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Improved Predictions of Drug–Drug Interactions Mediated by Time-Dependent Inhibition of CYP3A

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Abstract

Time-dependent inactivation (TDI) of cytochrome P450s (CYPs) is a leading cause of clinical drug–drug interactions (DDIs). Current methods tend to overpredict DDIs. In this study, a numerical approach was used to model complex CYP3A TDI in human-liver microsomes. The inhibitors evaluated included troleandomycin (TAO), erythromycin (ERY), verapamil (VER), and diltiazem (DTZ) along with the primary metabolites *N*-demethyl erythromycin (NDE), norverapamil (NV), and *N*-desmethyl diltiazem (NDD). The complexities incorporated into the models included multiple-binding kinetics, quasi-irreversible inactivation, sequential metabolism, inhibitor depletion, and membrane partitioning. The resulting inactivation parameters were incorporated into static in vitro–in vivo correlation (IVIVC) models to predict clinical DDIs. For 77 clinically observed DDIs, with a hepatic-CYP3A-synthesis-rate constant of $0.000\ 146\ \text{min}^{-1}$, the average fold difference between the observed and predicted DDIs was 3.17 for the standard replot method and 1.45 for the numerical method. Similar results were obtained using a synthesis-rate constant of $0.000\ 32\ \text{min}^{-1}$. These results suggest that numerical methods can successfully model complex in vitro TDI kinetics and that the resulting DDI predictions are more accurate than those obtained with the standard replot approach.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.8b00129. Values (fixed or estimated) of all the rate constants for the schemes in Figures 1–7, details of all 77 clinical-DDI studies, predictions from the TDI parameters (K_I and k_{inact}) obtained from both the numerical and replot methods using $k_{\text{deg}} = 0.000\ 146\ \text{min}^{-1}$ and $k_{\text{deg}} = 0.000\ 32\ \text{min}^{-1}$, references from the clinical-DDI studies, and comparison of the replot- method-parameter estimates used in this study with their literature values (PDF)

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Notes

The authors declare no competing financial interest.

Keywords

numerical method; time-dependent inhibition; drug–drug interactions; enzyme kinetic models

INTRODUCTION

Cytochrome P450s (CYPs) are an important superfamily of drug-metabolizing enzymes (DMEs) with 57 functional genes in humans.¹ These enzymes catalyze endogenous as well as xenobiotic metabolism. More than 90% of xenobiotics are metabolized by CYP1, –2, and –3 family members.² Because CYPs are the major DMEs, inhibition of CYPs can lead to severe drug–drug interactions. One type of enzyme inhibition is irreversible inhibition, which occurs through several molecular mechanisms and ultimately results in an inactive protein.^{3,4} In time-dependent inhibition (TDI), the magnitude of inhibition increases as the contact time between the enzyme and inactivator increases. TDI is characterized by loss of activity with increases in time and concentration of the inactivator. There are several examples of drugs that demonstrate nonlinear accumulation and increased half-lives in humans after multiple doses because of enzyme inactivation. These include diltiazem (DTZ),⁵ verapamil (VER), paroxetine (PAR), ticlopidine, and delavirdine.^{6,7} Inactivation of CYPs can lead to drug–drug interactions (DDIs)^{8–10} and adverse reactions,^{11–16} which can result in the withdrawal of drugs from the market (e.g., mibefradil, cerivastatin, and soruvudine).

TDI represents a more severe form of inhibition than reversible inhibition because the enzyme is permanently inactivated. In order to restore the activity of the enzyme, de novo synthesis is required. Because of this, a DDI may only be observed after multiple doses. Furthermore, the effects of irreversible inhibition can persist even after the inactivator is removed from the body.¹⁷

These characteristics make predictions of TDI-based DDIs more critical and difficult. Predictions of TDI-based DDIs require the use of the enzyme parameters, such as the natural degradation rate of the enzyme (k_{deg}), and the TDI parameters (the inactivation constant, K_I , and the rate of inactivation, k_{inact}). Current in vitro–in vivo correlation (IVIVC) methods tend to overpredict DDIs.^{18–20} One possible reason for poor predictions is improper in vitro analysis, resulting in inaccurate TDI parameters.

Experimental methods to evaluate TDI range from simple screening assays to the determination of the TDI parameters (K_I and k_{inact}).^{21–28} The replot method is the standard method for the analysis of in vitro data, but it has some inherent assumptions that may not always hold true. This can lead to inaccurate estimations of TDI parameters. Some aspects that should be considered while analyzing in vitro TDI data include the metabolism of the inactivator and nonspecific microsomal partitioning. Burt et al.²⁵ developed a new method for TDI data analysis wherein inactivator concentrations were monitored and incorporated into the model to account for inactivator metabolism. However, the DDI predictions were not performed using the TDI parameters obtained from progress-curve data. Inactivators that are highly partitioned into microsomes have lower-than-expected unbound concentrations in the primary- incubation step. Upon dilution for the secondary incubation, a shift in the

partitioning equilibrium results in inconsistencies between competitive inhibition and inactivation.

CYP kinetics can also be complicated by non-Michaelis–Menten (MM) kinetics (e.g., multiple-binding, quasi-irreversible, and partial-inactivation kinetics).^{29,30} Furthermore, sequential metabolism, in which the metabolite of a drug inactivates the enzyme, can further complicate DDI predictions.³¹ Therefore, models incorporating complex molecular mechanisms of CYPs may better estimate TDI parameters. A recently published numerical method^{32,33} has been used to explore complex kinetics and better estimate TDI parameters.

The current study uses a numerical method for the analysis of the in vitro data of four CYP3A inactivators, DTZ, erythromycin (ERY), troleandomycin (TAO), and VER, along with the primary metabolites *N*-desmethyl diltiazem (NDD), *N*-demethyl erythromycin (NDE), and norverapamil (NV). We previously published studies^{34,35} wherein TAO and podophyllotoxin were evaluated in rats, and DDI predictions were performed. The present study uses numerical methods to predict clinical DDIs for the compounds above. Moreover, novel models for sequential metabolism and inactivation due to metabolites were developed. DDI predictions were performed using TDI parameters obtained from replot as well as numerical methods, and comparative analyses were performed.

MATERIALS AND METHODS

Materials

All the solvents used for LC-MS/MS were obtained from Honeywell (B&J, AC/HPLC-certified solvents) and were of analytical grade. DTZ hydrochloride, midazolam (MDZ), and 1-hydroxy midazolam (1-OH MDZ) were obtained from Sigma-Aldrich. ERY, NDD, NDE, and NV were purchased from Toronto Research Chemicals. TAO was obtained from Enzo Life Sciences. Pooled ($n = 35$ livers) human-liver microsomes (HLM) and NADPH solution A and solution B were obtained from Corning Life Sciences.

Methods

In Vitro TDI Incubations—Several CYP3A inactivators (DTZ, ERY, NDD, NDE, NV, TAO, and VER) were tested using a standard two-step approach for determining TDI inhibition of CYPs in the pooled HLM. MDZ was used as a probe substrate. Briefly, eight concentrations of inactivators with 2-fold dilution schemes (0–40 μM DTZ, 0–50 μM ERY, 0–10 μM NDD, 0–50 μM NDE, 0–40 μM NV, 0–10 μM TAO, and 0–40 μM VER) were incubated at 37 °C in a 1 mg/mL suspension of HLM in 0.1 M potassium phosphate buffer (pH 7.4) as the primary incubation. After 5 min of preincubation, the reaction was initiated by the addition of the NADPH-regenerating system (final concentrations of 1.3 mM NADP⁺, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, and 3.3 mM magnesium chloride). At specific time points, aliquots (7.5 μL) of the primary incubations were added to the secondary incubations (142.5 μL), which contained 50 μM MDZ and the NADPH-regenerating system (final concentrations of 1.3 mM NADP⁺, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, and 3.3 mM magnesium

chloride). The primary incubation was run for 0–60 min, with data collected at 12–15 time points. The secondary incubation was allowed to run for 2 min and was followed by a quenching step with ice-cold acidified acetonitrile containing DTZ as the internal standard (VER was used as the internal standard when DTZ was used as the inactivator). After centrifugation at 10 000 rpm for 8 min, the supernatant was removed so that the amount of 1-OH MDZ in the supernatant could be measured. LC-MS/MS was used for the analysis of the supernatant. Each assay was conducted in duplicate. Stock solutions of the inactivators and substrates were prepared in methanol. The final methanol concentrations in the primary incubations were less than 0.1% (v/v). The assays were also performed without the inactivators to assess the nonspecific loss of enzyme activity.

