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ARTICLE

Prediction of CYP-mediated DDIs involving inhibition: Approaches to address the requirements for system qualification of the Simcyp Simulator

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Abstract

Physiologically-based pharmacokinetic (PBPK) modeling is being increasingly used in drug development to avoid unnecessary clinical drug–drug interaction (DDI) studies and inform drug labels. Thus, regulatory agencies are recommending, or indeed requesting, more rigorous demonstration of the prediction accuracy of PBPK platforms in the area of their intended use. We describe a framework for qualification of the Simcyp Simulator with respect to competitive and mechanism-based inhibition (MBI) of CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5. Initially, a DDI matrix, consisting of a range of weak, moderate, and strong inhibitors and substrates with varying fraction metabolized by specific CYP enzymes that were susceptible to different degrees of inhibition, were identified. Simulations were run with 123 clinical DDI studies involving competitive inhibition and 78 clinical DDI studies involving MBI. For competitive inhibition, the overall prediction accuracy was good with an average fold error (AFE) of 0.91 and 0.92 for changes in the maximum plasma concentration (C_{max}) and area under the plasma concentration (AUC) time profile, respectively, as a consequence of the DDI. For MBI, an AFE of 1.03 was determined for both C_{max} and AUC. The prediction accuracy was generally comparable across all CYP enzymes, irrespective of the isozyme and mechanism of inhibition. These findings provide confidence in application of the Simcyp Simulator (V19 R1) for assessment of the DDI potential of drugs in development either as inhibitors or victim drugs of CYP-mediated interactions. The approach described herein and the identified DDI matrix can be used to qualify subsequent versions of the platform.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Regulatory agencies are recommending, or indeed requesting, more rigorous demonstration of the prediction accuracy of physiologically-based pharmacokinetic

(PBPK) platforms in the area of their intended use to support regulatory submissions.

WHAT QUESTION DID THIS STUDY ADDRESS?

This work describes a framework for the qualification of the Simcyp Simulator with respect to competitive and mechanism-based inhibition of CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

These findings provide confidence in application of the Simcyp Simulator (V19 R1) for assessment of the drug–drug interaction (DDI) potential of prospective new drugs in development either as inhibitors or victim drugs of CYP-mediated interactions.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The qualification of DDIs for multiple CYP enzymes in this work supports the use of PBPK modeling, using the Simcyp Simulator, for prospective DDI assessments of new drugs which can be included in regulatory submissions.

INTRODUCTION

Physiologically-based pharmacokinetic (PBPK) models help to make optimal use of available data by combining the complex interplay of physiological parameters with characteristics relating to the absorption, distribution, metabolism, and excretion of a specific drug, thus representing a mechanistic approach to predict the pharmacokinetics (PKs) of the drug. PBPK modeling has been increasingly used for various applications to guide decision making in drug development on assessment of drug–drug interaction (DDI) liability, to design clinical studies, dose extrapolation in special populations including pediatrics, and investigation of formulation and food effects.[1] PBPK models that have demonstrated a good predictive performance, particularly in support of quantitative prediction of DDIs, are often submitted to regulatory agencies.[2-4] Once reviewed and accepted by health authorities, they have been used to inform the prescription drug label for untested clinical scenarios. Indeed, global regulators endorse the use of this approach for assessment of the potential DDI risk of investigational new drugs as both victim and perpetrator drugs; over the past 5 to 10 years, they have issued guidance documents or published best practice approaches for the application of PBPK in regulatory submissions.[5-8]

The US Food and Drug Administration (FDA) has stated in its current PBPK guidance document that it should be demonstrated that "the PBPK model is appropriate for the intended uses."[9] Recent European Medicines Agency (EMA) guidelines have taken this one step further by presenting a framework linking the required level of qualification to the intended use of PBPK models based on low, medium and high impact on regulatory applications.[10] More recently, the FDA reported that there is no consensus on how to establish and assess model credibility, and proposed a "risk-informed credibility assessment framework" for PBPK modeling and simulation, which includes the terms verification (of the code and calculations) and validation (of the model).[7] In any case, irrespective of the terminology used, if a PBPK model is being used to waive a clinical DDI study involving CYP3A4/5, as an example, considered to be high impact or high risk, the platform is expected to be qualified for prediction of CYP3A4/5-mediated DDIs. For this purpose, a prespecified qualification dataset using reference CYP3A4/5 sensitive substrates and a range of inhibitors is needed to evaluate the performance of the PBPK platform prior to DDI prediction for the new compound. Substrate and inhibitor PBPK models that have been verified for their intended purpose should form part of this comprehensive DDI matrix that can be applied to qualify an existing software platform and requalify subsequent versions.

The aim of this study was to identify a DDI matrix for CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5 interactions and utilize existing verified compound library files within the Simcyp Simulator to qualify the software platform for CYP-mediated competitive inhibition and mechanism-based inhibition (MBI). A secondary aim, was to provide guidance on key elements that form part of the qualification strategy, such as appropriate verification for each of the compound files.

METHODS

Data sources and software

The University of Washington Drug Interaction Database was used to identify controlled clinical DDI studies involving CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5 where observed increases in plasma exposure of substrates >20% (as a consequence of the DDI) were reported.

DDI studies were then selected to form part of the DDI qualification matrix if compound files for both substrate and inhibitor were available as compound files within the Simcyp Simulator. It should be noted that the substrates and inhibitors included as compound files within the Simcyp Simulator had previously been selected for development based on the FDA recommendations for reference index substrates and inhibitors.[11] Where possible, another criterion for selection of DDI studies was to ensure the inclusion of a range of weak, moderate, and strong inhibitors and substrates that were susceptible to differing degrees of inhibition (Table S1). A summary of the DDI qualification matrix for CYP3A4/5 is shown in Figure 1 as an example.

Development and verification of compound files

Although describing the development and verification of each of the files is beyond the scope of the current publication, it is important to indicate the robustness of the process (i.e., application of a best-practice approach which is described in a number of publications).[8,12] Prior to integration within the platform, a rigorous feasibility assessment was conducted for each compound to ensure that there were sufficient in vitro and clinical data available to develop and verify the files for their intended use (i.e., quantitative prediction of CYP-mediated DDIs either as a victim and/or perpetrator).

