Projections of Drug-Drug Interactions Caused by Time-Dependent Inhibitors of Cytochrome P450 1A2, 2B6, 2C8, 2C9, 2C19, and 2D6 Using In Vitro Data in Static and Dynamic Models

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Abbreviations: ADME, absorption, distribution, metabolism, and excretion; AUC, area under the concentration vs time curve; AUC_i, area under the concentration vs time curve when co-administered with an inhibitor; AUCR, area under the plasma concentration-time curve ratio in the inhibited and control state; C_{max}, maximum concentration values; C_{avg}, average concentration values; C_{max,u}, unbound maximum concentration values; C_{avg,u}, unbound average concentration values; CYP, cytochrome P450; DDI, drug-drug interaction; f_u, fraction unbound; HHEP, human hepatocyte; HLM, human liver microsome; IC_{50}, inhibitory concentration at 50%; [I], inhibitor concentration; [I]_g, intestinal inhibitor concentration; [I]_h, liver inhibitor concentration; K, reversible inhibition constant; K_i, time-dependent inhibition constant; k_{inact}, maximal rate of enzyme inactivation; k_{obs}, rate constant for inhibition; K_{p,uu}, unbound partition coefficient in hepatocytes; LC-MS/MS, liquid chromatography-tandem mass
spectrometry; m/z, mass to charge ratio; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PBPK, physiologically-based pharmacokinetic modeling; TDI, time-dependent inhibition; WEM, William’s E medium.
ABSTRACT

In vitro time-dependent inhibition (TDI) kinetic parameters for cytochrome P450 (CYP) 1A2, 2B6, 2C8, 2C9, 2C19, and 2D6, were determined in pooled human liver microsomes for 19 drugs (and 2 metabolites) for which clinical drug-drug interactions (DDI) are known. In vitro TDI data were incorporated into the projection of the magnitude of DDIs using mechanistic static models and Simcyp®. Results suggest that for the mechanistic static model, use of estimated average unbound exit concentration of the inhibitor from the liver resulted in a successful prediction of observed magnitude of clinical DDIs and was similar to Simcyp®. Overall, predictions of DDI magnitude (i.e., fold increase in AUC of a CYP-specific marker substrate) were within 2-fold of actual values. Geometric mean-fold errors were 1.7 and 1.6 for static and dynamic models, respectively. Projections of DDI from both models were also highly correlated to each other (r² = 0.92). This investigation demonstrates that DDI can be reliably predicted from in vitro TDI data generated in HLM for several CYP enzymes. Simple mechanistic static model equations as well as more complex dynamic PBPK models can be employed in this process.
SIGNIFICANCE STATEMENT:

Cytochrome P450 time-dependent inhibitors (TDI) can cause drug-drug interactions (DDI). An ability to reliably assess the potential for a new drug candidate to cause DDI is essential during drug development. In this report, TDI data for 19 drugs (and 2 metabolites) were measured and used in static and dynamic models to reliably project the magnitude of DDI resulting from inhibition of CYP1A2, 2B6, 2C8, 2C9, 2C19, and 2D6.
INTRODUCTION

Drug-drug interactions (DDI) can arise when one drug inhibits or induces the expression of a
drug metabolizing cytochrome P450 (CYP) enzyme and thus affects the exposure to a second drug that is
cleared by that enzyme. There are myriad examples of this: some that cause marked changes that can
impact efficacy and safety and others that can cause measurable changes in pharmacokinetics but with no
meaningful clinical impact (Certara Drug Interaction Database (DIDB; www.druginteractionsolutions.org)). In the design of new drugs, considerable effort is expended on this
phenomenon, with a goal to bring forth candidate compounds that do not inhibit or induce CYP activities
so that DDI will not occur. In vitro assays are employed to test newly synthesized compounds for
inhibition, inactivation, and induction of those human CYP enzymes most frequently involved in drug
metabolism (Grimm et al., 2009). Data from these assays are used to either make binary decisions on
candidate compound viability or, when used in conjunction with other information, to make estimates of
the magnitude of DDI that could be anticipated in the clinic.

Among the various mechanisms by which CYP activities can be altered, time-dependent
inhibition has proven to be the most challenging for which to accurately project DDI from in vitro data.
This has been investigated most thoroughly for CYP3A4 (Mayhew et al., 2000; Obach et al., 2007; Vieira
et al., 2014). Many compounds can demonstrate TDI against CYP3A4 in vitro, however many do not
cause clinically significant DDI (Zimmerlin et al., 2011; Eng et al., 2020). This can be due to a variety of
reasons, the most obvious of which is that in vivo concentrations of the TDI are not high enough to cause
a meaningful decrease in CYP3A4 activity. Fundamental models used to project the magnitude of in vivo
DDI (AUC/AUC) from in vitro data require three basic parameters as per the equation originally put
forth by Rowland and Matin (Rowland and Matin, 1973): (1) the fraction of total clearance of the affected
drug that is catalyzed by the affected enzyme, $f_m$; (2) the concentration of the inhibitor at the affected
enzyme, $[I]_{in vivo}$; and (3) the intrinsic potency of the inhibitor, $K_i$. In the case of TDI, the latter term is
more complicated in that the maximal rate constant of time-dependent inhibition ($k_{inact}$) caused by the
inhibitor and the basal rate of enzyme re-synthesis (which at steady-state is equal to the natural rate of enzyme degradation, $k_{deg}$) must also be included:

$$\frac{AUC_i}{AUC} = \frac{1}{f_m + \left(\frac{k_{inact} \cdot [I]_{in \, vivo}}{k_{deg} \cdot K_I}\right) + (1 - f_m)}$$

A key to success in projecting in vivo DDI from in vitro TDI data using this approach is the selection of the most appropriate value for $[I]_{in \, vivo}$. It was previously shown that simply employing estimates of total intestinal lumen concentrations and total liver concentrations yields marked over-projections of in vivo DDI (Obach et al., 2006; Einolf, 2007; Vieira et al., 2014). Consideration of plasma protein binding greatly improves the agreement between actual DDI and values projected from in vitro data. Furthermore, it was shown that using estimates of unbound inhibitor concentrations egging from the target tissues (i.e. systemic for liver and portal vein for intestine) yielded the most reliable projections of in vivo DDI for CYP3A4 TDI (Tseng et al., 2021). For this to work it must be presumed that free concentrations within the target organ are equal to free concentrations in plasma emanating from that organ and this may not always be the case for TDI that are good substrates for active transporters. Nevertheless, using this approach yielded the most accurate projections of DDI from in vitro TDI data, and when defining a clinically meaningful DDI as one wherein the drug exposure increases by 1.25-fold, there were no false negatives and only a ~25% rate in false positive predictions using TDI data generated in pooled human liver microsomes. However in that analysis, TDI was only evaluated for CYP3A4 and while that enzyme is the CYP most frequently subject to TDI, there are some known instances of TDIs for other CYPs that generate meaningful clinical DDI (e.g. paroxetine for CYP2D6; zileuton for CYP1A2 and so forth; (Granneman et al., 1995; Alderman et al., 1997).