Microsomal Partitioning and Human-Plasma-Protein Binding—Equilibrium dialysis was performed to determine the microsomal partitioning of all the inactivators except TAO in HLM and human plasma. For TAO, the unbound fractions in HLM ($f_{u,mic}$) and human plasma ($f_{u,p}$) reported in the literature were used.^{36,37} Briefly, 0.5 mg/mL HLM suspensions were spiked with DTZ, ERY, NDD, NDE, NV, and VER at final concentrations of 2 μ M in separate experiments ($n = 5$ replicates). The unbound fractions in plasma were determined for the parent inactivators only (DTZ, ERY, and VER) using a similar approach. A 96-well equilibrium dialyzer (Harvard Apparatus) was used to perform the dialyses with inactivator-spiked HLM suspensions or human plasma on one side and blank phosphate buffer (pH 7.4) on the other side at 37 °C for 20 h with 5% CO₂. The samples on each side of the membrane were analyzed by LC-MS/MS for inactivator concentrations. The unbound fractions (f_u) were calculated by using the following equation:

$$f_u = \frac{C_{buffer}}{C_{matrix}} \quad (1)$$

where f_u , C_{matrix} , and C_{buffer} represent the unbound fraction, the total concentration in the matrix (either the HLM suspension or human plasma), and the total concentration in the phosphate buffer, respectively. The $f_{u,mic}$ value was scaled to 1 mg/mL using the equation³⁸

$$f_{u,mic(1mg/mL)} = \frac{\frac{1}{D}}{\left(\frac{1}{f_{u,mic(0.5mg/mL)}} - 1\right) + \frac{1}{D}} \quad (2)$$

where $f_{u,mic(1mg/mL)}$ is the scaled unbound fraction at 1 mg/mL microsomal protein, and $f_{u,mic(0.5mg/mL)}$ is the unbound fraction experimentally measured at 0.5 mg/mL. $D = 2$ is the dilution factor.

LC-MS/MS—The samples from in vitro TDI assays were analyzed by LC-MS/MS. Calibration curves were prepared from 0.05 mg/mL HLM in phosphate buffer (pH 7.4) spiked with analyte standards, which were precipitated with acetonitrile. The supernatants were analyzed by LC-MS/MS. The LC system used was an Agilent 1100 series HPLC

system. For the chromatographic separation of 1-OH MDZ, a Phenomenex Luna-C18 (3 μ m, 30 $\times$ 2 mm) analytical column coupled with a C18 guard column (4 $\times$ 2.0 mm) was used. Sample volumes of 5 μ L were injected into the system. An AB Sciex API 4000 LC-MS/MS system was used for analyzing the plasma samples in positive-ion mode with the following MRM transitions: 342.092–324.100 m/z for 1-OH MDZ and 415.500–178.400 m/z for diltiazem (IS). The LC-MS solvents consisted of 0.1% formic acid in water, the aqueous mobile phase (A), and 0.1% formic acid in acetonitrile, the organic mobile phase (B). The flow rate was 0.7 mL/min with a gradient elution programmed from 10 to 90% B in 0.5 min, maintained at 90% until 1.1 min, returned to the initial condition at 2 min, and maintained until 6 min. The total run time was 6 min, and the retention time was 2.48 min.

In Vitro TDI Model Development—The concentrations of 1-OH MDZ obtained from the in vitro TDI experiments were converted to logarithmic percent-remaining-activity plots (PRA plots) and further evaluated for model development. All the inactivators evaluated in this study are known to be MIC-forming compounds^{39–42} by quasi-irreversible mechanisms. On the basis of the reported mechanism of inactivation³⁴ and the data sets generated, kinetic models for CYP3A TDI were developed. Concave upward curvature is indicative of either quasi-irreversible or partial inactivation, as shown previously.³² Using the numerical method,^{32,33} the kinetic models were fit to the data, and the kinetic parameters were estimated. The initial estimates of the rate constants were obtained from analyzing the data as detailed in previous publications.^{32–34} Briefly, nonspecific loss of enzyme activity was incorporated into the model if activity loss over time was observed in the absence of inactivators (0 μ M inactivator). The initial estimate for the rate constant for nonspecific enzyme loss (k_9) was obtained by fitting a first-order degradation model to the 0 μ M inactivator data. All the active enzyme species were assumed to degrade with first-order kinetics. Furthermore, a competitive-inhibition model was fit to the 0 and 60 min time point data to obtain an initial estimate for K_I . A difference in the initial estimate of K_I from the 0 min data versus that from the 60 min data was indicative of multiple binding. As shown previously,³⁴ MIC formation is a complex multistep process involving the formation of Fe^{3+} :carbene and Fe^{2+} :carbene. Hence, enzyme inactivation was modeled with three types of rate constants. For example, in Figure 1A, k_6 and k_{12} were used for Fe^{3+} :carbene formation, k_7 was used for the reformation of the active enzyme, and k_8 was used for Fe^{2+} :carbene formation.

First, two models, denoted as MIC-IL and MIC-EII-IL, were developed and evaluated. MIC refers to a metabolite- intermediate complex, and IL refers to inhibitor lipid partitioning, in which I and L are the inhibitor and lipid concentrations in microsomes, respectively. The latter model, MIC-EII-IL, refers to two molecules of an inactivator binding simultaneously in the active site. These models were tested individually for each of the inhibitors and their primary metabolites, because some primary metabolites are known to be the inactivating species.^{41,43,44} Next, the information obtained from the model fittings of the primary metabolites was used to build sequential- metabolism models for the parent drugs that captured CYP3A inactivation by the in situ formed metabolites upon incubation with the inhibitor. While the sequential (seq) metabolism models (seq-MIC-IL or seq-MIC-EII-IL) were fitted, the rate constants obtained from the primary-metabolite models were fixed in the

subsequent inhibitor models. For example, while the sequential-metabolism models were fitted to the DTZ data, fixed values for the rate constants, obtained from the NDD fits, were used.

The association-rate constants (k_1 , k_4 , and k_{10}) were fixed at $270 \mu\text{M}^{-1} \text{min}^{-1}$, and initial estimates for the dissociation-rate constants were obtained from the data.³⁴ For MDZ, the association- (k_1) and dissociation-rate constants (k_2) were fixed at $270 \mu\text{M}^{-1} \text{min}^{-1}$ and 1350min^{-1} , respectively (assuming a K_m of $5 \mu\text{M}$). Lipid partitioning was also incorporated into the models to account for microsomal partitioning. The association- rate constant for the lipids was set at $2000 \mu\text{M}^{-1} \text{min}^{-1}$, and the dissociation-rate constant was calculated using the following equation:⁴⁵

$$k_{\text{off}} = \frac{f_{\text{u,mic}} k_{\text{on}}}{1 - f_{\text{u,mic}}} \quad (3)$$

where k_{on} is the association-rate constant, k_{off} is the dissociation- rate constant, and $f_{\text{u,mic}}$ is the unbound fraction in the microsomes.