Typically, development of an in vitro–in vivo extrapolation linked PBPK model aims to describe concentration-time profiles from clinical datasets based on in vitro data alone. Model development was performed initially using intravenous data (if available) with a focus on the distribution and elimination parameters. Thereafter, absorption-related parameters were introduced into the PBPK models for each compound to predict plasma concentration–time profiles following oral administration. A first-order absorption

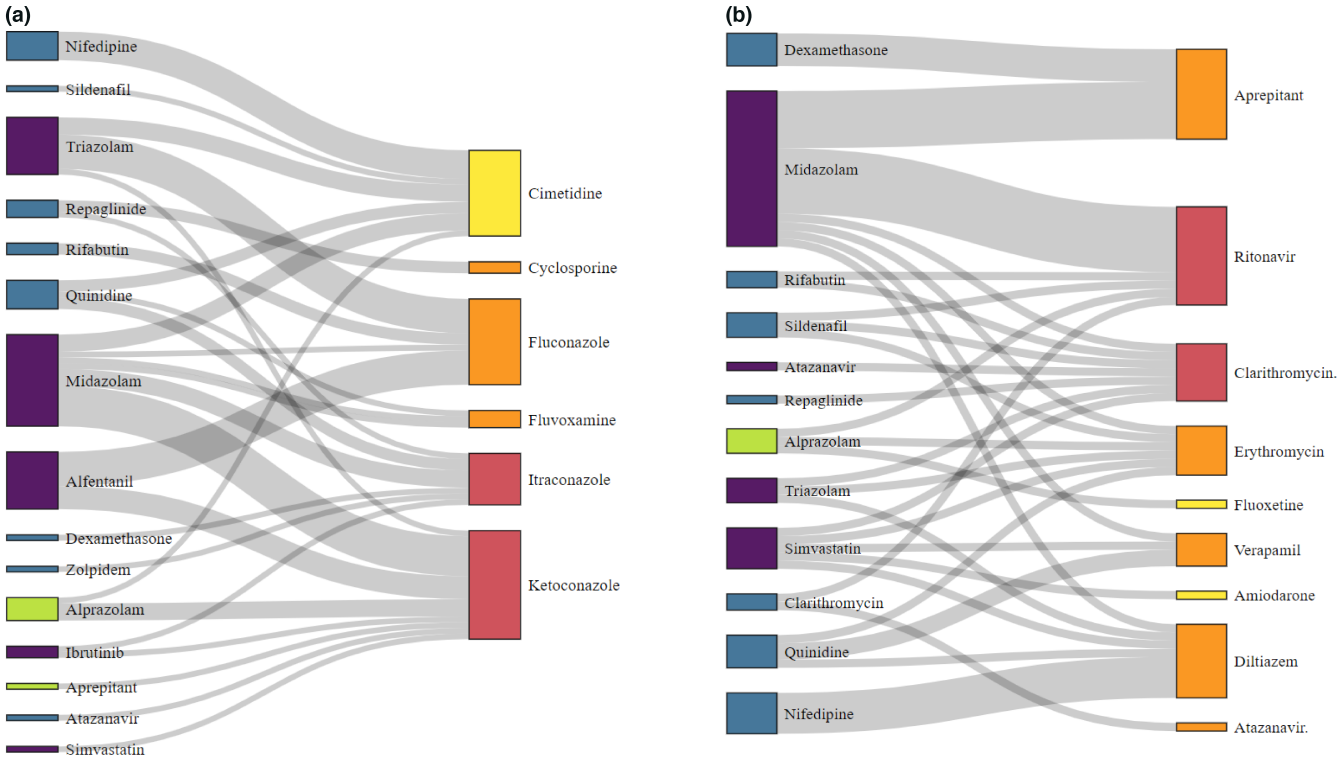

FIGURE 1 Drug–drug interaction matrix for CYP3A4, including available substrate and inhibitor pairings to evaluate competitive (a) and mechanism based (b) inhibition within the Simcyp simulator (V19R1). Colors indicate the clinical sensitive substrates (purple), moderate sensitive substrates (green), subStrates of 3A4 (blue), strong inhibitors (red), moderate inhibitors (orange), and weak inhibitors (yellow)

model was applied for 32 of the 34 substrates and for 20 of the 24 inhibitors. The more complex Advanced Dissolution, Absorption, and Metabolism model was used to describe the absorption of ibrutinib, flurbiprofen, ciprofloxacin, gemfibrozil, ritonavir, and verapamil.

At each stage, optimization of relevant parameters was performed using clinical data, if necessary, to ensure accurate recovery of observed data. For a victim drug (substrate), it was important to characterize the clearance routes and demonstrate that when inhibited, the observed increase in exposures was accurately captured. For a perpetrator (inhibitor), it was necessary to ensure that after integration of the inhibitory parameters into the PBPK model, they led to accurate prediction of clinical DDIs. This process and the input data are captured in a compound file summary, which is version specific. In addition, the source of the input data and the clinical DDI studies, as well as the level of verification that has been performed are included. An example of such a document can be found in Figure S1.

Simulations

For the current analysis, all simulations were performed using the Simcyp Simulator (version 19, release 1). The program allows simple extrapolation of in vitro enzyme kinetic data in multiple organs, including the liver and intestines, to predict PK changes in vivo in virtual populations.[13,14] Genetic, physiological, and demographic variables relevant to the prediction of DDIs are generated for each individual using correlated Monte-Carlo methods and equations derived from population databases obtained from literature sources. To ensure that the characteristics of the virtual subjects were matched closely to those of the subjects studied in vivo, numbers, age range, ethnicity, and sex ratios were replicated in 10 simulated trials and for the number of subjects in each clinical trial. Qualification of the DDI matrix was performed based on prediction of the observed clinical interactions for the respective drug pairings (Table S1).