In this report, in vitro time-dependent inhibition data for several drugs were determined for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6. These hepatic enzymes represent the
set that are major contributors to human drug metabolism, other than the aforementioned CYP3A4. The objective was to determine if the previously described methods that were developed to project clinical DDI from in vitro time-dependent inhibition data for CYP3A4 (Tseng et al., 2021) will also work for the other major human CYP enzymes. These findings build on a recent report (Ramsden et al., 2022) and comparisons of those data and conclusions with projections of clinical DDI for non-CYP3A enzymes generated in this study.
MATERIALS AND METHODS

Materials. Research was conducted on human tissue acquired from a vendor that was verified as compliant with internal policies, including IRB/IEC approval. Pooled HLM (prepared contractually for Pfizer), consisting of 36 male and 14 female donors, were purchased from Sekisui XenoTech (Kansas City, KS). Pooled mix-gender human plasma collected with K$_3$EDTA was purchased from BioIVT (Westbury, NY). Monobasic and dibasic potassium phosphate buffers, magnesium chloride, β-nicotinamide adenine dinucleotide phosphate reduced form (NADPH), Dulbecco’s phosphate-buffered saline (DPBS), DMSO, acetaminophen, amodiaquine HCl, diclofenac, dextromethorphan hydrobromide, dextrophan tartrate, and phenacetin were purchased from Sigma-Aldrich (St. Louis, MO). S-Mephenytoin was purchased from Toronto Research Chemicals (North York, Ontario, Canada). $^{[13]C_6}$-4’ hydroxydiclofenac and $^{[2}H_3$-dextrophan were purchased from Cerilliant (Round Rock, Texas). $^{[2}H_7$-acetaminophen, bupropion, hydroxybupropion, $^{[3}H_8$-hydroxybupropion, N-desethylamodiaquine, $^{[2}H_3$ N-desethylamodiaquine, 4’-hydroxydiclofenac, 4’-hydroxymephenytoin, $^{[2}H_3$-4’-hydroxymephenytoin were synthesized at Pfizer (Groton, CT). Commercially obtained chemicals and solvents were of high-performance liquid chromatography or analytical grade. Tested drugs (typical purity >95%) were either synthesized internally at Pfizer (Groton, CT) or purchased from one of the following sources: Sigma-Aldrich (St. Louis, MO), Toronto Research Chemicals (North York, Ontario, Canada), MedChemExpress (Monmouth Junction, NJ), TCI (Portland, OR), USP. United States Pharmacopeia (Rockville, MD), or APEXBio (Houston, TX).

Identification of Test Drugs. The Certara Drug Interaction Database (DIDB; www.druginteractionsolutions.org) was used to compile a list of drugs for which clinical inhibition interaction studies were conducted (Table 1). Studies in which the CYP probe substrate was dosed via the oral route were chosen. The magnitude of DDI (AUCR) was determined based on the ratio of the probe substrate AUC in the presence (AUC$_i$) and absence (AUC) of the test
inhibitor drug. When more than one clinical DDI study existed for a given substrate-inhibitor pair, the study demonstrating the greatest AUCR was used for in vitro-in vivo comparison.

**Binding to Plasma and Liver Microsomes.** Binding of the test drugs to human plasma and liver microsomes (0.81 mg/mL) were determined based on methods essentially as previously described (Di et al., 2017). Briefly, binding experiments were performed by equilibrium dialysis (EqD). Binding matrix was mixed with 2 µM test drug (donor side) and allowed to dialyze against Dulbecco’s phosphate buffered saline DPBS (receiver side) for a duration of 6 hours. The membrane had 12000-14000 molecular weight cutoff and was incubated in a humidified incubator supplemented with 5% CO₂ at 37°C. All incubations were performed in quadruplicate. At equilibrium, matrix and buffer samples were collected and matrix matched before addition of 3.3-volumes of acetonitrile containing a mixture of internal standards (50 ng/mL indomethacin and 5 ng/mL terfenadine). Samples were vortexed and centrifuged for 5 minutes at approximately 2300 x g at room temperature. The supernatant was collected and analyzed directly by LC-MS/MS. Determination of the fraction unbound (f_u) in human plasma and liver microsomes, were determined as previously described (Riccardi et al., 2017). Fraction unbound in microsomes was scaled appropriately (Austin et al., 2002) to microsomal concentrations used in either IC₅₀ or TDI experiments prior data analysis. For some compounds, plasma protein binding values were obtained from the Certara DIDB.

**IC₅₀ determination in Human Liver Microsomes.** HLM was used to determine the inhibitory concentration of the test drug to result in 50% (IC₅₀) of CYP1A2, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 enzyme activity remaining. HLM incubations of 0.03 or 0.1 mg/mL were supplemented with MgCl₂ (3.3 mM) and NADPH (1.3 mM) in potassium phosphate buffer (100 mM, pH 7.4). Drug stocks, at incrementing concentrations, were prepared at 100-times the final incubation concentration (up to 100 µM final) in a mixture of organic solvent (such as acetonitrile) and water. The probe substrate was prepared at 10x the final concentration (corresponding to K_M in HLM; Supp. Table 1) in potassium phosphate buffer. The final total organic solvent in the incubations was ≤ 1%. The incubation was
initiated with the addition of drug stock immediately followed by the probe substrate. After a substrate incubation in HLM ranging 10-20 minutes, depending on the CYP isoform, the incubation was terminated by the addition of two volumes of acetonitrile containing internal standard. All incubations were carried out at 37°C, at a final volume of 200 µL, in duplicate. Samples were vortexed and centrifuged for 5 minutes at approximately 2300 x g at room temperature. A standard curve of the respective metabolite and inhibitor interference quality control (iQC) was prepared in duplicate at the final protein concentration in the assay for HLM. The inhibitor concentration included in the iQC was determined based on the highest concentration tested in the assay. Standards and iQC samples were processed in the same manner as the incubation samples.

**Time-dependent Inhibition in Human Liver Microsomes.** Time-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 was measured in HLM at 0.3 or 0.5 mg/mL depending on the isoform tested and supplemented with MgCl₂ (3.3 mM) and NADPH (1.3 mM) in potassium phosphate buffer (100 mM, pH 7.4). Drug stock solutions, typically up to 300 µM (final), were prepared as described above (Supp. Table 2). The final total solvent in the primary incubations was ≤ 1%. The incubation was initiated with the addition of drug stock to the microsomal mixture. At various time points, generally up to 40 minutes, an aliquot of the mixture was transferred to an activity incubation mixture containing the probe substrate at 5-20-fold Kᵟ in HLM, MgCl₂ (3.3 mM), and NADPH (1.3 mM) in potassium phosphate buffer (100 mM, pH 7.4), generally resulting in a 20 to 60-fold dilution. After the predetermined reaction time, the activity reaction was terminated by the addition of two volumes of acetonitrile containing internal standard (Supp. Table 1). All reactions were carried out at 37°C, in a final volume of 200 µL, in duplicate. Samples were vortexed and centrifuged for 5 minutes at approximately 2300 x g at room temperature. Depending on the substrate probe, the supernatant was either first dried down under nitrogen stream or mixed with an equal volume of 5/95 acetonitrile/water containing 0.1% formic acid and analyzed directly by LC-MS/MS. A standard curve for the respective metabolite and an iQC of each tested drug was prepared in duplicate at the final protein
concentration in the assay for HLM. The inhibitor concentration included in the iQC was determined based on the highest concentration tested in the assay divided by the fold-dilution of the primary to secondary incubation. Standards and iQC samples were processed in the same manner as the incubation samples. For several drugs, assay conditions were modified due to rapid inactivation or observation of potent inhibition at the initial time point. Modifications to the incubation conditions can be found in Supp. Table 2.