The K_I values were estimated from the ratios of the association- and dissociation-rate constants (assuming rapid equilibrium). The K_I obtained from the numerical method is the same as the unbound one, $K_{I,u}$ (i.e., $K_{I,u} = K_I$), because lipid partitioning was incorporated into the model. The inactivation parameters (k_{inact}) were calculated using the partition method as described previously;⁴⁶ for example, for the scheme in Figure 2, k_{inact} can be calculated as a net rate constant:

$$k_{\text{inact}} = \frac{1}{\frac{1}{k_8} + \frac{k_8 + k_7}{k_6 k_8}} \quad (4)$$

For TAO (e.g., see Figure 1) and NV, k_{inact} was described as

$$k_{\text{inact}} = \frac{1}{\left(\frac{1}{k'_6} + \frac{1}{k_8} + \frac{k'_{10}}{k'_6 k'_{12}} + \frac{1}{k'_{10}} + \frac{1}{k'_{12}} + \frac{1}{k_8} + \frac{k'_6}{k'_{10}} \right)} \quad (5)$$

where

$$k'_6 = \frac{k_6 k_8}{k_7 + k_8}$$

$$k'_{10} = \frac{k_{10} k'_{12}}{k_{10} + k'_{12}}$$

$$k'_{12} = \frac{k_{10}k_8}{k_7 + k_8}$$

The parameter errors for net rate constants were calculated with error propagation for individual rate constants. AICc⁴⁷ and adjusted R^2 were used to compare the different models for each data set.

The replot method²² was also used to analyze the in vitro TDI data sets. The data were analyzed either by using all the data (data not shown) or only the linear parts of the PRA plots.²² The following replot equation was used to obtain the estimates of K_I and k_{inact} :

$$k_{\text{obs}} = \frac{k_{\text{inact}}[I]}{K_I + [I]} \quad (6)$$

For the comparison with the numerical method, the K_I obtained from the replot method was corrected for microsomal partitioning by multiplying K_I with $f_{\text{u,mic}}$ to obtain $K_{I,u}$. Model fitting was conducted with Mathematica 11.0.1.0 (Wolfram Research). The NonlinearModelFit function was used to fit the model to the data with a PrecisionGoal of 10, finite difference derivatives of an order of 4, and 1/Y weighting. The WhenEvent function was used to incorporate the dilution step in the model.

In Vitro–In Vivo Correlation (IVIVC)—DDI predictions using $K_{I,u}$ and k_{inact} obtained from either the replot or numerical method were performed using the following static equations.³⁷ Equation 7 was used when the probe substrate was dosed through an intravenous route, and eq 8 was used when probe substrate was dosed orally.

$$\frac{\text{AUC}_i}{\text{AUC}} = \left(\frac{1}{\left(\frac{k_{\text{deg,h}}}{k_{\text{deg,h}} + \left(\frac{[I]_h k_{\text{inact}}}{[I]_h + K_I} \right)} \right) f_{\text{m,CYP3A}} + (1 - f_{\text{m,CYP3A}})} \right) \quad (7)$$

$$\frac{AUC_i}{AUC} = \left(\frac{1}{F_g + (1 - F_g) \left(\frac{k_{deg,g}}{k_{deg,g} + \left(\frac{[I]_g k_{inact}}{[I]_g + k_I} \right)} \right)} \right) \times \left(\frac{1}{\left(\frac{k_{deg,h}}{k_{deg,h} + \left(\frac{[I]_h k_{inact}}{[I]_h + k_I} \right)} \right) f_{m,CYP3A} + (1 - f_{m,CYP3A})} \right) \quad (8)$$

where AUC_i and AUC are the areas under the plasma- concentration–time curves of the probe substrates in the presence and absence of the inactivator, respectively; $f_{m,CYP3A}$ is the fraction of MDZ metabolized by CYP3A; F_g is the fraction that escapes gut metabolism (Table 1); $[I]_h$ and $[I]_g$ are the inactivator concentrations in hepatic portal vein and gut, respectively; and $k_{deg,h}$ and $k_{deg,g}$ are the degradation-rate constants of hepatic ($0.000146 \text{ min}^{-1}$)⁴⁸ and gut ($0.000481 \text{ min}^{-1}$)⁴⁹ CYP3A, respectively. It was assumed that K_I and k_{inact} were equivalent for hepatic and gut enzymes. Different values were used for in vivo inactivator concentrations depending on the clinical-DDI-study design. In cases where the victim drug was given orally, $[I]_{h,u}$ and $[I]_{g,u}$ were used for the in vivo inhibitor concentrations, as given by the following equations:⁵⁰

$$[I]_h = \left([I]_{max} + \frac{DK_a F_a F_g}{Q_h BP} \right) \quad (9)$$

$$[I]_{h,u} = f_{u,g} [I]_h \quad (10)$$

$$[I]_{g,u} = f_{u,g} \left(\frac{DK_a F_a}{Q_g} \right) \quad (11)$$

where $[I]_{max}$ is the maximum concentration of the inactivator in the plasma after an oral dose; D is the dose; F_a is the fraction absorbed; k_a is the first-order absorption-rate constant; Q_g (0.3 L/min) and Q_h (1.5 L/min) are the gut and hepatic blood flows, respectively; $f_{u,p}$ is

the unbound fraction in the plasma; $f_{u,g}$ (assumed to be equal to 1) is the unbound fraction in the enterocytes; and BP is the blood-to-plasma-partition ratio. In cases where the victim drug was administered intravenously, the maximum unbound concentration, $[I]_{\max,u}$, was used as the inactivator concentration for the DDI prediction. $[I]_{\max,u}$ was given by the following equation:⁵¹

$$[I]_{\max,u} = f_{u,p} \frac{[I]_{\text{sys}} k \tau}{1 - e^{-k\tau}} \quad (12)$$

where τ is the dosing interval, and k is the elimination-rate constant, which is calculated by the following equation:

$$k = \frac{CL_s}{V_{ss}} \quad (13)$$

where CL_s is the systemic clearance, and V_{ss} is the volume of distribution at steady state.

$[I]_{\text{sys}}$ is the average systemic inactivator concentration:

$$[I]_{\text{sys}} = \frac{FD}{\tau CL_s} \quad (14)$$

where F is the bioavailability.

$[I]_{\text{sys},u}$ was calculated using the following equation:

$$[I]_{\text{sys},u} = [I]_{\text{sys}} f_{u,p} \quad (15)$$

The values reported in the literature for the substrate-dependent parameters (F_g and $f_{m,CYP3A}$) are listed in Table 1. F_g and $f_{m,CYP3A}$ values were calculated for substrates for which literature values were not available. The inactivator pharmacokinetic (PK) parameters (including the experimentally measured $f_{u,mic}$ and $f_{u,p}$ values) used for calculating in vivo plasma inactivator concentrations are listed in Table 2. DDI predictions were also performed with other substrates besides MDZ, and it was assumed that there were no substrate-specific interactions. Clinical-DDI studies in which the inactivator and substrate were coadministered were not considered for the DDI predictions. The DDI predictions using the parameters from both the numerical and replot methods were compared with the observed DDIs.

RESULTS

In Vitro TDI Models

Several kinetic models were developed for each inactivator depending upon the reported mechanism of inactivation and the observed data set. A best-fit model for each inactivator

was chosen on the basis of the AICc values. In those cases (e.g., DTZ and VER) where multiple models resulted in equally good fits (based on AICc), all the best-fit models were reported.

For TAO, because there was no activity loss observed in the 0 μ M incubation, enzyme loss was not incorporated into the model. The observed PRA plots show concave upward curvature, suggesting either quasi-irreversible or partial inactivation (Figure 1). Analysis of the observed PRA plots shows that the different TAO concentrations plateau at different levels, indicating quasi-irreversible mechanisms rather than partial inactivation.³² An MIC model with EII formation (MIC-EII-IL) provided the best fit.

The concave upward curvature observed even at the highest inhibitor concentration indicates that inhibitor depletion is not the cause of the curvature in the PRA plots. An MIC-EII-IL model with an inhibitor-depletion step did not converge unless the inhibitor-depletion-rate constant was fixed at $<0.001 \text{ min}^{-1}$ (data not shown), suggesting minimal inhibitor loss. Estimates of $K_{I,u}$ and k_{inact} from the numerical and replot methods for TAO are shown in Table 3. Our experimental replot parameters agree with the literature values (see the Supporting Information, Table S6). The numerical method yields a 3.02-fold lower inactivation efficiency ($k_{inact}/K_{I,u}$) for TAO than that from the replot method.

