Evaluation of the use of static and dynamic models to predict drug-drug interaction and its associated variability: Impact on drug discovery and early development

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Abbreviations

**AUC**  
Area under the concentration-time curve

**AUCR_{hp}**  
AUC ratio from hepatic first pass

**AUC_{i}**  
Area under the curve in the presence of an inhibitor

**CL_{substrate}**  
Clearance of the substrate compound in the absence of the inhibitor

**CL_{int}**  
Intrinsic metabolic clearance

**CL_{po}**  
Oral clearance (CL/F)

**CL_{i,substrate}**  
Clearance of the substrate when co-administered with an inhibitor

**DDI**  
Drug-drug interaction

**Ind_{max}**  
Fold increase in response (induction) over vehicle

**IndC_{50}**  
Inducer concentration at 50% Ind_{max}.

**E_{g}**  
Fraction of compound extracted by enzymes in the gut wall

**f_{mg}**  
Fraction of gut metabolism mediated by an induced enzyme

**f_{abs}**  
Fraction absorbed of an orally administered drug

**f_{up}**  
Fraction of drug unbound in plasma

**f_{u,emic}**  
Fraction of drug unbound in microsomal incubation

**f_{gut}**  
Fraction of absorbed drug escaping gut metabolism

**f_{gut}^{*}**  
Fraction of absorbed drug escaping gut metabolism in the presence of an inhibitor

**f_{gut}^{*}**  
Fraction of victim drug unbound in the gut

**f_{m}**  
Fraction metabolized in the liver

**f_{m,CYP3A4}**  
Fraction metabolized by CYP3A4

**f_{v}**  
Fraction of orally administered victim drug escaping hepatic first pass

**f_{v}^{*}**  
Fraction of orally administered victim drug escaping hepatic first pass in the presence of the inhibitor

**f_{m}^{*}**  
Fraction of orally administered victim drug escaping hepatic first pass

**F**  
Oral bioavailability

**I_{sys}**  
Average steady-state systemic inhibitor concentration

**I_{max}**  
Maximum steady-state systemic inhibitor concentration
DMD #44602

\( I_{inlet} \)  Inhibitor concentrations from hepatic artery and hepatic portal vein
\( k \)  Elimination rate constant
\( k_a \)  First order absorption rate constant
\( k_{deg} \)  First order enzyme degradation rate constant
\( K_i \)  Reversible inhibition constant
\( k_{inact} \)  Maximal enzyme inactivation rate constant measured for a time-dependent inhibitor
\( K_I \)  Unbound inhibitor concentration at 50% \( k_{inact} \)
\( log P \)  Logarithm of octanol water partition coefficient
\( MW \)  Molecular weight
\( pKa \)  Negative logarithm of acid dissociation constant
\( P_{app} \)  Measured apparent permeability from Caco-2 or MDCK cell lines
\( Q_{villi} \)  Blood flow rate to villi in intestine
\( Q_{pv} \)  Blood flow rate in hepatic portal vein
\( Q_{LI} \)  Total blood flow rate to liver
\( R \)  Blood to plasma concentration ratio
\( R_{ss} \)  \( AUC \) ratio in static model under steady-state conditions within Simcyp
\( V_{ss} \)  Steady-state volume of distribution
Abstract

Simcyp®, a population-based simulator is widely used for evaluating DDI risks in healthy and disease populations. We compare the prediction performance of Simcyp with mechanistic static models using different types of inhibitor concentrations, with an aim to understanding their strengths/weaknesses and recommending an optimal use of tools in drug discovery/early development. The inclusion of an additional term to static equations to consider the contribution of hepatic first pass to DDI ($AUC_{Rhfp}$) has also been examined. A second objective was to assess Simcyp’s estimation of variability associated with DDI. The dataset used for the analysis comprises 19 clinical interactions from 11 proprietary compounds. Except gut interaction parameters, all other input data were identical for Simcyp and static models. Static equations using unbound average steady-state systemic inhibitor concentration ($I_{sys}$), a fixed fraction of gut extraction and neglecting gut extraction in the case of induction interactions, performed better than Simcyp (84% compared to 58% of the interactions predicted within 2-fold). Differences in the prediction outcomes between the static and dynamic models are attributable to differences in first-pass contribution to DDI. The inclusion of $AUC_{Rhfp}$ in static equations leads to systematic overprediction of interaction, suggesting a limited role for hepatic first pass in determining inhibition-based DDI for our dataset. Our analysis supports the use of static models when elimination routes of victim compound and role of gut extraction for the victim and/or inhibitor in human are not well-defined. A fixed variability of 40% of predicted mean $AUC$ ratio is recommended.
Introduction

Drug-drug interactions (DDIs) impact the exposure of a substrate (victim) drug via inhibition/induction of its metabolic pathways by a co-administered inhibitor/inducer (perpetrator) drug. Regulatory guidelines (FDA, 2012, EMA, 2010) recommend that initial DDI risk assessments are done using in vitro data, as they have been shown to predict interaction within 2-fold of that observed for reversible CYP inhibition- (Brown et al, 2005; Ito et al, 2005; Obach et al, 2006), mechanism-based inhibition- (Kanamitsu et al., 2000a; Mayhew et al, 2000; Yamano et al., 2001) and induction- (Ripp et al., 2006; Shou et al., 2008) based DDI. Improved DDI predictions are possible by incorporating in vitro data into mechanistic static or physiologically-based models.

Estimation of AUC ratios has traditionally been done with static equations. The prediction accuracy of these models relies on employing an appropriate surrogate for the inhibitor concentration at the active site of the enzyme. Although the use of unbound inhibitor concentration is considered to be most relevant (Einolf, 2007), FDA recommends total inhibitor concentration in order to avoid false negatives. Average steady-state systemic concentration ($I_{sys}$), maximum steady-state systemic concentration ($I_{max}$) and hepatic inlet concentration ($I_{inlet}$) have all been evaluated for use (Ito et al, 2004) in static models. For an orally administered inhibitor, $I_{inlet}$ is an appropriate measure of drug exposure to enzyme during the absorption phase of the inhibitor in the absence of transporter involvement. However, as the absorption phase is short compared to the dosing interval for a once daily drug, the use of $I_{inlet}$ may lead to over-estimation of DDI risk, especially for a short half-life inhibitor. The use of $I_{sys}$ on the other hand could underestimate DDI risk (Ito et al, 2004) as it neglects the higher-than-systemic inhibitor concentration associated with hepatic first pass, especially for high clearance substrates.

In recent years, Simcyp® (Simcyp Limited, Sheffield UK, www.simcyp.com), a dynamic, population-based model is used to predict DDI risk over the entire PK profile of a substrate with dynamically varying inhibitor concentrations. Simcyp enables inclusion of inhibitory metabolites, simultaneous mechanisms of interaction (e.g., inhibition and induction), dose staggering,
multiple inhibitors and inhibition of multiple enzymes. By incorporating sources of variability (enzyme/transporter polymorphism, demography and differences in ethnicities/disease states) Simcyp aids the design of DDI studies and helps identify individuals who are at extreme risk from DDI (Cubitt et al, 2011) in any population. However, confidence in DDI and variability estimates can be confounded by the large uncertainties associated with the input data in early stages (Fig. 1). Prediction performance of static and dynamic approaches depends on the quality of in vitro data and on the certainty in understanding elimination/metabolic routes and gut extraction for a substrate. Failure to understand the role of transporters or to identify the existence of inhibitory metabolites, concurrent inhibition/induction, compensatory elimination mechanisms or active metabolites that are potential victims of DDI can confound predictions. In the pre-clinical stage, uncertainties in the predicted clearance of victim/inhibitor and/or predicted inhibitor dose can further limit prediction accuracy. In addition, non-CYP, renal and biliary routes identified for the victim in preclinical species may be irrelevant to man.