Data analysis

The ratio of the area under the curve (AUC) in the absence and presence of inhibitor (AUC_i/AUC, where AUC_i and AUC are the $AUC_{[0-\infty]}$ values of the substrate in the presence and absence of inhibitor, respectively) is commonly used as a basis for prediction of metabolic DDIs. In addition, the ratio of the maximum plasma concentration (C_{max}) in the presence and absence of inhibitor can also be used. Accordingly, the mean C_{max} and AUC ratios from the 10 simulated trials were compared against the mean ratios from each clinical study. Equations 1 and 2 were used to calculate the average fold error (AFE) and absolute average fold error (AAFE) as described by Shimizu,[15] which were used to assess the bias and precision of the predictions, respectively.

$$\mathrm{AFE} = 10^{\frac{1}{n}\Sigma\left(\log\frac{\text{Predicted DDI}}{\text{Observed DDI}}\right)} \quad (1)$$

$$\mathrm{AAFE} = 10^{\frac{1}{n}\Sigma\left|\log\frac{\text{Predicted DDI}}{\text{Observed DDI}}\right|} \quad (2)$$

Predictions were assessed as to whether they fell within 1.5-fold of observed data, in addition, as some of the clinical DDIs resulted in weak to moderate inhibitors, the validation criteria were calculated using the method proposed by Guest et al.[16] to avoid a misleading judgment using the more relaxed two-fold criteria for a successful prediction. The data were analyzed according to type of inhibition (competitive vs. MBI) and also according to CYP enzyme.

RESULTS

Substrates and inhibitors

In total, 34 substrates were identified for inclusion in the DDI matrix for qualification of CYP-mediated inhibition using the Simcyp Simulator (V19R1; Table 1). For CYP1A2, caffeine, theophylline, and tizanidine were available with fraction metabolized (f_m) by CYP1A2 ranging from 75.4% to 97.9%. Three substrates were included to evaluate CYP2C19 with $f_{mCYP2C19}$ ranging from 37.7% to 88.5% for imipramine and S-mephenytoin, respectively. Repaglinide (64.4%) and rosiglitazone (53.8%) were included as substrates of CYP2C8. Four CYP2C9 substrates were included, with $f_{mCYP2C9}$ ranging from 73.1% to 98.3% for phenytoin and S-warfarin, respectively. Six substrates were evaluated for CYP2D6-mediated DDIs with $f_{mCYP2D6}$ ranging from 74.3% to 86.9%. The largest range of f_m values was observed for the substrates that were used to evaluate CYP3A4/5-mediated DDIs with $f_{mCYP3A4}$ ranging from 35.4% for repaglinide up to 95.4% for nifedipine.

Across all substrates, the predicted bioavailability (F) ranged from 0.03 to 0.92 for simvastatin and rosiglitazone, respectively. Simvastatin had the lowest predicted fraction escaping first-pass metabolism in the gut (F_g) at 0.03 and this increased up to a maximum value of one for a number of substrates, including caffeine, theophylline, tizanidine, rosiglitazone, and phenytoin.

TABLE 1 Mean f_m, F_g, and F for each substrate according the enzyme of interest as calculated in a population 10 trials and 10 individuals in Simcyp Simulator V19 (R1)

Enzyme	Substrate	f_m %	F_g	F
CYP1A2	Caffeine	97.9	1.00	0.83
	Theophylline	75.4	1.00	0.86
	Tizanidine	96.5	1.00	0.18
CYP2C19	S-Mephenytoin	88.5	0.89	0.35
	Omeprazole	80.5	0.96	0.49
	Imipramine	37.7	0.99	0.38
CYP2C8	Repaglinide	64.4	0.91	0.75
	Rosiglitazone	53.8	1.00	0.92
CYP2C9	Celecoxib	83.5	0.77	0.51
	Flurbiprofen	74.5	0.96	0.90
	Phenytoin	73.1	1.00	0.81
	S-Warfarin	98.3	0.99	0.88
	Tolbutamide	96.5	0.99	0.86
CYP2D6	Atomoxetine	76.7	0.92	0.64
	Desipramine	80.6	0.96	0.49
	Dextromethorphan	86.9	0.91	0.25
	Metoprolol	74.3	0.97	0.47
	Nebivolol	85.7	0.92	0.18
	Tolterodine	81.9	0.99	0.34
CYP3A4/5	Alfentanil	91.8	0.51	0.32
	Alprazolam	70.6	0.99	0.85
	Aprepitant	86.0	0.60	0.48
	Atazanavir	73.8	0.93	0.37
	Clarithromycin	73.7	0.83	0.51
	Dexamethasone	86.2	0.99	0.77
	Ibrutinib	95.2	0.36	0.03
	Midazolam	85.9	0.56	0.28
	Nifedipine	95.4	0.64	0.40
	Quinidine	71.8	0.94	0.68
	Rifabutin	64.9	0.16	0.13
	Repaglinide	33.6	0.92	0.76
	Sildenafil	85.9	0.64	0.36
	Simvastatin	88.9	0.11	0.03
	Triazolam	88.4	0.72	0.50
	Zolpidem	47.8	0.94	0.78

Abbreviations: F, bioavailability; F_g, fraction escaping gut metabolism; f_m, fraction metabolized.

Across all CYP enzymes, there were 24 inhibitors available for qualification of the platform (Table S2). Some inhibitors included inhibition values for multiple CYP enzymes. Out of the inhibitors and metabolites included in the analysis, 63% had interaction parameters based on in vitro data and the remainder were optimized based on clinical data. The full spectrum of strong, moderate, and weak inhibitors was only available for CYP2D6 and CYP3A4.

Competitive versus mechanism-based inhibition

The prediction accuracy of DDIs across all of the CYP enzymes investigated is indicated in Figure 2 and Table 2 for 23 competitive and 18 mechanism-based inhibitors.

Clinical DDIs using competitive inhibitors were investigated for a total of 123 studies for CYP1A2 (20 studies), CYP2C8 (4 studies), CYP2C9 (16 studies), CYP2C19 (4 studies), CYP2D6 (17 studies), and CYP3A4 (62 studies). The overall prediction accuracy was good with a bias of 0.91 and precision of 1.20 for the C_{max} ratio and values of 0.92 and 1.19, respectively, for the AUC ratio. Across the 123 DDIs investigated with competitive inhibitors, 10% fell outside the 1.5-fold of the observed C_{max} ratio with only three of 125 falling outside two-fold from the observed C_{max} ratio. Prediction of the AUC ratio was comparable with 8% falling outside 1.5-fold of the predicted AUC ratio and only one DDI investigated falling outside of two-fold of the observed AUC ratio.

