	1.2
Saquinavir	5.2	0.35	2.1	**2.5**
Troglitazone	0.62	5.0	1.8	**3.0**
Troleandomycin	3.8	1.3	1.1	**3.4**
Geometric mean fold error				**3.7**

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Table 3: Summary of induction data (Induction Term from Equation 9) for the compounds tested and the DDI predicted AUC ratios. The values used for [I] in vivo were estimated total systemic plasma concentration. GFE is the geometric fold error in predicting DDI

Precipitant	DDI (Observed)	EC_{50} (μM)	E_{max}	Predicted AUC'_{PO}/AUC_{PO} (induction)	**GFE**
Avasimibe	0.27	3.3	14.9	0.40	1.5
Avasimibe	0.06	3.3	14.9	0.22	**3.6**
Bosentan	0.66	3.4	38.8	0.07	**9.3**
Carbamazepine	0.26	56	34.3	0.09	**2.9**
Ethinyl estradiol	1.2	20	69	0.97	1.2
Fluoxetine	0.87	0.5	2.1	0.60	1.4
Fluoxetine	1.0	0.5	2.1	0.60	1.7
Mibefradil	8.9	4.1	6.5	0.46	**19**
Mibefradil	8.4	4.1	6.5	0.46	**18**
Modafinil	0.42	26	8.9	0.18	**2.3**
Nafcillin	0.37	9.0	5.1	0.23	1.6
Nelfinavir	6.1	0.75	23.2	0.06	**106**
Nifedipine	1.0	18	34.3	0.70	1.5
Phenobarbital	0.39	159	49.1	0.10	**4.1**
Phenytoin	0.06	24	15.8	0.10	1.6
Pioglitazone	0.98	2.0	7.0	0.24	**4.1**
Pleconaril	0.65	16.5	51	0.17	**3.9**
Rifampin	0.03	1.9	14.9	0.09	**3.2**
Rifampin	0.08	1.9	14.9	0.09	1.1
Rifampin	0.12	1.9	14.9	0.09	1.3
Rifampin	0.05	1.9	14.9	0.09	1.8
Ritonavir	27	1.0	68.5	0.02	**1378**
Rosiglitazone	0.88	14	23.9	0.69	1.3
Saquinavir	5.2	0.87	34.6	0.10	**52**
Troglitazone	0.62	2.0	9.8	0.16	**4.0**
Troleandomycin	3.8	0.3	15.9	0.19	**20**
Verapamil	2.9	0.16	16.4	0.12	**24**
Verapamil	4.7	0.16	16.4	0.12	**40**
Geometric mean fold error					**61**

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Table 4: Summary of the CYP3A4 inactivation data and the DDI predicted values of AUC ratio based on time-dependent Inactivation data only (TDI Term in Equation 9) vs observed values. The values used for [I] in vivo were estimated total systemic plasma concentration

Precipitant	DDI (Observed)	k_i (μM)	k_{inact} (min^{-1})	Predicted AUC'_{PO}/AUC_{PO} (TDI)	**GFE**
Azithromycin	1.1	19	0.005	1.2	1.1
Azithromycin	1.0	19	0.005	1.2	1.2
Clarithromycin	3.6	14	0.028	7.3	**2.0**
Clarithromycin	7.0	14	0.028	7.9	1.1
Clarithromycin	5.3	14	0.028	7.0	1.3
Clarithromycin	10	14	0.028	9.1	1.1
Cyclosporine	8.0	8.9	0.025	7.6	1.1
Cyclosporine	2.6	8.9	0.025	4.3	1.7
Diltiazem	3.8	3.3	0.070	6.1	1.6
Diltiazem	3.0	3.3	0.070	6.1	**2.0**
Diltiazem	4.8	3.3	0.070	8.1	1.7
Diltiazem	2.8	3.3	0.070	6.1	**2.2**
Erythromycin	3.3	13	0.025	3.0	1.1
Erythromycin	4.1	13	0.025	4.5	1.1
Erythromycin	3.8	13	0.025	4.5	1.2
Erythromycin	6.2	13	0.025	4.5	1.4
Erythromycin	2.1	13	0.025	7.9	**3.7**
Ethinyl estradiol	1.2	18	0.040	1.1	1.1
Fluoxetine	0.9	5.3	0.017	3.5	**4.1**
Fluoxetine	1.0	5.3	0.017	3.5	**3.5**
Mibefradil	8.9	2.3	0.400	14	1.6
Mibefradil	8.4	2.3	0.400	14	1.7
Nelfinavir	6.1	4.0	0.260	14	**2.3**
Pioglitazone	1.0	10	0.011	5.5	**5.6**
Raloxifene	1.0	9.9	0.160	1.0	1.0
Ritonavir	27	0.04	0.330	14	1.9
Rosiglitazone	0.88	12	0.020	2.4	**2.7**
Roxithromycin	1.5	72	0.023	4.3	**2.9**
Saquinavir	5.2	0.35	0.170	14	**2.6**
Troglitazone	0.62	5.0	0.335	14	**22**
Troleandomycin	3.8	1.3	0.032	6.4	1.7
Verapamil	2.9	2.9	0.150	10	**3.5**
Verapamil	4.7	2.9	0.150	10	**2.2**
Geometric mean fold error					**2.7**