A comparison of dynamic and static models covering a wide range of inhibitors, substrates and enzymes (Einolf, 2007) was disadvantaged by the use of inconsistent input parameters in the two models. A combined static model was compared with Simcyp for 30 DDI involving midazolam and various CYP3A4 inhibitors (Fahmi et al, 2009). Wang compared the models for 54 interactions perpetrated by mechanism-based inhibitors of CYP3A4 (Wang, 2010). Guest et al used 35 DDIs to compare Simcyp ® V8’s time-based model with its implementation of static models (Guest et al, 2011). All evaluations showed a comparable performance of the two models. Our objective is to employ a diverse proprietary dataset of 19 DDIs to compare the mechanistic static equations with Simcyp ® V11, ensuring consistent pre-clinical input parameters in the models to understand the reasons for any differences in performance. In order to avoid the impact that the quality of PK predictions can have on the performance of the tools, clinically-observed clearance and distribution parameters have been used for all the 11 AZ compounds. We have not fit the dynamic model to observed, as we position our evaluation at the end of the preclinical phase. We also examine the use of an additional term in static equations to consider the contribution from hepatic first pass to DDI. Together with an assessment of Simcyp’s estimates of variability, we suggest an optimal use of DDI prediction
tools in drug discovery/early development.
Methods

Compounds used in the study

The 11 proprietary compounds chosen for this retrospective analysis were either victims or perpetrators of reversible or time-dependent inhibition or induction of CYP3A4 or inhibition of CYP2D6. Clinical interaction studies that involved the inhibition of drug transporters were not included. The selected compounds are from 7 disease areas and their chemical space encompasses a medium to high molecular weight (350 to 592), log P ranging from about 1 to 5.6 and the polar surface area from about 40 to 140 Å².

Clinical pharmacokinetic and drug interaction data

Intravenous (iv) PK data were obtained from Phase I studies. Clinical PK parameters obtained from study reports were calculated using compartmental or non-compartmental analysis. Metabolic clearance was estimated by subtracting any measured renal clearance from total clearance. Biliary clearance was not reported for any of the compounds studied. When iv data was not available, total clearance was obtained by taking the product of per oral clearance and bioavailability (based on the best estimated internal data). Fraction of compound metabolized \((f_m)\) was obtained by subtracting from 1 the fraction of dose excreted as parent drug in urine. Clinical drug interaction data was collected from in-house clinical DDI trials. The study designs for the 19 interactions from 11 compounds are reported in Table 1. With the exception of AZ10 in the AZ10-carbamazepine interaction, the substrate (victim) dose was typically a single oral dose administered after several days of oral administration of the inhibitor (perpetrator) to ensure attainment of steady-state. The duration of inhibitor administration was long enough to cover the elimination of the substrate drug. When the perpetrator was an inducer, it was administered for a sufficient duration to ensure both steady-state and maximum enzyme induction. Drug analyses were carried out with validated LC-MS/MS. AZ10-carbamazepine interaction study was done in psychiatric patients who were otherwise healthy. All other clinical studies were done in healthy volunteers. Studies have been carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all individuals participating in the studies, prior to initiation of the studies.
Determination of in vitro interaction parameters

In-house methods for the in vitro evaluation of cytochrome P450 reaction phenotyping, competitive and time-dependent inhibition as well as induction have been described (Zhou, et al., 2011). The panel of recombinant CYPs in CYP reaction phenotyping included the isoforms 1A2, 2C9, 2C19, 2D6 and 3A4ddid but not CYP3A5 isoform. Midazolam is used as a probe substrate for CYP3A4 inhibition and induction assays. Positive controls were used for demonstrating the validity and reproducibility of the methods. For CYP3A4-mediated competitive and time-dependent inhibition and induction these are ketoconazole, verapamil and rifampicin respectively. Inhibition constant $K_i$ were obtained from measured $IC_{50}$ values using the Cheng-Prusoff equation (Cheng and Prusoff, 1973), assuming competitive inhibition. Induction parameters were normalized with rifampicin data generated in-house. Plasma drug concentrations were analyzed using LC-MS/MS. In vitro parameters that were used as input in Simcyp and static models for substrates and inhibitors are listed in Tables 2 and 3 respectively. For AZ4, the measured $K_i$ is >35 µM. In this case, estimation of DDI was carried out using 35 µM.

DDI predictions with Simcyp

Simcyp Population-based ADME Simulator V11 was used in DDI evaluations. As previously described, (Jamei et al., 2009; Tucker et al., 2001; Yang et al, 2005) competitive inhibition, induction, and mechanism-based inactivation can be investigated using this software. Reference substrates and inhibitors used in the clinical studies were all available in the Simcyp compound library. No changes were carried out on the PK models or data provided by Simcyp for these compounds, except in the case of ketoconazole, where dose dependency of clearance was taken into consideration for the 400 mg dose (Huang et al., 1986). In vitro intrinsic clearance ($CL_{int}$) from recombinant CYPs, inhibition constant ($K_i$), fraction unbound in plasma ($f_{up}$), Caco-2 permeability and physicochemical data such as molecular weight, calculated $logP$, $pKa$ and blood-plasma ratio ($R$) were employed as input in Simcyp. $pKa$ values were obtained using the ACD/pKa algorithm (ACD/Labs, 2011). When a measured value of $R$ was not available, it was assumed to be 0.55 for acids and 1 for bases and neutrals. First order absorption model was used along with predicted Caco-2 or MDCK permeability. Minimal PBPK
model was chosen for distribution and user-input values of steady-state volume of distribution ($V_{ss}$) derived from single ascending dose or other Phase I studies were used. Renal and metabolic clearances used as inputs in Simcyp were also obtained from clinical studies. All physicochemical data and PK parameters that were used in the evaluation are presented in Table 4. As the clearance of a victim drug will influence the extent of interaction during hepatic first pass, Simcyp’s retrograde calculator was employed to compute $CL_{int}$ in µL/min/pmol enzyme using the clinically-observed metabolic clearance as well as the percentage contribution from each of the CYPs obtained from in vitro CYP reaction phenotyping assay. For CYP3A substrates, the CYP3A4 isoform is assumed to be the only isoform involved in their metabolism. Simcyp predictions were done using trial designs consistent with the clinical interaction studies with respect to healthy volunteers, size of population, and proportion of females, doses of victims and perpetrators as well as fasted/fed condition of subjects. The number of trials was kept at 10 instead of the single trial in the clinic, to ensure reliability of Simcyp’s variability prediction. The fraction of victim drug unbound in the gut ($f_{u,g}$) was set to unity, as this was shown to give the best predictions (Yang et al, 2007). No user input values were provided for blood flow to gut ($Q_{gut}$). The fraction unbound in microsome incubation ($f_{u,mic}$) was calculated (Austin et al, 2002) and used to correct in vitro parameters derived from microsomes. Apart from the time-based $AUC$ ratios, Simcyp also provides an estimate of $AUC$ ratio with mechanistic static equations, $R_{ss}$, for which either the hepatic inlet or outlet (systemic) inhibitor concentration can be chosen. The hepatic outlet inhibitor concentration, which is the default option was used for our analysis to enable comparison with the spreadsheet-based static equations described below.

**DDI predictions with static equations**

$AUC$ ratio estimates from the mechanistic static equations in this section differ from static estimates from Simcyp, which are specifically referred to as $R_{ss}$. Henceforth, any future reference to $AUC$ ratios from static equations refers only to results from using the equations in this section. The following static equations were used in the prediction of $AUC$ ratios for reversible inhibition, time-dependent inhibition (TDI) (Obach, 2009) and enzyme induction (Almond et al, 2009).
Reversible inhibition:

\[
\frac{AUC_i}{AUC} = \frac{f_{\text{gut}}^i \times 1}{f_{\text{gut}} \sum f_m \times f_{\text{m,CYP}} + (1 - \sum f_m \times f_{\text{m,CYP}})} + \sum \frac{I_u}{K_{i,u}}
\]

\[\text{Eq. 1}\]

\(AUC\) is the area under the concentration-time curve of a victim drug in the absence of inhibitor; \(AUC_i\) is the area under the curve of victim when inhibitor is present; \(f_{\text{gut}}^i\) is the fraction escaping gut metabolism in the presence of perpetrator; \(f_{\text{gut}}\) is the fraction escaping gut metabolism in the absence of perpetrator; \(f_m\) is the fraction of total clearance due to hepatic metabolism; \(f_{\text{m,CYP}}\) is the fraction of total hepatic metabolism due to the inhibited CYP enzyme; \(K_{i,u}\) is the inhibition constant after correcting for microsomal binding; \(I_u\) is the unbound inhibitor concentration with \(I\) representing either \(I_{\text{sys}}\) (Eq. 2), \(I_{\text{max}}\) (Eq. 3) or \(I_{\text{inlet}}\) (Eq. 4) (Kanamitsu et. al, 2000b).

\[I_{\text{sys}} = \frac{F \times \text{dose}}{\tau \times CL}
\]

\[\text{Eq. 2}\]

\[I_{\text{max}} = \frac{I_{\text{sys}} \times k \tau}{1 - e^{-k \tau}}
\]

\[\text{Eq. 3}\]

\[I_{\text{inlet}} = I_{\text{sys}} + \frac{k_u \times f_{\text{abs}} \times f_{\text{gut}} \times \text{dose}}{Q_{pv}}
\]

\[\text{Eq. 4}\]