Clinical DDIs involving MBI were investigated for CYP2C8 (8 studies), CYP2C9 (4 studies), CYP2C19 (5 studies), CYP2D6 (9 studies), and CYP3A4/5 (52 studies). The prediction accuracy was good across all CYPs investigated with a bias of 1.03 for both C_{max} and AUC ratios and a precision of 1.20 and 1.26 for C_{max} and AUC ratios, respectively. For the C_{max} ratio, 6% fell outside of 1.5-fold of the observed C_{max} from the clinical studies, with two out of the 62 studies falling outside of two-fold for interactions using simvastatin as a substrate of CYP3A4/5. Prediction of AUC ratios had a slightly higher number of studies falling 1.5-fold outside of the observed AUC ratio with 23% of the DDIs investigated not meeting these criteria, however, only three predictions fell outside two-fold of the observed AUC ratio for omeprazole (CYP2C19 substrate), quinidine, and simvastatin (CYP3A4 substrates).

Individual enzymes by substrate

The DDI matrix for each enzyme was evaluated for the prediction accuracy against and the clinical data and this is shown in Figures 3, 4, and Table 1. The prediction accuracy was generally comparable across all of the CYP enzymes studies in the qualification of the platform. For both CYP2C8 and CYP2C9, all of the predictions fell within 1.5-fold of the observed clinical values for both

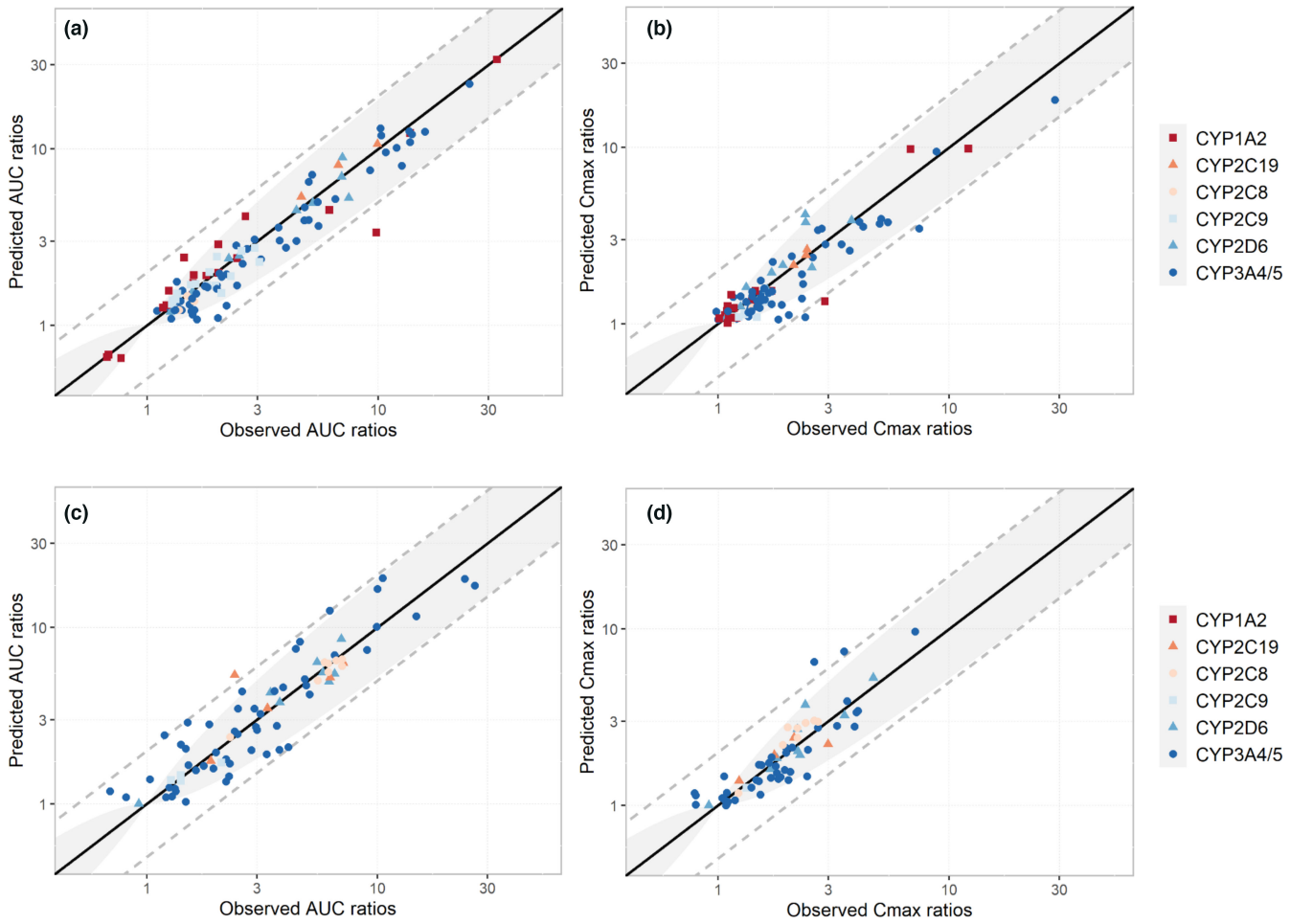

FIGURE 2 Comparison of predicted and observed AUC and C_{max} ratios for competitive (a, b) and mechanism based (c, d) inhibitors for CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. AUC, area under the curve; C_{max}, maximum plasma concentration

TABLE 2 Precision (AFE) and bias (AAFE) of the DDI predictions for the different interaction matrices evaluated

		C_{max}		AUC	
Scenario	***n***	**AFE (bias)**	**AAFE (precision)**	**AFE (bias)**	**AAFE (precision)**
Competitive inhibitors	123	0.91	1.20	0.92	1.19
MBI inhibitors	78	1.03	1.20	1.03	1.26
CYP1A2	20	0.98	1.17	1.01	1.21
CYP2C8	12	1.08	1.16	0.94	1.08
CYP2C9	20	0.88	1.13	0.98	1.12
CYP2C19	9	1.02	1.11	1.09	1.22
CYP2D6	26	1.03	1.20	0.95	1.17
CYP3A4	114	0.93	1.11	0.95	1.26

Abbreviations: AAFE, absolute average fold error; AFE, average fold error; AUC, area under the curve; C_{max}, maximum plasma concentration; DDI, drug-drug interaction; MBI, mechanism-based inhibition.