For the other inactivators (ERY, DTZ, and VER), TDI was also evaluated for their primary metabolites (NDE for ERY, NDD for DTZ, and NV for VER). The primary metabolites of TAO were not available commercially and were not evaluated. When sequential metabolism was considered, the model for the metabolite was first constructed. This model was then incorporated into the parent TDI model.

Of all the models tested for NDE, the MIC-IL-M model (M refers to inhibitor metabolism in the model) was the best-fit model (Figure 2). Inhibitor metabolism (k_{13} in Figure 2A) and nonspecific enzyme loss (k_9 in Figure 2A) were incorporated into this model.

For ERY, both the MIC-IL and seq-MIC-IL models were evaluated. However, MIC-IL gave a better fit than the seq-MIC-IL model on the basis of AICc (AICc = -606.65 for MIC-IL vs -400.2 for seq-MIC-IL). The numerical- and replot-model fits for ERY are shown in Figure 3. The data sets for both NDE and ERY show concave upward curvature, indicating quasi-irreversible mechanisms. The TDI kinetic parameters for NDE and ERY are shown in Table 3. The $k_{inact}/K_{I,u}$ values for NDE and ERY from the numerical method were found to be approximately 7.5- and 13-fold lower, respectively, than those from the replot method. NDE was observed to be a more efficient inactivator than ERY.

For NDD, an MIC-I₂L model (Figure 4) was evaluated (I₂ denotes that NDD is a metabolite of DTZ and is itself an inhibitor). While the MIC-I₂L model was being fitted to the NDD data set, it was not possible to parametrize k_8 because of its covariance with other parameters. Therefore, a sensitivity analysis was performed with a range of k_8 values between 0 and 0.05 min^{-1} . Values greater than 0.02 min^{-1} did not predict the experimental data (inactivation was overpredicted) on the basis of visual observations and analysis of the residuals. Equally good fits were observed when values were fixed at 0.02 min^{-1} . Hence, 0.02 min^{-1} was considered the upper limit, and 0 min^{-1} was considered the lower limit for

k_8 . Figure 4B shows that the MIC- I_2L model fits the NDD data set assuming $k_8 = 0.02 \text{ min}^{-1}$. The k_{inact} for NDD with an upper limit of k_8 as 0.02 min^{-1} was estimated to be $0.009 \pm 0.001 \text{ min}^{-1}$ using the numerical method, and $0.036 \pm 0.001 \text{ min}^{-1}$ using the replot method with linear data points. The estimates of $K_{I,u}$ for NDD were $0.62 \pm 0.04 \mu\text{M}$ using the MIC- I_2L model and $1.08 \pm 0.21 \mu\text{M}$ using the replot method.

For DTZ (denoted by I_1 in Figure 5), three tested models provided equally good fits. These models were the sequential MIC EI_1I_1 model with lipid partitioning (Seq-MIC- EI_1I_1 - I_1L - I_2L , Figure 5A), the sequential MIC EI_1I_1 model with lipid partitioning with the addition of inhibitor metabolism from EI_1 (Seq-MIC- EI_1I_1 -M- I_1L - I_2L , Figure 5C), and the sequential MIC EI_1I_1 model with lipid partitioning with the addition of inhibitor metabolism from both EI_1 and EI_1I_1 (Seq-MIC- EI_1I_1 -MM- I_1L - I_2L , Figure 5E). While these sequential models were being fitted to the DTZ data set, the rate constants k_{5b} , k_6 , k_7 , and k_8 were fixed from the model fitting of the NDD data set. The k_8 value was fixed at 0.02 min^{-1} . The numerical- and replot-model fits to the DTZ data set are shown in Figure 5B,D,F. For all the models tested, double-binding-site models gave better fits to the observed data. The two binding constants, $K_{I1,u}$ and $K_{I2,u}$, ranged from 7.09 to 7.72 and 6.66 to $15.96 \mu\text{M}$, respectively, according to the numerical method. According to the replot method, $K_{I,u}$ was observed to be 2.98 ± 0.68 (Table 4). The estimate of $K_{I,u}$ from the numerical method was 2.4- to 2.6-fold higher than that from the replot method. The k_{inact} estimate from the numerical method was approximately 2.5-fold lower than or equal to that from the replot method. For the calculations of the inactivation efficiencies and DDI predictions, $K_{I1,u}$ was used. The inactivation efficiency of NDD was found to be around 12.44-fold higher than that of DTZ according to the numerical method, whereas it was only 4.6-fold higher according to the replot method.

NV is known to be an inactivator upon VER administration and was characterized in addition to VER. For NV, the MIC- EI_2I_2 - I_2L model was found to be the best-fit model. The $K_{I1,u}$ and k_{inact} obtained from the numerical method were $10.2 \pm 2.54 \mu\text{M}$ and $0.01 \pm 0.003 \text{ min}^{-1}$, respectively. The $K_{I,u}$ and k_{inact} obtained from the replot method were $4.99 \pm 0.72 \mu\text{M}$ and $0.174 \pm 0.007 \text{ min}^{-1}$, respectively (Table 3). The $k_{\text{inact}}/K_{I,u}$ value from the numerical method was 35-fold lower than that from the replot method. The MIC- EI_2I_2 - I_2L model (Figure 6A) captured the observed concave upward curvature and atypical kinetics in the NV data set (Figure 6B).

For VER, three models showed comparably good fits: a sequential MIC model forming a double binding site for I_1 and I_2 with lipid partitioning (Seq-MIC- EI_1I_1 - EI_2I_2 - I_1L - I_2L , Figure 7A), a sequential MIC model forming a double binding site for I_1 and I_2 with lipid partitioning and inhibitor loss from EI_1 (Seq-MIC- EI_1I_1 - EI_2I_2 -M- I_1L - I_2L , Figure 7C), and a sequential MIC model forming a double binding site for I_1 and I_2 with lipid partitioning and inhibitor loss from EI_1 and EI_1I_1 (Seq-MIC- EI_1I_1 - EI_2I_2 -MM- I_1L - I_2L , Figure 7E). While the different models were being fitted to the VER data set, the rate constants k_{5b} , k_{5d} , k_{14} , k_6 , k_7 , and k_8 were not parametrized, and their estimates were fixed at values obtained from model fitting of NV data set. Models incorporating inhibitor loss (i.e., Seq-MIC- EI_1I_1 - EI_2I_2 -M- I_1L - I_2L and Seq-MIC- EI_1I_1 - EI_2I_2 -MM- I_1L - I_2L) gave better fits than the Seq-MIC- EI_1I_1 - EI_2I_2 - I_1L - I_2L model. However, both of the inhibitor-loss models gave similar fits and similar

AICc (−732.2 and −732.1, respectively). The TDI parameters obtained from the simpler (Seq-MIC-EI₁I₁-EI₂I₂-M-I₁L-I₂L) of the two inhibitor-loss models was used for the DDI predictions. The $K_{I1,u}$ and $K_{I2,u}$ estimates ranged from 5.03 to 5.31 and from 5.44 to 6.19 μ M respectively. $K_{I,u}$ was estimated to be 1.79 ± 0.45 μ M using the replot method. The estimate of $K_{I1,u}$ from the numerical method was 2.8- to 3-fold higher than that from the replot method, whereas k_{inact} was 7.4-fold lower as compared with that from the replot method (Table 5). The $k_{inact}/K_{I,u}$ value of NV was 2-fold lower as compared with that of VER according to the numerical method, whereas it was 1.2-fold lower according to the replot method.