\(F\) is the clinically-observed oral bioavailability of the inhibitor; \(\tau\) is the inhibitor dosing interval; \(CL\) is the clinically-observed total clearance of the inhibitor; \(k\) is the elimination rate constant obtained from \(CL\) and \(V_{ss}\); \(k_u\) is the absorption rate constant, adopted from Simcyp V11 for reference compounds and for AZ compounds. \(f_{\text{abs}}\) is the fraction absorbed assumed as 1 (a valid assumption based on the physicochemical characteristics of the compounds); and \(Q_{pv}\) is the blood flow rate in the hepatic portal vein, whose value 18L/h is adopted from Simcyp V11 for consistency. In the absence of intravenous PK data, per oral clearance, \(CL_{po}\) is used directly in Eq. 2 for \(CL/F\).
Time-dependent inhibition:

\[
\frac{\text{AUC}_i}{\text{AUC}} = \frac{f_{\text{gut}}^i}{f_{\text{gut}}} \times \frac{1}{\sum f_m \times f_{m,\text{CYP}} + \left(1 - \sum f_m \times f_{m,\text{CYP}}\right) + \left(1 + \sum \frac{k_{\text{inact}} \times I_u}{k_{\text{deg}} \times (K_{I,u} + I_u)}\right)}
\]

\(k_{\text{inact}}\) is the maximal enzyme inactivation rate constant; \(K_{I,u}\) is the unbound inhibitor concentration at 50\% \(k_{\text{inact}}\) and \(k_{\text{deg}}\) is the endogenous degradation rate constant of enzyme. \(k_{\text{deg}}\) for CYP3A4 is 0.0193 h\(^{-1}\), a value adopted from Simcyp V11 for consistency.

In Eqs 1 and 5, \(f_{\text{gut}}\) was set to 1, a reasonable assumption considering the high inhibitor concentrations that are likely in the gut. The use of maximal inhibition of intestinal CYP3A4 has been suggested as a pragmatic indicator of the intestinal contribution to the drug-drug interactions for CYP3A4 cleared drugs (Galetin et al., 2007). \(f_{\text{gut}}\) can be estimated from hepatic extraction and systemic bioavailability, assuming fraction absorbed to be 1. The bioavailability of an orally administered drug is the product of fraction absorbed, \(f_{\text{gub}}\) and hepatic bioavailability (which is given by 1 - hepatic extraction). Dose linearity was ensured (Table 2) in deriving \(f_{\text{gub}}\) from clinical data. In the absence of sufficient information to estimate \(f_{\text{gub}}\), it is assumed to be 0.5 for CYP3A substrates. Apart from being a central value, nearly a third of the 25 compounds with known \(f_{\text{gut}}\) estimates, have a value around 0.5 (in the range 0.4 to 0.6) (Gertz et al, 2010). Since the extent of CYP3A4 interaction in the gut can be extensive, a good estimate of \(f_{\text{gut}}\) can be crucial in obtaining a realistic estimate of interaction risk.

Induction:

\[
\frac{\text{AUC}_i}{\text{AUC}} = \frac{f_{\text{gut}}^i}{f_{\text{gut}}} \times \frac{1}{\sum f_m \times f_{m,\text{CYP}} \times \left(1 + \sum \frac{E_{\text{max}} \times I_u}{E_{50} + I_u}\right) + \left(1 - \sum f_m \times f_{m,\text{CYP}}\right)}
\]

where,
\[ f_{gut}^i = \frac{1}{f_{gut} + (1 - f_{mg}) \times E_g + f_{mg} \times E_g \times (1 + \frac{E_{max} \times I_{gut}}{E_{C50} + I_{gut}})} \]  

(6b)

and

\[ I_{gut} = \frac{Dose \times k_a \times f_{abs}}{Q_{villi} \times N} \]  

(6c)

\( E_{max} \) and \( E_{C50} \) are the calibrated maximum fold induction over vehicle and the calibrated inducer concentration at 50% \( E_{max} \) respectively. Eq. 6b has been described previously in the literature (Wang et al., 2004). No ‘d’ correction as suggested by (Fahmi et al., 2009) was applied for induction. However, in order to maintain consistency with the input provided for Simcyp, induction parameters were normalized with positive control. \( E_g \) is the basal gut wall extraction, and \( f_{mg} \) is the fraction of gut metabolism mediated by the induced enzyme and is taken as 1 for compounds in this study, as the only known gut enzyme mediating gut metabolism for the victim compounds is CYP3A4. The inhibitor concentration in the small intestine, \( I_{gut} \), is calculated using Eq. 6c (Obach et al, 2007). \( N \) is the number oral doses per day. \( k_a \) is the first order absorption rate constant; \( f_{abs} \) is the fraction of dose absorbed; \( Q_{villi} \) is the blood flow rate to the villi and is adopted as 6 L/h from Simcyp V11 for consistency. The fraction of CYP3A4-mediated intestinal metabolism was assumed to be 0.57 and 0.66 for midazolam and simvastatin respectively, as reported previously (Obach et al., 2007).

Proposed multiplier to account for contribution to \( AUC \) ratio from hepatic first pass (\( AUCR_{hfp} \)):

For an orally administered inhibitor, the concentrations at the enterocytes during its absorption phase is likely to be much higher compared to its concentration in the liver during hepatic first pass, which in turn is likely to be higher compared to its systemic concentration at steady-state. Eqs 1 and 5 account for contributions to DDI from gut and after steady-state is attained, but ignores contribution to DDI from hepatic first pass, if \( I_{sys} \) is used. To mitigate the risk for underestimation of DDI due to the neglect of hepatic first pass effect for orally co-administered
inhibitor and victim, an additional multiplier term that accounts for the contribution to DDI from hepatic first pass \((AUC_{R_{hfp}})\) was introduced into Eqs 1 and 5, such that the overall \(AUC\) ratio is a product of contributions to \(AUC\) ratio from gut \((AUC_{R_{gut}})\), hepatic first pass and systemic \((AUC_{R_{systemic}})\) inhibition of the affected enzyme. Thus,

\[
\frac{AUC_{i}}{AUC} = AUC_{R_{gut}} \times AUC_{R_{hfp}} \times AUC_{R_{systemic}}
\]

The contribution to DDI during hepatic first pass of an orally administered inhibitor, \(AUC_{R_{hfp}}\), can be given by Eq 7, similar to gut contribution during intestinal first pass:

\[
AUC_{R_{hfp}} = \frac{f_{h}}{f_{h}^{'}} = \frac{1-(CL_{i,substrate}/Q_{L})}{1-(CL_{substrate}/Q_{L})}
\]

(7)

Where, \(f_{h}\) and \(f_{h}^{'}\) are the fractions of the victim drug escaping hepatic first pass metabolism in the absence and presence of inhibitor respectively. \(Q_{L}\) is the hepatic blood flow rate and \(CL_{substrate}\) is the plasma clearance of the substrate compound in the absence of the inhibitor, estimated from clinical PK data as described before. In the absence of intravenous PK, \(CL_{substrate}\) is obtained from the best estimate of bioavailability and \(CL_{p,v}\). \(CL_{i,substrate}\) is the plasma clearance of the substrate when co-administered with an inhibitor given by Eqs.8 and 9 for the reversible and time-dependent inhibition respectively.

\[
CL_{i,substrate} = CL_{substrate} \times \left( \frac{\sum f_{m,CYP}}{1+\sum \frac{I_{u,inlet}}{K_{i,u}}} + (1-\sum f_{m,CYP}) \right)
\]

(8)

\[
CL_{i,substrate} = CL_{substrate} \times \left( \frac{\sum f_{m,CYP} \times k_{inact} \times I_{u,inlet}}{1+\frac{K_{i,u}}{k_{deg} \times (K_{f,in} + I_{u,inlet})}} + (1-\sum f_{m,CYP}) \right)
\]

(9)
The mechanistic, static models (Eqs 1, 5 and 6a and incorporating Eq 7 into Eqs 1 and 5) were all compiled in a spreadsheet to enable easy calculation of AUC ratios. Using this spreadsheet, AUC ratios can be calculated for different types of inhibitor concentrations ($I_{sys}$, $I_{max}$ and $I_{inlet}$).