C_{max} and AUC. The prediction of AUC ratios was good for both enzymes with AFE 0.94 and 0.98 and precision of 1.08 and 1.12 for CYP2C8 and CYP2C9, respectively. The prediction of C_{max} ratios was also close to unity with an AFE of 1.09 for CYP2C8. However, a slight trend toward underprediction of the C_{max} ratio was observed for the 20 CYP2C9 clinical studies that were evaluated in the qualification with a bias of 0.88.

For CYP1A2, the prediction accuracy was good with an AFE of 0.98 and 1.01 and an AAFE of 1.17 and 1.21

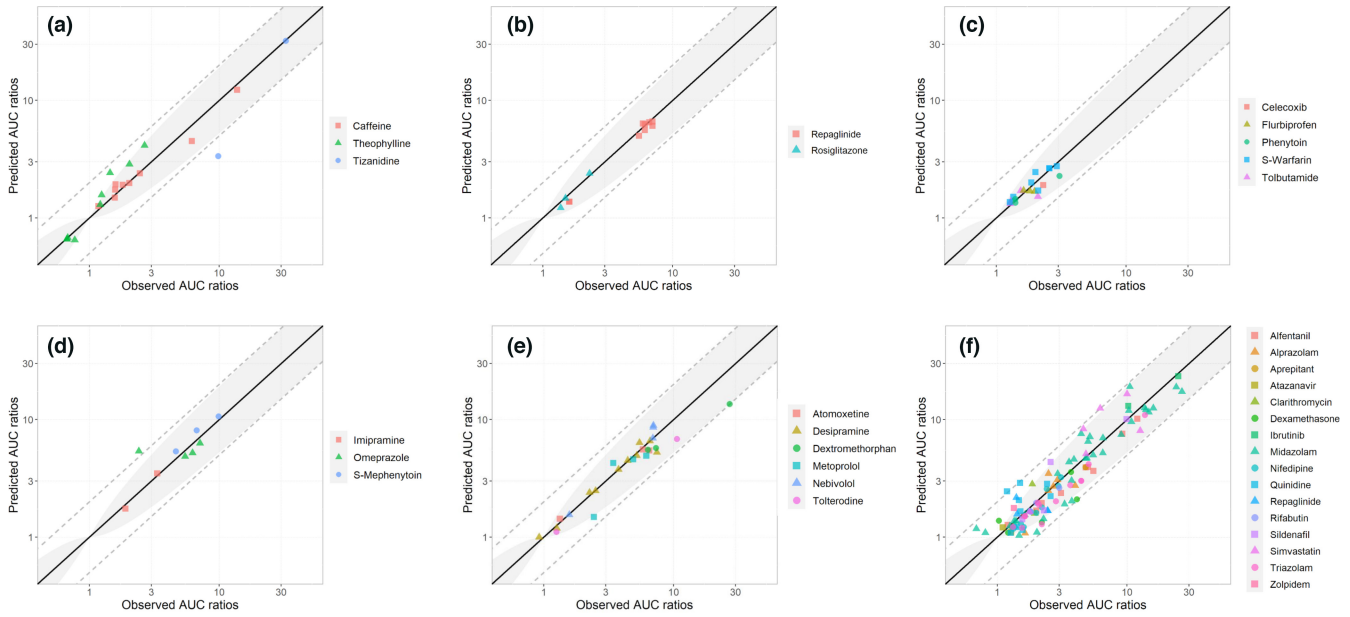

FIGURE 3 Predicted and observed AUC ratios for the qualification of CYP1A2 (a), CYP2C8 (b), CYP2C9 (c), CYP2C19 (d), CYP2D6 (e), and CYP3A4 (f) mediated competitive and mechanism-based inhibition using the Simcyp Simulator (V19R1). AUC, area under the curve

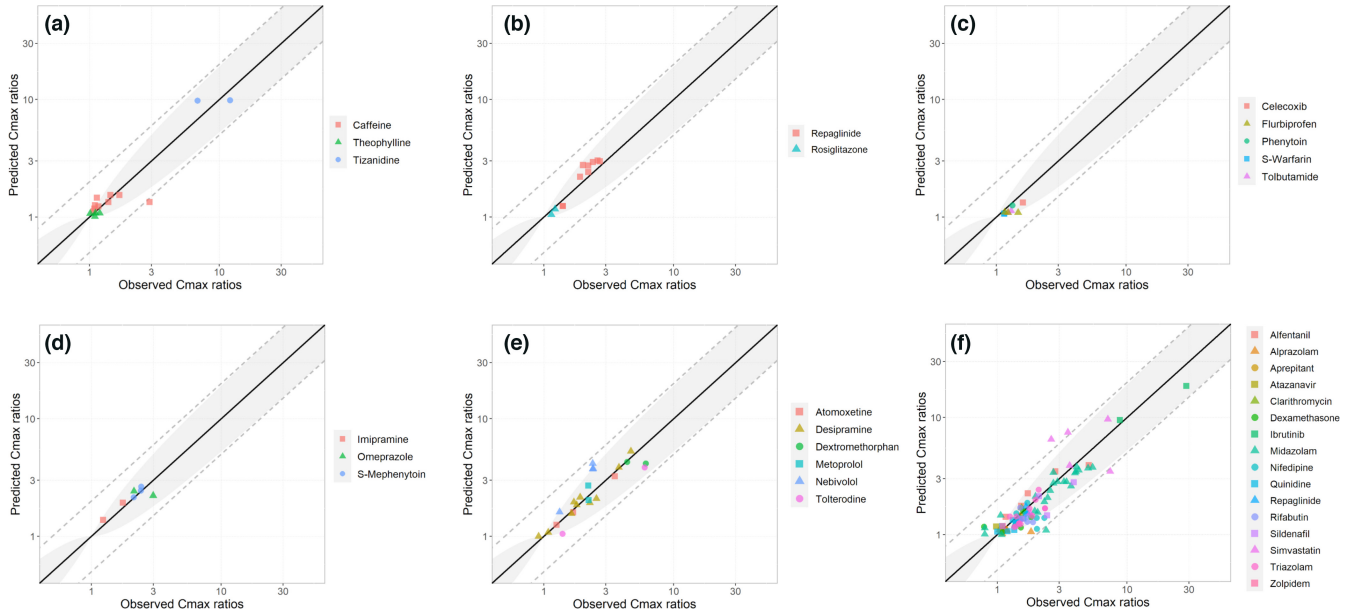

FIGURE 4 Predicted and observed C_{max} ratios for the qualification of CYP1A2 (a), CYP2C8 (b), CYP2C9 (c), CYP2C19 (d), CYP2D6 (e), and CYP3A4 (f) mediated competitive and mechanism-based inhibition using the Simcyp Simulator (V19R1). C_{max}, maximum plasma concentration

for C_{max} and AUC, respectively. Out of the 20 DDIs studied, only three fell outside the 1.5-fold prediction accuracy from observed AUC ratio data and one against the C_{max} ratio data. The clinical studies involved interactions between caffeine and fluvoxamine (1 instance), theophylline and fluvoxamine (2 studies), and tizanidine and ciprofloxacin (1 study).