In Vitro–In Vivo Correlation (IVIVC)

The DDI predictions were performed using the standard static model as recommended by the FDA.⁵² Because there is a wide range of $k_{deg,h}$ values reported in the literature,^{48,49,53–58} three different values for $k_{deg,h}$ (ranging from 0.0004 to 0.000 082 5 min^{−1}) were used for the DDI predictions. The results for two $k_{deg,h}$ values ($k_{deg,h} = 0.000 146$ min^{−1}, an average of the reported values, and $k_{deg,h} = 0.000 32$ min^{−1}, a commonly used value in the literature) are listed in Table 6. In the cases of VER and DTZ, DDI predictions were performed only using the $K_{I1,u}$ and k_{inact} from the simplest of the three models. The DDI predictions using the $K_{I,u}$ and k_{inact} from the replot method were higher than those using the $K_{I,u}$ and k_{inact} from the numerical method. The average fold differences between the observed and predicted values were 3.64 for the replot method and 1.41 for the numerical method when a hepatic k_{deg} value of 0.000 146 min^{−1} was used. The average fold differences between the observed and predicted values were 2.8 for the replot method and 0.95 for the numerical method when a hepatic k_{deg} value of 0.0003 min^{−1} was used.

Finally, DDIs with substrates other than MDZ were additionally predicted, and Figure 8 shows the plots of the observed clinical interactions with CYP3A substrates versus those predicted for a total of 77 clinical DDIs (details in Supporting Information). The average fold differences between observed and predicted values were 3.17 for the replot method and 1.45 for the numerical method when a hepatic k_{deg} value of 0.000 146 min^{−1} was used. The average fold differences between observed and predicted were 2.62 for the replot method and 1.12 for the numerical method when a hepatic k_{deg} value of 0.0003 min^{−1} was used.

DISCUSSION

In the current study, the irreversible inhibition of CYP3A was characterized for four inactivators using a numerical method in which the system is modeled directly from kinetic differential equations. All the studied inactivators are known to form MICs. These MICs require sequential metabolism, and when available, primary metabolites were additionally evaluated. Furthermore, mechanistic kinetic models were developed to account for different phenomena, including the aging of MICs, which is critical in the determination of k_{inact} ,³⁴ atypical kinetics, nonspecific partitioning into microsomes, and inhibitor depletion during the inactivation phase. Clinical-DDI predictions were performed using the parameters obtained from the numerical method and compared with the predictions from the replot method. This is the first study to use these numerical methods for clinical-DDI predictions.

Because this modeling approach does not assume steady-state kinetics and irreversibility, the curvature observed in the PRA plots for the MIC- forming inactivators can be modeled. For VER and DTZ, the models were also successful in capturing the lag due to the sequential metabolism observed in the PRA plots.

One disadvantage of the numerical method is the number of data points required for complex models. In the present study, the total number of time points for the various inactivators ranged from 12 to 15. However, similar parameters were obtained with comparable errors from an 8×8 matrix (8 inhibitor concentrations and 8 time points) as compared to those from 8×12 and 8×15 matrices (data not shown). Generating an 8×8 matrix or more might be challenging in the early drug-discovery phase. However, an 8×8 matrix was sufficient to solve the complex models presented here. Both very early time points (<5 min) and longer primary-incubation time points (>30 min) are required in order to capture lags in activation, such as those observed with sequential metabolism and the aging of MICs.

It was shown earlier by Barnaba et al.³⁴ that the aging of the MIC is critical in determining the rate of inactivation. It was observed that the Fe^{3+} :carbene intermediate can either get reduced to Fe^{2+} :carbene, which is terminally inactive, or it can release the carbene to free the active enzyme, resulting in a branched pathway (e.g., k_6 , k_7 , and k_8 in all the kinetic models). Because all the inactivators studied here are known to form MICs, the aging of MICs was incorporated into all the kinetic models. Furthermore, sequential metabolism was incorporated into the models to account for the formation of the primary metabolite (I_2). However, for ERY, it was observed that the simpler models gave better fits than the sequential models. In the absence of TAO-metabolite data, a sequential model was not evaluated. Sequential metabolism is required to form the MICs for both TAO and ERY, but lags in inactivation (i.e., initially concave downward PRA plots) are not observed. This suggests that sequential metabolism occurs without the release of the metabolite intermediates.

In the case of TAO, the two-binding-site model was able to capture the competitive inhibition (~8% decrease in activity at $10 \mu\text{M}$), resulting in a better fit than the single-binding-site model. Competitive inhibition is evident from the Y -intercept of the PRA plot (Figure 1). The use of an EII model was also supported by deviation in the competitive K_i and inactivation K_i . Although TAO is a large molecule (molecular weight, MW, of 814), it is known that CYP3A4 is capable of substrate-induced conformational changes. For instance, the binding of ERY dramatically expanded⁵⁹ the substrate-binding pocket from ~520 to ~2000 Å³. Also, much larger molecules (e.g., cyclosporine with an MW of 1203) are metabolized by CYP3A4. Hence, it is possible that two molecules of TAO can bind to CYP3A4. It is also possible that more complex kinetic events, such as enzyme–enzyme interactions, are responsible for the observed kinetic behavior. However, EII is the simplest model that captures the observed kinetics.

In case of ERY, the TDI kinetics for the metabolite NDE were also studied in order to characterize ERY inactivation kinetics. Although the primary metabolite of ERY, NDE, has to be formed in order to inactivate CYP3A, a simpler model (MIC-IL) gave a better fit to the

ERY data than the sequential models. The sequential models, which allow for the formation of the primary metabolite, did not improve the model fit. Again, the absence of a lag time suggests that NDE predominantly stays in the active site where it inactivates CYP3A. Although a literature report suggests the possibility of multiple bindings of ERY,⁶⁰ multiple-binding events could not be observed in this study.

For VER and DTZ, a lag in inactivation was observed, suggesting that the primary metabolites (the actual inactivators) were released from the active site. The sequential models were able to capture the lag in the inactivation as well as the atypical kinetics observed during the inactivation. VER is known to exhibit atypical kinetic behavior,⁶¹ and atypical behavior (multiple-inhibitor binding) could be observed from the PRA plots in Figure 7. For both VER and DTZ, of all the models developed, three models were able to explain the observed data by accounting for sequential metabolism, atypical kinetics, and inhibitor depletion. All the models gave equally good fits to the data. The deviation from hyperbolic saturation kinetics observed in the PRA plot (e.g., the sigmoidal saturation curve in Figure 7g) could be the result of multiple bindings, inhibitor depletion, or both. The models shown for DTZ and VER include both multiple bindings and inhibitor depletion. To further refine the models, more data (e.g., inhibitor concentrations during the preincubation phase) will be required. Because inhibitor-loss data was not measured for either DTZ or VER, model identifiability was not possible. Measurement of inhibitor loss during the inactivation phase is important, as evidenced from studies in which inactivation-phase inhibitor concentrations were measured and used to characterize TDI kinetics. Burt et al.²⁵ used the progress-curve method in which inhibitor concentrations are used in a mechanistic model to estimate TDI parameters. Zhao et al.⁴³ used a static time-averaged inhibitor concentration while estimating TDI parameters for DTZ. Berry et al.⁶² measured inhibitor loss and corrected the IC₅₀ shift with the inhibitor-loss data. It is evident from these examples that the use of inhibitor concentrations will improve the TDI-parameter estimates. Studies are currently underway to include inhibitor-concentration data in complex TDI modeling.

In all the models, nonspecific microsomal partitioning was modeled to provide accurate unbound-inhibitor concentrations. The advantages of the dynamic modeling of microsomal partitioning are predominantly seen for highly partitioned compounds. There is a shift in the equilibrium after dilution, which can lead to inconsistent inactivator concentrations in the secondary incubation. This shift in the equilibrium can be accounted for by incorporating dynamic partitioning into the model. Moreover, because the estimates for k_{on} and k_{off} (eq 3) are obtained from measurements of $f_{u,mic}$ and fixed during the modeling process, they do not impact the number of parameters that can be estimated from the model.