**Data analysis**

90% confidence interval (CI) was chosen as measure of variability as it was the statistical parameter used to describe the clinical data. 90% confidence intervals were computed for both AUC and $C_{max}$ ratios using the output data generated in Simcyp. If $N$ is the size of the population, $df$, the number of degrees of freedom is $N-1$. The upper and lower limits of confidence interval are then given by:

$$
\ln (CI) = \text{mean}(\ln(AUC \, \text{ratio})) \pm t_{df} \times \frac{S\ln (AUC \, \text{ratio})}{\sqrt{N}}
$$

(10)

Where $t_{df}$ is the t-distribution corresponding to $df$ and $SD$ is the standard deviation. A similar equation was used to describe confidence intervals for the $C_{max}$ ratio.

Prediction performance of Simcyp (time-based and $R_{ss}$) as well as static equations outlined in this Section were judged by the percentage of compounds that were predicted within 2-fold of the observed clinical interaction parameters. When predictions indicate no DDI, contrary to clinical observation, it is considered as an under prediction, even if the observed AUC ratio is below 2. Prediction precision was assessed with root mean square error (RMSE):

$$
RMSE = \sqrt{\frac{\sum_{predicted\, DDI - observed\, DDI}^2}{\text{Number of predictions(n)}}}
$$

(11)

In order to assess Simcyp’s estimate of variability associated with DDI predictions, the ratio of variance (square of standard deviation) of estimated to clinically-observed was calculated.
Variance ratio \((\text{Simcyp: observed})\) = \frac{\text{Variance of estimated AUC (or } C_{\text{max}} \text{) ratio}}{\text{Variance of clinically-observed AUC (or } C_{\text{max}} \text{) ratio}} \tag{12}

Variance ratio was also plotted against the geometric mean (GM) of the geometric means of the estimated and clinically-observed interaction ratios which is given by:

\[GM \ (\text{Simcyp, observed}) = \sqrt{GM_{\text{Simcyp-estimated ratio}} \times GM_{\text{clinically-observed ratio}}} \tag{13}\]

A plot of variance ratio vs. GM (estimated and clinic) should reveal any systematic dependencies of estimated variability on DDI magnitude.
Results

The 19 DDI clinical trials from the 11 AstraZeneca compounds were sub-divided into three categories:

A. AstraZeneca compounds are perpetrators of reversible or irreversible CYP inhibition

B. AstraZeneca compounds are victims of reversible or irreversible CYP inhibition

C. AstraZeneca compounds are victims or perpetrators of CYP induction

Table 5 summarizes the clinically-observed interaction parameters as well as predicted using Simcyp and mechanistic static equations for all the 19 interactions falling into 3 categories.

Table 6 lists the RMSE for all static and dynamic predictions of DDI. The use of unbound $I_{sys}$ in static equations (Eqs. 1, 5) is associated with the lowest RMSE. This is also evident from Fig. 2 which shows a comparison of $AUC$ ratios from static equations using the 3 different types of inhibitor concentrations - $I_{sys}$, $I_{inlet}$ and $I_{max}$, for the inhibition-based interactions. $I_{inlet}$ and $I_{max}$ tend to over-predict the interaction risk. Therefore, $I_{sys}$ was used in all evaluations using the static equations for comparison with the time-based Simcyp predictions. With the inclusion of hepatic first pass correction (Eq. 7) into mechanistic static equations 1 and 5, systemic inhibition with $I_{sys}$, hepatic first pass inhibition with $I_{inlet}$ and gut enzyme inhibition with the $f_{gut}$ would be considered. However, inclusion of $AUCR_{hfp}$ tends to over-predict the DDI risk. The overall accuracy decreased (see RMSE in Table 6).

Clinically-observed interactions for the 11 compounds were moderate ($AUC$ ratio $\leq 5$) for Category A compounds and moderate to strong (strong being $AUC$ ratio $>5$ for inhibition and $<0.2$ for induction) for the Categories B and C. No clinical $AUC$ ratios were $>10$-fold. This is also evident in Fig. 3, in which time-based $AUC$ ratios and $R_{ss}$ from Simcyp are plotted against the clinically-observed $AUC$ ratios for all 3 categories of compounds. While the clinical $AUC$ ratios of the interactions studied range between 0.1 and 10, Simcyp-predicted ratios (both time-based and $R_{ss}$) exceed this range, especially for larger $AUC$ ratios. Since the hepatic outlet (or systemic) inhibitor concentration was used in the estimation of $R_{ss}$, it should be comparable to results from static models with unbound $I_{sys}$, presented in the last column of Table 5. However, $R_{ss}$ consistently over predicts DDI compared to $AUC$ ratios from Eqs. 1-3, possibly due to differences in the estimation of gut contributions to DDI. This is especially true for CYP3A4-
mediated interactions. Therefore, only time-based predictions from Simcyp have been used for the comparative analysis with static equations. Fig. 4 shows the predicted and observed $AUC$ ratios for the 3 categories of compounds. Prediction performance of Simcyp V11 and the best of static equations ($I_{sys}$ with and without hepatic first pass correction) are shown in Table 7.

Among the 19 interactions studied, AZ5-metoprolol interaction is the only non-CYP3A4 interaction for which the predicted $AUC$ ratio is > 1 and for which the $AUC$ ratios from both Simcyp and mechanistic static models match. As gut contribution to DDI is irrelevant for CYP2D6-mediated interactions, the static and Simcyp predictions for these interactions can be expected to be similar. This suggests that differences in $f_{gut}$ between the 2 methods may be the reason for differences in their prediction of $AUC$ ratios. A comparison of $f_{gut}$ used in static equations with those estimated in Simcyp for the 8 victim compounds in this study, show no correlation between the two (Fig. 5). If $f_{gut}$ predicted by Simcyp is low, as in the case of simvastatin and AZ7 (see Table 2), then DDI interactions mediated by the inhibition of gut enzymes will be over-predicted by Simcyp, leading to a large $AUC$ ratio.

In Category A, two out of the six interactions are mediated by CYP2D6, and the remaining by CYP3A4. The over-prediction of AZ1-simvastatin interaction by Simcyp is probably due to the low gut bioavailability (0.09) predicted by Simcyp for the victim compound, simvastatin, as explained above. For the AZ4-metoprolol interaction, both Simcyp and static models predict no $AUC$ change, whereas a less than 2-fold $AUC$ change is observed clinically. Obviously, in this case, the measured in vitro $K_i$ ($K_i > 35 \mu M$) did not capture the observed interaction. This interaction has been included in the analysis only to illustrate that a negative in vitro result need not necessarily hold in vivo. AZ5 being lipophilic, the predicted $f_{u,mic}$ is very low. Any error in this prediction could lead to substantial changes in the estimated $AUC$ ratio. A sensitivity analysis was therefore carried out (Supplemental Figure 1). The $AUC$ ratio goes from about 1.3 to 1 in the full range of $f_{u,mic}$. In the case of AZ5, an imprecise prediction of $f_{u,mic}$ had little impact on the overall result.
In Category B, AZ compounds were victims of CYP3A4 inhibition. The product $f_m \times f_{m,CYP}$ is nearly 1 for all except for AZ9. The AZ6-ketoconazole interaction is the only one where the ketoconazole dose was 400 mg. Ketoconazole exhibits dose-dependent PK (Huang et al., 1986), possibly due to autoinhibition. A lower per oral clearance of 7.4 L/h (Huang et al., 1986) at 400 mg was used both in Simcyp and static equations. The $AUC$ ratio predicted by static equations improves slightly from 6.4 to 8.1 with the use of lower hepatic clearance. The interaction of AZ7 with ketoconazole is over-predicted by Simcyp. The fraction escaping gut metabolism ($f_{gut}$) estimated by Simcyp was 0.08 compared to 0.43 used in static equations. AZ7 is a highly bound ($f_{up} = 0.001$) compound and in this case, fixing $f_{u,g} = 1$ may not be valid. The sensitivity of $f_{gut}$, $CL_{po}$ and mean $AUC$ ratio to $f_{u,g}$ for the AZ7-ketoconazole interaction is presented in the supplementary material (Supplemental Figure 2). Fixing $f_{u,g}$ closer to the $f_{up}$ of AZ7, gave a more accurate $AUC$ ratio of 4 for the AZ7-ketoconazole interaction (communications with staff at Simcyp Ltd). For the AZ7-diltiazem interaction, the additional contribution to CYP3A4 inhibition from the metabolite of diltiazem (Rowland Yeo et al, 2010) is built into Simcyp. Similarly, for the AZ9-itraconazole interaction, inhibition by the metabolite of itraconazole is considered in Simcyp. These were not considered in static equations. AZ9 is not a very sensitive substrate as it has a low $f_m \times f_{m,CYP}$ (~0.4), and low clearance. Thus, although the metabolites are known to be more potent in vitro compared to the parent and account for ~50% of the overall CYP3A inhibition in vivo (Guest et al, 2011, Templeton et al, 2008), their impact on AZ9-itraconazole interaction is low. The interactions of AZ8 with ketoconazole are under-predicted only by Simcyp. As highlighted earlier, gut-mediated DDI are likely to be different in the 2 approaches because of differences in $f_{gut}$. The $f_{gut}$ estimated by Simcyp for AZ8 was 0.86 compared to 0.23 used in static equations, which might explain the differences in the $AUC$ ratio. Difference in estimated $AUC$ ratios between static and dynamic models for the AZ10-ketoconazole interaction is probably attributable to differences in the treatment of intestinal and hepatic first pass in the 2 models. As AZ10 has very high $CL_{po}$ difference between the 2 approaches is also large. Unlike Simcyp, the mechanistic static models do not consider interaction during hepatic first pass. The inclusion of $AUC_{Rhfp}$ in the static DDI estimation should provide a value closer to that from Simcyp. Simcyp reports an estimated
systemic clearance of 33 L/h from CL_po and f_gut (0.5) provided as input, whereas static equation uses 13.6 L/h, a low value arising from the low bioavailability of AZ10. Consequently, the estimated $AUC_{Rhfp}$ correction is low (1.12). Using a clearance value of 33 L/h, the $AUC_{Rhfp}$ correction is 1.4 and the $AUC$ ratio using the static model becomes 5.6.