There were nine DDI studies available to evaluate the prediction of CYP2C19 DDIs with three substrates, S-mephenytoin, omeprazole, and imipramine. The C_{max} was predicted well across all nine studies with an AFE 1.02 and AAFE 1.11 and all studies fell within 1.5-fold of the observed clinical data. There was also a good prediction of the AUC ratio across the substrate's studies with a

bias of 1.09 and precision 1.22. One of the two predictions with omeprazole and the MBI inhibitor ticlopidine fell outside the two-fold criteria against the observed clinical data.

CYP2D6 predictions were assessed for 26 clinical studies involving the six substrates; there was a good prediction accuracy with AFE of 1.03 and 0.93 and AAFE of 1.20 and 1.17 for C_{max} and AUC ratios, respectively. Four of the predictions fell outside of 1.5-fold of the observed C_{max} ratios for the substrates nebivolol and tolterodine. Three of the predictions fell outside of 1.5-fold for the AUC ratios for the substrates dextromethorphan, metoprolol, and tolterodine.

CYP3A4/5 by substrate

The F_g values ranged from 0.11 to 0.99 and the F ranged from 0.03 to 0.85 for the 16 substrates used to evaluate CYP3A4 mediated DDIs (Table 1) which were consistent with observed data (when available). The CYP3A4 interactions and precision and bias are shown in Figures 3, 4, and Table 2. A total of 114 DDIs were evaluated for CYP3A4 mediated interactions and there was a slight trend toward an underprediction of the C_{max} and AUC ratios across the entire dataset with a bias of 0.93 and 0.95 for both the C_{max} and AUC ratios, respectively. Overall, the precision of the predictions was good for both C_{max} and AUC ratios with an AAFE of 1.22 and 1.26, respectively. The predictions were within 1.5-fold of observed C_{max} ratios for all except nine of the interactions where four of these also fell outside two-fold of the observed C_{max} ratio. The AUC ratio was predicted well with 81% of the predictions falling within 1.5-fold of the observed data, only two predictions were outside of two-fold from the observed AUC ratio for simulations with quinidine and erythromycin, and simvastatin and erythromycin.

DISCUSSION

In this study, we identified a DDI matrix involving substrates and inhibitors of CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4, which was then used for qualification of the Simcyp Simulator (V19R1). The DDI qualification matrix consisted of compound files that were already available within the Simcyp Simulator and had previously been selected for development based on the FDA recommendations for reference index substrates and inhibitors.[11] This included a range of weak, moderate, and strong inhibitors that caused DDIs via competitive or MBI and substrates that were susceptible to differing degrees of inhibition. In total, 34 substrates and 24 inhibitors were identified for inclusion in the DDI qualification matrix. Although describing the development and verification of each of the files is beyond the scope of the current publication, it is important to note that compound file summaries are prepared for all substrates and inhibitors and include details of the predicted versus observed PK parameters and DDIs. An example compound summary for midazolam is shown in Figure S1. These summaries can easily be regenerated in later versions of the Simcyp Simulator. Similarly, the DDI matrix can be applied to requalify subsequent versions.

Of the 123 simulated DDIs involving competitive inhibition (20 CYP1A2; 4 CYP2C8; 16 CYP2C9; 4 CYP2C19; 17 CYP2D6; and 62 CYP3A4), the prediction accuracy was good with a bias of 0.91 and precision of 1.20 for the C_{max} ratio and 0.92 and 1.19, respectively, for the AUC ratio. The prediction accuracy was similar across all CYP enzymes studied. Only 8% of the simulated DDIs were outside of the 1.5-fold predicted/observed AUC ratios. Thus, based on the predictive performance of the platform, and the fact that all CYP enzymes are likely to behave the same mechanistically, the Simcyp Simulator (V19R1) can be considered to be qualified with respect to CYP-mediated competitive inhibition.

For an investigational new drug (IND), it is pertinent to provide some guidance for assessing the DDI potential of the drug either as a victim or perpetrator based on the findings of our study. Indeed, the CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 inhibitors presented here can be used with confidence to assess the CYP-mediated drug interaction potential of novel drugs as victims. However, it is important to recognize, that during model development, for a number of substrates included in the DDI qualification matrix, a clinical DDI study was used to optimize their f_mCYP values and was then verified using an independent clinical DDI study (if available). Thus, for drugs in development, even though initial simulations can be carried out to assess the DDI potential as victim drugs, it is likely that a clinical DDI study with a strong inhibitor (typically) or mass balance study is warranted to refine the relative contributions of clearance routes.[1,4] Thereafter, the qualification dataset described herein can be used to support untested DDI scenarios involving moderate or weak inhibitors of the relevant CYP enzyme as has typically been the case.[4,17]

The results presented here indicate that the CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 substrates included in this analysis can be applied with confidence to assess the CYP-mediated drug interaction potential of novel drugs as perpetrators. First, as performed here, it is essential to demonstrate that the PBPK model developed for the perpetrator is able to capture the observed plasma concentration–time profiles and PK parameters at

clinically relevant doses.[18] Second, the in vitro determined inhibitory parameters of the drug may require some calibration or optimization prior to assessing the DDI potential of the compound, as described below.