To our knowledge, this is the first effort to model TDI due to sequential metabolism with a numerical method. The models developed in this study could successfully capture the observed lag in inactivation for the parent inhibitors. Parent- and metabolite-inactivation data were used together in order to develop a single model for sequential metabolism and inactivation. An obvious limitation of these models is that metabolite concentrations in vitro may not mimic intracellular in vivo concentrations. This could be due to vast differences in

the distributions of the metabolite in the two systems as well as the elimination of the metabolite. The impacts of these model limitations on DDI predictions are discussed below.

DDI predictions were performed using the static models summarized in the FDA guidelines.⁵² As seen from Table 6 and Figure 8, the TDI parameters obtained from the numerical method were able to predict the observed DDIs with CYP3A substrates within an average of 1.45-fold, as compared with 3.17-fold from the replot method. Other research groups have also shown that the TDI parameters from the replot method tend to overpredict DDIs.^{37,63–65} One possible reason for the overprediction is that the replot method is unable to capture the curvature in the PRA plots, leading to an overestimation of k_{inact} . The k_{inact} estimates from the numerical method are 2.51-fold (for DTZ) to 21.1-fold (for TAO) lower than those from the replot method. Furthermore, because the replot method assumes Michaelis–Menten kinetics, it is unable to account for atypical kinetics. The estimates of $K_{\text{I,u}}$ for the inactivators that show Michaelis–Menten kinetics (NDE, ERY, and NDD) are similar to those from both the numerical and replot methods, whereas for inactivators that show non-Michaelis–Menten kinetics (NV, DTZ, VER, and TAO), the estimates are 0.14-fold (for TAO) to 3-fold (for VER) lower when the numerical method is used.

DDI predictions were calculated for 77 observed CYP3A-victim-perpetrator-drug pairs. A majority of the DDI predictions are within 2-fold when the parameters from the numerical method are used. However, 11 out of 77 the DDI interactions (e.g., ERY–atorvastatin, VER–buspirone, and TAO–triazolam), were overpredicted by more than 2-fold, and 1 interaction (VER–dabigatran, which has possible P-gp involvement) was underpredicted by more than 2-fold. In comparison, 44 interactions were overpredicted by more than 2-fold with the replot method, and 1 (VER–dabigatran) was underpredicted by more than 2-fold. Possible reasons for the poor DDI predictions with the numerical method include inaccurate pharmacokinetic parameters (e.g., absorption-rate constants); parent- and metabolite-distribution kinetics, including the influences of transporters; and differences in the in vitro versus in vivo metabolite-elimination kinetics.

The enzyme-synthesis-rate constants, $k_{\text{deg,h}}$ and $k_{\text{deg,g}}$, are important parameters in the DDI-prediction equation. One reason for the lack of IVIVC could be the use of inaccurate $k_{\text{deg,h}}$ and $k_{\text{deg,g}}$ values. There is wide variability in the reported values for $k_{\text{deg,h}}$ in the literature. The estimates for these rate constants can be derived from either in vitro or in vivo studies. The $k_{\text{deg,h}}$ values derived from in vitro systems (e.g., hepatocytes) tend to be higher ($t_{1/2} \sim 36$ h, more rapid enzyme syntheses),^{53,55,57,66} whereas the $k_{\text{deg,h}}$ values derived from in vivo studies are lower ($t_{1/2} \sim 80$ h, slower enzyme syntheses),^{56,58,67,68} indicating system- or method-specific biases in k_{deg} estimates. The values for CYP3A half-lives reported in the literature range from 28 to 140 h. An average $k_{\text{deg,h}}$ value of 79 h⁴⁸ was chosen for the present study. Recent literature reports^{63,65,69} have used values for $k_{\text{deg,h}}$ resulting in an enzyme half-lives of 26–36 h. Because any DDI prediction will be decreased by a faster $k_{\text{deg,h}}$, there may have been a tendency to use higher $k_{\text{deg,h}}$ values to mitigate the general overprediction of DDIs. For MIC-forming inactivators, much of the overprediction was likely due to the use of initial-phase- inactivation data.³⁴ The most appropriate value of $k_{\text{deg,h}}$ should be revisited, given recent advances in TDI data analysis. The data in Table 6

and Figure 8 indicate that irrespective of the $k_{deg,h}$ value used, the numerical approach results in better clinical-DDI predictions compared with the replot method.

In the present study, IVIVC was conducted with standard static equations. With the static IVIVC method, DDI predictions can be performed without in vivo parent- and metabolite-concentration–time profiles. However, static methods suffer from the following deficiencies: (1) a static model utilizes a single drug concentration instead of a concentration–time profile; (2) static models are not able to differentiate between different inactivator-dosing regimens; (3) for sequential metabolism, metabolite concentrations cannot be included in static IVIVC methods; and (4) models of the complexity described in this work (e.g., multiple binding sites) are not easily incorporated into static equations. Another issue with all IVIVC methods is the difference between drug and metabolite dispositions in vitro and those in vivo. In a “closed” in vitro incubation, compounds are restricted to the incubation volume for the duration of the experiment. In vivo, the “open” system allows for the distribution of the compounds out of the hepatocytes and enterocytes and for their irreversible elimination by multiple pathways.

Although these issues cannot be easily resolved, dynamic models can begin to incorporate some of these complexities into the modeling process. Thus, PBPK and semi-PBPK models can be used to incorporate the target-site concentrations of the parent and the primary metabolite when predicting TDI-mediated DDIs. There are several reports in the literature using PBPK and semi-PBPK models for DDI prediction.^{70–74} However, all the reported models assume Michaelis–Menten kinetics when incorporating TDI parameters. The incorporation of complex TDI schemes into dynamic DDI models with the numerical method is in fact facile, because both PBPK models and numerical TDI models consist of collections of ordinary differential equations. We have successfully modeled membrane partitioning and diffusion into and out of cells using an explicit membrane-modeling approach.^{75,76} This suggests that in addition to membrane partitioning, permeability data could be utilized to model parent and metabolite distribution. Efforts to incorporate the TDI models presented here into a physiological framework are currently underway in our laboratory.

Finally, the categorization of CYP3A inhibitors (weak, moderate, and strong) in the current FDA guidelines⁵² is based on K_I and k_{inact} values from the replot method. It is obvious from Figure 8 that the numerical method can result in improved categorizations of DDI predictions. However, changes in regulatory guidelines will require additional studies to support the incorporation of lipid partitioning, non-MM kinetics, and sequential metabolism.

CONCLUSIONS

In conclusion, a numerical approach was used to model complex CYP3A TDI in human-liver microsomes. A number of MIC-forming TDIs and sequential metabolites were evaluated. The complexities incorporated into the models included multiple-binding kinetics and quasi-irreversible inactivation and for the first time sequential metabolism, inhibitor depletion, and membrane partitioning. The resulting inactivation parameters were incorporated into static in vitro–in vivo correlation (IVIVC) models to predict clinical DDIs.

For 77 clinically observed DDIs, using a hepatic CYP3A-synthesis-rate constant of 0.000 146 min⁻¹, the average fold difference between the observed and predicted DDIs was 3.17 for the standard replot method and 1.45 for the numerical method. Similar results were obtained using a synthesis-rate constant of 0.000 32 min⁻¹. These results suggest that numerical methods can successfully model complex in vitro TDI kinetics and that the resulting DDI predictions are more accurate than those obtained with the standard replot approach. The incorporation of these complex kinetic models into dynamic IVIVC models is currently underway and is expected to improve TDI-mediated DDI predictions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CYPs	cytochromes P450
DDI	drug–drug interactions
DME	– drug-metabolizing enzymes
DTZ	diltiazem
ERY	erythromycin
HLM	human liver microsomes
IVIVC	in vitro–in vivo correlation
MIC	metabolite-intermediate complex
MM	Michaelis–Menten
NDD	<i>N</i> -demethyl diltiazem
NDE	<i>N</i> -demethyl erythromycin
NV	norverapamil
PAR	paroxetine
PBPK	physiologically based pharmacokinetic models
TAO	troleandomycin
TDI	time-dependent inhibition
VER	verapamil

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Figure 1.