It is worthwhile to note that for 15 out of the 16 interactions for which $C_{max}$ ratios were available, the DDI risk indicated by clinical $C_{max}$ ratios were less than or comparable (AZ8-ketoconazole interaction) to that corresponding to $AUC$ ratios. This is in keeping with the smaller range of $C_{max}$ ratios compared to $AUC$ ratios reported for 54 clinical DDI involving mechanism-based CYP3A inhibitors (Wang, 2010). Assuming that $C_{max}$ is affected by first pass, this reflects a reduced importance of first pass (hepatic, intestinal or both) in DDI. One explanation could be that while the magnitude of $AUC$ ratio depends only on the extent of intestinal and/or hepatic extraction, the magnitude of $C_{max}$ ratio would in addition to these, depend on the region of gastrointestinal tract where maximum absorption of substrates occur. Substrates of an inhibited intestinal enzyme that have high permeability are more likely to have $C_{max}$ ratios that are comparable to their $AUC$ ratios, if like CYP3A4, the inhibited enzyme is expressed mainly in the small intestine (Paine et al., 2006). AZ6-ketoconazole interaction has a relatively large deviation of $C_{max}$ ratio from its $AUC$ ratio. Comparing this with AZ8-ketoconazole interaction, we note that the victim drugs in both interactions have similar clearance. However, the permeability of AZ8 is very much higher compared to AZ6, which supports the hypothesis that a maximum absorption in the jejunal region where CYP3A4 expression is maximum would result in AZ8 having a $C_{max}$ ratio comparable to its $AUC$ ratio.

Category C represents induction mediated interactions. Static equation calculations have been done using $I_{sys}$ with and without the gut contributions to DDI. Inclusion of gut interaction seems to overestimate the risk, especially for interactions with rifampicin. For carbamazepine, Simcyp uses calibrated slope of the fold induction vs. concentration plot in the linear range of concentrations, $Ind_{slope}$. For carbamazepine the value of $Ind_{slope}$ used in Simcyp is 0.16. Static equations used $E_{max}$ and $EC_{50}$ values (7.7 fold and 40µM respectively) from the literature (McGinnity et al., 2009). It is clear from Table 5 that prediction of $AUC$ ratios from Simcyp and
static equations are comparable. Many previous evaluations (Einolf, 2007; Fahmi et al., 2009; Perdaems et al., 2010; Youdim et al., 2008) have shown similar prediction success with Simcyp.

Geometric means, 90% confidence interval and variance for the 19 interactions are shown in Table 4. Figs. 6a and 6b show that the variance of \( AUC \) ratio and \( C_{\text{max}} \) ratio are not independent of their corresponding geometric means. The greater the deviation of the \( AUC \) ratio from 1, greater is the variance, which is to be expected. This is also evident from Fig. 4, where the absolute extent of variability is seen to be proportional to the mean values of \( AUC \) and \( C_{\text{max}} \) ratios. Fig. 4 also shows that the clinical variability in terms of 90% confidence interval ranges roughly between 10% and 40% of observed \( AUC \) ratios, whereas estimated variability covers a broader range. Fig. 6c shows variance ratio (Simcyp:observed) plotted against GM (Simcyp, observed). This ratio should be 1 for all interactions, if the estimated variability matches the observed. Setting arbitrary acceptance limits of 2-fold of observed (shown by dotted lines in Fig 6c), it can be seen that Simcyp tends to over- or under-estimate the variability for a considerable number of interactions, depending on the mean values. In addition to this, over-prediction of mean \( AUC \) ratio by Simcyp, as in the case of AZ1-simvastatin or AZ7-ketoconazole can further exaggerate its variability estimation.

Clinical and Simcyp-predicted PK parameters for all AZ compounds have been provided as supplemental data (Supplemental Tables 1 and 2).
Discussion

Static equations using unbound average steady-state systemic inhibitor concentration ($I_{sys}$) has been shown to perform better with respect to accuracy compared to Simcyp V11 for the 19 interactions studied in this report (84% and 58% of the interactions predicted within 2-fold respectively). Other retrospective validations (Wang, 2010; Guest et al, 2011; Shardlow et al, 2011) indicate comparable predictions. Guest et al reported that Simcyp and static models predicted 71% and 77% of the DDIs within two-fold respectively. The authors attributed the comparability to high potency and large dosing of the inhibitors in their study. The higher prediction accuracy of Simcyp reported by Guest et al. could also be because the validation compounds in their study were all well-characterized azole inhibitors and benzodiazepine substrates.

Differences in the prediction outcomes between static and dynamic models can be attributed to differences in the treatment of hepatic and intestinal first pass and to differences in inhibitor concentration. Also, the neglect of metabolite inhibition and auto-inactivation of the affected enzyme by a time-dependent inhibitor in static models can lead to an underestimation of DDI risk. AUC ratios from Simcyp exceeding 10-fold should be treated with caution, as clinical interactions rarely exceed that limit. This study has identified a significant risk for over-estimating DDI liability with Simcyp, largely attributable to uncertainty in CYP3A-mediated intestinal DDI. This is consistent with a recent report (Sinha et al., 2012). In the gut, CYP3A represents the principal drug-metabolizing CYP enzyme (Paine et al., 1997, 2006). The high gut concentrations of an orally administered inhibitor and the significant intestinal extraction of a substrate despite the low gut CYP3A4 content of just ~1% of that found in liver (Paine et al., 1997) translate to a significant gut contribution to DDI. In addition, although CYP3A4 (a low affinity, high capacity enzyme) mediated DDI could be limited by alternative metabolic/elimination pathways in the liver, intestinal CYP3A4-mediated DDI could still be high, as CYP3A is almost the only CYP enzyme in the gut. Thus difficulty to assess $f_{gut}$ of a substrate or inhibitor in Simcyp due to uncertainties in $f_{ug}$, and/or due to quality of in vitro data could result in a substantial deviation of predicted DDI from the observed. An underestimation of substrate $f_{gut}$ would mean underestimation of DDI risk (due to underestimation of substrate...
gut metabolism), while underestimation of inhibitor $f_{gut}$ would mean overestimation of DDI risk (due to higher inhibitor concentration resulting from neglect of gut extraction). Information on human-relevant gut metabolism is sparse in drug discovery. Even during clinical development, such information requires an additional intravenous clinical PK study to be done, in order to be able to distinguish between gut and hepatic first-pass. When multiple gut enzymes (e.g., CYP3A4 and UGT2B7) are involved, an assessment of relative contribution to $f_{gut}$ is an additional challenge. The use of an estimated $f_{gut}$ in static equations is therefore an attractive alternative to using the dynamic model for predicting the $AUC$ ratio of CYP3A-mediated DDI. In the absence of an $f_{gut}$ estimate in human, an assumption of $f_{gut} = 0.5$ is suggested for CYP3A substrates (unpublished analysis). As competition with permeability is likely to limit gut metabolism, compounds with fairly good permeability cannot have very high extraction in the gut. Thus, if extent of gut metabolism is not capacity-limited, a central value of 0.5 can be rationalized. Interactions involving substrates with large deviations of $f_{gut}$ from those used in static were either under- or over-predicted by Simcyp, which further lends support for an $f_{gut}$ of 0.5.