**LC-MS/MS Methodology for the Quantitation of Substrate Marker Assay.** LC-MS/MS analysis was conducted on either a Sciex 5500 or 6500 triple quadrupole mass spectrometer (Framingham, MA) fitted with an electrospray ion source operated in positive ion mode using multiple reaction monitoring. An Agilent 1290 binary pump (Santa Clara, CA) with a CTC Leap autosampler (Leap Technology, Carrboro, NC) was programmed to inject 10 µL of sample on column. A binary gradient was employed using 0.1% (v/v) formic acid in water (mobile phase A) and 0.1% (v/v) formic acid in acetonitrile (mobile phase B) at a flow rate of 0.5 mL/min. Mass-to-charge (m/z) transitions for analytes and column information can be found in Supp. Table 1. Analytes were quantified against a standard curve using Analyst software (Sciex). A linear regression with either a weighting of 1/x or 1/x^2 was used. Standards and iQCs were accepted if the calculated concentrations were ± 25% of their nominal concentration. Acceptance of the iQC demonstrates that the inhibitor did not interfere with the respective metabolite signal on the MS.

**Data Analysis**

**Estimation of K_i.** Percent activity remaining of the CYP enzyme was determined by normalizing the concentration of marker metabolite in the presence of varying concentrations of test drug to the concentration of marker metabolite in the solvent control. The concentration of inhibitor corresponding to a 50% decrease in activity (IC_{50}) was generated using GraphPad Prism 9 (La Jolla, CA). Since inhibition experiments were conducted at the K_M of the substrate, K_i was estimated as IC_{50} divided by two (IC_{50}/2).
assuming competitive inhibition (Cheng and Prusoff, 1973). Free $K_i$ was determined by correcting $K_i$ with the free fraction determined in microsomes ($f_{u,mic}$) for HLM. Measured IC$_{50}$ values are listed in Supp. Table 6.

**Estimation of $K_I$ and $k_{\text{inact}}$.** Data analysis methods previously described by Yates and co-workers (Yates et al., 2012) were used for the estimation of $k_{\text{obs}}$, $K_I$, and $k_{\text{inact}}$. Briefly, $k_{\text{obs}}$ was determined by normalizing the marker metabolite concentration in each sample to that of the mean solvent control concentration in the initial time point, plotting the natural log of percent remaining activity versus preincubation time, and then calculating the slope of the line ($-k_{\text{obs}}$) using the initial linear portion of the curve. A statistical test was done at each concentration of test drug to determine if $k_{\text{obs}}$ was statistically different from the within-experiment solvent control, i.e. a parallel lines test, shown in eq. 1.

$$Z = \frac{|k_{\text{obs}}[I] - k_{\text{obs}}[0\mu M]|}{\sqrt{SE^2_{k_{\text{obs}}[I]} + SE^2_{k_{\text{obs}}[0\mu M]}}} \quad \text{(eq. 1)}$$

Here $k_{\text{obs}}[I]$, $k_{\text{obs}}[0\mu M]$, and SE represent the inactivation rate for an inhibitor at a single concentration, inactivation rate with solvent control, and standard error, respectively. A statistically significant TDI is defined when the parallel lines test yields a p-value of <0.05. When possible, $K_I$ and $k_{\text{inact}}$ parameters were determined using nonlinear regression of the three-parameter Michaelis-Menten equation below:

$$k_{\text{obs}} = k_{\text{obs}}[0\mu M] + \frac{k_{\text{inact}} \times [I]}{K_I + [I]} \quad \text{(eq. 2)}$$

where $[I]$ represents the concentrations of the test drug in the primary incubation, $k_{\text{inact}}$ is the maximal inactivation rate, and $K_I$ is the inactivator concentration at half $k_{\text{inact}}$.

For compounds where substrate inhibition was observed at the higher concentrations, nonlinear regression of a four-parameter substrate inhibition model (eq. 3) was used to fit the curve:
\[
k_{o bs} = k_{o bs[0\mu M]} + \frac{k_{i na c t} \times [I]}{K_I + [I]} (\text{eq. 3})
\]

where, \( K_I \) is a dissociation constant for binding enabling a better fit of the data.

In six instances, the relationship between \( k_{o bs} \) and \([I]\) did not yield enough of a hyperbola (saturable kinetics) to yield reliable values for \( K_I \) and \( k_{i na c t} \). For these, a composite slope was determined that represents the ratio of \( k_{i na c t}/K_I \). The static model equations and Simcyp require individual parameters as input values. To accomplish this, \( K_{I,u} \) was arbitrarily set at a high value of 1 mM, and the slope was used to calculate \( k_{i na c t} \). This value for \( k_{i na c t} \) along with the value of 1 mM for \( K_{I,u} \) were then used as input values for DDI projections, under the reasonable assumption that \([I]_{in \, v i v o} << 1 \, mM\). For compounds where \( K_I \) can be determined, \( K_{I,u} \) was determined by correcting \( K_I \) with \( f_{u,mi c} \) for HLM. Analyses were performed using Microsoft Excel (Redmond, WA) and GraphPad Prism 9 (La Jolla, CA).

**Predicting Magnitude of DDIs.** The magnitude of DDI (AUCR) can be described as a ratio of \( \text{AUC}_i \) divided by \( \text{AUC} \). Mathematical models to determine the extent of DDI while incorporating competitive inhibition and time dependent inactivation in the liver have been extensively described (Rowland and Matin, 1973; Mayhew et al., 2000; Wang et al., 2004; Obach et al., 2006; Obach et al., 2007; Fahmi et al., 2008) and is summarized in the equation below (eq. 4):

\[
AUCR = \frac{\text{AUC}_i}{\text{AUC}} = \left( \frac{1}{1 + \left( \frac{[I]_{h}}{K_I} \right) \left( \frac{k_{i na c t} \times [I]_{h}}{K_I + [I]_{h} \times k_{de g,CYPX,h}} \right)} \times f_{m(CYPX)} \right) + (1 - f_{m(CYPX)}) (\text{eq. 4})
\]

In the above equation, \( k_{de g} \) represents the hepatic (degradation rates of the CYP enzyme). The hepatic \( k_{de g} \) for CYP1A2, CYP2C8, CYP2C19, and CYP2D6, were based on the turnover half-lives estimated from clinical DDI studies. CYP2B6 and CYP2C9 hepatic \( k_{de g} \) were based on half-lives determined from in vitro methods (Supp. Table 3). The fraction of the victim drug metabolized by the CYP\(_X\) enzyme is represented as \( f_{m(CYPX)} \) and listed in Supp. Table 4. The term \([I]_{h}\) represents the estimated unbound
concentration of the inhibitor in the liver to which the target CYP enzyme is exposed. Unbound test drug concentrations ($C_u$) resulting in enzyme inhibition in the liver were estimated as unbound steady state maximum ($C_{max,hepatic\ inlet,u}$) or average ($C_{avg,hepatic\ inlet,u}$) hepatic inlet concentration using the equation below (eq. 5a and 5b) (Kanamitsu et al., 2000) or unbound steady state maximum ($C_{max,systemic,u}$) or average ($C_{avg,systemic,u}$) systemic concentrations. Calculated [I]$h$ concentrations are listed in Supp. Table 36.

\[
C_{max,hepatic\ inlet,u} = f_{u,p} \times (C_{max} + \frac{F_a \times F_g \times k_a \times \text{Dose}}{BPR \times Q_h}) \quad (eq. \ 5a)
\]

\[
C_{avg,hepatic\ inlet,u} = f_{u,p} \times \left( C_{avg} + \frac{F_a \times F_g \times k_a \times \text{Dose}}{\tau \times BPR \times Q_h} \right) \quad (eq. \ 5b)
\]

In the equation above, $k_a$ is the oral absorption rate of the test drug (or 0.1 min$^{-1}$), $F_a$ is the fraction of test drug absorbed following oral administration (assume unity), $F_g$ is the fraction of test drug escaping first-pass intestinal metabolism (assume unity), $f_{u,p}$ is the free fraction of the test drug in plasma, BPR is the blood-to-plasma ratio (assume 1), tau is the inhibitor dosing interval, and $Q_h$ is the liver blood flow (1617 mL/min (Yang et al., 2007)). All inputs used towards the prediction of DDIs can be found in Supp. Table 5. The $k_a$ was calculated as $0.693/(t_{max,\ in\ vivo}/5)$ assuming rate of absorption equals rate of elimination at $t_{max}$ and absorption is >90% complete after 5 absorption half-lives as describe by Holford (Holford, 2016). $t_{max}$ of each inhibitor was obtained from clinical studies reported in the Certara Drug Interaction Database. Alternatively, for a test drug, the predicted $k_a$ or $t_{max}$ from human PK predictions could be used.