Kinetic scheme for CYP3A inhibition by TAO (10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 μM) in HLM. (A) Kinetic scheme for the MIC-EII-IL model. E, enzyme; I, inhibitor; L, lipid; P, product; S, substrate; k , rate constant. (B) Experimental (points) and MIC-EII-IL-model-fitted (solid lines) PRA plots. (C) Plot of k_{obs} vs [I] for the standard replot method with linear data points ($n = 4$ points).

Figure 2.

Kinetic scheme for CYP3A inhibition by NDE (50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0 μM) in HLM. (A) Kinetic scheme for the MIC-IL-M model. E, enzyme; I, inhibitor; L, lipid; M, inhibitor metabolite; P, product; S, substrate; k , rate constant. (B) Experimental (points) and MIC-IL-M-model-fitted (solid lines) PRA plots. (C) Plot of k_{obs} vs [I] for the standard replot method with linear data points ($n = 4$ points).

Figure 3.

Kinetic scheme for CYP3A inhibition by ERY (50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0 μM) in HLM. (A) Kinetic scheme for the MIC-IL model. E, enzyme; I, inhibitor; L, lipid; P, product; S, substrate; k , rate constant. (B) Experimental (points) and MIC-IL-model-fitted (solid lines) PRA plots. (C) Plot of k_{obs} vs [I] for the standard replot method with linear data points ($n = 7$ points).

Figure 4.

Kinetic scheme for CYP3A inhibition by NDD (10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 μM) in HLM. (A) Kinetic scheme for the MIC-I₂L model. E, enzyme; I₂, metabolite inhibitor; L, lipid; P, product; S, substrate; *k*, rate constant. (B) Experimental (points) and MIC-I₂L-model-fitted (solid lines) PRA plots. (C) Plot of *k*_{obs} vs [I] for the standard replot method with linear data points (*n* = 7 points).

Figure 5.

Kinetic schemes for CYP3A inhibition by DTZ (40, 20, 10, 5, 2.5, 1.25, 0.625, and 0 μ M) in HLM. (A) Kinetic scheme for the Seq-MIC- EI_1I_1 - I_1L - I_2L model. (B) Experimental (points) and Seq-MIC- EI_1I_1 - I_1L - I_2L -model-fitted (solid lines) PRA plots. (C) Kinetic scheme for the Seq-MIC- EI_1I_1 -M- I_1L - I_2L model. (D) Experimental (points) and Seq-MIC- EI_1I_1 -M- I_1L - I_2L -model-fitted (solid lines) PRA plots. (E) Kinetic scheme for the Seq-MIC- EI_1I_1 -MM- I_1L - I_2L model. (F) Experimental (points) and Seq-MIC- EI_1I_1 -MM- I_1L - I_2L -model-fitted (solid lines) PRA plots. (G) Plot of k_{obs} vs [I] for the standard replot method with linear data

points. E, enzyme; I_1 , parent inhibitor; I_2 , metabolite inhibitor; L, lipid; M, metabolite; P, product; S, substrate; k , rate constant.

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Figure 6. Kinetic scheme for CYP3A inhibition by NV (40, 20, 10, 5, 2.5, 1.25, 0.625, and 0 μM) in HLM. (A) Kinetic scheme for the MIC-EI₂I₂-I₂L model. E, enzyme; I₂, metabolite inhibitor; L, lipid; P, product; S, substrate; k , rate constant. (B) Experimental (points) and MIC-EI₂I₂-I₂L-model-fitted (solid lines) PRA plots. (C) Plot of k_{obs} vs $[I]$ for the standard replot method with linear data points ($n = 4$ points).

Figure 7.

Kinetic schemes for CYP3A inhibition by VER (40, 20, 10, 5, 2.5, 1.25, 0.625, and 0 μM) in HLM. (A) Kinetic scheme for the Seq-MIC-EI₁I₁-EI₂I₂-I₁L-I₂L model. (B) Experimental (points) and Seq-MIC-EI₁I₁-EI₂I₂-I₁L-I₂L-model-fitted (solid lines) PRA plots. (C) Kinetic scheme for the Seq-MIC-EI₁I₁-EI₂I₂-M-I₁L-I₂L model. (D) Experimental (points) and Seq-MIC-EI₁I₁-EI₂I₂-M-I₁L-I₂L-model-fitted (solid lines) PRA plots. (E) Kinetic scheme for the Seq-MIC-EI₁I₁-EI₂I₂-MM-I₁L-I₂L model. (F) Experimental (points) and Seq-MIC-EI₁I₁-EI₂I₂-MM-I₁L-I₂L-model-fitted (solid lines) PRA plots. (G) Plot of k_{obs} vs [I] for the

standard replot method with linear data points. E, enzyme; I_1 , parent inhibitor; I_2 , metabolite inhibitor; L, lipid; M, metabolite; P, product; S, substrate; k , rate constant.

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Figure 8.

Observed DDIs versus predicted DDIs with different hepatic k_{deg} . (A) $k_{\text{deg}} = 0.00015 \text{ min}^{-1}$ ($t_{1/2} = 79 \text{ h}$). (B) $k_{\text{deg}} = 0.00032 \text{ min}^{-1}$ ($t_{1/2} = 36 \text{ h}$). The solid lines are the lines of unity, the dashed lines (—) are the 1.25-fold lines, and dot-dashed lines (— · — · —) are the 2-fold lines. The tables in the figure show the averages $\pm$ the standard deviations of the predicted fold difference for both the methods.

Table 1 F_g and $f_{m,CYP3A}$ Parameters Used for the DDI Predictions

compound	F_g^a	$f_{m,CYP3A}^b$
almotriptan ^{77-80,c}	0.82	0.12
alprazolam ^{81,82}	0.86	0.83
antipyrine ^{83-86,c}	1	0.38
atenolol ^{83,87,88,c}	1	0.05
atorvastatin ⁸⁹⁻⁹¹	0.24	0.64
buspirone ^{92,93}	0.21	0.94
cerivastatin ^{83,91}	0.69	0.18
cyclosporin ^{90,94}	0.65	0.8
dabigatran ^{95,96,c}	1	0.02
diazepam ^{83,90}	1	0.24
felodipine ^{92,97}	0.45	0.81
fexofenadine ^{98,c}	0.4	0.18
flunitrazepam ^{99-101,c}	0.91	0.85
imipramine ¹⁰²	1	0.02
lignocaine ^{103-108,c}	1	0.42
losartan ^{109,83,c}	0.66	0.1
lovastatin ^{83,110,111,c}	0.22	0.6
metoprolol ^{83,102}	0.84	0.15
midazolam ^{112,113}	0.57	0.93
nifedipine ^{91,92}	0.78	0.78
pravastatin ^{83,114,115,c}	0.8	0.22
propranolol ^{83,116,117,c}	0.5	0.17
quinidine ^{92,97}	0.91	0.76
risperidone ^{83,118,c}	0.85	0.11
ropivacaine ^c		0.45
sildenafil ⁹²	0.54	0.85
simvastatin ^{92,119}	0.66	0.92
terfenadine ^{71,94}	0.4	0.74
theophylline ^{83,120,c}	0.99	0.14
triazolam ^{97,121}	0.75	0.92
zopiclone ^{83,122}	0.93	0.5

^aThe F_g values were calculated by an iv or oral method as described in Varma et al.⁸³

^bThe $f_{m,CYP3A}$ values were calculated in-house on the basis of the dispositions of the drugs upon intravenous dosings as reported in the literature and CYP-reaction phenotyping.

^cCalculated in-house on the basis of the values reported in the literature.