Hepatic first pass correction to overcome under-prediction of DDI when using static equations with $I_{sys}$, resulted in systematic over-prediction of DDI risk. However, this may be a useful approach to estimate a maximum expected risk, especially for high clearance drugs where it can make a substantial difference. The importance of hepatic first pass may depend on the nature of the interacting compounds. $C_{max}$ is seen as critical when summarizing the DDI risk. However, the clinical interaction data used in this study shows that $C_{max}$ ratios are generally less than the $AUC$ ratios. Thus, $AUC$ ratio prediction should provide an adequate estimate of the maximum DDI risk.

In order to further understand the basis for the differences in $AUC$ ratios predicted by Simcyp (time-based), Simcyp $R_{as}$ and the proposed static equations, we need to consider the differences in inhibitor concentrations in the 3 approaches. As clinical DDI studies aim to achieve steady-state inhibitor concentration, prior to administration of the victim, differences in average inhibitor concentrations and therefore DDI can be expected to be minimal in the 3
approaches for inhibitors administered intravenously. However, for orally-administered inhibitors that are not metabolized in the gut, the higher-than-systemic hepatic concentrations during the absorption phase implies that the use of a single uniform inhibitor concentration in static equations is likely to under- or over-estimate DDI, depending on whether systemic or hepatic inlet concentration is employed, as in the estimation of $R_{ss}$. The proposed static equations using $I_{sys}$ and incorporating $AUC_{R_{hp}}$, should be expected to perform better. In the case of time-based Simcyp, the high levels of inhibitor concentrations in the portal vein are valid only during the absorption phase, reflecting the reality. In our analysis, since $I_{sys}$ has been chosen for $R_{ss}$ estimation, we should expect Simcyp $R_{ss}$ and static equations with $I_{sys}$ to give similar results for non-CYP3A substrates. Finally, for orally-administered inhibitors that are metabolized in the gut, high inhibitor concentrations in the gut need to be additionally considered. In the $R_{ss}$ method, $I_{gut}$ is a constant high value, explaining its tendency to over-predict CYP-3A-mediated interaction whereas in the static equations proposed in this study, it is simply dependent on an estimated $I_{gut}$ and on the validity of assuming maximal inhibition of gut enzymes by the inhibitor. In Simcyp (time-based), the dynamically varying inhibitor concentration starts off at a high value, but drops substantially over the absorption phase. Thus, it is differences in the inhibitor concentration ([I]) during hepatic and/or intestinal first pass that makes the 3 approaches different. Simcyp’s dynamic treatment of [I] should be expected to provide a better prediction of DDI under conditions when inhibitor concentration is not at steady-state (eg, the intended human dose schedule is not chronic). However, uncertainties in the input used in the 2 models (see Fig. 1) can dominate prediction performance and can offset any advantages of a dynamic approach. Simple models with fewer input data are therefore preferable for DDI predictions.

The neglect of autoinhibition and autoinactivation of the affected enzyme by AZ1 – AZ5 would lead to an under-prediction of DDI risk, as it amounts to neglecting the prolonged high concentrations of the inhibitor. Simcyp accounts for the autoinactivation by the mechanism-based inhibitors, AZ1 and AZ3, but with the input provided, it cannot consider the autoinhibition by the reversible inhibitors, AZ2, AZ4 and AZ5. Static equations do not consider either of these. As there is no information on the clinical relevance of autoinhibition it is difficult to quantify its impact.
With the exception of AZ10-carbamazepine interaction, all induction interactions were predicted well with static equations, if gut contribution to DDI is ignored. Inclusion of gut contribution results in an overestimation of DDI risk. A rationale for this could be that an increase in gut enzymes due to induction may not really impact the extent of metabolism of the victim compound, as there is a competition between permeability and metabolism in the enterocytes. Therefore, enzyme capacity may have a lesser role than might be anticipated in the absence of a competition from permeability. Victim compounds of induction in this analysis have log P of at least 0.88 and good permeability. As dynamic models can consider the effect of permeability on the rate and extent of gut metabolism, Simcyp’s estimation of AUC ratios agree with clinically-observed values.

This study demonstrated a tendency for Simcyp–estimated variability to be under- or over-predicted depending on the mean value. Thus, when the mean AUC ratios are themselves over-predicted by Simcyp, the associated variability are likely to be exaggerated for extreme individuals in a population. Since the variability associated with clinical DDI parameters is generally < 40% of the mean values, this study recommends a conservative estimate of 40% of predicted mean AUC ratio estimated by mechanistic static equations, as a rule of thumb. This is consistent with the proposed coefficient of variation (CV) for CYP3A4 content of 41% (Cubitt et al, 2011) and 33% (Kato et al, 2010). A fixed variability that is slightly higher than clinically-observed margins will have the advantage of covering for any prediction uncertainty and/or higher clinical variability.

In conclusion, this analysis highlights the importance of characterizing the gut and hepatic metabolism of a substrate as well as its major elimination routes in human. This is possible only through having at least the intravenous clinical PK of the substrate. In the absence of relevant information, the use of unbound \( l_{sys} \), \( f_{u,mic} \), \( AUC_{Rbh} \), and an estimated \( f_{gut} \) in mechanistic static equations with a neglect of gut interactions for induction-mediated DDI can provide reasonable predictions. Considering the possibility for large deviations of Simcyp-predicted AUC ratios from the observed, a fixed measure of variability around the mean predicted AUC ratios appears to be preferable over a population-based approach during early development phases, for assessing the
potential for extreme individuals to experience adverse events. However, during later clinical development, a population-based approach can be valuable in simulating the simultaneous impact of disease, ethnicity, age, multiple inhibitors (including potent metabolites) as well as enzyme and transporter polymorphism on DDI.
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Authorship contributions

Participated in research design: Peters

Conducted experiments: none

Contributed new reagents or analytic tools: none

Performed data analysis: Peters, Schroeder and Giri

Wrote or contributed to the writing of the manuscript: Peters, Schroeder and Dolgos
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Footnotes

The outcome of this work has been presented at a workshop jointly organized by the FDA and Drug metabolism Leadership Group of the IQ consortium (IQ DMLG) on June 6-7, 2011.

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Legends for Figures

Fig. 1. Sources of uncertainty associated with DDI evaluation in discovery and preclinical phases.

Fig. 2. Mechanistic static equation predictions of $AUC$ ratios vs. clinically-observed for CYP-based drug interactions in Category A and Category B compounds. Line of unity and 2-fold limits are indicated. $I_{sys}$, $I_{inlet}$ and $I_{max}$ are the average systemic steady-state, hepatic inlet and maximum systemic steady-state inhibitor concentrations. $AUCR_{HFP}$: $AUC$ ratio with inclusion of hepatic first pass.

Fig. 3. Simcyp time-based $AUC$ ratios and $R_{ss}$ predictions vs. clinically-observed for 19 drug interactions. Line of unity and 2-fold limits are indicated.

Fig. 4. $AUC$ ratio predictions with Simcyp V11 (time-based) and static equations using $I_{sys}$ with and without inclusion of $AUC$ ratio for hepatic first pass for (a) Category A (b) Category B and (c) Category C interactions along with the clinically-observed. 90% confidence intervals are also shown.

Fig. 5. Comparison of fraction escaping gut metabolism ($I_{gut}$) used in static models with those generated in Simcyp V11 for victims of CYP inhibition.

Fig. 6. Variance ratios (Simcyp V11:clinic) for $AUC$ and $C_{max}$ ratios vs. geometric means of the geometric mean values of Simcyp-estimated and clinically-observed. Dotted lines indicate arbitrary limits of acceptable variability (2-fold). Trend lines are also shown.
Table 1. Overview of the clinical trial design for the 19 drug interaction studies