**Simcyp Modeling.** Simcyp version 20 release 1 (20.0; Certara, Princeton, NJ) was used to simulate the time course of victim and perpetrator concentrations in plasma. Simulations were conducted using a design of 10 trials with 10 subjects using the age range of 20–50 years and 1:1 male to female ratio. Simulations were performed in a virtual population library of healthy volunteers supplied by Simcyp (Sim-Healthy Volunteers). The hepatic $k_{deg}$ for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 were 0.0183, 0.0099, 0.0267, 0.0067, 0.0301 and 0.0217 hr$^{-1}$, respectively. In HV population, frequency of CYP1A2 and CYP2C8 extensive metabolizers (EMs) were set to 100%
The frequency of CYP2C9, CYP2C19, CYP2D6 and CYP2B6 extensive metabolizers (EMs) were changed to 100% during the related CYP DDI simulations. DDI modeling of ticlopidine with omeprazole was conducted in a Japanese population with 100% CYP2C19EM subjects, similar to that reported by Ieiri et al. (Ieiri et al., 2005). The intestinal concentrations were the enterocyte exit concentration (portal vein) with $f_{u,\text{int}} = f_{u,\text{plasma}}$, whereas liver concentrations were the liver exit concentration. A summary of the trial designs for all simulations are listed in Supp. Table 7. In the DDI studies, the fold-increase in AUCR (e.g., $\text{AUC}_{\infty}$ ratios in the single-dose studies and AUC, ratios in the multiple-dose studies) was calculated from the ratios of the simulated values in treatment groups relative to control groups. Geometric means of pharmacokinetic parameters generated from Simcyp simulations were compared with the clinically observed geometric mean parameters.

For perpetrator drugs ticlopidine, fluvoxamine, paroxetine, esomeprazole, gemfibrozil and gemfibrozil acyl glucuronide compound files were qualified by Simcyp. To maintain consistent with the use of input parameters generated internally (i.e., $f_{u,p}$, $K_I$, $k_{\text{inact}}$, etc.), the steady-state volume of distribution ($V_{ss}$) and oral clearance were adjusted to adequately recover the clinically observed AUC and $C_{\text{max}}$ at the dose used in the DDI study. In predicting CYP2D6 inhibitory effect of amiodarone administration, we have incorporated the inhibitory effects of its primary metabolite ($N$-desethylamiodarone). Amiodarone Simcyp compound file was obtained from literature and further qualified by adjusting $V_{ss}$ and CL (Kimoto et al., 2019).

Input parameters of 19 perpetrators (plus two metabolites) are summarized in Supp. Tables 8-25. Briefly, physicochemical properties (pKa, logP) are obtained from in silico software (ACD/pKa; BioByte). Plasma binding ($f_{u,p}$) was obtained from Certara DIDB or internal experimental data (Supp. Table 5). The apparent permeability ($P_{\text{app}}$) values used were either default values included as part of the qualified Simcyp files (Simcyp) or calculated from in silico models (Keefer et al., 2013) and extrapolated to effective permeability in human ($P_{\text{eff,man}}$). $k_{u}$, $V_{ss}$ and $\text{CL}_{\text{po}}$ were fitted manually to adequately recover the clinically observed results at the doses used in the DDI studies (Supp. Table 38). These values were internally consistent with those used in static projections (model 4).
For victim drugs, caffeine, theophylline, dextromethorphan, metoprolol, desipramine, omeprazole, S-warfarin, repaglinide and bupropion, Simcyp library compound files were qualified by Simcyp and used without modification. The pantoprazole Simcyp model was developed by FDA using Simcyp version 15 and was changed from ADAM to FO absorption model. The pantoprazole model could reasonably recover both PK and $f_{m(CYP2C19)}$. Input parameters for the victim drugs are summarized in Supp. Tables 26-35.

**Accuracy of Predictions.** Accuracy of the various prediction models were assessed by the average fold error (AFE) or bias (eq. 6). Precision of the predictions were evaluated using geometric mean absolute fold error (GMFE, eq. 7) and root mean square fold error (RMSFE, eq. 8).

\[
AFE = 10^{ \frac{\sum \log \frac{\text{predicted DDI}}{\text{actual DDI}}}{N} } \quad (\text{eq. 6})
\]

\[
GMFE = 10^{ \frac{\sum |\log \frac{\text{predicted DDI}}{\text{actual DDI}}|}{N} } \quad (\text{eq. 7})
\]

\[
RMSFE = \sqrt{ \frac{\sum \left(\log \frac{\text{predicted DDI}}{\text{actual DDI}}\right)^2}{N} } \quad (\text{eq. 8})
\]

where $N$ is the total number of predictions.
RESULTS

**Inhibition in Human Liver Microsomes.** Nineteen compounds (and two metabolites) with reported clinical DDIs identified from the Certara DIDB (https://www.druginteractionsolutions.org/) were chosen to evaluate reversible inhibition and TDI for CYP 1A2, 2B6, 2C8, 2C9, 2C19 or 2D6 (Table 1). In vitro measurement of reversible and TDI kinetic parameters were generated in human liver microsomes. While most of the compounds were selective to a singular CYP isoform, compounds such as ticlopidine, fluvoxamine, and amiodarone were evaluated across multiple CYPs based on reported drug interaction studies. Additionally, for compounds such as gemfibrozil and amiodarone, where metabolites are known to exhibit TDI (Yu et al., 2015), inhibition kinetics of the metabolite were also generated. Reversible and time-dependent inhibition kinetic parameters along with free fraction in microsomes are listed in Table 2 and the % control activity vs incubation time and \(k_{\text{obs}}\) vs [I] plots for each drug are shown in Supp. Figures 1-25. In general, the \(K_i\), \(K_I\), and \(k_{\text{inact}}\) values generated were within 3-fold of those in the published literature. Minor potency differences were likely due to variation in study protocols.