Table 2

PK Parameters Used for the DDI Predictions

inhibitor	$f_{u,mic}(1mg/mL)$	$f_{u,p}$	PK values
DTZ ^{83,123–126}	0.852	0.299 ± 0.027	$k_a = 1.04 \text{ h}^{-1}$ $F_a F_g = 0.45$ $F = 0.42$ $CL_s = 48.3 \text{ L/h}$ $V_{ss} = 777 \text{ L}$ $BP^a = 1$
ERY ¹²⁷	0.563	0.285 ± 0.010	$k_a = 0.66 \text{ h}^{-1}$ $F_a F_g = 0.33$ $F = 0.15$ $CL_s = 39.8 \text{ L/h}$ $V_{ss} = 55.5 \text{ L}$ $BP = 1.3$
NDD	0.550		
NDE	0.510		
NV	0.710	0.145 ± 0.023	
TAO ¹²⁸	0.730^b	0.038^b	$k_a = 0.46 \text{ h}^{-1}$ $[I]_{max} = 2.44 \mu M$ $F_a F_g = 1^c$ $BP = 1^c$
VER ^{94,126,129–132}	0.320	0.104 ± 0.017	$k_a = 1.93 \text{ h}^{-1}$ $F_a F_g = 0.71$ $F = 0.18$ $CL_s = 51.6 \text{ L/h}$ $V_{ss} = 267 \text{ L}$ $BP = 0.77$

^aBlood-to-plasma-partition ratio.^bObtained from the literature.^cAssumed to be 1.

Table 3

TDI Parameters for the Inactivators from the Numerical and Replot Methods

inactivator	numerical method			replot method		
	$K_{I,u}$ (μM)	k_{inact} (min^{-1})	$k_{\text{inact}}/K_{I,u}^a$ ($\mu\text{M}/\text{min}^{-1}$)	$K_{I,u}$ (μM)	k_{inact} (min^{-1})	$k_{\text{inact}}/K_{I,u}^a$ ($\mu\text{M}/\text{min}^{-1}$)
ERY	5.64 ± 0.45	0.004 ± 0.003	0.0007 ± 0.0004	6.06 ± 0.74	0.053 ± 0.001	0.009 ± 0.001
NDD	0.62 ± 0.04	0.009 ± 0.001	0.014 ± 0.001	1.08 ± 0.21	0.036 ± 0.001	0.033 ± 0.007
NDE	1.94 ± 0.35	0.004 ± 0.005	0.003 ± 0.004	2.45 ± 0.85	0.073 ± 0.005	0.030 ± 0.010
NV	$K_{I1,u} = 10.17 \pm 2.54$ $K_{I2,u} = 1.85 \pm 0.33$	0.010 ± 0.003	0.001 ± 0.0004	4.99 ± 0.72	0.174 ± 0.007	0.034 ± 0.005
TAO	$K_{I1,u} = 0.75 \pm 0.25$ $K_{I2,u} = 1.98 \pm 0.35$	0.007 ± 0.003	0.009 ± 0.005	5.30 ± 0.62	0.148 ± 0.010	0.028 ± 0.004

^a For TAO and NV, the $K_{I1,u}$ values were used to calculate $k_{\text{inact}}/K_{I,u}$.

Table 4TDI Kinetic Parameters for CYP3A Inhibition by DTZ^a

parameter	numerical method			replot method
	Seq-MIC-EI ₁ I ₁ -I ₁ L-I ₂ L	Seq-MIC-EI ₁ I ₁ -M-I ₁ L-I ₂ L	Seq-MIC-EI ₁ I ₁ -MM-I ₁ L-I ₂ L	
$K_{I1,u}$ (μ M)	7.72 ± 3.29	7.27 ± 2.75	7.09 ± 2.62	2.98 ± 0.68
$K_{I2,u}$ (μ M)	6.66 ± 1.97	15.96 ± 6.9	14.77 ± 6.2	
k_{inact} (min^{-1}) ^b	0.009 ± 0.001	0.009 ± 0.001	0.009 ± 0.001	0.022 ± 0.001
$k_{inact}/K_{I1,u}$	0.001 ± 0.0005	0.001 ± 0.0005	0.001 ± 0.0005	0.007 ± 0.002
AICc	-728.87	-732.2	-732.1	
adjusted R^2	0.9992	0.9993	0.9993	
MSE ^c	3.99	3.33	3.33	

^aThe data are presented as parameter estimates $\pm$ SD.^b k_8 was fixed at 0.02 min^{-1} .^cMean-square error.

Table 5TDI Parameters for VER by the Numerical Method for Three Models and also by the Replot Method^a

parameters	numerical method			replot method
	Seq-MIC-EI ₁ I ₁ -EI ₂ I ₂ -I ₁ L-I ₂ L	Seq-MIC-EI ₁ I ₁ -EI ₂ I ₂ -M-I ₁ L-I ₂ L	Seq-MIC-EI ₁ I ₁ -EI ₂ I ₂ -MM-I ₁ L-I ₂ L	
$K_{I1,u}$ (μM)	5.63 $\pm$ 2.56	5.31 $\pm$ 2.11	5.03 $\pm$ 1.94	1.79 $\pm$ 0.45
$K_{I2,u}$ (μM)	1.54 $\pm$ 0.58	5.44 $\pm$ 3.03	6.19 $\pm$ 4.21	
k_{inact} (min^{-1})	0.010 $\pm$ 0.003	0.010 $\pm$ 0.003	0.010 $\pm$ 0.003	0.074 $\pm$ 0.005
$k_{inact}/K_{I1,u}$	0.002 $\pm$ 0.001	0.002 $\pm$ 0.001	0.002 $\pm$ 0.001	0.041 $\pm$ 0.011
AICc	-556.03	-568.38	-566.578	
adjusted R^2	0.999	0.998	0.999	
MSE ^b	3.88	3.23	3.23	

^aThe data are presented as parameter estimates $\pm$ SD.^bMean-square error.

Table 6

DDI Predictions According to the Numerical and Replot Methods for TAO, ERY, DTZ, and VER with MDZ as the Victim^a

midazolam dosing route	inactivator	observed DDI	predicted DDI		predicted fold difference	
			numerical	replot	numerical/observed	replot/observed
$k_{deg} = 0.000\ 146\ \text{min}^{-1}$						
iv ⁷⁴	DTZ	1.72	1.78	3.56	1.03	2.07
po ⁷⁴	DTZ	4.01	6.24	15.66	1.56	3.91
po ¹³³	ERY	4.41	5.52	19.34	1.25	4.39
iv ¹³³	ERY	2.2	2.74	9.9	1.25	4.5
iv ¹³⁴	TAO	4.65	4.57	8.31	0.98	1.79
po ¹³⁴	TAO	14.82	11.07	18.76	0.75	1.27
po ⁹²	VER	3.5	12.2	23.34	3.49	6.67
iv ⁹²	VER	1.45	1.45	6.53	1.0	4.50
$k_{deg} = 0.000\ 32\ \text{min}^{-1}$						
iv ⁷⁴	DTZ	1.72	1.22	2.3	0.71	1.34
po ⁷⁴	DTZ	4.01	4.01	11.25	1.0	2.81
po ¹³³	ERY	4.41	3.57	15.48	0.81	3.51
iv ¹³³	ERY	2.2	1.86	7.38	0.85	3.36
iv ¹³⁴	TAO	4.65	2.91	5.76	0.63	1.24
po ¹³⁴	TAO	14.82	7.24	14.65	0.49	0.99
po ⁹²	VER	3.5	8.12	21.71	2.32	6.20
iv ⁹²	VER	1.45	1.21	4.26	0.83	2.94

^aClinical studies with iv and po MDZ dosings are included, and additional reports are included in the Supporting Information. For all the DDI predictions, the $K_{1,u}$ and k_{inact} values listed in Table 3 were used, except those for DTZ, whose $K_{1,u}$ and k_{inact} values were listed in Table 4, and VER, whose $K_{1,u}$ and k_{inact} values were listed in Table 5.