<table>
<thead>
<tr>
<th>Category</th>
<th>Interacting drug</th>
<th>Perpetrator oral dose (mg)</th>
<th>Perpetrator dosing regimen</th>
<th>Victim oral dose (mg)</th>
<th>Victim dosing regimen</th>
<th>fed/ fasted</th>
<th>N</th>
<th>Age range</th>
<th>Proportion of females</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>AZ1 Midazolam</td>
<td>400</td>
<td>5 days, qd</td>
<td>7.5</td>
<td>single, fasted</td>
<td>28</td>
<td>18-65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AZ1 Simvastatin</td>
<td>400</td>
<td>5 days, qd</td>
<td>40</td>
<td>single, fasted</td>
<td>10</td>
<td>20-50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AZ2 Midazolam</td>
<td>400</td>
<td>4 days, qd</td>
<td>7.5</td>
<td>single, fasted</td>
<td>30</td>
<td>18-65</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AZ3 Midazolam</td>
<td>125</td>
<td>11 days, qd</td>
<td>7.5</td>
<td>single, fasted</td>
<td>12</td>
<td>20-49</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AZ4 Metoprolol</td>
<td>750</td>
<td>6 days, bid</td>
<td>100</td>
<td>single, fasted</td>
<td>14</td>
<td>18-65</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AZ5 Metoprolol</td>
<td>500</td>
<td>28 days, qd</td>
<td>50</td>
<td>single, fasted</td>
<td>18</td>
<td>44-55</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>AZ6 Ketoconazole</td>
<td>400</td>
<td>5 days, qd</td>
<td>125</td>
<td>single, fasted</td>
<td>22</td>
<td>18-65</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AZ6 Verapamil</td>
<td>240</td>
<td>7 days, qd</td>
<td>125</td>
<td>single, fasted</td>
<td>23</td>
<td>18-65</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>CYP inhibition</td>
<td>Compound</td>
<td>Dose</td>
<td>Duration</td>
<td>Day</td>
<td>Fed Status</td>
<td>CYP Inhibition</td>
<td>Induction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
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<td></td>
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<tr>
<td>AZ7 Ketoconazole</td>
<td>200</td>
<td>10 days, bid</td>
<td>90</td>
<td>single, day 4</td>
<td>fasted</td>
<td>14</td>
<td>20-45</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>AZ7 Diltiazem</td>
<td>240</td>
<td>14 days, qd</td>
<td>90</td>
<td>single, day 8</td>
<td>fasted</td>
<td>17</td>
<td>20-45</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>AZ8 Ketoconazole</td>
<td>200</td>
<td>4 days, qd</td>
<td>3</td>
<td>single, day 4</td>
<td>fed</td>
<td>4</td>
<td>22-40</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>AZ8 Ketoconazole</td>
<td>100</td>
<td>4 days, bid</td>
<td>3</td>
<td>single, day 4</td>
<td>fed</td>
<td>8</td>
<td>20-40</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>AZ9 Itraconazole</td>
<td>200</td>
<td>7 days, qd</td>
<td>10</td>
<td>single, day 4</td>
<td>fasted</td>
<td>6</td>
<td>30-49</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>AZ10 Ketoconazole</td>
<td>200</td>
<td>4 days, qd</td>
<td>25</td>
<td>single, day 4</td>
<td>fed</td>
<td>12</td>
<td>24-42</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>AZ5 Rifampicin</td>
<td>600</td>
<td>16 days, qd</td>
<td>500</td>
<td>single, day 10</td>
<td>fasted</td>
<td>18</td>
<td>27-56</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>AZ7 Rifampicin</td>
<td>600</td>
<td>12 days, qd</td>
<td>180</td>
<td>single, day 12</td>
<td>fasted</td>
<td>16</td>
<td>22-49</td>
<td>0.375</td>
<td></td>
</tr>
<tr>
<td>AZ9 Rifampicin</td>
<td>600</td>
<td>13 days, qd</td>
<td>15</td>
<td>single, day 10</td>
<td>fasted</td>
<td>12</td>
<td>20-61</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>AZ10 Carbamazepine</td>
<td>600</td>
<td>25 days, qd</td>
<td>300</td>
<td>35 days bid</td>
<td>fed</td>
<td>18</td>
<td>29-63</td>
<td>0.2</td>
<td></td>
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<tr>
<td>AZ11 Midazolam</td>
<td>10</td>
<td>12 days, qd</td>
<td>5</td>
<td>single, day 12</td>
<td>fed</td>
<td>24</td>
<td>20-49</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
qd: quaque die or once daily; bid: bis in die or twice daily.

Interactions of AZ4 and AZ5 with metoprolol are mediated by CYP2D6; All other interactions are CYP3A4 mediated.
### Table 2: Substrate-related parameters for victims of CYP3A4

<table>
<thead>
<tr>
<th>Victim</th>
<th>fm</th>
<th>fm,CYP3A4</th>
<th>fgut (static model)</th>
<th>Linear dose range from clinical Phase I studies (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZ5</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>50 - 500; small departure from linearity at 500 mg</td>
</tr>
<tr>
<td>AZ6</td>
<td>0.87</td>
<td>1</td>
<td>0.56</td>
<td>50-500</td>
</tr>
<tr>
<td>AZ7</td>
<td>1</td>
<td>1</td>
<td>0.43</td>
<td>70 - 220</td>
</tr>
<tr>
<td>AZ8</td>
<td>1</td>
<td>1</td>
<td>0.23</td>
<td>upto 180</td>
</tr>
<tr>
<td>AZ9</td>
<td>0.53</td>
<td>0.16</td>
<td>0.5</td>
<td>3 - 15</td>
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<tr>
<td>AZ10</td>
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<td></td>
<td>0.5</td>
<td>upto 400</td>
</tr>
<tr>
<td>midazolam</td>
<td>0.94</td>
<td>1</td>
<td>0.57</td>
<td>-</td>
</tr>
<tr>
<td>simvastatin</td>
<td>0.93</td>
<td>0.99</td>
<td>0.66</td>
<td>-</td>
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</table>

*fm*: fraction metabolized in the liver; *fm,CYP3A4*: fraction metabolized by CYP3A4.

*fgut*: fraction escaping gut metabolism, assumed 0.5 for AZ5, AZ9 and AZ10.
### Table 3: Inhibitor-related pharmacokinetic and interaction parameters

<table>
<thead>
<tr>
<th>Perpetrator drug</th>
<th>Type of perpetrator</th>
<th>Affected enzyme</th>
<th>$f_{abs}$</th>
<th>$k_a$ (min$^{-1}$)</th>
<th>$f_{gut}$</th>
<th>$k$ (min$^{-1}$)</th>
<th>t-half (h)</th>
<th>$f_{u,mic}$</th>
<th>$K_i$ (µM) / $K_I$ (µM); $k_{inact}$ (min$^{-1}$) / $E_{max}$ (fold), $EC_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZ1</td>
<td>TDI</td>
<td>CYP3A4</td>
<td>0.88</td>
<td>0.053</td>
<td>0.72</td>
<td>0.00119</td>
<td>9.7</td>
<td>0.39</td>
<td>$K_i$ 9.4; $k_{inact}$ 0.056</td>
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<tr>
<td>AZ2</td>
<td>competitive inhibitor</td>
<td>CYP3A4</td>
<td>1</td>
<td>0.033</td>
<td>0.5</td>
<td>0.00002</td>
<td>571</td>
<td>0.54</td>
<td>$K_i$ 3.86</td>
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<tr>
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<td>TDI</td>
<td>CYP3A4</td>
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<td>0.033</td>
<td>0.5</td>
<td>0.00064</td>
<td>18</td>
<td>0.28</td>
<td>$K_i$ 11.1; $k_{inact}$ 0.051</td>
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<tr>
<td>AZ4</td>
<td>competitive inhibitor</td>
<td>CYP2D6</td>
<td>0.64</td>
<td>0.005</td>
<td>0.52</td>
<td>0.00289</td>
<td>4</td>
<td>0.7</td>
<td>$K_i$ &gt; 35</td>
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<tr>
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<td>competitive inhibitor</td>
<td>CYP2D6</td>
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<td>0.013</td>
<td>1</td>
<td>0.0003</td>
<td>38.6</td>
<td>0.04</td>
<td>$K_i$ 3.79</td>
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<td>0.00134</td>
<td>8.6</td>
<td>0.23</td>
<td>$E_{max}$ 28; $EC_{50}$ 1.3</td>
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<td>0.00154</td>
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<td>0.97</td>
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<td>CYP3A4</td>
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<td>0.020</td>
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<td>0.00136</td>
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<td>1</td>
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<td>0.100</td>
<td>0.8</td>
<td>0.00289</td>
<td>4</td>
<td>1</td>
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<td>0.010</td>
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<td>50</td>
<td>1</td>
<td>$K_i$ = 0.0013</td>
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<td>Inducer</td>
<td>CYP3A4</td>
<td>( k_a )</td>
<td>( f_{gut} )</td>
<td>( E_{\text{max}} )</td>
<td>( EC_{50} )</td>
<td>( E_{\text{max}} )</td>
<td>( f_{\text{abs}} )</td>
<td>( f_{u,mic} )</td>
<td>( E_{\text{max}} )</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------</td>
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<td>-------------</td>
<td>----------------</td>
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<td>rifampicin</td>
<td>CYP3A4</td>
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<td>0.009</td>
<td>0.5</td>
<td>0.00385</td>
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<td>1</td>
<td>Indmax 8</td>
<td>IndC50 0.32</td>
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<td>CYP3A4</td>
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<td>0.008</td>
<td>0.96</td>
<td>0.00031</td>
<td>37.5</td>
<td>1</td>
<td>Ind slope = 0.16</td>
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</table>