**CYP1A2.** Furafylline, zileuton, ticlopidine, isoniazid, rofecoxib, and fluvoxamine were evaluated for their reversible and TDI potential in human liver microsomes using phenacetin O-deethylase as the probe reaction. Values for \(K_i\), \(K_I\), and \(k_{\text{inact}}\) ranged from 0.011 – 194 μM, 0.05-87.0 μM, and 0.0566-0.175 min\(^{-1}\), respectively. Isoniazid was shown to be a weak reversible (\(K_{i,u}\) 194 μM) and time dependent inhibitor (\(k_{\text{inact}}/K_{i,u}\) 0.150 mL∙min\(^{-1}\)∙µmol\(^{-1}\)). Conversely, the inhibition observed by fluvoxamine was highly potent with a \(K_i\), \(K_I\), and \(k_{\text{inact}}\) of 0.011 μM, 0.0514 μM, and 0.0661 min\(^{-1}\), respectively. To adequately measure TDI for fluvoxamine, a 60-fold dilution of the inactivation incubation was necessary to further dilute reversible inhibition effects during the substrate activity measurement. Potent reversible inhibitors such as fluvoxamine may often mask the potential for TDI if appropriate experimental considerations are not taken.
**CYP2B6.** Ticlopidine and clopidogrel were evaluated for their reversible and TDI potential in human liver microsomes using bupropion hydroxylase as the probe reaction. Both compounds demonstrated similar reversible and time-dependent inhibition. Values for $K_i$, $K_I$, and $k_{inact}$ ranged 0.162 – 0.201 μM, 0.122 – 0.352 μM, and 0.243 - 0.354 min$^{-1}$, respectively.

**CYP2C8.** Gemfibrozil glucuronide was evaluated for reversible and TDI potential in human liver microsomes using amodiaquine $N$-deethylase as the probe reaction. It demonstrated reversible and time-dependent inhibition with $K_i$, $K_I$, and $k_{inact}$ values of 13.1 μM, 18.9 μM, and 0.214 min$^{-1}$, respectively. Substrate inhibition was observed at higher concentrations and kinetic parameters were fitted using equation 3. It has been reported that gemfibrozil glucuronide metabolite, and not gemfibrozil itself, is responsible for CYP 2C8 TDI (Ogilvie 2006). Therefore, evaluating the potency of the metabolite directly is advantageous over assessment of gemfibrozil alone. The assumption should not be made that the amount of the metabolite generated in an in vitro system will equate that which is observed in vivo to elicit the same extent of inhibition.

**CYP2C9.** Tienilic acid, amiodarone, mifepristone, and fluvoxamine were evaluated for their reversible and TDI potential in human liver microsomes using diclofenac 4'-hydroxylase as the probe reaction. While all compounds demonstrated potent reversible inhibition towards CYP2C9, no TDI was observed for mifepristone and fluvoxamine. Values for $K_i$, $K_I$, and $k_{inact}$ ranged from 0.118 – 2.71 μM, 0.715 – 6.91 μM, and 0.00772 - 0.257 min$^{-1}$, respectively. Amiodarone demonstrated substrate inhibition at higher concentrations and kinetic parameters were fitted using equation 3.

**CYP2C19.** Ticlopidine, esomeprazole, fedratinib, osilodorstat, and fluvoxamine were evaluated for their reversible and TDI potential in human liver microsomes using (S)-mephenytoin 4'-hydroxylase as the probe reaction. Fedratinib, while shown to be a TDI, demonstrated only weak reversible potency against CYP2C19 with $K_i > 50.0$ μM. Fluvoxamine did not exhibit TDI as inactivation parameters were
not measurable, but it was a potent reversible inhibitor. Values for $K_i$, $K_r$, and $k_{\text{inact}}$ for the six drugs evaluated ranged from $0.129 - >50.0 \mu M$, $1.09 - 15.9 \mu M$, and $0.00673 - 0.114 \text{ min}^{-1}$. Ticlopidine, esomeprazole, and fedratinib demonstrated substrate inhibition at higher concentrations and kinetic parameters were fitted using equation 3.

**CYP2D6.** Paroxetine, MDMA, amiodarone, $N$-desethylamiodarone, hydralazine, mirabegron, and dronedarone were evaluated for their reversible inhibition and TDI potential for CYP2D6 in human liver microsomes using dextromethorphan $O$-demethylase as the marker reaction. Hydralazine demonstrated weak reversible potency against CYP2D6 with $K_i >50.0 \mu M$. Hydralazine and dronedarone did not exhibit TDI potential and inactivation parameters were not measurable. Amiodarone and its oxidative metabolite $N$-desethylamiodarone were both evaluated. $N$-desethylamiodarone displayed greater inhibition potency than parent amiodarone. This is another example which emphasizes the need to evaluate the metabolite rather than depending on the formation of the metabolite in an incubation with the parent drug. Values for $K_i$, $K_r$, and $k_{\text{inact}}$ ranged from $0.615 - 15.4 \mu M$, $1.09 - 15.9 \mu M$, and $0.00925 - 0.444 \text{ min}^{-1}$, respectively.

**Projection of DDI from TDI Data in Liver microsomes: Static Method.** Projections of DDI were made using the mechanistic static model (Equation 4), targeting the overall ratio of AUC values for the victim drug in the inhibited and control state. The mechanistic static model represents a more relevant assessment compared to the basic R2 model (FDA, 2020) through the incorporation of victim and perpetrator specific input parameters. In addition to experimentally derived inhibition parameters reported here, input values such as $f_{\text{inc}}$ and $f_{\text{plasma}}$ were also experimentally derived unless noted. Additional parameters such as $f_m$ of each substrate, $k_{\text{deg}}$, and organ inlet and outlet blood flows, were all input parameters gathered from literature (Supp. Tables 3-5).

The four models evaluated are represented in Table 3 and Figure 1, where current input parameters as recommended in the U.S. FDA regulatory guidance document on drug-drug interactions.
was also evaluated (referred to as Model 1). Models 1 and 2 represent inhibitor concentrations calculated from entrance \(C_{\text{max}}\) and \(C_{\text{avg}}\) concentrations into the liver, respectively. Model 1, as recommended in regulatory guidance, used a very rapid rate of absorption (proximate to the rate of gastric emptying) and yielded poor performance and consistent over-projection of DDI from TDI with a mean fold error of 2.25. In model 2, the rate of absorption was adjusted for each perpetrator and \(C_{\text{avg}}\) hepatic inlet concentrations were used for predictions instead of \(C_{\text{max}}\). The predictions were much improved with a mean fold error of 1.70. Models 3 and 4 represent exit \(C_{\text{max}}\) and \(C_{\text{avg}}\) concentrations from the liver, respectively. Using \(C_{\text{max}}\), model 3 performance was improved with a mean fold error of 1.76 compared to entrance concentrations used in model 1. However, model 4 performed similarly to model 2, with a mean fold error of 1.72.

Incorporation of parameters to represent the calculation of average hepatic inlet concentrations in model 2 appear to have minor contribution towards inlet concentration and therefore performs very similarly to model 4.

Compounds such as hydralazine (CYP2D6), dronedarone (CYP2D6), fluvoxamine (CYP2C19, CYP2C9), and mifepristone (CYP2C9), were not included in the overall GMFE analysis since they were determined to be reversible inhibitors rather than TDIs. They generally underpredicted observed DDI in models 2-4 and only when estimated unbound hepatic inlet concentrations were used (model 1), then the prediction was more aligned with observed. This is in agreement with Obach et al. (Obach et al., 2006) that the hepatic inlet unbound \(C_{\text{max}}\) provided the more accurate predictions for reversible inhibitors.

TDI data generated by metabolites such as \(N\)-desethylamiodarone and gemfibrozil glucuronide performed better than their parent drugs at predicting DDI for CYP2D6 and CYP2C8, respectively. Observed versus predicted AUCR for the individual compounds and models are listed in Supp. Table 37.