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Table 5: Predicted net DDI vs. that observed and associated geometric fold error assuming total plasma concentrations and interaction in the liver only (Equation 9) or interaction in the liver plus intestine (Equation 16), using all in vitro data (reversible inhibition, time-dependent inactivation and induction)

Precipitant	Range of DDI (Observed)	$[I]_H$ (μM)	AUC' / AUC (Predicted) ($[I]_H$)	GFE	$[I]_G$ (μM)	AUC' / AUC (Predicted) ($[I]_H$+$[I]_G$)	GFE
Avasimibe	0.27	0.50	0.63	**2.3**	12.1	1.4	**5.4**
Avasimibe	0.06	1.5	0.60	**10**	181	2.0	**33**
Azithromycin	0.87-1.3	0.27	1.2	1.1	80.8	2.1	1.9
Azithromycin	1.0	0.27	1.2	1.2	40.4	1.6	1.6
Bosentan	0.66	2.6	0.09	**7.4**	25.3	0.12	**5.5**
Carbamazepine	0.26	34	0.09	**2.8**	154	0.07	**3.9**
Cimetidine	1-2.2	3.3	1.1	1.4	144	1.7	1.1
Clarithromycin	3.6	2.4	7.8	**2.2**	40.4	14	**3.8**
Clarithromycin	5.5-8.4	3.0	8.6	1.2	80.9	15	**2.1**
Clarithromycin	5.3	2.2	7.5	1.4	80.9	10	1.9
Clarithromycin	10	4.8	10	1.0	80.9	15	1.5
Cyclosporine	8.0	1.9	8.2	1.0	26.8	12	1.6
Cyclosporine	2.6	0.58	4.5	1.8	23.8	6.8	**2.7**
Diltiazem	3.8	0.14	6.2	1.6	17.5	11	**2.9**
Diltiazem	3.0	0.14	6.2	**2.1**	17.5	8.0	**2.7**
Diltiazem	4.8	0.27	8.4	1.7	35.0	13	**2.6**
Diltiazem	2.3-3.4	0.14	6.2	**2.2**	17.5	8.3	**3.0**
Erythromycin	3.3	0.44	3.0	1.1	33.0	5.3	1.6
Erythromycin	3.8-4.4	0.95	4.7	1.2	82.4	8.3	**2.0**
Erythromycin	3.8	0.95	4.7	1.2	82.4	6.3	1.7
Erythromycin	6.2	0.95	4.7	1.3	82.4	7.2	1.2
Erythromycin	2.1	3.3	8.6	**4.1**	54.9	11	**5.5**
Ethinyl estradiol	1.1-1.2	0.01	1.0	1.1	20.4	1.7	1.5
Fluconazole	3.6	7.2	1.9	1.9	59.3	3.1	1.2
Fluconazole	3.6-4.9	29	4.1	1.1	158	7	1.6
Fluconazole	2.7	22	3.5	1.3	79.1	4.3	1.6
Fluconazole	2.1-4.4	7.2	1.9	1.7	39.5	2.4	1.3
Fluoxetine	0.87	0.36	2.5	**2.8**	21.0	5.4	**6.2**
Fluoxetine	1.0	0.36	2.5	**2.5**	21.0	4.1	**4.1**
Itraconazole	6.2-10.8	0.27	9.7	1.2	34.3	17	**2.2**
Itraconazole	10	0.27	9.7	1.0	34.3	15	1.5
Itraconazole	3.1-4.5	0.27	9.7	**2.5**	34.3	13	**3.3**
ketoconazole	14	5.4	14	1.0	45.6	24	1.8
ketoconazole	5.2-8.7	1.9	13	1.9	45.6	23	**3.4**
ketoconazole	9.5-15.9	2.8	14	1.1	91.1	20	1.6
ketoconazole	9.2-22.4	5.4	14	1.1	45.6	19	1.2
Mibefradil	8.9	1.2	14	1.5	24.4	24	**2.7**
Mibefradil	8.4	1.2	14	1.6	12.2	19	**2.2**