TDI: Time-dependent inhibitor; \( f_{\text{abs}} \): fraction absorbed; \( k_a \): absorption rate constant; \( f_{gut} \): fraction escaping gut metabolism, assumed 0.5 for AZ2, AZ3, ketoconazole, and rifampicin; \( k \): elimination rate constant; \( f_{u,mic} \): fraction of drug unbound in microsomal incubation; \( K_i \): reversible inhibition constant; \( k_{\text{inact}} \): maximal enzyme inactivation rate constant measured for a time-dependent inhibitor; \( K_i \): unbound inhibitor concentration at 50% \( k_{\text{inact}} \); \( E_{\text{max}} \): fold increase in response over vehicle; \( EC_{50} \): Inducer concentration at 50% \( E_{\text{max}} \).
### Table 4. Compound input parameters for dynamic and static modeling

|      | MW | logP | area | Polar surface | Acid/Base | logpKa | R | fup (x10^6 cm s⁻¹) | Caco-2 / MDCK P_app | Intraveno -us dose* (mg) | Vss (L/Kg) | Total CL (L/h) | Renal CL (L/h) | Observed/assumed bioavailability (%)
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<td>AZ1</td>
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<td>3.4</td>
<td>129</td>
<td>B</td>
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<td>1</td>
<td>0.025</td>
<td>15.3</td>
<td>na</td>
<td>(estimated)</td>
<td>65.1 (po)</td>
<td>0.007</td>
<td>(assumed)</td>
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<td>4.49 (MDCK)</td>
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<td>(estimated)</td>
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<td>AZ9</td>
<td>424</td>
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<td>124</td>
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<td>11.3 (MDCK)</td>
<td>na</td>
<td>(estimated)</td>
<td>20 (po)</td>
<td>1.04</td>
<td>(assumed)</td>
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</tbody>
</table>

This article has not been copyedited and formatted. The final version may differ from this version.
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<tr>
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<th>MW</th>
<th>log P</th>
<th>pKa</th>
<th>R</th>
<th>fup</th>
<th>fgut</th>
<th>Caco-2 / MDCK Papp</th>
<th>Vss</th>
<th>CL</th>
<th>na</th>
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<td>6.9</td>
<td>1</td>
<td>0.14</td>
<td>55</td>
<td>(estimated) 138 (po)</td>
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<td>(assumed)</td>
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<td>25</td>
<td>(estimated) 28.4 (po)</td>
<td>None</td>
<td>(assumed)</td>
<td></td>
</tr>
</tbody>
</table>

MW: molecular weight; log P: Octanol water partition coefficient; pKa: negative logarithm of acid dissociation constant; R: blood to plasma concentration ratio; fup: fraction of drug unbound in plasma; fgut: fraction of absorbed drug escaping gut metabolism; Caco-2 / MDCK Papp: Measured apparent permeability from Caco-2 or MDCK cell lines; Vss: steady-state volume of distribution; CL: clearance; na: not available.
Table 5. Summary of observed and predicted (Simcyp V11 and static equations with $I_{sys}$) DDI

<table>
<thead>
<tr>
<th>Interaction</th>
<th>AUC ratio Geom Mean</th>
<th>90% CI lower</th>
<th>90% CI upper</th>
<th>Var</th>
<th>AUC ratio Geom Mean</th>
<th>90% CI lower</th>
<th>90% CI upper</th>
<th>Var</th>
<th>AUC ratio Geom Mean</th>
<th>90% CI lower</th>
<th>90% CI upper</th>
<th>Var</th>
<th>AUC ratio Geom Mean</th>
<th>90% CI lower</th>
<th>90% CI upper</th>
<th>Var</th>
<th>AUC ratio Geom Mean</th>
<th>90% CI lower</th>
<th>90% CI upper</th>
<th>Var</th>
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<td>AZ9- itraconazole</td>
<td>1.3</td>
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<td>1.5</td>
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<td>0.7</td>
<td>0.6</td>
<td>0.8</td>
<td>0.005</td>
<td>1.4</td>
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<td>0</td>
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<td>AZ10- ketokonazole le</td>
<td>5.9</td>
<td>4.5</td>
<td>7.8</td>
<td>0.021</td>
<td>3.4</td>
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<td>4.5</td>
<td>0.024</td>
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<td>6.8</td>
<td>7.9</td>
<td>0.019</td>
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<td>0.0155</td>
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<tr>
<td>AZ5- esfampolin</td>
<td>0.17</td>
<td>0.14</td>
<td>0.2</td>
<td>0.008</td>
<td>0.35</td>
<td>0.29</td>
<td>0.42</td>
<td>0.008</td>
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<td>0.0082</td>
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<td>0.0202</td>
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<td>0.004</td>
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<td>0.17</td>
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<td>0.04</td>
<td>0.13</td>
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<td>0.003</td>
<td>0.84</td>
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<td>0.0023</td>
<td>0.16</td>
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<td>AZ11- midazolam</td>
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<td>0.64</td>
<td>1.02</td>
<td>0.018</td>
<td>na</td>
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<td>na</td>
<td>0.16</td>
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<td>0.76</td>
<td>0.0004</td>
<td>0.06</td>
<td>0.89</td>
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</table>

CI: Confidence interval; Var: variance; Geom. Mean: geometric mean; Rss: AUC ratio from Simcyp V11 using the default average systemic inhibitor concentration at steady-state.

Interactions of AZ4 and AZ5 with metoprolol are mediated by CYP2D6; All other interactions are CYP3A4 mediated; na: not available

*First pass refers to hepatic first pass contributions for the CYP inhibition interactions, and intestinal gut contributions for CYP induction interactions (the last 5 interactions)
Table 6: RMSE for static and dynamic DDI predictions

<table>
<thead>
<tr>
<th></th>
<th>Root mean square error (RMSE)</th>
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</thead>
<tbody>
<tr>
<td>Simcyp AUC ratio - $R_{SS}$</td>
<td>43.7</td>
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<tr>
<td>Simcyp AUC ratio – time-based</td>
<td>12.9</td>
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<tr>
<td>Simcyp $C_{max}$ ratio – time-based</td>
<td>5.9</td>
</tr>
<tr>
<td>Static AUC ratio $I_{max}$ without first pass</td>
<td>4.7</td>
</tr>
<tr>
<td>Static AUC ratio $I_{inlet}$ without first pass</td>
<td>14.9</td>
</tr>
<tr>
<td>Static AUC ratio $I_{sys}$ without first pass</td>
<td>2.4</td>
</tr>
<tr>
<td>Static AUC ratio $I_{sys}$ with first pass</td>
<td>3.8</td>
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</table>
Table 7: Summary of prediction performance (with respect to AUC ratios) of Simcyp V11 (time-based) and static equations with \( I_{sys} \)

<table>
<thead>
<tr>
<th>N</th>
<th>Simcyp V11 (time-based)</th>
<th>Static equations (without hepatic first correction)</th>
<th>Static equations (with hepatic first correction)</th>
<th>Simcyp V11 (time-based)</th>
<th>Static equations (without hepatic first correction)</th>
<th>Static equations (with hepatic first correction)</th>
<th>Simcyp V11 (time-based)</th>
<th>Static equations (without hepatic first correction)</th>
<th>Static equations (with hepatic first correction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted within 2-fold</td>
<td>Over-predicted</td>
<td>Under-predicted</td>
<td>Predicted within 2-fold</td>
<td>Over-predicted</td>
<td>Under-predicted</td>
<td>Predicted within 2-fold</td>
<td>Over-predicted</td>
<td>Under-predicted</td>
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<td>4</td>
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<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

nc: not calculated.
Figure 1

**Over-prediction**
1. Inhibitor: efflux transporter-mediated reduction in intracellular concentrations
2. Simultaneous mechanisms: concurrent inhibition and induction of enzyme

**Under-prediction**
1. Inhibitor: uptake transporter-mediated increase in intracellular concentrations
2. Simultaneous mechanisms: inhibitory metabolites, multiple inhibitors, enzyme and transporter inhibition

**DDI risk prediction in preclinical**

% contribution of CYPs from rCYPs

1A2
2C8
2C9
2C19
2D6
3A4

Ki from IC50: assumption of reversible inhibition, Michaelis-Menten kinetics
Figure 2
Figure 5
Figure 6c

AUC ratio
Cmax ratio

Variance ratio (Simcyp V1:Clinic)

Geometric mean of geometric means