**Projection of DDI from TDI Data in Liver microsomes: Dynamic PBPK Method.** These in vitro data were also used as input values for projecting DDI using Simcyp. The observed and predicted
inhibitor $C_{\text{max}}$ and AUC using Simcyp are listed in Supp. Table 39. In this case, various input parameters such as in vivo [I] values, absorption extent and rate values, and enzyme degradation rates are embedded in the algorithms. The output DDI projections were evaluated as above for numerical accuracy of DDI projections (Table 4). Use of SimCYP yielded overall accuracy for projections of DDI from in vitro data with mean fold error of 1.61 from microsomes (Figure 2). These performance characteristics are slightly better than the best of the static models, although prediction agreement between the two are still very good (Model 4, Figure 3).
DISCUSSION

A reliable ability to convert in vitro TDI data for CYP enzymes to projections of clinical DDI is highly desirable. Early efforts generally yielded over-predictions of DDI (Obach et al., 2007), albeit it was recognized that selection of the value for in vivo concentration (i.e. unbound systemic $C_{\text{max}}$) of the TDI was critical. Current guidance on this topic from government regulatory agencies suggests that the maximum unbound organ entry concentrations for the TDI should be used to project DDI, however this can yield overestimations of the magnitude of DDI as well as mis-categorize drugs as perpetrators of DDI when they show no effect. Recent efforts in our lab focused on projections of DDI for CYP3A4 TDI (Tseng et al., 2021) and the use of various values for in vivo $[I]$ in static models was explored as well as the performance of dynamic PBPK modelling using Simcyp. In contrast to the CYP enzymes evaluated in the present work, CYP3A TDI has the added complexity of inhibition occurring in intestine during first pass. That work showed that the most accurate projections of DDI magnitude could be obtained applying a static model using estimates of average unbound organ exit concentrations from liver and intestine, and that this method matched well to projections made using dynamic PBPK modelling in Simcyp. Use of estimates of maximum organ entry concentrations as proposed in aforementioned current regulatory guidance yielded marked over-projections of DDI. On this backdrop, the objective of the present effort was to determine whether the approach that worked well for predicting DDI for CYP3A TDI would also work for DDI involving other major drug-metabolizing CYP enzymes. Unlike CYP3A, these other CYPs are not highly expressed in the intestine, and thus the approach could be simplified by solely focusing on TDI in the liver.

Results from this investigation showed that DDI can be reliably projected from CYP TDI data. Compared to CYP3A4, there are not nearly as many DDI known to be caused by TDI of other individual CYP enzymes and this precludes making robust conclusions for any single enzyme. However, taken in its entirety, the data showed that DDI can be projected for TDI for all CYPs. As with the previous work with CYP3A TDI, use of unbound average organ exit concentrations of the TDI for $[I]_h$ in equation 4
yielded good performance with a GMFE of 1.72 (Table 3). Additionally, use of other \([I]_h\) values towards DDI predictions were also comparable for the non-CYP3A enzymes. Specifically, model 2, which uses unbound average organ entry concentrations yielded comparable performance to model 4. This observation is unlike that in our previous work with CYP3A TDI where unbound average organ entry concentrations resulted in over predictions. The likely difference between the two assessments includes the incorporation of \([I]_e\) (and the concentrations used towards calculations) in CYP3A predictions.

The greatest over-prediction of DDI was generated when using the unbound hepatic inlet \(C_{\text{max}}\) assuming a very rapid absorption rate as the \([I]_h\) values (Model 1). Dynamic PBPK modelling yielded even more accurate projections of DDI than the static models with a GMFE of 1.61 (Table 4). The statistical assessments of the results from Simcyp modeling were also comparable to that of a recent publication from Kilford et al. (Kilford et al., 2022) where the bias of mechanism-based inhibitors was 1.03 compared to the value of 1.13 observed in this assessment.

Previous evaluations by us (Tseng et al., 2021) and others (Mao et al., 2011; Ramsden et al., 2022) examined whether CYP TDI data generated in human hepatocytes were superior to human liver microsomes for projecting DDI. Use of hepatocytes is compelling because of the perceived similarity to in vivo with regard to cell structure, cofactor concentrations, active transport, etc. In our previous investigation of CYP3A4 TDI, we found no meaningful difference in predictive performance using TDI data from hepatocytes and liver microsomes (Tseng et al., 2021) thus we focused on liver microsomes for the current investigation since their application is simpler and takes less resources than human hepatocytes. It should be noted that recent work has shown that for some compounds being evaluated for intrinsic clearance in hepatocytes, the rate of membrane permeation can be slower than the rate of metabolism and thus yield an artificially low estimate of hepatic metabolic intrinsic clearance (Keefer et al., 2020). The same can be true for inactivation rates, and it is not infrequent to measure \(k_{\text{obs}}\) values in hepatocytes that are lower than in microsomes (Eng et al., 2020). Furthermore, a reversible inhibitor can mistakenly appear to be a TDI in hepatocyte incubations if the rate of permeation is slow relative to the
incubation time used for the marker substrate assay; that is, concentrations of the inhibitor inside the cell to which the target enzyme is exposed can be increasing over time and cause greater inhibition over time. Conclusions made by Ramsden et al. (Ramsden et al., 2022) suggested that human TDI data suitable for prediction of DDI for non-CYP3A enzymes can be obtained in human hepatocytes. However, with the data reported herein, we have shown that reliable DDI projections can also be made using TDI data from human liver microsomes, using either static or dynamic models. It is worthy to note that while the replot method was employed to generate TDI parameters, the intricacies of estimating TDI parameters could benefit from use of more mechanistic forms of data fitting, such as the numerical method proposed by Nagar et al. (Nagar et al., 2014) to describe the data, leading to less error in parameter estimates.

One important factor to consider in some cases is whether the TDI is caused by the parent drug or a metabolite. For example, in the case of gemfibrozil, it is the glucuronide metabolite that is the TDI for CYP2C8 (Ogilvie et al., 2006) and testing gemfibrozil itself in human liver microsomes does not yield data that can be projected to a clinical DDI. It is tempting to think that conducting TDI in hepatocytes may be better for an example like gemfibrozil because the glucuronide could be generated in situ during the incubation, however that would require that the in vitro system yields a suitable representation of the in vivo exposure to the metabolite. This may not suffice, and it is better to measure TDI parameters for the metabolite directly and use those data in models to predict DDI.

In conclusion, clinical DDI caused by TDI of CYP enzymes can be reliably projected using in vitro data in human liver microsomes. Key to success in this endeavor is the selection of the most appropriate input value for in vivo concentration of the TDI which is most relevant to that which the target enzyme is exposed. Average unbound organ exit concentrations (i.e. systemic free C_{avg} for liver and portal free C_{avg} for intestine) of the TDI yield the most accurate projections of DDI magnitude (AUCR). These can be applied not only for CYP3A4 TDI but also for TDI for other important drug metabolizing CYP enzymes. While simple static models yield good projections of DDI, the more sophisticated approach of PBPK modelling offers even better projections of DDI from in vitro TDI data.
ACKNOWLEDGEMENTS

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DATA AVAILABILITY STATEMENT

The authors declare that all the data supporting the findings of this study are available within the paper and its Supplemental Data.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: Tseng, Lin, Goosen, Obach.

Conducted experiments: Tseng, Strelevitz, DaSilva.

Performed data analysis: Tseng, Lin, Strelevitz, DaSilva, Goosen, Obach.

Wrote or contributed to the writing of the manuscript: Tseng, Lin, DaSilva, Goosen, Obach.
Table 1. Summary of observed clinical drug-drug interactions for CYP 1A2, 2B6, 2C8, 2C9, 2C19, and 2D6 cleared drugs.