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Table 5 (Continued): Predicted net DDI vs. that observed and associated geometric fold error assuming total plasma concentrations and interaction in the liver only (Equation 9) or interaction in the liver plus intestine (Equation 16), using all in vitro data (reversible inhibition, time-dependent inactivation and induction)

Precipitant	Range of DDI (Observed)	$[I]_H$ (μM)	AUC' / AUC (Predicted) ($[I]_H$)	GFE	$[I]_G$ (μM)	AUC' / AUC (Predicted) ($[I]_H$+$[I]_G$)	GFE
Modafinil	0.42	49	0.19	**2.2**	133	0.26	1.6
Nafcillin	0.37	44	0.24	1.5	133	0.37	1.0
Nelfinavir	6.1	7.1	12	**2.0**	246	20	**3.3**
Nifedipine	1.0	0.29	0.71	1.5	21.0	0.42	**2.5**
Phenobarbital	0.39	52	0.10	**3.9**	52.1	0.13	**3.0**
Phenytoin	0.06	70	0.11	1.8	71.9	0.10	1.6
Pioglitazone	0.98	2.8	2.2	**2.2**	13.9	5.4	**5.5**
Pleconaril	0.65	2.4	0.17	**3.9**	122	0.05	**12**
Raloxifene	1.0	0.001	1.0	1.0	16.1	1.8	1.8
Rifampin	0.016-0.041	12	0.09	**3.3**	88.2	0.08	**2.8**
Rifampin	0.08	12	0.09	1.1	88.2	0.11	1.4
Rifampin	0.09-0.14	12	0.09	1.3	88.2	0.09	1.3
Rifampin	0.05	12	0.09	1.8	88.2	0.11	**2.1**
Ritonavir	20.4 to 40.7	12	14	1.9	33.6	19	1.4
Rosiglitazone	0.88	0.34	1.8	2.0	2.04	2.6	**2.9**
Roxithromycin	1.5	5.1	4.5	**3.1**	43.4	8	**5.3**
Saquinavir	5.2	0.43	12	**2.3**	216	23	**4.4**
Troglitazone	0.62	4.8	13	**21**	110	21	**34**
Troleandomycin	3.8	0.15	2.0	1.9	149	4.7	1.3
Verapamil	2.9	0.22	3.3	1.2	21.3	10	**3.5**
Verapamil	4.7	0.22	3.3	1.4	21.3	9	1.9
Geometric mean fold error				**1.8**			**2.5**

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Table 6: Predicted net effect vs. observed AUC ratios and associated geometric fold error assuming unbound plasma concentrations and interaction in the liver only (Equation 9), and interaction in the liver plus intestine (Equation 16)