A range of inhibition constant (K_i) values from different in vitro sources were available for each of the inhibitors included in this analysis and were determined using pooled human liver microsomes (HLMs) or recombinant systems (supersomes, baculosomes, or bactosomes). After correction for nonspecific microsomal binding at the relevant protein concentration, an average K_i value was determined for each of the inhibitors. Of the inhibitors and metabolites included in the DDI qualification matrix, 63% had interaction parameters from in vitro data and the remainder were optimized based on clinical data (in vivo values). Thus, the qualification dataset described here can fully support untested scenarios (comedications and less sensitive substrates) for perpetrators of CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 typically when a clinical study has been performed to assess the DDI potential of the drug using a sensitive substrate, thus allowing optimization of the relevant in vitro K_i value if needed.

Of the 18 CYP3A4 inhibitors used in our study, only three had optimized K_i values. Thus, if an IND is identified as a CYP3A4 inhibitor and the K_i value for the positive control determined in the same incubation is similar to that used in our simulations (Table S2), it may be pertinent to use the qualification dataset described here to support untested scenarios involving CYP3A4-mediated inhibition without conducting a clinical DDI study. However, it will be entirely dependent on the predicted magnitude of interaction and whether it is likely to be clinically significant or not. In the former case, a clinical DDI study may still be warranted, whereas, in the latter case, a sensitivity analysis using an appropriate range of K_i values (see below) may suffice.

Among the 37% of inhibitors where an optimized K_i value was used, with the exception of two weak inhibitors, the median difference between the optimized and in vitro K_i values was about 10-fold. Reasons for the differences between in vivo and in vitro K_i values have been discussed in the literature previously and include possible inhibition by metabolites, general environmental differences between in vitro and in vivo enzyme systems, partitioning into organelles (e.g., lysosomal distribution) or cellular membranes, and active uptake processes altering local concentrations.[19-21] As it is not always possible to identify these mechanisms a priori, we suggest conducting appropriate sensitivity analyses for compounds in development to assess the impact of a range of K_i values on the magnitude of interaction. Thus, when in vitro K_i values of CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 perpetrators indicate negligible DDI liability with a sensitive substrate, we recommend predicting the impact of K_i values up to 10-fold lower. If the DDI potential remains low, then it is likely that the clinical study can be waived especially if supported by the qualification dataset presented here, which includes a range of weak inhibitors.

Of the 78 simulated DDIs involving mechanism-based inhibitors (8 CYP2C8; 4 CYP2C9; 5 CYP2C19; 9 CYP2D6; and 52 CYP3A4), the prediction accuracy was good with a bias of 1.03 for both C_{max} and AUC ratios and a precision of 1.20 and 1.26 for C_{max} and AUC ratios, respectively. With the exception of two cases, all inactivation parameters used in the simulations were based on in vitro data. This finding is in line with that of a recent publication where data from both HLM and human hepatocytes were shown to give good predictions of clinical DDIs.[22] However, given the uncertainty often associated with inactivation parameters, it is likely that the qualification described here can only fully support untested scenarios (comedications and less sensitive substrates) for perpetrators of CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 when a clinical study has been performed to assess the DDI potential of the drug. In addition to inactivation parameters for the inhibitors, estimates of enzyme turnover in the liver (k_{deg}) are required for DDI predictions. In vivo enzyme levels are governed by the rates of de novo enzyme synthesis and degradation which differ for CYP enzymes and thus, result in different enzyme turnovers.[23] Thus, it is important to indicate which values were used for each enzyme; values were 0.0183 (CYP1A2), 0.0301 (CYP2C8), 0.0067 (CYP2C9), 0.0267 (CYP2C19), 0.0099 (CYP2D6), and 0.0193 h^{-1} (CYP3A4).[24] Based on the predictive performance of the platform, and the fact that all CYP enzymes are likely to behave the same mechanistically, the Simcyp Simulator (V19R1) can be considered to be qualified with respect to CYP-mediated MBI.

The overall prediction accuracy of the Simcyp Simulator in terms of CYP3A4-mediated DDIs was reported previously using an earlier version of the platform (V15). Marsousi et al.[25] simulated 74 CYP3A4-mediated DDIs involving ketoconazole, itraconazole, clarithromycin, and rifampicin; a geometric mean fold error (GMFE) of 1.5 was obtained when the predicted AUC ratios were compared against corresponding observed data. Furthermore, a recently published summary of the current drug interaction guidance from the EMA contained an example of a platform qualification for prediction of CYP3A4-mediated MBI, which was finally accepted by the EMA.[17] This analysis consisted of 27 clinical DDI study designs, including the inhibitors diltiazem, erythromycin, fluoxetine, and ritonavir and five different CYP3A4 victim substrates; a GMFE of 1.3 was reported based on a comparison of predicted versus observed AUC ratios. Interestingly, it was indicated by the EMA reviewers, that qualification is only

valid for simulations or scenarios covered by the qualification dataset. Qualification examples or approaches involving other PBPK platforms have also been published for CYP3A4-mediated DDIs[26] and CYP1A2 or CYP2C19 interactions,[27] respectively.

In summary, our analysis demonstrates that the Simcyp Simulator can be used with confidence to assess the DDI potential of INDs as victims or perpetrators of CYP-mediated interactions involving competitive inhibition or MBI.

CONFLICT OF INTEREST

At the time of the work, all of the authors were employees of Certara UK Limited and may hold shares in Certara.

AUTHOR CONTRIBUTIONS

Wrote the manuscript: P.K., I.G., K.R.Y. *Designed the research*: P.K., K.-F.C., K.C., O.H., A.K., S.N., M.Z., K.R.Y. *Performed the research*: P.K., K.-F.C., K.C., O.H., A.K., S.N., M.Z. *Analyzed the data*: P.K., K.-F.C., K.C., I.G., O.H., A.K., S.N., M.Z., K.R.Y.

DISCLAIMER

As an Associate Editor of *CPT: Pharmacometrics & Systems Pharmacology*, Karen Rowland Yeo was not involved in the review or decision process for this paper.