<table>
<thead>
<tr>
<th>CYP</th>
<th>Drug Name</th>
<th>Inhibitor Dose</th>
<th>Victim Drug</th>
<th>Clinical Interaction (AUCR)</th>
<th>Clinical Interaction Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>Furafylline</td>
<td>90 mg single dose</td>
<td>Caffeine</td>
<td>~10.0</td>
<td>(Tarrus et al., 1987)</td>
</tr>
<tr>
<td></td>
<td>Zileuton</td>
<td>800 mg BID, 5d</td>
<td>Theophylline</td>
<td>1.92</td>
<td>(Granneman et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>Ticlopidine</td>
<td>250 mg BID, 10d</td>
<td>Theophylline</td>
<td>1.58</td>
<td>(Colli, 1987)</td>
</tr>
<tr>
<td></td>
<td>Isoniazid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>400 mg QD, 15d</td>
<td>Theophylline</td>
<td>1.27</td>
<td>(Samigun et al., 1990)</td>
</tr>
<tr>
<td></td>
<td>Rofecoxib</td>
<td>50 mg QD, 7d</td>
<td>Theophylline</td>
<td>1.60</td>
<td>(Bachmann et al., 2003)</td>
</tr>
<tr>
<td></td>
<td>Fluvoxamine</td>
<td>100 mg QD 6d</td>
<td>Theophylline</td>
<td>3.33</td>
<td>(Rasmussen et al., 1997)</td>
</tr>
<tr>
<td>2B6</td>
<td>Ticlopidine</td>
<td>250 mg BID, 4d</td>
<td>Bupropion</td>
<td>1.81</td>
<td>(Turpeinen et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Clopidogrel</td>
<td>75 mg QD, 4d</td>
<td>Bupropion</td>
<td>1.36</td>
<td>(Turpeinen et al., 2005)</td>
</tr>
<tr>
<td>2C8</td>
<td>Gemfibrozil</td>
<td>900 mg single dose</td>
<td>Rapaglinide</td>
<td>8.23</td>
<td>(Honkalammi et al., 2011)</td>
</tr>
<tr>
<td>2C9</td>
<td>Tienilic acid</td>
<td>250 mg QD, 19d</td>
<td>S-warfarin</td>
<td>2.92</td>
<td>(O'Reilly, 1982)</td>
</tr>
<tr>
<td></td>
<td>Amiodarone</td>
<td>200 mg BID, 10d</td>
<td>S-warfarin</td>
<td>2.11</td>
<td>(O'Reilly et al., 1987)</td>
</tr>
<tr>
<td></td>
<td>Mifepristone</td>
<td>1200 mg QD, 7d</td>
<td>Fluvastatin</td>
<td>4.21</td>
<td>(FDA, 2019a)</td>
</tr>
<tr>
<td></td>
<td>Fluvoxamine</td>
<td>150 mg QD, 5d</td>
<td>Tolbutamide</td>
<td>1.50</td>
<td>(Madsen et al., 2001)</td>
</tr>
<tr>
<td>2C19</td>
<td>Ticlopidine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>200 mg QD, 8d</td>
<td>Omeprazole</td>
<td>6.22</td>
<td>(Ieiri et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Esomeprazole</td>
<td>80 mg BID, 8d</td>
<td>(R)-Pantoprazole</td>
<td>4.91</td>
<td>(Kaufman et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>Fedratinib</td>
<td>500 mg QD, 15d</td>
<td>Omeprazole</td>
<td>2.95</td>
<td>(FDA, 2019a)</td>
</tr>
<tr>
<td></td>
<td>Osilodrostat</td>
<td>50 mg single dose</td>
<td>Omeprazole</td>
<td>1.91</td>
<td>(FDA, 2019b)</td>
</tr>
<tr>
<td></td>
<td>Fluvoxamine</td>
<td>25 mg BID, 6d</td>
<td>Omeprazole</td>
<td>5.62</td>
<td>(Yasui-Furukori et al., 2004)</td>
</tr>
<tr>
<td>2D6</td>
<td>Paroxetine</td>
<td>20 mg BID, 1d</td>
<td>Dextromethorphan</td>
<td>14.3</td>
<td>(Storelli et al., 2018)</td>
</tr>
<tr>
<td></td>
<td>MDMA (CS)</td>
<td>1.5 mg/kg single dose</td>
<td>Dextromethorphan</td>
<td>9.50</td>
<td>(OMathúna et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Amiodarone</td>
<td>1.2 g QD, 6d</td>
<td>Metoprolol</td>
<td>1.80</td>
<td>(Werner et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Hydralazine</td>
<td>25 mg BID, 4d</td>
<td>Metoprolol</td>
<td>1.38</td>
<td>(Lindeberg et al., 1988)</td>
</tr>
<tr>
<td></td>
<td>Mirabegron</td>
<td>100 mg QD, 19d</td>
<td>Desipramine</td>
<td>3.41</td>
<td>(FDA, 2012)</td>
</tr>
<tr>
<td></td>
<td>Dronedarone</td>
<td>800 mg BID, 8d</td>
<td>Metoprolol</td>
<td>2.53</td>
<td>(FDA, 2009)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Selected study from slow-acetylator population.

<sup>b</sup>Selected study in Japanese population.

QD, once a day; BID, twice a day; d, number of days of dosing.
Table 2. Total and free inhibition parameters determined in human liver microsomes.

<table>
<thead>
<tr>
<th>CYP</th>
<th>Drug Name</th>
<th>Reversible Inhibition</th>
<th>Time-dependent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$K_i$</td>
<td>$c_{u,mic}$</td>
</tr>
<tr>
<td>1A2</td>
<td>Furafylline</td>
<td>16.5</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>Zileuton</td>
<td>16.4</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>Ticlopidine</td>
<td>4.93</td>
<td>0.925</td>
</tr>
<tr>
<td></td>
<td>Isoniazid</td>
<td>194</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>Rofecoxib</td>
<td>0.345</td>
<td>0.985</td>
</tr>
<tr>
<td></td>
<td>Fluvoxamine</td>
<td>0.0110</td>
<td>0.971</td>
</tr>
<tr>
<td>2B6</td>
<td>Ticlopidine</td>
<td>0.162</td>
<td>0.925</td>
</tr>
<tr>
<td></td>
<td>Clopidogrel</td>
<td>0.201</td>
<td>0.954</td>
</tr>
<tr>
<td>2C8</td>
<td>Gemfibrozil acyl glucuronide</td>
<td>13.1</td>
<td>0.985</td>
</tr>
<tr>
<td>2C9</td>
<td>Tienilic acid</td>
<td>0.118</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>Amiodarone</td>
<td>3.77</td>
<td>0.178</td>
</tr>
<tr>
<td></td>
<td>Mifepristone</td>
<td>2.14</td>
<td>0.850</td>
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<td>Fluvoxamine</td>
<td>2.71</td>
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<td>2C19</td>
<td>Ticlopidine</td>
<td>0.165</td>
<td>0.788</td>
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<td></td>
<td>Esomeprazole</td>
<td>1.29</td>
<td>0.934</td>
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<td></td>
<td>Fedatinib</td>
<td>&gt;50.0</td>
<td>0.182</td>
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<td></td>
<td>Osilodrostat</td>
<td>4.45</td>
<td>0.973</td>
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<tr>
<td></td>
<td>Fluvoxamine</td>
<td>0.129</td>
<td>0.909</td>
</tr>
<tr>
<td>2D6</td>
<td>Paroxetine</td>
<td>0.198</td>
<td>0.735</td>
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<td></td>
<td>MDMA (CS)</td>
<td>1.92</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>Amiodarone</td>
<td>13.1</td>
<td>0.178</td>
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<tr>
<td></td>
<td>Hydralazine</td>
<td>&gt;50.0</td>
<td>0.998</td>
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<td></td>
<td>Mirabegron</td>
<td>1.63</td>
<td>0.985</td>
</tr>
<tr>
<td></td>
<td>Dronedarone</td>
<td>0.351</td>
<td>0.083</td>
</tr>
</tbody>
</table>

*a* Calculated as measured IC$_{50}$/2.