Precipitant	Range of DDI (Observed)	$[I]_{H,u}$ (μM)	AUC' / AUC (Predicted) $[I]_{H,u}$	GFE	AUC' / AUC (Predicted) $[I]_{H,u}+[I]_G$	GFE
Avasimibe	0.27	0.001	1.00	**3.7**	1.4	**5.2**
Avasimibe	0.06	0.003	0.99	**17**	1.7	**29**
Azithromycin	0.87-1.3	0.193	1.2	1.1	2.0	1.8
Azithromycin	1.0	0.193	1.2	1.2	1.5	1.5
Bosentan	0.66	0.091	0.51	1.3	0.38	1.7
Carbamazepine	0.26	8.50	0.19	1.4	0.1	**2.4**
Cimetidine	1-2.2	2.58	1.1	1.5	1.7	1.1
Clarithromycin	3.6	1.30	5.8	1.6	10	**2.8**
Clarithromycin	5.5-8.4	1.62	6.5	1.1	11	1.6
Clarithromycin	5.3	1.19	5.6	1.1	7.4	1.4
Clarithromycin	10	2.59	8.1	1.2	12	1.2
Cyclosporine	8.0	0.193	2.4	**3.3**	3.7	**2.2**
Cyclosporine	2.6	0.058	1.5	1.7	2.2	1.2
Diltiazem	3.8	0.035	2.9	1.3	5.0	1.3
Diltiazem	3.0	0.035	2.9	1.0	3.7	1.2
Diltiazem	4.8	0.068	4.2	1.2	6.3	1.3
Diltiazem	2.3-3.4	0.035	2.9	1.0	3.8	1.4
Erythromycin	3.3	0.101	1.5	**2.2**	2.7	1.2
Erythromycin	3.8-4.4	0.219	2.1	2.0	3.7	1.1
Erythromycin	3.8	0.219	2.1	1.8	2.8	1.4
Erythromycin	6.2	0.219	2.1	**3.0**	3.2	2.0
Erythromycin	2.1	0.754	4.2	2.0	5.5	**2.6**
Ethinyl estradiol	1.1-1.2	0.010	1.0	1.1	1.7	1.4
Fluconazole	3.6	6.41	1.8	2.0	3.0	1.2
Fluconazole	3.6-4.9	26.2	3.8	1.1	6.5	1.5
Fluconazole	2.7	19.6	3.2	1.2	4.0	1.5
Fluconazole	2.1-4.4	6.41	1.8	1.8	2.3	1.4
Fluoxetine	0.87	0.029	1.2	1.3	2.1	**2.4**
Fluoxetine	1.0	0.029	1.2	1.2	1.6	1.6
Itraconazole	6.2-10.8	0.004	1.4	**5.7**	2.4	**3.2**
Itraconazole	10	0.004	1.4	**7.2**	2.1	**4.7**
Itraconazole	3.1-4.5	0.004	1.4	**2.8**	1.9	**2.1**
ketoconazole	14	0.054	4.2	**3.2**	7.4	1.8
ketoconazole	5.2-8.7	0.019	2.3	**2.9**	4.0	1.7
ketoconazole	9.5-15.9	0.028	2.9	**4.4**	4.3	**2.9**
ketoconazole	9.2-22.4	0.054	4.2	**3.6**	5.6	**2.7**
Mibefradil	8.9	0.041	8.9	1.0	16	1.8
Mibefradil	8.4	0.041	8.9	1.1	12	1.4

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Table 6 (Continued): Predicted net effect vs. observed AUC ratios and associated geometric fold error assuming unbound plasma concentrations and interaction in the liver only (Equation 9), and interaction in the liver plus intestine (Equation 16)

Precipitant	Range of DDI (Observed)	$[I]_{H,u}$ (μM)	AUC' / AUC (Predicted) $[I]_{H,u}$	GFE	AUC' / AUC (Predicted) $[I]_{H,u}+[I]_G$	GFE
Modafinil	0.42	19.6	0.22	1.9	0.28	1.5
Nafcillin	0.37	4.84	0.37	1.0	0.48	1.3
Nelfinavir	6.1	0.106	4.2	1.4	10	1.6
Nifedipine	1.0	0.015	0.97	1.1	0.44	**2.4**
Phenobarbital	0.39	26.0	0.13	**2.9**	0.16	**2.4**
Phenytoin	0.06	5.25	0.27	**4.5**	0.19	**3.2**
Pioglitazone	0.98	0.028	1.00	1.0	1.5	1.6
Pleconaril	0.65	0.024	0.93	1.4	0.13	**4.8**
Raloxifene	1.0	0.00005	1.0	1.0	1.8	1.8
Rifampin	0.016-0.041	2.38	0.11	**4.0**	0.09	**3.0**
Rifampin	0.08	2.38	0.11	1.4	0.13	1.6
Rifampin	0.09-0.14	2.38	0.11	1.0	0.10	1.2
Rifampin	0.05	2.38	0.11	**2.2**	0.12	**2.4**
Ritonavir	20.4 to 40.7	0.176	14	2.0	19	1.5
Rosiglitazone	0.88	0.001	1.0	1.1	1.2	1.4
Roxithromycin	1.5	0.718	1.6	1.1	2.8	1.9
Saquinavir	5.2	0.009	6.3	1.2	12	**2.3**
Troglitazone	0.62	0.041	5.3	**8.6**	8.7	**14**
Troleandomycin	3.8	0.006	1.1	**3.5**	1.7	**2.3**
Verapamil	2.9	0.013	1.3	**2.2**	3.5	1.2
Verapamil	4.7	0.013	1.3	**3.5**	3.0	1.6
Geometric mean fold error				1.9		2.0