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REFERENCES

1. Shebley M, Sandhu P, Emami Riedmaier A, et al. Physiologically based pharmacokinetic model qualification and reporting procedures for regulatory submissions: a consortium perspective. *Clin Pharmacol Ther*. 2018;104:88-110.
2. Grimstein M, Yang Y, Zhang X, et al. Physiologically based pharmacokinetic modeling in regulatory science: an update from the US Food and Drug Administration's Office of Clinical Pharmacology. *J Pharm Sci*. 2019;108:21-25.
3. Luzon E, Blake K, Cole S, Nordmark A, Versantvoort C, Berglund EG. Physiologically based pharmacokinetic modeling in regulatory decision-making at the European Medicines Agency. *Clin Pharmacol Ther*. 2017;102:98-105.
4. Zhang X, Yang Y, Grimstein M, et al. Application of PBPK modeling and simulation for regulatory decision making and its impact on US prescribing information: an update on the 2018-2019 submissions to the US FDA's Office of Clinical Pharmacology. *J Clin Pharmacol*. 2020;60:S160-S178.
5. US Food and Drug Administration. *In Vitro Drug Interaction Studies—Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry*. US Food and Drug Administration. 2020. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions [cited 18 December 2020].
6. European Medicines Agency. Guideline on the investigation of drug interactions. 2012. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions-revision-1_en.pdf [cited 18 December 2020].
7. Kuemmel C, Yang Y, Zhang X, et al. Consideration of a credibility assessment framework in model-informed drug development: potential application to physiologically-based pharmacokinetic modeling and simulation. *CPT:PSP*. 2020;9:21-28.
8. Zhao P, Rowland M, Huang SM. Best practice in the use of physiologically based pharmacokinetic modeling and simulation to address clinical pharmacology regulatory questions. *Clin Pharmacol Ther*. 2012;92:17-20.
9. US Food and Drug Administration. Physiologically based pharmacokinetic analyses — Format and content guidance for industry. 2018. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/physiologically-based-pharmacokinetic-analyses-format-and-content-guidance-industry [cited 18 December 2020].
10. European Medicines Agency. Reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation. 2018. https://www.ema.europa.eu/en/reporting-physiologically-based-pharmacokinetic-pbpk-modelling-simulation [cited 18 December 2020].
11. US Food and Drug Administration Drug development and drug interactions: table of substrates, inhibitors and inducers. 2020. https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers [cited 18 December 2020].
12. Ke A, Barter Z, Rowland-Yeo K, Almond L. Towards a best practice approach in PBPK modeling: case example of developing a unified efavirenz model accounting for induction of CYPs 3A4 and 2B6. *CPT:PSP*. 2016;5:367-376.
13. Jamei M, Bajot F, Neuhoff S, et al. A mechanistic framework for in vitro–in vivo extrapolation of liver membrane transporters: prediction of drug–drug interaction between rosuvastatin and cyclosporine. *Clin Pharmacokinet*. 2014;53:73-87.
14. Yeo KR, Jamei M, Yang J, Tucker GT, Rostami-Hodjegan A. Physiologically based mechanistic modelling to predict complex drug–drug interactions involving simultaneous competitive and time-dependent enzyme inhibition by parent compound and its metabolite in both liver and gut—the effect of diltiazem on the time-course of exposure to triazolam. *Eur J Pharm Sci*. 2010;39:298-309.
15. Shimizu H, Yoshida K, Nakada T, et al. Prediction of human distribution volumes of compounds in various elimination phases using physiologically based pharmacokinetic modeling and experimental pharmacokinetics in animals. *Drug Metab Dispos*. 2019;47:114-123.
16. Guest EJ, Aarons L, Houston JB, Rostami-Hodjegan A, Galetin A. Critique of the two-fold measure of prediction success for ratios: application for the assessment of drug-drug interactions. *Drug Metab Dispos*. 2011;39:170-173.
17. Cole S, Kerwash E, Andersson A. A summary of the current drug interaction guidance from the European Medicines

Agency and considerations of future updates. *Drug Metab Pharmacokinet*. 2020;35:2-11.
18. Jones H, Rowland-Yeo K. Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT Pharmacometrics Syst Pharmacol*. 2013;2:e63.
19. Yao C, Kunze KL, Kharasch ED, et al. Fluvoxamine–theophylline interaction: gap between in vitro and in vivo inhibition constants toward cytochrome P4501A2. *Clin Pharmacol Ther*. 2001;70:415-424.
20. Yao C, Kunze KL, Trager WF, Kharasch ED, Levy RH. Comparison of in vitro and in vivo inhibition potencies of fluvoxamine toward CYP2C19. *Drug Metab Dispos*. 2003;31:565-571.
21. Zheng YF, Bae SH, Huang Z, et al. Lack of correlation between in vitro and in vivo studies on the inhibitory effects of ()-sophoranone on CYP2C9 is attributable to low oral absorption and extensive plasma protein binding of ()-sophoranone. *Pharmaceutics*. 2020;12:328-345.
22. Tseng E, Eng H, Lin J, et al. Static and dynamic projections of drug-drug interactions caused by cytochrome P450 3A time-dependent inhibitors measured in human liver microsomes and hepatocytes. *Drug Metab Dispos*. 2021;49:947-960.
23. Yang J, Liao M, Shou M, et al. Cytochrome p450 turnover: regulation of synthesis and degradation, methods for determining rates, and implications for the prediction of drug interactions. *Curr Drug Metab*. 2008;9:384-394.
24. Rowland Yeo K, Walsky RL, Jamei M, Rostami-Hodjegan A, Tucker GT. Prediction of time-dependent CYP3A4 drug-drug interactions by physiologically based pharmacokinetic modelling: impact of inactivation parameters and enzyme turnover. *Eur J Pharm Sci*. 2011;43:160-173.
25. Marsousi N, Desmeules JA, Rudaz S, Daali Y. Prediction of drug-drug interactions using physiologically-based pharmacokinetic models of CYP450 modulators included in Simcyp software. *Biopharm Drug Dispos*. 2018;39:3-17.
26. Frechen S, Solodenko J, Wendl T, et al. A generic framework for the physiologically-based pharmacokinetic platform qualification of PK-Sim and its application to predicting cytochrome P450 3A4–mediated drug–drug interactions. *CPT:PSP*. 2021;10:633-644.
27. Kanacher T, Lindauer A, Mezzalana E, et al. A Physiologically-Based Pharmacokinetic (PBPK) model network for the prediction of CYP1A2 and CYP2C19 drug–drug–gene interactions with fluvoxamine, omeprazole, S-mephenytoin, moclobemide, tizanidine, mexiletine, ethinylestradiol, and caffeine. *Pharmaceutics*. 2020;12:1191.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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