*b* $c_{u,mic}$ was calculated based on 0.1 mg/mL in reversible inhibition studies or 0.5 mg/mL in TDI studies.

*c* $c_{u,mic}$ at HLM concentration used in the study was calculated from $f_{u,mic}$ measured or predicted at 0.81 mg/mL (n=3-4) using equation from (Austin et al., 2002).

*d* reported as $k_{inact}/K_{I,u}$ (SE) composite slope

%CV, percent coefficient of variation; SE, standard error; ND, not determined (assume 1); NR, not reported (see data analysis section for the estimation of $k_{max}/K_{I,u}$)
Table 3. Numerical accuracy of DDI predictions determined from human liver microsomes using mechanistic static models.

<table>
<thead>
<tr>
<th>Relevant [I]_liver</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>entrance</td>
<td>entrance</td>
<td>exit</td>
<td>exit</td>
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</table>

**Fixed Input Parameters**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;a&lt;/sub&gt; x F&lt;sub&gt;g&lt;/sub&gt;</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP k&lt;sub&gt;deg,h&lt;/sub&gt;</td>
<td>Fixed for each CYP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q&lt;sub&gt;h&lt;/sub&gt;</td>
<td>1617 mL/min</td>
<td>1617 mL/min</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Varied Input Parameters**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>k&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.1 min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>custom</td>
<td>custom</td>
<td>custom</td>
</tr>
<tr>
<td>[I]&lt;sub&gt;h&lt;/sub&gt;</td>
<td>C&lt;sub&gt;max,hepatic inlet,u&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;avg,hepatic inlet,u&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>C&lt;sub&gt;max,systemic,u&lt;/sub&gt;</td>
<td>C&lt;sub&gt;avg,systemic,u&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

**Performance:**

<table>
<thead>
<tr>
<th>Human Liver Microsomes</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias (CI&lt;sub&gt;90%&lt;/sub&gt;)</td>
<td>2.05 (1.6-2.6)</td>
<td>1.16 (0.92-1.5)</td>
<td>1.38 (1.1-1.8)</td>
<td>1.15 (0.90-1.4)</td>
</tr>
<tr>
<td>GMFE (CI&lt;sub&gt;90%&lt;/sub&gt;)</td>
<td>2.25 (1.8-2.8)</td>
<td>1.70 (1.5-2.0)</td>
<td>1.76 (1.5-2.1)</td>
<td>1.72 (1.5-2.0)</td>
</tr>
<tr>
<td>RMSFE</td>
<td>2.71</td>
<td>1.95</td>
<td>2.10</td>
<td>1.95</td>
</tr>
<tr>
<td>% Within 2-fold</td>
<td>43</td>
<td>67</td>
<td>71</td>
<td>67</td>
</tr>
<tr>
<td>% Within 3-fold</td>
<td>67</td>
<td>86</td>
<td>81</td>
<td>86</td>
</tr>
<tr>
<td>% Outside 10-fold</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>As calculated per equation 5a.

<sup>b</sup>As calculated per equation 5b

NA, not applicable; [I]<sub>h</sub>, liver inhibitor concentration; F<sub>a</sub>, fraction absorbed following oral dose; F<sub>g</sub>, fraction escaping intestinal metabolism; k<sub>deg,h</sub>, hepatic degradation rate; Q<sub>h</sub>, liver blood flow; k<sub>a</sub>, inhibitor absorption rate constant; C<sub>max,hepatic inlet,u</sub>, unbound maximum hepatic inlet concentration; C<sub>avg,hepatic inlet,u</sub>, unbound average hepatic inlet concentration; C<sub>max,systemic,u</sub> unbound maximum systemic concentration; C<sub>avg,systemic,u</sub> unbound average systemic concentration; GMFE, geometric mean fold error; CI90%, 90% confidence interval; RMSFE, root mean square fold error
**Table 4: Numerical accuracy of DDI predictions using Simcyp**

<table>
<thead>
<tr>
<th>Performance:</th>
<th>Human Liver Microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias (CI90%)</td>
<td>1.13 (0.91-1.4)</td>
</tr>
<tr>
<td>GMFE (CI90%)</td>
<td>1.61 (1.4-1.8)</td>
</tr>
<tr>
<td>RMSFE</td>
<td>1.83</td>
</tr>
<tr>
<td>% Within 2-fold</td>
<td>80</td>
</tr>
<tr>
<td>% Within 3-fold</td>
<td>90</td>
</tr>
<tr>
<td>% Outside 10-fold</td>
<td>0</td>
</tr>
</tbody>
</table>

GMFE, geometric mean fold error; RMSFE, root mean square fold error
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FDA (2019b) Isturisa (osilodrostat) NDA 212801 https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/212801Orig1s000ClinPharmR.pdf, in: Medical review(s) [Internet] (FDA drug approval package) (Research CfDEa ed, US Food and Drug Administration, Rockville (MD).


Simcyp Certera Simcyp Library Files. [https://members.simcyp.com/account/libraryFiles](https://members.simcyp.com/account/libraryFiles).


Footnotes

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Declaration of interest:

The authors are employees of Pfizer and report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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FIGURE LEGENDS

Figure 1. Predicted vs Observed AUC ratios from mechanistic static models 1-4 (Panels A-D).

Model 1 (A), model 2 (B), model 3 (C), and model 4 (D) are results using human liver microsome-generated inactivation parameters. Solid black lines represent unity, dotted lines represent the bias. Open symbols represent underpredictions compared to observed.

Figure 2. Predicted vs Observed AUC ratios from Simcyp modeling.

Results using human liver microsome-generated inactivation parameters. Solid black lines represent unity, dotted lines represent the bias. Open symbols represent underpredictions compared to observed.

Figure 3.

Correlation of predicted AUC ratios from mechanistic static model 1 (A), model 2 (B), model 3 (C), model 4 (D) and Simcyp modeling.

Data represents predictions using human liver microsome-generated inactivation parameters. The solid black line represents unity.
Figure 1

A

B

C

D

Predicted AUCR

Observed AUCR

Predicted AUCR

Observed AUCR

Predicted AUCR

Observed AUCR

Predicted AUCR

Observed AUCR

- CYP1A2
- CYP2D6
- CYP2C19
- CYP2C9
- CYP2C8
- CYP2B6
- Unity
- Bias

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Figure 2

The figure shows a scatter plot comparing predicted AUCR against observed AUCR. The data points are color-coded and represent different isoforms of the CYP enzymes: black circles (CYP1A2), red squares (CYP2D6), green triangles (CYP2C19), purple inverted triangles (CYP2C9), and pink diamonds (CYP2C8). The plot also includes a unity line (solid black line) and a bias line (dashed blue line).
Figure 3

A

B

C

D

Model 1

Model 2

Model 3

Model 4

\$r^2=0.88\$

\$r^2=0.93\$

\$r^2=0.94\$

\$r^2=0.92\$

- CYP1A2
- CYP2D6
- CYP2C19
- CYP2C9
- CYP2C8
- CYP2B6
- Unity