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Table 7: Predicted net DDI vs. observed and associated geometric fold error assuming total plasma concentrations and interaction in the liver only (Equation 9). Predictions were made assuming an exclusively competitive mechanism, a TDI mechanism and a mixed mechanism

Precipitant	DDI (Observed)	DDI predicted (Competitive Mechanism)	GFE	DDI predicted (TDI mechanism)	**GFE**	DDI predicted (mixed mechanism)	GFE
Avasimibe	0.27	1.6	**5.8**	1.0	**3.7**	0.63	**2.3**
Avasimibe	0.06	2.5	**42**	1.0	**17**	0.60	**10**
Bosentan	0.7	1.2	1.9	1.0	1.5	0.09	**7.4**
Carbamazepine	0.26	1.0	**4.0**	1.0	**3.8**	0.09	**2.8**
Ethinyl estradiol	1.2	1.0	1.1	1.1	1.1	1.0	1.1
Fluoxetine	0.87	1.1	1.2	3.5	**4.1**	2.5	**2.8**
Fluoxetine	1.0	1.1	1.1	3.5	**3.5**	2.5	**2.5**
Mibefradil	8.9	1.5	**6.0**	14	1.6	14	1.5
Mibefradil	8.4	1.5	**5.6**	14	1.7	14	1.6
Modafinil	0.42	1.0	**2.5**	1.0	**2.4**	0.19	**2.2**
Nafcillin	0.4	1.0	**2.8**	1.0	**2.7**	0.24	1.5
Nelfinavir	6.1	2.5	**2.5**	14	**2.3**	12	**2.0**
Nifedipine	1.0	1.0	1.0	1.0	1.0	0.71	1.5
Phenobarbital	0.39	1.0	**2.7**	1.0	**2.6**	0.10	**3.9**
Phenytoin	0.06	1.1	**18**	1.0	**17**	0.11	1.8
Pioglitazone	0.98	1.2	1.3	5.5	**5.6**	2.2	**2.2**
Pleconaril	0.7	1.0	1.5	1.0	1.5	0.17	**3.9**
Rifampin	0.03	1.0	**36**	1.0	**36**	0.09	**3.3**
Rifampin	0.08	1.0	**12**	1.0	**12**	0.09	1.1
Rifampin	0.12	1.0	**8.8**	1.0	**8.7**	0.09	1.3
Rifampin	0.05	1.0	**20**	1.0	**20**	0.09	1.8
Ritonavir	27	13.7	**2.0**	14	1.9	14	1.9
Rosiglitazone	0.88	1.0	1.2	2.4	**2.7**	1.8	2.0
Saquinavir	5.2	2.1	**2.5**	14	**2.6**	12	**2.3**
Troglitazone	0.62	1.8	**3.0**	14	**22**	13	**21**
Troleandomycin	3.8	1.1	**3.4**	6.4	1.7	2.0	1.9
Verapamil	2.9	1.1	**2.7**	10	**3.5**	3.3	1.2
Verapamil	4.7	1.1	**4.4**	10	**2.2**	3.3	1.4
Geometric Mean Fold Error			**7.1**		**6.7**		**3.2**

Figure 1: