QUANTIFICATION OF THE EFFECTS OF INVESTIGATIONAL DRUGS AS VICTIMS OR PERPETRATORS OF CYP-MEDIATED DRUG INTERACTIONS INVOLVING INHIBITION IN THE SIMCYP SIMULATOR

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Table of Contents

Abbreviations

ADAM	Advanced dissolution, absorption and metabolism model
ADME	Absorption, distribution, metabolism and excretion
AFE	Average fold error
AUC	Area under the plasma versus concentration time curve
C_{max}	Maximum plasma concentration
CL_{int}	Intrinsic clearance
COU	Context of use
DDI	Drug-drug interaction
ELF	Epithelial lining fluid
EM	Extensive metaboliser phenotype
EMA	European Medicines Agency
Fm	Fraction of a compounds systemic clearance occurring via a particular enzyme/mechanism
fu	Fraction unbound
FDA	Food and Drug Administration
FTIH	First time in human
GMFE	Geometric mean fold error
HLM	Human liver microsomes
IM	Intermediate metaboliser phenotype
IND	Investigational new drug
IV	Intravenous
IVIVE	*In vitro-in vivo* extrapolation
K_{deg}	Enzyme turnover in the liver
K_m	Michaelis constant
Kp	Tissue:plasma partition coefficient
PBPK	Physiologically-based pharmacokinetics
PD	Pharmacodynamics
Peff	Effective permeability
PK	Pharmacokinetics
pKa	pH where a drug exists as 50% ionized and 50% unionised forms
PM	Poor metaboliser phenotype
PO	Oral dosing (per os)
QSAR	Quantitative structure–activity relationship
SD	Standard deviation

TB	Tuberculosis
UAA	Upper airway alveoli/air
V_{ss}	Apparent volume of distribution at steady state

Tables and Figures

List of Tables

List of Figures

1. Executive Summary

Physiologically-based pharmacokinetic (PBPK) modelling is being increasingly used in drug development to inform drug labels and untested clinical drug-drug interaction (DDI) scenarios i.e. replace clinical studies. Thus, regulatory agencies are recommending 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® (V19 R1) with respect to competitive and mechanism-based inhibition (MBI) of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5. Certara UK Ltd requests an EMA Qualification Opinion for the proposed Contexts of Use.

METHODS: Along with an internal curated clinical DDI database that has been collated for compound files during development and verification of the respective compound files for integration within the Simcyp Simulator over a period of 20 years, the University of Washington Drug Interaction Database (DIDB) was used to identify controlled clinical DDI studies involving CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 where observed increases in plasma exposure of substrates greater than 20% (as a consequence of the DDI) were reported. Studies were then selected to form part of the DDI qualification matrix if compound files for both substrate and inhibitor were available within the Simcyp Simulator. The DDI matrix, consisting of a range of weak, moderate, and strong inhibitors and substrates with varying fraction metabolised by specific CYP enzymes (fmCYP) that were susceptible to different degrees of inhibition, were identified. Simulations were run with 122 clinical DDI studies involving competitive inhibition and 83 clinical DDI studies involving mechanism-based inhibition (MBI).

Using the Simcyp Simulator (V19 R1), ten trials of virtual subjects with demography matching that of the subjects recruited into each of the clinical studies were generated and the precise study designs were applied. The default values for the compound files (V19 R1) were used in simulations and are indicated in the compound file summaries (see Appendix 2).

The ratio of 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, as is the maximum plasma concentration (C_{max}). Accordingly, the mean C_{max} and AUC ratios from the 10 simulated trials were compared against the mean AUC ratios from each *in vivo* study.

RESULTS: For competitive inhibition, the overall prediction accuracy was good with an average fold error (AFE) of 0.92 and 0.95 for changes in the maximum plasma concentration (C_{max}) and area under the plasma concentration (AUC) time profile, respectively, because of the DDI. For MBI, AFE values of 1.01 and 0.98 were 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.

CONCLUSION: 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 involving inhibition.

2. Background

Certara UK Ltd is submitting this briefing book for EMA qualification opinion on the suitability of the Simcyp Simulator, as a tool to predict the cytochrome P50 (CYP)-mediated drug-drug interaction (DDI) potential of victim and perpetrator drugs involving inhibition following oral and IV administration. Certara UK Ltd is considering this approach as a candidate for novel methodology qualification, based upon the EMA draft guideline entitled *"Guideline on the qualification and reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation*" (July 21, 2016; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC500211315.pdf) and is seeking the EMA opinion on the use of the Simcyp Simulator (V19 R1) as a tool to address the intended purpose as proposed in the context of use (COU) statements for high impact decisions, as per the draft EMA guideline document.

PBPK models make optimal use of available data by combining the complex interplay of physiological parameters with characteristics relating to the Absorption, Distribution, Metabolism and Excretion (ADME) of a specific drug to predict its pharmacokinetics (PK). PBPK modelling has been increasingly used for various applications to guide decision making in drug development on assessment of DDI liability, to design clinical studies, dose extrapolation in special populations including paediatrics, and investigation of formulation and food effects.[1] PBPK models that have demonstrated a good predictive performance, particularly in support of quantitative prediction of drug-drug interactions (DDIs), are often submitted to regulatory agencies.[2,3,4] Once reviewed and if accepted by health authorities they have been used to inform the prescription drug label for untested clinical scenarios. Global regulators have issued guidance documents or published best practice approaches for the application of PBPK in regulatory submissions.[5,6,7,8] Furthermore, regulatory agencies are recommending, or indeed requesting, more rigorous demonstration of the prediction accuracy of PBPK platforms in the area of their intended use.[9,10,11]

3. Proposed Context of Use (COU) Statements

1. **The Simcyp Simulator (V19 R1) can be used to predict the effects of weak and moderate CYP inhibitors on the exposure of a drug administered orally under fasted conditions or intravenously in healthy subjects when a clinical study with a strong CYP inhibitor has been conducted (and used to verify the fmCYP).**
2. **The Simcyp Simulator (V19 R1) can be used to predict the CYP-mediated inhibitory effect of a drug on the exposure of other CYP substrates administered orally under fasted conditions or intravenously in healthy subjects when a clinical study with a sensitive CYP substrate has been conducted (and used to verify the competitive inhibition effect *in vivo*).**
3. **The Simcyp Simulator (V19 R1) can be used to predict the CYP-mediated MBI effect of a drug on the exposure of other CYP substrates administered orally under fasted conditions or intravenously in healthy subjects when a clinical study with a sensitive CYP substrate has been conducted (and used to verify the MBI effect *in vivo*).**
4. **For scenarios where no clinical studies have been conducted, the Simcyp Simulator (V19 R1) can be used to predict the CYP-mediated inhibitory effect of a drug on the exposure of relevant CYP substrates (V19 files) to waive a clinical DDI study if a**

significant interaction is not predicted. Sensitivity analyses on relevant parameters including unbound fraction (fu) and inhibitory potency of the drug should be conducted to assess the risk of predicting a false negative. For fu, the range should be determined by the variability associated with the *in vitro* determination of the parameter. For the inhibitory potency, the range can be determined by a comparison of the inhibitory potency of the positive control from the *in vitro* study *versus* that of the compound file used in the Simcyp Simulator (V19).

NOTE: No complex DDIs involving transporters/enzymes or inhibition/induction are included in the qualification.

A separate document called “Non-CYP DDI-Feb2023" has been provided to support the fourth COU.

4. Modeling Analysis Plan

The objective of this submission to the EMA is to receive qualification opinion on the suitability of the Simcyp Simulator to address the stated COU scenarios. Thus, one of the initial aims was to identify a DDI matrix that could be used for CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 interactions to qualify the platform for CYP-mediated competitive inhibition and mechanism-based inhibition (MBI) via these enzymes. Such an analysis was conducted and published previously by Kilford *et al.* [12]. As several compound files were in development at the time, the simulations were repeated, and the updated analysis is described herein.

4.1 Simcyp Simulator Platform (V19 R1)

For the current analysis, all simulations were performed using the Simcyp Simulator (V19 R1). The program allows simple extrapolation of *in vitro* enzyme kinetic data in multiple organs, including liver and intestine, to predict pharmacokinetic changes *in vivo* in virtual populations.[13,14] Thus, in order to assess clearance predictions in a specific population, data are required for the population variables as well as for the *in vitro* metabolism of the test drug and its observed clearance in the population of interest. 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. Default Simcyp parameter values for creating a virtual North European Caucasian population (physiological parameters including liver volume and blood flows, enzyme abundances) have been described previously.[15] With the exception of demographic data, all parameter values for the healthy volunteer (HV) population are the same as those used for the Caucasian population.

Scaling of *in vitro* data relevant to the ADME processes for integration into PBPK models is described in detail in Appendix 1 and briefly, hereafter. A minimal PBPK model, which considers both liver and intestinal metabolism, is incorporated in the Simcyp Simulator.[16] The model can also be expanded to a full PBPK model by inclusion of additional tissues such as adipose, brain, bone, heart, lung, muscle and skin.[13,14] Several absorption models are available within the Simcyp Simulator including a first-order absorption model and the advanced dissolution absorption metabolism (ADAM) model.[17] Changes in metabolic clearance due to reversible inhibition of enzyme activity, or changes in enzyme levels due to mechanism-based inactivation are handled

using mechanistic dynamic models within the Simcyp Simulator. The underlying assumptions and operating differential equations relevant to prediction of DDI have been described in detail elsewhere.[16]

4.2 Historical Compound File Development and Clinical DDI Database

The compound files within the Simcyp Simulator (V19 R1) have been developed and added over the past 20 years. Substrates and inhibitors included as compound files were selected based on the FDA and EMA recommendations for reference index substrates and inhibitors. These compounds were seen as a priority as they are typically used in drug development to assess the DDI potential of substrates and inhibitors being developed. In addition to reference substrates and inhibitors, so-called "sensitive" substrates have also been included as well as weak, moderate, and strong inhibitors (when possible).

Throughout this 20-year period of development, clinical DDI studies for each compound were identified on an individual basis using UOW and literature searches. Each of the clinical studies were reviewed to determine whether they should be included or excluded from the development and verification of the compound file. Clinical DDI studies were included if they were randomised controlled clinical DDI studies and were excluded if they were:

- Conducted in patients
- Case studies
- Cocktail studies
- Micro-dosing studies

During the development of each compound file, a set of clinical DDI studies was derived for each substrate based on the above inclusion/exclusion criteria. Typically, when developing PBPK models for compounds, some clinical studies are used to help develop and optimise the compound files (training sets) and other independent clinical studies are then used to verify the model (verification set). If clinical studies were used to optimise parameters relevant to prediction of DDIs, including fmCYP and inhibitory parameters, they were then not used for verification. Compound file summaries describing the development and verification of each substrate and inhibitor have been provided in Appendix 2 of the briefing document. In summary, a carefully curated database of clinical DDI studies for CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 involving key substrates and inhibitors has been collated during development of the Simcyp Simulator and was used as a source for the DDI qualification matrix. for the DDI qualification matrix.

4.3 UOW Searches and DDI Qualification Matrix

In addition, the University of Washington Drug Interaction Database (DIDB) was applied to identify clinical DDI studies involving CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19 and CYP3A4/5 where observed increases in plasma exposure of substrates greater than 20% (because of the DDI) were reported (Figure 1). DDI studies were flagged if both substrate and inhibitor were available as compound files within the Simcyp Simulator (V19 R1).

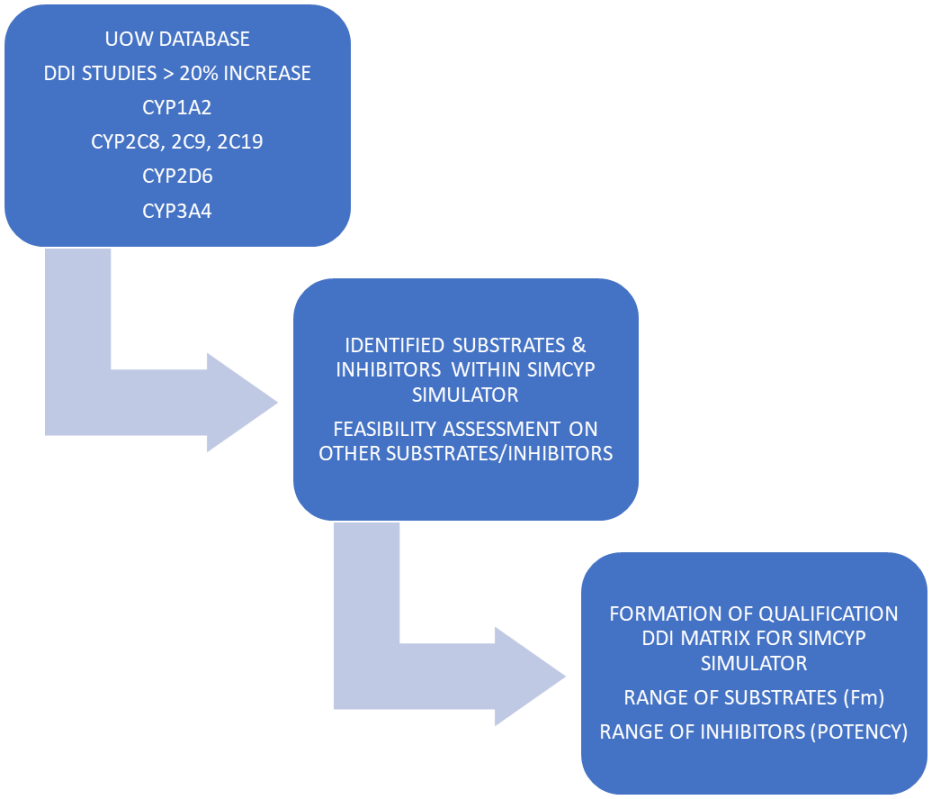


Figure 1. The workflow used to identify substrates and inhibitors for the DDI Qualification Matrix.

Where possible, another criterion for selection of compounds/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. In DDI clinical studies, it is customary to use inhibitors which are known to have a strong effect. However, the inhibitory effect of a perpetrator is also dependent on the metabolic characteristics of a victim, i.e., affinity to the principal enzyme, relative contribution of a specific enzyme to overall metabolism or PK behavior of a drug, and alternative enzymatic and excretory clearance routes. Consequently, the interaction outcome of a "strong" perpetrator may be strong, moderate, or weak, depending on the victim drug. Thus, the intensity of inhibition is defined by the FDA based on the AUC change of the victim drug. Strong, moderate, and weak inhibitors give rise to an increase in AUC of a victim drug by at least 5-fold, between 2- and 5-fold, and 1.25- to 2-fold, respectively.

In addition to reference substrates and inhibitors, so-called "sensitive" substrates were also included. Usually, sensitive substrates are metabolised almost completely or to a significant extent (>25%) by the CYP enzyme concerned, so that the inhibition by a specific inhibitor will lead to a significant increase in the exposure of the victim drug.

4.4 Development and verification of compound files within the Simulator

Prior to integration within the platform, a rigorous feasibility assessment is conducted for each compound to ensure that there are 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. As part of this process, relevant information on physicochemical properties, cell permeability, protein and blood binding, *in vitro* metabolism and clinical PK is collated. Where multiple values for data are available, a meta-analysis approach is used as described in Howgate *et al.*[15] to obtain a weighted geometric mean value and variance for a particular parameter. Development and verification of each compound file is performed according to best practice approaches described in several publications and briefly below (Figure 2).

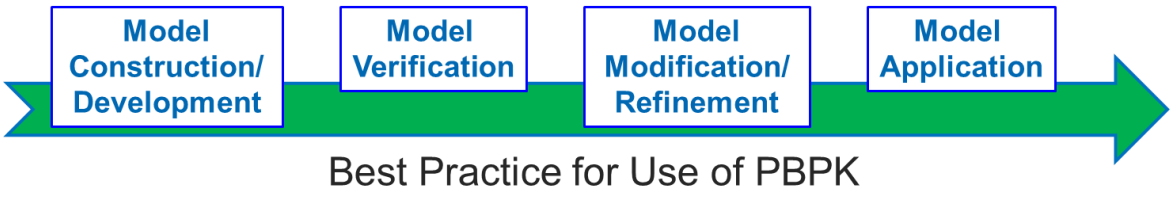


Figure 2. A typical workflow for PBPK compound file development.

Simulations using each of the compound files aims to describe concentration-time profiles from clinical datasets based on *in vitro* data alone, at least in the initial stages. Model development is performed initially using intravenous data (if available) with a focus on the distribution and elimination parameters. Thereafter, absorption related parameters are introduced into the PBPK models for each compound to predict plasma concentration-time profiles following oral administration. Of the compounds included in the qualification matrix, a first-order absorption model was applied for 31 of the 33 substrates and for 20 of the 24 inhibitors. The ADAM model was used to describe the absorption of ibrutinib, flurbiprofen, ciprofloxacin, gemfibrozil, ritonavir and verapamil.

At each stage, optimisation of relevant parameters is performed using clinical data, if necessary, to ensure accurate recovery of observed data. For a victim drug (substrate), it is important to characterise the clearance routes and demonstrate that when inhibited, the observed increase in exposures is accurately captured. For a perpetrator (inhibitor), it is necessary to ensure that after integration of the inhibitory parameters into the PBPK model, they lead to accurate prediction of clinical DDIs.

This process and the input data are captured in a compound file summary, which is version specific. The source of the input data and the clinical DDI studies for each compound, as well as the level of verification that has been performed are included in a document specific to that compound. Each compound that has been used in the qualification dataset has a compound file summary that can be found in Appendix 2

4.5 Simulations

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.

4.6 Data Analysis

The ratio of the area-under-the-curve of the plasma concentration-time profile (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 is also 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 *et al.*[18], which were used to assess the bias and precision of the predictions, respectively.

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

The data were analysed according to type of inhibition (competitive *versus* MBI) and also according to the CYP enzyme.

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 interactions, the validation criteria proposed by Guest *et al.*[19] were also indicated on the graphs.

5. Results

5.1 DDI Qualification Matrix

In total, 33 substrates and 24 inhibitors were identified for inclusion in the DDI matrix for qualification of CYP-mediated inhibition using the Simcyp Simulator (V19R1) (Figure 3). There were 122 clinical studies involving competitive inhibition and 83 clinical studies involving time-dependent inhibition (MBI) and 99 unique pairs of substrates (Figure 4).

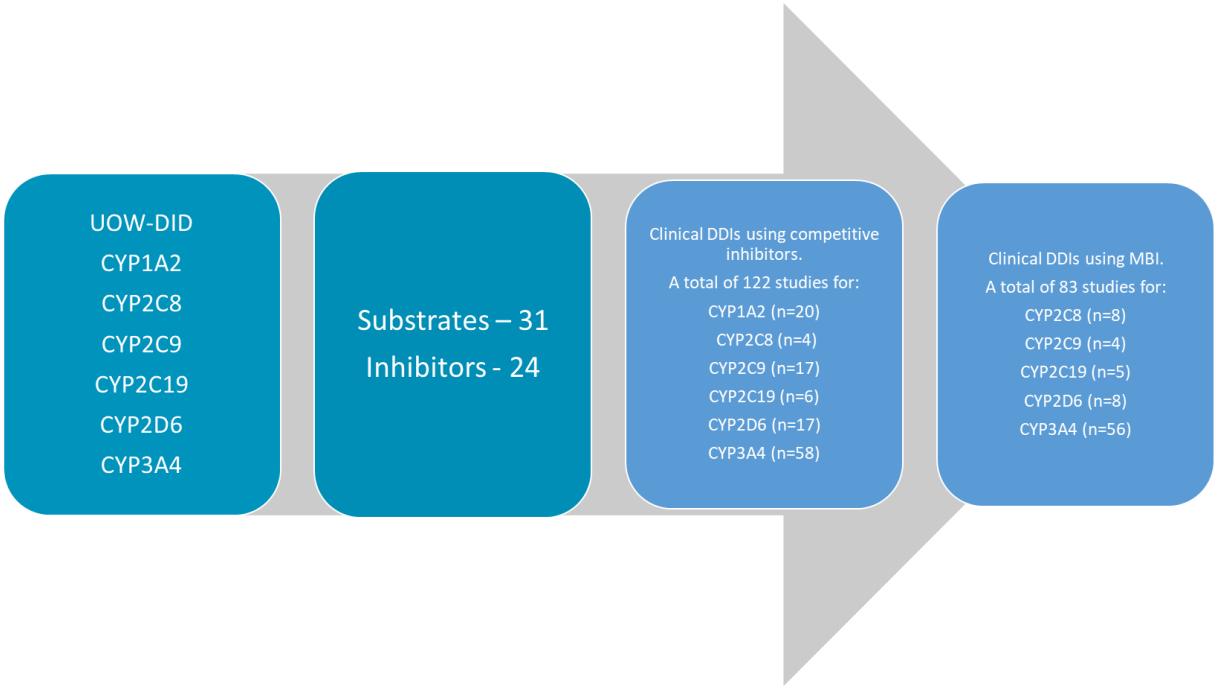


Figure 3. An overview of the DDI qualification matrix

Unique pairs

	Substrate	Inhibitor
1	Midazolam	Clarithromycin
2	Alfentanil	Fluconazole
3	Alfentanil	Ketoconazole
4	Alprazolam	Ketoconazole
5	Alprazolam	Ritonavir
6	Alprazolam	Fluoxetine
7	Alprazolam	Cimetidine
8	Atazanavir	Ketoconazole
9	Atazanavir	Clarithromycin

Unique pairs

	Substrate	Inhibitor
51	Tolbutamide	Fluconazole
52	Phenytoin	Fluconazole
53	Celecoxib	Fluconazole
54	Tolbutamide	Fluvoxamine
55	Phenytoin	Sulphaphenazole
56	Tolbutamide	Sulphaphenazole
57	Atomoxetine	Fluvoxamine
58	Atomoxetine	Paroxetine
59	Desipramine	Paroxetine

10	Clarithromycin	Atazanavir	60	Desipramine	Fluoxetine
11	Clarithromycin	Ritonavir	61	Desipramine	Bupropion
12	Midazolam	Fluconazole	62	Desipramine	Ritonavir
13	Midazolam	Itraconazole	63	Dextromethorphan	Quinidine
14	Midazolam	Ritonavir	64	Dextromethorphan	Fluoxetine
15	Midazolam	Diltiazem	65	Metoprolol	Paroxetine
16	Midazolam	Verapamil	66	Metoprolol	Quinidine
17	Midazolam	Ketoconazole	67	Tolterodine	Fluoxetine
18	Midazolam	Cimetidine	68	Repaglinide	Trimethoprim
19	Midazolam	Erythromycin	69	Repaglinide	Gemfibrozil
20	Midazolam	Fluvoxamine	70	Rosiglitazone	Trimethoprim
21	Phenytoin	Amiodarone	71	Rosiglitazone	Gemfibrozil
22	Nifedipine	Cimetidine	72	Caffeine	Ciprofloxacin
23	Nifedipine	Diltiazem	73	Caffeine	Fluvoxamine
24	Quinidine	Verapamil	74	Theophylline	Ciprofloxacin
25	Quinidine	Diltiazem	75	Theophylline	Fluvoxamine
26	Quinidine	Cimetidine	76	S-Mephenytoin	Fluvoxamine
27	Quinidine	Itraconazole	77	Omeprazole	Fluvoxamine
28	Quinidine	Erythromycin	78	Omeprazole	Fluoxetine
29	Quinidine	Fluvoxamine	79	Imipramine	Fluoxetine
30	Repaglinide	Itraconazole	80	Omeprazole	Ticlopidine
31	Repaglinide	Cyclosporine	81	Tizanidine	Fluvoxamine
32	Repaglinide	Clarithromycin	82	Tizanidine	Ciprofloxacin
33	Sildenafil	Clarithromycin	83	Aprepitant	Ketoconazole
34	Sildenafil	Erythromycin	84	Dexamethasone	Itraconazole

35	Sildenafil	Ritonavir	85	Dexamethasone	Aprepitant
36	Sildenafil	Cimetidine	86	Ibrutinib	Ketoconazole
37	Simvastatin	Diltiazem	87	Ibrutinib	Itraconazole
38	Simvastatin	Erythromycin	88	Midazolam	Aprepitant
39	Simvastatin	Verapamil	89	Rifabutin	Fluconazole
40	Simvastatin	Ketoconazole	90	Rifabutin	Ritonavir
41	Simvastatin	Clarithromycin	91	Rifabutin	Clarithromycin
42	Triazolam	Diltiazem	92	Simvastatin	Amiodarone
43	Triazolam	Fluconazole	93	Flurbiprofen	Fluconazole
44	Triazolam	Ketoconazole	94	S-Warfarin	Amiodarone
45	Triazolam	Clarithromycin	95	Desipramine	Cinacalcet
46	Triazolam	Cimetidine	96	Nebivolol	Paroxetine
47	Triazolam	Erythromycin	97	Nebivolol	Bupropion
48	Triazolam	Itraconazole	98	Nebivolol	Fluvoxamine
49	Zolpidem	Itraconazole	**99**	Nebivolol	Fluoxetine
50	S-Warfarin	Fluconazole			

Figure 4. An overview of the unique pairs included in DDI qualification matrix

For some of the drug interaction pairs, several different CYP enzymes and inhibitory mechanisms are involved. Thus, a table presented in Appendix 3 is provided to give an overview of all reported interaction mechanisms for each pair of substances. In addition, it is also indicated in the same table whether these additional interaction mechanisms were taken into account (i.e., implemented in the Simcyp Simulator V19 R1) for the purpose of the present platform qualification.

5.1.1 Substrates

The 33 substrates identified for inclusion in the DDI matrix are shown in

Table **1**. The verification of the contributions of the CYP enzymes to each substrate is indicated in each compound file summary (Appendix 2).

For CYP1A2, caffeine, theophylline and tizanidine were available with fraction metabolised (fm) values ranging from 75.8 to 97.9%.

Three substrates were included to evaluate CYP2C19 with $fm_{CYP2C19}$ values ranging from 38.3 to 85.8% for imipramine and S-mephenytoin, respectively.

Repaglinide (66.1%), rosiglitazone (56.1%) and amiodarone (32.2%) were included as substrates of CYP2C8.

Five CYP2C9 substrates were included, with fm_{CYP2C9} ranging from 73.1 to 98.4% for phenytoin and S-warfarin, respectively.

Six substrates were evaluated for CYP2D6-mediated DDIs with fm_{CYP2D6} ranging from 73.9 to 87.7%.

Not surprisingly, the largest range of fm values was observed for the substrates that were used to evaluate CYP3A4/5-mediated DDIs with fm_{CYP3A4} ranging from 33.7% for repaglinide up to 99.8% for nifedipine.

Across all substrates, the predicted bioavailability (F) ranged from 0.04 to 0.92 for simvastatin and rosiglitazone, respectively. Simvastatin had the lowest predicted fraction escaping first-pass metabolism in the gut (Fg) at 0.12 and this increased up to a maximum value of 1 for a number of substrates including caffeine, theophylline, tizanidine, rosiglitazone, alprazolam and phenytoin. Gertz *et al* [18] reported observed values of 0.6, 0.9, 0.51, 0.74, 0.9, 0.21, 0.89, 0.54, 0.14, 0.75 and 0.79 for alfentanil, alprazolam, midazolam, nifedipine, quinidine, rifabutin, repaglinide, sildenafil, simvastatin, triazolam and zolpidem, respectively. With the exception of rifabutin, which was within 1.5-fold of the observed value, all other predicted Fg values were within 1.25-fold.

Table 1. Mean fraction metabolised (fm), fraction escaping gut metabolism (Fg) and bioavailability (F) for each substrate according to the enzyme of interest as calculated in the Simcyp Simulator V19 (R1).

Enzyme	Substrate	fm%	Fg	F
CYP1A2	Caffeine	97.9	1	0.81
	Theophylline	75.8	1	0.83
	Tizanidine	96.6	1	0.16
CYP2C19	S-Mephenytoin	85.8	0.89	0.34
	Omeprazole	77.9	0.96	0.5
	Imipramine*	38.31	1	0.38
CYP2C8	Repaglinide	66.1	0.92	0.76
	Rosiglitazone	56.1	1	0.93
CYP2C9	Celecoxib	83.5	0.77	0.51
	Flurbiprofen	81.9	0.96	0.92
	Phenytoin	73.1	1	0.79
	S-Warfarin	98.4	0.99	0.86
	Tolbutamide	96.8	0.99	0.84
CYP2D6	Atomoxetine	78.6	0.91	0.61
	Desipramine	81.8	0.95	0.44

	Dextromethorphan	87.7	0.9	0.21
	Metoprolol	73.9	0.97	0.45
	Nebivolol	85.7	0.92	0.17
	Tolterodine	82.7	0.99	0.29
CYP3A4/5	Alfentanil	91.8	0.54	0.34
	Alprazolam	71	1	0.83
	Aprepitant	85.2	0.6	0.48
	Atazanavir	80.2	0.94	0.36
	Clarithromycin	73.6	0.85	0.51
	Dexamethasone	86.1	0.99	0.76
	Ibrutinib	95	0.4	0.04
	Midazolam	96.2	0.6	0.29
	Nifedipine	99.8	0.67	0.42
	Quinidine	71.7	0.95	0.66
	Rifabutin	66	0.14	0.11
	Repaglinide	33.7	0.92	0.76
	Sildenafil	86	0.67	0.38
	Simvastatin	88.7	0.12	0.04
	Triazolam	97.1	0.74	0.51
	Zolpidem	48.2	0.95	0.79

* For Imipramine the V20 setting was used in V19

Simulation of 10 subjects in 10 trials each for the Sim-Healthy Volunteer population as a single dose for 96h using V19.0.96.0 (V19 Release 1).

5.1.2 Inhibitors

Across all CYP enzymes there were 24 inhibitors available for qualification of the platform (Appendix 4); some had inhibition parameters for multiple CYP enzymes. A range of Ki 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 (HLM) or recombinant systems (supersomes, baculosomes, or bactosomes). After correction for nonspecific microsomal binding at the relevant protein concentration, an average Ki value was determined for each of the inhibitors.

Out of the inhibitors and metabolites included in the analysis, 63% had interaction parameters based on *in vitro* data and the remainder were optimised based on clinical data.

The full spectrum of strong, moderate and weak inhibitors was only available for CYP2D6 and CYP3A4.

5.2 Analysis - Competitive and Mechanism Based Inhibition Level

The results of all the individual simulations (n=205) are shown in Appendix 5.

Predicted *versus* observed changes in AUC and C_{max} across all the CYP enzymes investigated are shown in Figure 5 for competitive inhibition (n=122 DDIs) and in Figure 6 for mechanism-based inhibition (n=83 DDIs).

The prediction accuracy for the DDIs involving 23 competitive inhibitors and 18 mechanism-based inhibitors is shown in

Table 2 and Table 3, respectively.

Figures relating to prediction accuracy at an inhibition mechanism/CYP enzyme/substrate level are presented in Appendix 6.[12]

Table 2. Prediction accuracy for competitive inhibition

Competitive inhibition (V19)	C_{max} Ratio	AUC Ratio
AFE (bias)	0.92	0.95
AAFE (precision)	1.20	1.19
Number Studies	94	122

Table 3. Prediction accuracy for MBI

Mechanism-based inhibition (V19)	C_{max} Ratio	AUC Ratio
AFE (bias)	1.01	0.98
AAFE (precision)	1.21	1.28
Number Studies	69	83

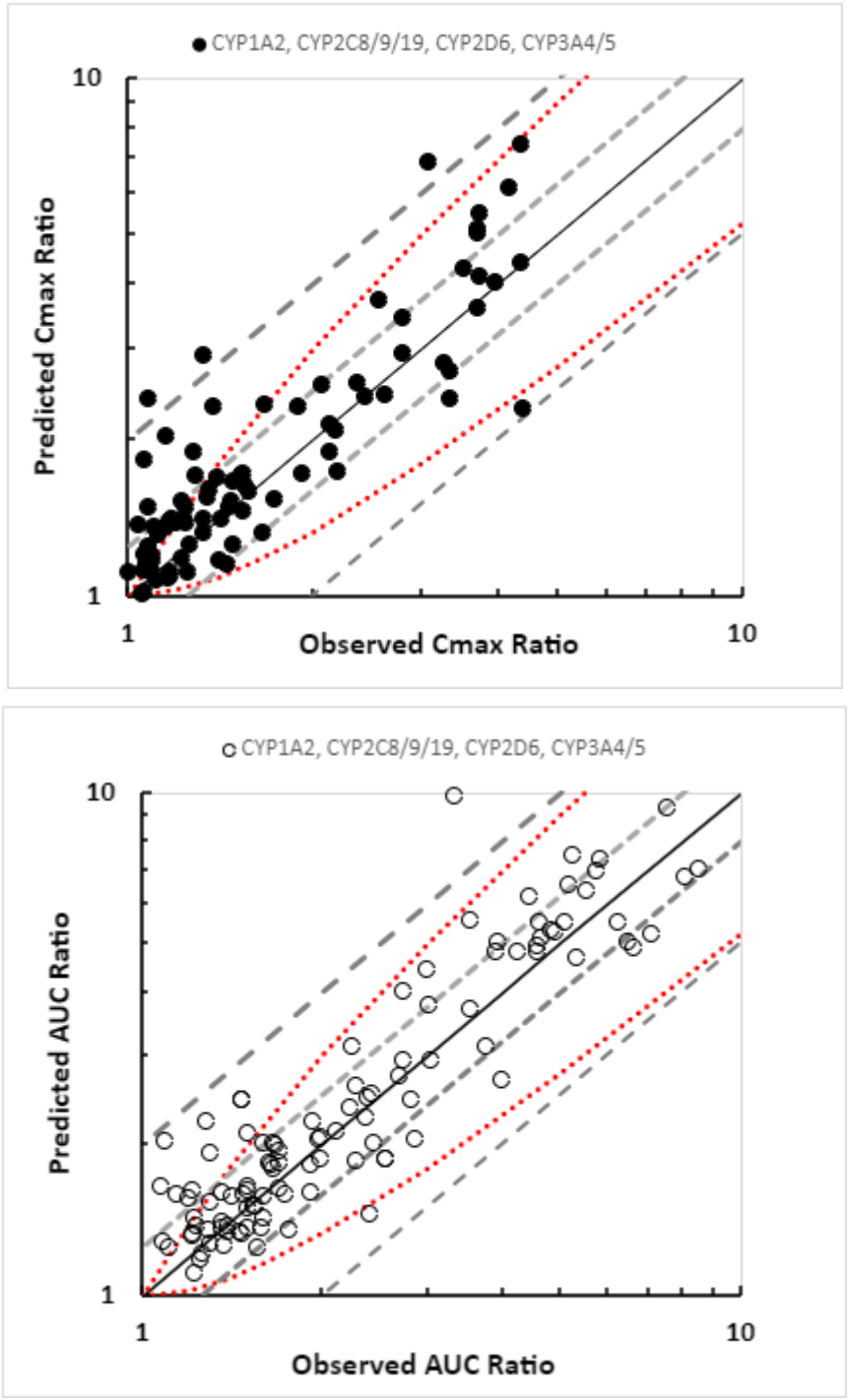


Figure 5. Predicted *versus* observed DDIs involving competitive inhibition

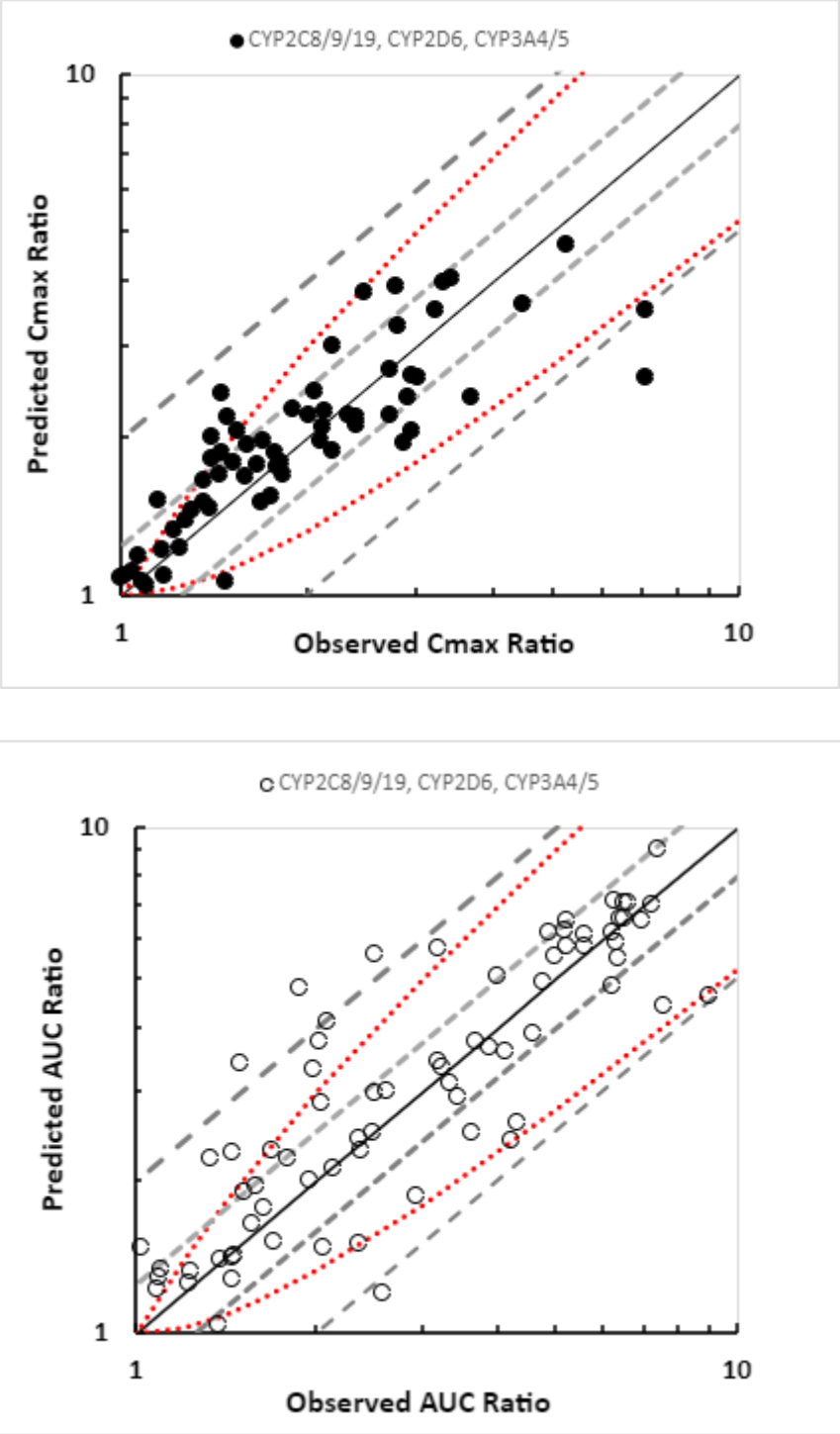


Figure 6. Predicted *versus* observed DDIs involving mechanism-based inhibition

5.2.1 Analysis – Individual CYP-Enzyme

For each enzyme, the prediction accuracy of the DDIs was evaluated against the clinical data and this is shown in Figure 7 (CYP1A2), Figure 8 (CYP2C8), Figure 9 (CYP2C9), Figure 10 (CYP2C19), Figure 11 (CYP2D6) and Figure 12 (CYP3A4/5). The prediction accuracy was generally comparable across all the CYP enzymes in the qualification DDI matrix.

For CYP1A2, the prediction accuracy was good with an AFE (bias) of 0.91 and 1.03 and an AAFE (precision) of 1.21 and 1.21 for C_{max} and AUC, respectively. Out of the 20 DDIs studied, only 3 fell outside the 1.5-fold prediction accuracy from observed AUC ratio data and 1 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).

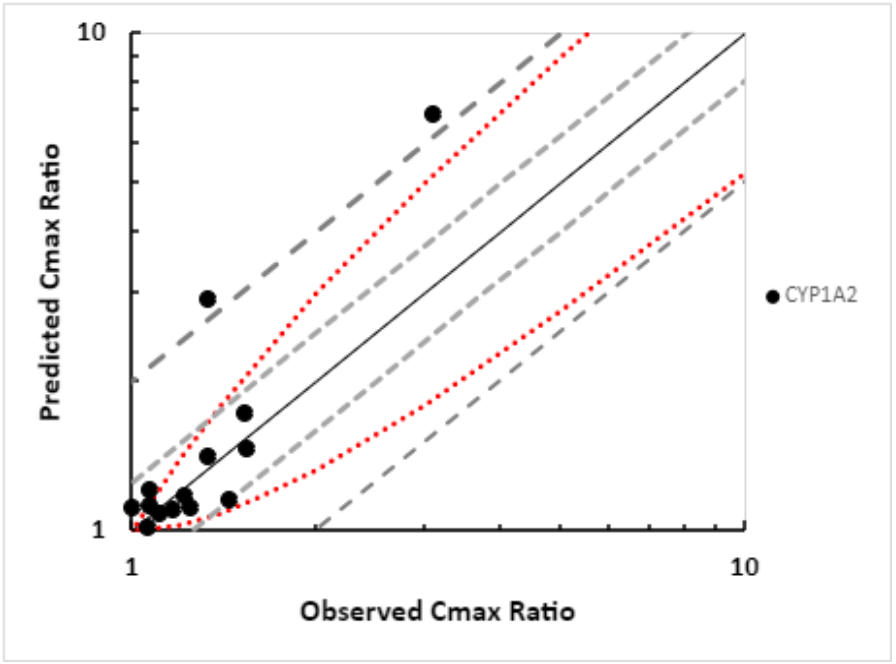
For CYP2C8 (n=16 studies), with the exception of 4 studies involving gemfibrozil, all the predictions fell within 1.5-fold of the observed clinical values for both C_{max} and AUC. In 2 of the studies, lower than normal doses of 100 mg gemfibrozil (600 mg BID typically used) were used. In the other 2 studies, it should be noted that complex study designs including a dose stagger of 12 hours was applied to assess the duration of mechanism-based inhibition. Overall, the prediction accuracy was good with an AFE of 1.08 and 0.81 and an AAFE of 1.19 and 1.28 for C_{max} and AUC, respectively.

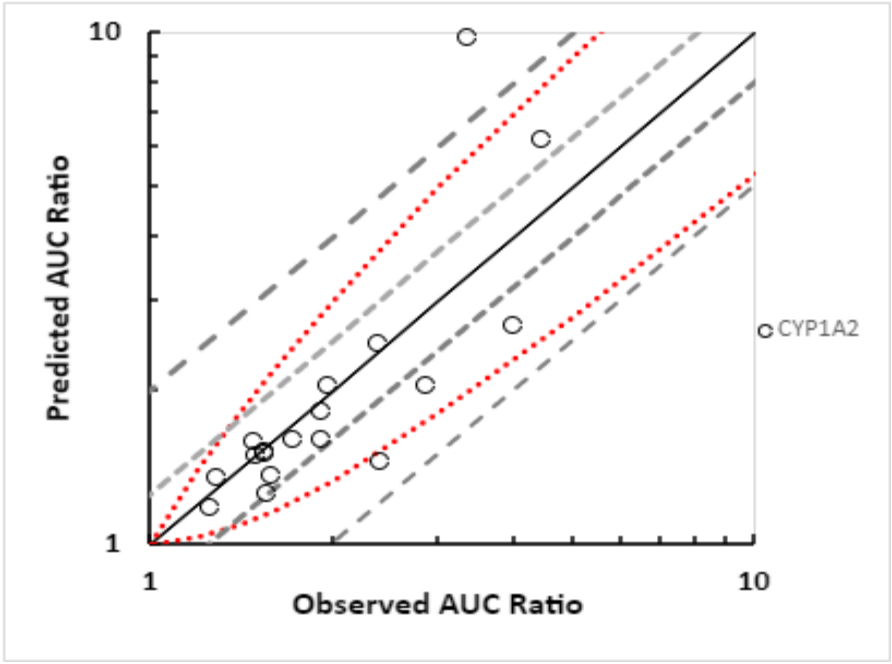
For CYP2C9 (n=21), all the predictions fell within 1.5-fold of the observed clinical values for both C_{max} and AUC. Overall, the prediction accuracy was good with an AFE of 0.89 and 1.03 and an AAFE of 1.13 and 1.15 for C_{max} and AUC, respectively.

There were 11 DDI studies available to evaluate the prediction of CYP2C19 DDIs with 3 substrates, S-mephenytoin, omeprazole and imipramine. The C_{max} was predicted with an AFE of 0.95 and AAFE of 1.14 and apart from 1, 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 studies with a bias of 1.03 and precision of 1.19.

CYP2D6 predictions were assessed for 26 clinical studies involving the 6 substrates; there was a good prediction accuracy with an AFE of 1.05 and 0.93 and an AAFE of 1.17 and 1.16 for C_{max} and AUC ratios, respectively. Three of the predictions fell outside of 1.5-fold of the observed C_{max} ratios for the substrates nebivolol and tolterodine. Two of the predictions fell outside of 1.5-fold for the AUC ratios for the substrates dextromethorphan and metoprolol.

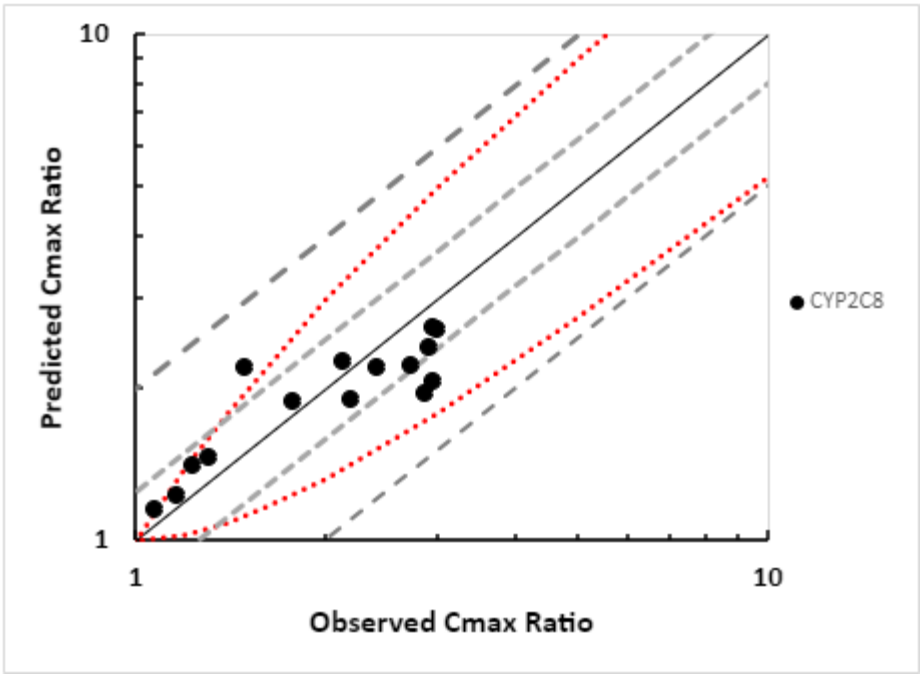
For CYP3A4/5 (n=111), the prediction accuracy was good with an AFE of 0.93 and 0.96 and an AAFE of 1.22 and 1.26 for C_{max} and AUC, respectively. The predictions were within 1.5-fold of observed C_{max} ratios for all except 9 of the interactions where 2 of these also fell outside 2-fold of the observed C_{max} ratio. The AUC ratio was predicted well with 81% of the predictions falling within 1.5-fold of the observed data, only 2 predictions were outside of 2-fold from the observed AUC ratio for simulations with quinidine and erythromycin, and simvastatin and erythromycin.

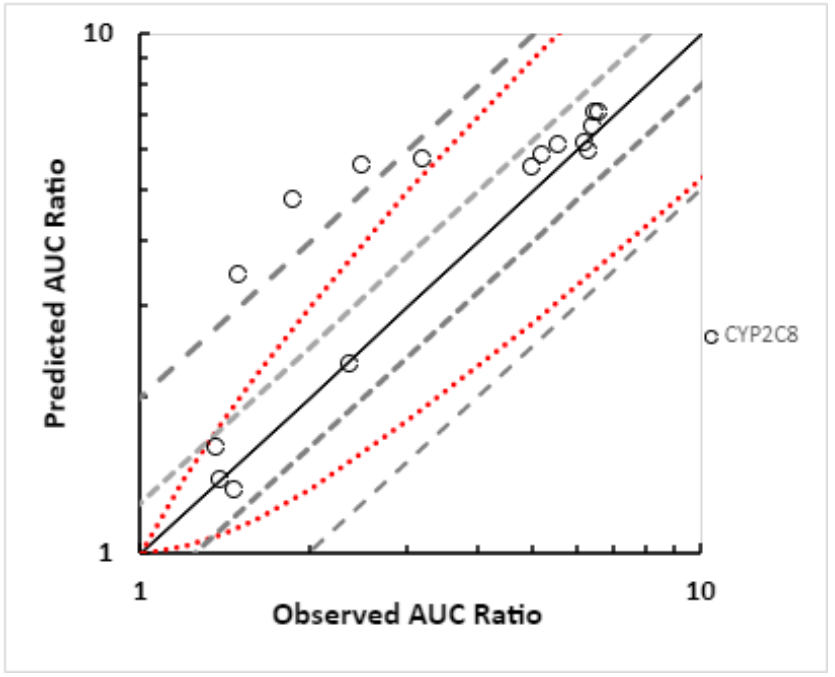




CYP1A2	V19R1	
	Cmax Ratio	**AUC Ratio**
AFE (bias)	0.91	1.03
AAFE (precision)	1.21	1.21
Number Studies	15	20

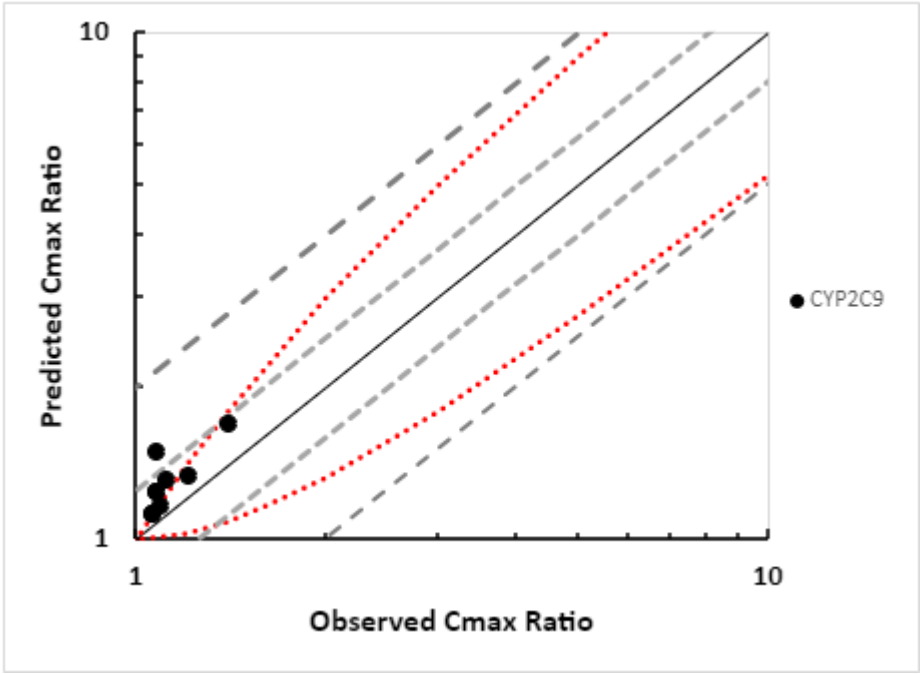
Figure 7. Predicted *versus* observed CYP1A2-mediated DDIs

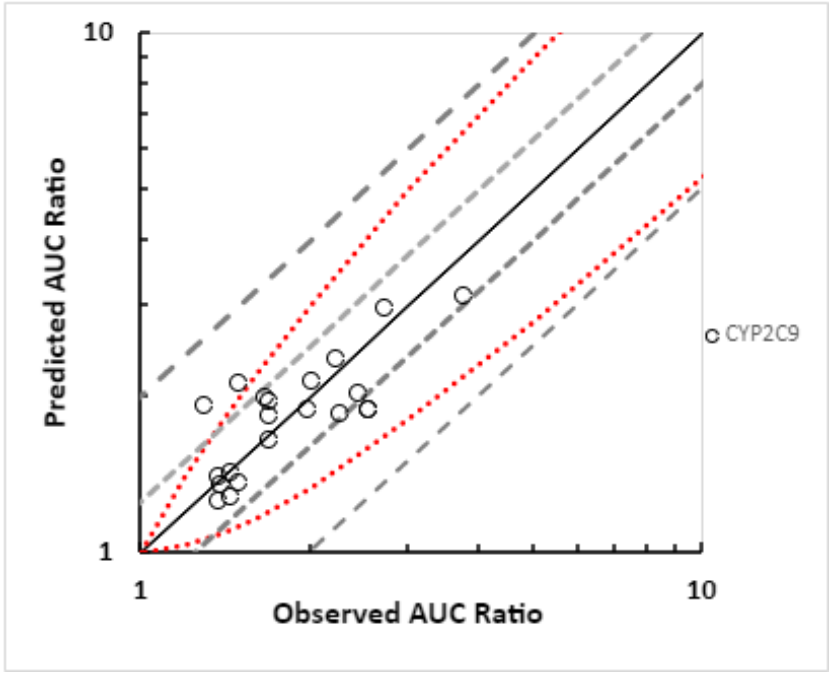




CYP2C8	V19R1	
	Cmax Ratio	**AUC Ratio**
AFE (bias)	1.08	0.81
AAFE (precision)	1.19	1.28
Number Studies	16	16

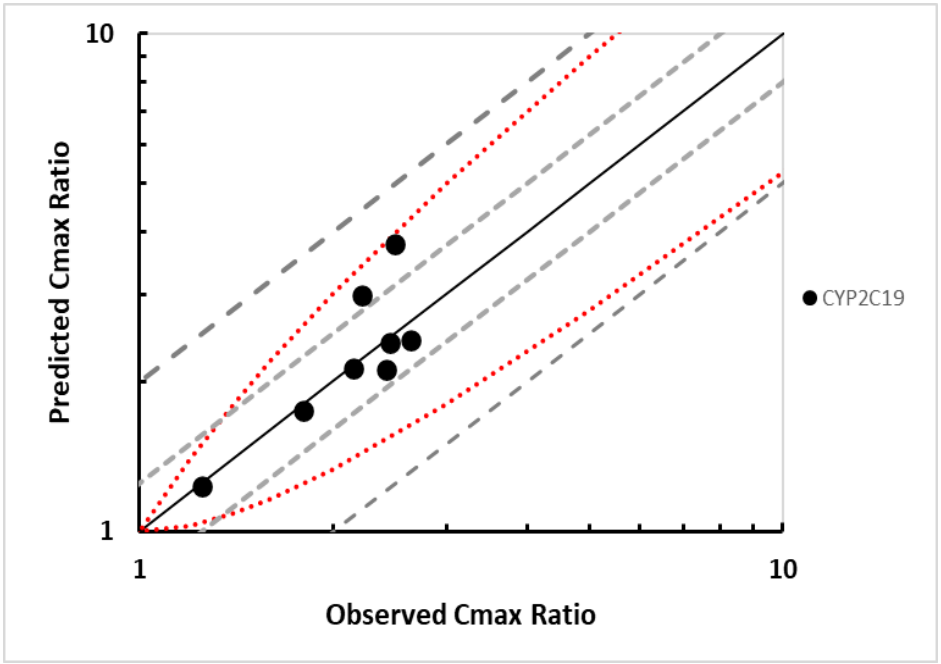
Figure 8. Predicted *versus* observed CYP2C8-mediated DDIs

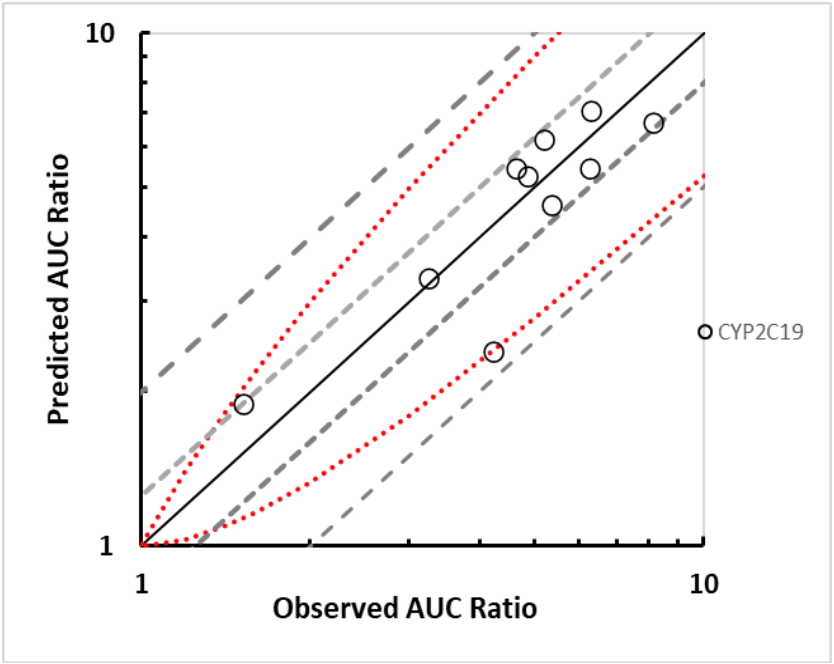




CYP2C9	V19R1	
	Cmax Ratio	**AUC Ratio**
AFE (bias)	0.89	1.03
AAFE (precision)	1.13	1.15
Number Studies	9	21

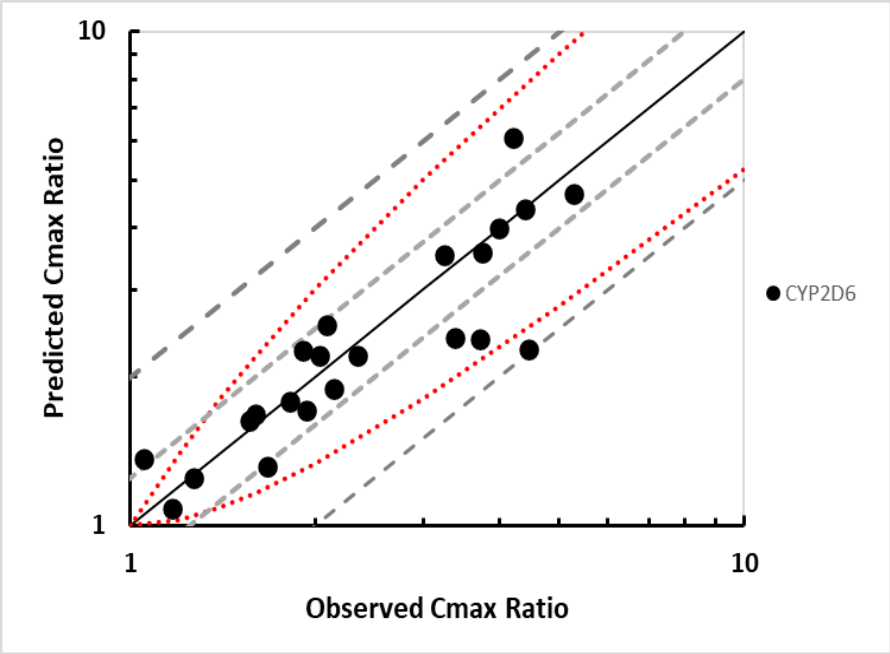
Figure 9. Predicted *versus* observed **CYP2C9-mediated** DDIs

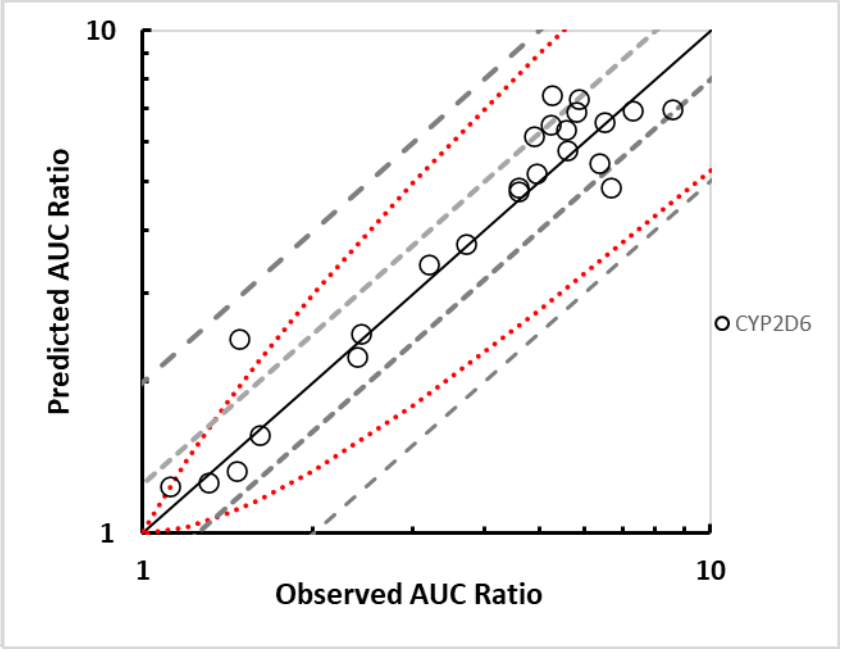




CYP2C19	V19R1	
	Cmax Ratio	**AUC Ratio**
AFE (bias)	0.95	1.03
AAFE (precision)	1.14	1.19
Number Studies	8	11

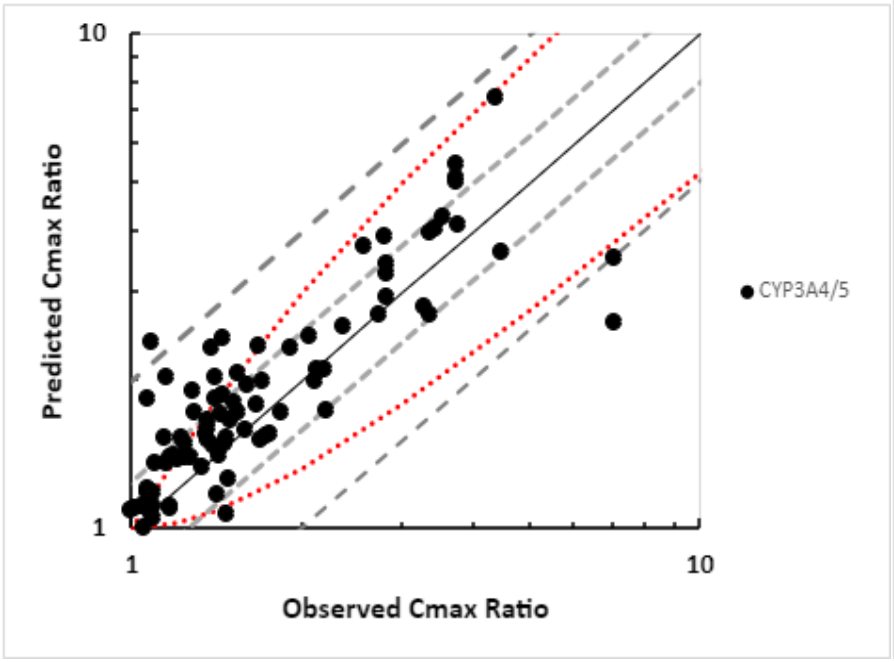
Figure 10. Predicted *versus* observed **CYP2C19-mediated** DDIs

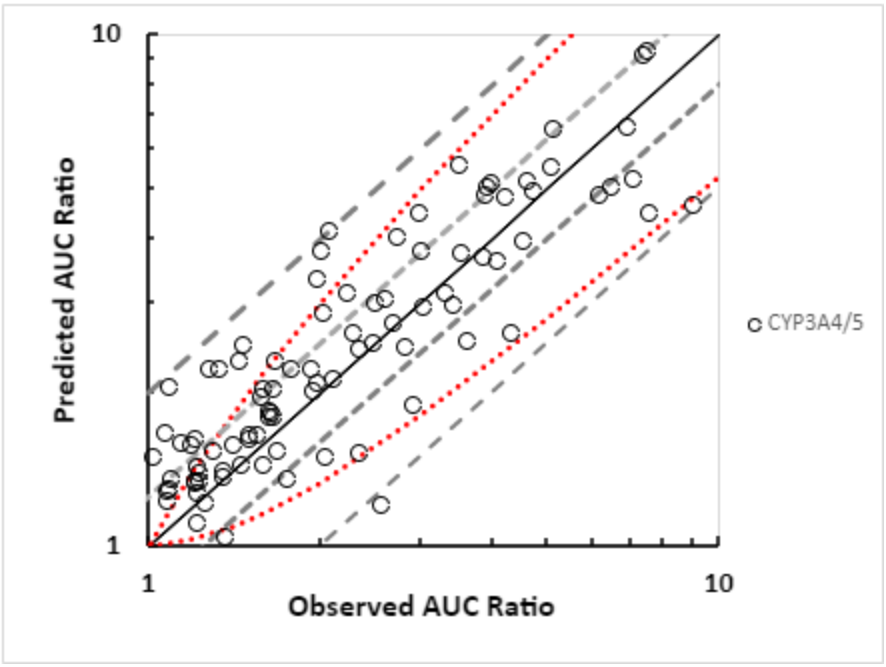




CYP2D6	V19R1 Built 96	
	Cmax Ratio	**AUC Ratio**
AFE (bias)	1.05	0.93
AAFE (precision)	1.17	1.16
Number Studies	23	26

Figure 11. Predicted *versus* observed **CYP2D6-mediated** DDIs





CYP3A4	V19R1 Built 96	
	Cmax Ratio	**AUC Ratio**
AFE (bias)	0.93	0.96
AAFE (precision)	1.22	1.26
Number Studies	94	111

Figure 12. Predicted *versus* observed **CYP3A4-mediated** DDIs

6. Version comparisons

In this analysis, we identified a DDI matrix involving substrates and inhibitors of CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 which was then used for qualification of the Simcyp Simulator (V19R1). In total, 33 substrates and 24 inhibitors were identified for inclusion in the DDI qualification matrix. Compound file summaries have been prepared for each of these compounds in V19 R1 (Appendix 2).

In addition to providing details relating to input parameters and PK predictions, the compound file summaries also contain examples of DDI predictions. These summaries have been generated in later versions of the Simcyp Simulator, including V20 and V21.

If new data become available for compounds, the files within the Simcyp Simulator are updated especially if they improve the performance of the models. Thus, any differences in PK and DDI predictions for a compound may be a consequence of changes in compound file parameters or system parameter updates.

Thus, two sets of comparisons are typically performed. Compound files developed in a new version e.g., V20 files in the V20 simulator would be compared against V20 files in the V19 simulator – this comparison gives an indication of the impact of the changes in compound files. In addition, V20 files in the V20 simulator would be compared against V19 files in the V19 simulator. This comparison would reflect changes in system parameters as well as changes in compound files. The former has been provided for V19/V20 and the latter for V20/V21 (Appendix 6). The changes in compound files which are shown in Appendix 7 are negligible as can be seen by the graphs shown in the documents included in Appendix 7.

7. Summary and Key Findings

For a compound in development, the CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 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 recognise, that during model development, for most of the substrates included in the DDI qualification matrix, a clinical DDI study was used to optimise their fmCYP 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] Thereafter, the qualification dataset described herein indicate that PBPK modelling can be used to support untested DDI scenarios involving moderate or weak inhibitors of the relevant CYP enzyme as has typically been the case.[11]

The results presented here indicate that the CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 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. Firstly, 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.

Secondly, the *in vitro* determined inhibitory parameters of the drug may require some calibration or optimisation, prior to assessing the DDI potential of the compound, as described below. Thus, the qualification dataset described here, indicate that PBPK modelling can be used to support untested scenarios (co-medications and less sensitive substrates) for perpetrators of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 typically when a clinical study has been performed to assess the DDI potential of the drug using a sensitive substrate, thus allowing optimisation of the relevant *in vitro* inhibitory parameters if needed.

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.[21]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).

Based on the predictive performance of the platform, the data presented here demonstrate that Simcyp Simulator (V19 R1) can be applied with reasonably accuracy to assess the CYP-mediated DDI potential of investigational new drugs (IND) as victim or perpetrators involving competitive inhibition or MBI.

8. Questions for EMA

Question 1: Does the Agency agree that the results presented here support our proposed COU statements for using the Simcyp Simulator V19 R1 to predict the DDI potential of drugs as victims or perpetrators of CYP-mediated interactions involving competitive inhibition or MBI for untested clinical DDI scenarios?

Certara UK Ltd Position: Certara UK Ltd believes that the results from our data analysis and that published previously[12], establish the high predictive accuracy of the Simcyp Simulator V19 R1 for clinical trial outcomes involving CYP-mediated inhibition. The platform can be used to optimise dose(s) and dosing schedules for the drug itself or drugs that are likely to be co-prescribed. In addition to the analysis presented here, the Simcyp Simulator has been applied to assess the DDI potential of drugs in development and the results have been used to inform clinical DDIs in the label.[1]

Question 2: What steps are needed for qualification of subsequent versions of the Simcyp Simulator?

Certara UK Ltd Position: A full analysis was conducted for the Simcyp Simulator V19 R1. Supporting documentation in the form of comparisons of outputs of PK parameters generated from compound files run in V19 *versus* v20 *versus* V21 are routinely generated. Furthermore, compound files summaries for each compound are generated in V19, V20 and V21. These could be used to support qualification of CYP-mediated inhibition in V20 and V21 in addition to examples that have been published using V20 and V21 of the platform.

9. 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, *et al.* 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. *The Journal of Clinical Pharmacology.*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 [cited 18 December 2020]Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions
6. European Medicines Agency. Guideline on the investigation of drug interactions. 2012 [cited 18 December 2020]Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions-revision-1_en.pdf
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 [cited 18 December 2020]Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/physiologically-based-pharmacokinetic-analyses-format-and-content-guidance-industry
10. European Medicines Agency. Reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation. 2018 [cited 18 December 2020]Available from: https://www.ema.europa.eu/en/reporting-physiologically-based-pharmacokinetic-pbpk-modelling-simulation

11. 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.

12. Kilford PJ, Chen KF, Crewe K, Gardner I, Hatley O, Ke AB, Neuhoff S, Zhang M, Rowland Yeo K. Prediction of CYP-mediated DDIs involving inhibition: Approaches to address the requirements for system qualification of the Simcyp Simulator. CPT Pharmacometrics Syst Pharmacol. 2022 Jul;11(7):822-832. doi: 10.1002/psp4.12794. Epub 2022 Apr 28. PMID: 35445542; PMCID: PMC9286715.

13. Jamei, M. *et al.* The simcyp population based simulator: architecture, implementation, and quality assurance. *In Silico Pharmacol* **1**, 9, doi:10.1186/2193-9616-1-9 (2013).

14. Jamei, M. *et al.* The Simcyp population-based ADME simulator. *Expert Opin Drug Metab Toxicol* **5**, 211-223, doi:10.1517/174252

15. Howgate EM, Rowland-Yeo K, Proctor NJ, Tucker GT and Rostami-Hodjegan A. Prediction of in vivo drug clearance from in vitro data: impact of interindividual variability. *Xenobiotica* 2006; 36 (6): 473-497.

16. 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.

17. Jamei M, Turner D, Yang J *et al.* Population-based mechanistic prediction of oral drug absorption. *AAPS J* 2009; 11, 225-237.

18. 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.

19. 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.

20. Gertz M, Harrison A, Houston JB, Galetin A. Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab Dispos*. 2010 Jul;38(7):1147-58. doi: 10.1124/dmd.110.032649. Epub 2010 Apr 5. PMID: 20368326.

10.Appendix 1

Scaling Methods and PBPK Models

10.1 Physiologically based pharmacokinetic models

A minimal physiologically based pharmacokinetic (PBPK) model, which considers both liver and intestinal metabolism (Figure A), is incorporated in the Simcyp Simulator. It includes a single adjusting compartment (SAC) that lumps all tissues excluding the intestine, liver and portal vein and can be used to represent those organs that make a significant contribution to the volume of distribution. The model can also be expanded to a full PBPK model by inclusion of additional tissues such as adipose, brain, bone, heart, lung, muscle and skin (Figure A2).

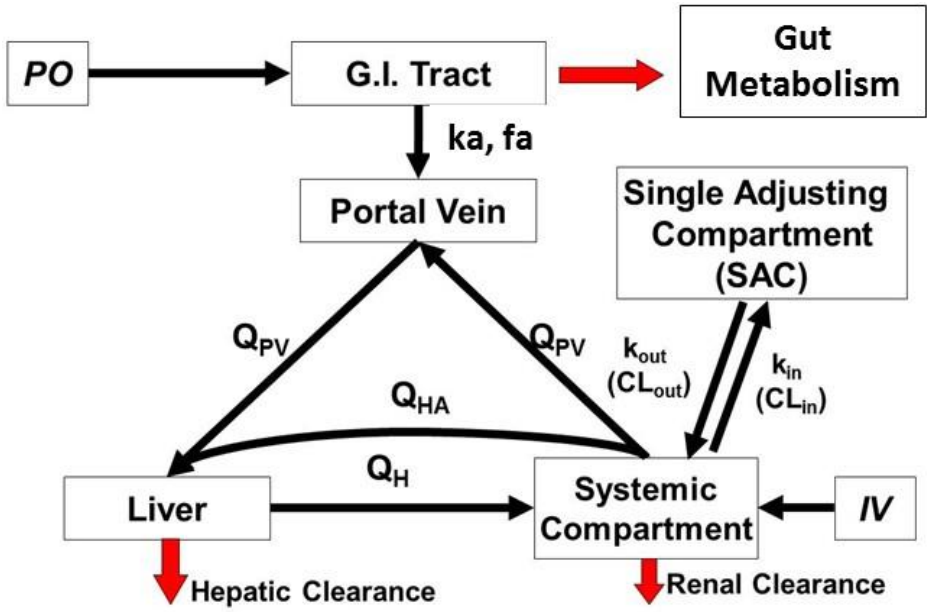


Figure A1. Minimal physiologically based pharmacokinetic model with single adjusting compartment. Q_H, Q_{PV}, and Q_{HA} are blood flows in the liver, portal vein, and hepatic artery, respectively; k_{in} and k_{out} are first order rate constants which act on the masses of drug within the systemic compartment and the SAC respectively; IV and PO are intravenous and oral dosing routes respectively; fa and ka are the fraction absorbed and the first order absorption rate constant, respectively.

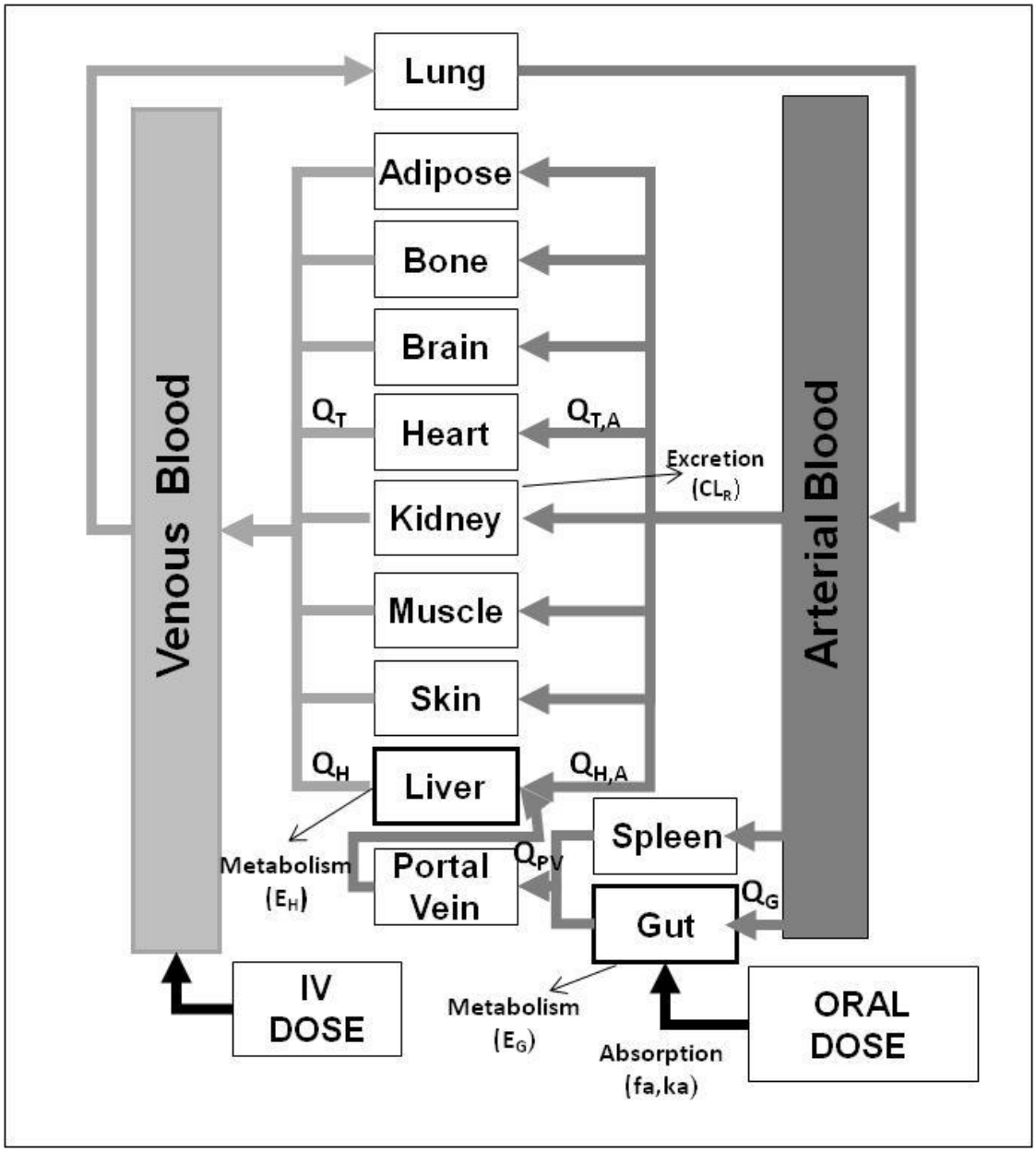


Figure A2. A physiologically based pharmacokinetic model. $Q_{H,}$ $Q_{H,A}$, $Q_{PV,}$ $Q_{G,}$ $Q_{T,A}$ and Q_T are blood flows in the hepatic vein, hepatic artery, hepatic portal vein, gut and blood flows into and out of the other tissue (T) compartments, respectively; E_G and E_H are the fractions undergoing first pass metabolism in the gut and liver, respectively; CL_R is the renal clearance; fa and ka are the fraction absorbed and the first order absorption rate constant, respectively.

10.2 Prediction of V_{ss}

For the minimal model, an *in vivo* V_{ss} value (and associated variability) can be used as an input or this parameter can be predicted using Equation 1 from Sawada *et al*. (1984):

$$V_{ss} = (\sum V_t \times P_{t:p}) + (V_e \times E:P) + V_p \quad \text{(Equation 1)}$$

Where V is the fractional body volume (L/kg) of a tissue (t), erythrocyte (e), and plasma (p), E:P is the erythrocyte:plasma ratio and $P_{t:p}$ is the partition coefficient for non-adipose and adipose components. Three methods are available for prediction of $P_{t:p}$, the first reported by Poulin and Theil (2002) and modified by Berezhkovskiy (2004) and the second by Rodgers and Rowland (2006). The third method extends the Rodgers and Rowland method to account for the impact of membrane potential on the permeation of ionised drugs using the Fick-Nernst-Planck equation (Gaohua *et al.*, 2016).

10.3 Absorption Models

Several absorption models are available within the Simcyp Simulator including a first-order absorption model and the advanced dissolution absorption metabolism (ADAM) model (Jamei *et al.,* 2009).

10.4 Prediction of first order absorption (fa) and associated rate constant (ka)

For the fraction absorbed (fa) and first order absorption rate constant (ka), *in vivo* values and associated variability can be used as inputs. Alternatively, Equation 2 and Equation 3 can be used to predict fa and ka from an estimate of *in vivo* permeability, $P_{eff,man}$, (Yu *et al*., 1998). Several methods can be used within the Simcyp Simulator to predict $P_{eff,man}$ for a given drug. These are based on apparent permeability data obtained with cell lines (Caco-2, MDCK, LLC-PK1) (Sun *et al.,* 2002, Tchaparian *et al*., 2008), the PAMPA system or from QSAR based on physicochemical properties (PSA and HBD, Winiwarter *et al.,* 1998).

$$ka = \frac{2 \times P_{eff,man}}{R} \quad \text{(Equation 2)}$$

$$fa = 1 - (1 + 0.54\ P_{eff,man})^{-7} \quad \text{(Equation 3)}$$

10.5 Prediction of Clearance

Clearance (CL) can be predicted from either human liver microsome (HLM) data or from human hepatocyte (HHep) data using Equation 4 and Equation 5.

$$CLu_{\text{int}H-pe} = \frac{CL_{\text{int}-pe}}{fu_{mic-pe}} \times Uptake \times MPPGL \times LiverWt \times 60 \times 10^{-6} \quad \text{(Equation 4)}$$

$$CLu_{\text{int}H-pe} = \frac{CL_{\text{int}-pe}}{fu_{inc-pe}} \times Uptake \times HPGL \times LiverWt \times 60 \times 10^{-6} \quad \text{(Equation 5)}$$

Where $CL_{intH\text{-}pe}$ is the CL in HLM by pathway 'p' by enzyme 'e' per mg microsomal protein, or CL in HHep by pathway 'p' by enzyme 'e' per million cells, MPPGL is the amount (mg) of microsomal protein per gram of liver, HPGL is the total number (in million) of hepatocytes per gram of liver, fu_{mic} is the free fraction of drug in the microsomal incubation, fu_{inc} is the free fraction of drug in the hepatocyte incubation, 'Uptake' is a factor that accounts for any active hepatic uptake (default value = 1) and 'LiverWt' is the liver weight of an individual, '60 x 10^{-6}' is a unit conversion factor.

Hence, total unbound intrinsic hepatic clearance ($CLu_{int,H\text{-}pe}$) is given by the sum of all intrinsic clearances by all enzymes and pathways (Equation 6).

$$CLu_{\text{int},H} = \sum_{p=1}^{n}\sum_{e=1}^{m} CLu_{\text{int},H-pe} \quad \text{(Equation 6)}$$

Where n is the number of pathways and m is the number of enzymes involved in the metabolism of the substrate. This intrinsic clearance value is applied in association with a prediction of drug distribution and through a number of differential equations (PBPK model) to generate a plasma drug concentration-time profile.

10.6 Prediction of First Pass Metabolism in the Gut (F_G)

To estimate intestinal availability (F_G), a model of 'first pass' metabolism, similar to the 'well-stirred liver', (Rostami-Hodjegan and Tucker, 2004) is used for substrates metabolised primarily by CYP3A but also by CYP2D6, CYP2C9 and CYP2C19 (Equation 7). In contrast to the 'well-stirred' liver model, the flow term (Q_{gut}) represents a nominal blood flow and is a hybrid parameter reflecting drug absorption rate from the gut lumen, removal of drug from the enterocyte by the enterocytic blood supply and the volume of enterocytes. The free fraction of drug within the enterocyte is represented by the fu_{gut} term.

$$F_G = \frac{Q_{gut}}{Q_{gut} + fu_{gut} \times CL_{uG,int}} \quad \text{(Equation 7)}$$

The Q_{gut} term can be expanded in terms of its fundamental parameters:

$$Q_{gut} = \frac{Q_{villi} \text{ x } CL_{perm}}{Q_{villi} + CL_{perm}} \quad \text{(Equation 8)}$$

Where Q_{villi} is the villous blood flow (6% of the cardiac output in the Simulator) and CL_{perm} is a clearance term defining the permeability through the enterocyte.

$$CL_{perm} = P_{eff,man} \text{ x } A \quad \text{(Equation 9)}$$

CL_{perm} is the product of the value for effective intestinal permeability in man ($P_{eff,man}$) and A is the net cylindrical surface area of the small intestine (Yang *et al*., 2007).

In the absence of any information on active drug uptake into the enterocyte, fu_{gut} is set at a default value of 1 (which assumes that there is insufficient time for plasma protein binding equilibrium or erythrocyte uptake before the drug is removed from the basolateral side of the enterocyte). However, it may also be set at fu_p which assumes that there is sufficient time for plasma protein binding equilibrium. Assuming that a proportional relationship exists between Q_{gut} and permeability (P_{app}) data obtained using Caco-2 cells, a Q_{gut} value can be estimated (Yang *et al.,* 2007). For calculation of gut intrinsic clearance ($CLu_{G,int}$), the CYP3A-mediated hepatic CLu_{int} is divided by the abundance of CYP3A in liver (137 pmol P450/mg protein) to obtain the CLu_{int} in terms of µl/min per pmol P450. Using a mean abundance of 70000 pmol CYP3A/total gut this value is scaled to a whole gut CLu_{int} value (Yang *et al*., 2004). The assumption that the intrinsic clearance per pmol CYP is the same in both gut and liver is supported by observations on a number of drugs, such that hepatic rather than intestinal microsomal data can be used (Yang *et al*., 2004).

10.7 Prediction of F_H and F

The 'well-stirred' model of hepatic clearance was used to estimate the fraction avoiding first-pass metabolism in the liver (F_H).

$$F_H = \frac{Q_H}{Q_H + fu_B \times CLu_{H.\text{int}}} \quad \text{(Equation 10)}$$

where Q_H (hepatic blood flow), fu_B (the fraction of drug unbound in blood) and $CLu_{H,int}$ (intrinsic metabolic clearance) are the primary determinants of net hepatic clearance (CL_H). Thus, following oral administration, bioavailability F can be estimated using Equation 11:

$$F = fa.F_G.F_H \quad \text{(Equation 11)}$$

10.8 Enzyme Dynamics and Inhibition

Changes in metabolic clearance due to reversible inhibition of enzyme activity, or changes in enzyme levels due to mechanism-based inactivation and/or induction can be handled using mechanistic dynamic models within the Simcyp Simulator. The underlying assumptions and operating differential equations have been described in detail elsewhere (Rowland Yeo *et al*., 2010; Rowland Yeo *et al*., 2011). Unbound concentrations of inhibitor in the liver and portal vein are used as the driving force for inhibition of metabolism in the liver and gut, respectively. Values of the intrinsic turnover of hepatic and gut CYP3A4 (k_{deg}) used in the simulations involving induction of CYP3A4 by rifampicin were 0.019 h^{-1} and 0.03 h^{-1}, respectively (Rowland Yeo *et al.,* 2011; Yang *et al*., 2008).

References

1. Berezhkovskiy LM (2004) Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. *J Pharm Sci* **93:**1628-1640.
2. Gaohua L, Turner DB, Fisher C, Emami Riedmaier A, Musther H, Gardner I and Jamei M A novel mechanistic approach to predict the steady state volume of distribution (Vss) using the Fick-Nernst-Planck equation [abstract], in: PAGE. Abstracts of the Annual Meeting of the Population Approach Group in Europe June 2016; Lisbon, Portugal. Abstr 5709.
3. Jamei M, Turner D, Yang J *et al.* Population-based mechanistic prediction of oral drug absorption. *AAPS J* 2009; 11, 225-237.
4. Poulin P and Theil FP. Prediction of pharmacokinetics prior to in vivo studies 1. Mechanism-based prediction of volume of distribution. *J Pharm Sci* 2002; 91:129-156.
5. Rodgers T and Rowland M. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. J Pharm Sci. 2006; 95(6):1238-57.
6. Rostami-Hodjegan A and Tucker GT. *'In silico'* simulations to assess the 'in vivo' consequences of *'in vitro'* metabolic drug-drug interactions. *Drug Discov Today: Technology* 2004;1(4):441-8.
7. Rowland-Yeo K, Jamei M, Yang J, Tucker GT and 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.

8. Rowland Yeo K, Walsky RL, Jamei M, Rostami-Hodjegan A and Tucker GT (2011) Prediction of time-dependent CYP3A4 drug-drug interactions by physiologically based pharmacokinetic modelling: impact of inactivation parameters and enzyme turnover. *Eur J Pharm Sci* **43:**160-173.
9. Sawada Y, Hanano M, Sugiyama Y, Harashima H and Iga T. Prediction of the volumes of distribution of basic drugs in humans based on data from animals. *J Pharmacokinet Biopharm* 1984; 12:587-596.
10. Sun D, Lennernas H, Welage LS, Barnett JL, Landowski CP, Foster D, Fleisher D, Lee KD and Amidon GL. Comparison of human duodenum and Caco-2 gene expression profiles for 12,000 gene sequences tags and correlation with permeability of 26 drugs. Pharmacol Res 2002; 19: 1400-06.
11. Tchaparian E, Tang L, Xu G, Huang T and Jin L Cell based experimental models as tools for the prediction of human intestinal absorption [abstract], in: ISSX Online Abstracts Supplement 3 (3); October 2008; San Diego, CA. Abstr 245.
12. Winiwarter S, Bonham NM, Ax F, Hallberg A, Lennernas H and Karlen A Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. J Med Chem 1998; 41:4939-4949.
13. Yang J, Tucker GT and Rostami-Hodjegan A. Cytochrome P450 3A expression and activity in the human small intestine. Clin Pharmacol Ther 2004; 76: 391.
14. Yang J, Jamei M, Rowland-Yeo K, Tucker GT and Rostami-Hodjegan A. Prediction of intestinal first-pass metabolism. *Curr Drug Metab* 8: 676-684 (2007).
15. Yang J, Liao M, Shou M, Jamei M, Rowland-Yeo K, Tucker GT and Rostami-Hodjegan A. 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 (2008).
16. Yu LX and Amidon GL. Saturable small intestine drug absorption in humans: modelling and interpretation of cefatrizine data. Eur J Pharm Biopharm 1998; 45: 199-203.

11.Appendix 2

Compound File Summaries

In total, 33 substrates and 24 inhibitors were identified for inclusion in the DDI matrix for qualification of CYP-mediated inhibition using the Simcyp Simulator (V19R1). Compound file summaries for each substrate and inhibitor relevant to V19 R1 can be found within the corresponding document cited below. The input parameters for each compound, predicted PK

parameters and verification of DDI as substrates and inhibitors are indicated within each compound file summary, all of which are saved in the accompanying documents shown below.

Inhibitor Summaries

Substrate Summaries

12. Appendix 3

Inhibitory mechanisms involved for each unique substrate-inhibitor pair

Details of the CYP enzymes involved in each drug-drug interaction pair as well as the inhibitory mechanisms investigated are provided in the document below.

Table-Inhibitor Matrix.pdf

13. Appendix 4

Inhibitor Characteristics

Table A1. Inhibitor information from Simcyp Simulator compound files (V19R1) showing classification, interaction parameters and driving mechanism of the interactions.

Inhibitor		FDA Classification	$K_{i,u}$ (µM)	K_{app} (µM)	K_{inact} (1/h)	Driving Mechanism	In Vitro/ Optimised	Ref
Ciprofloxacin	CYP1A2	Strong	1.84*	-	-	Competitive	Optimised	(1)
Fluvoxamine	CYP1A2	**Strong**	0.002*	-	-	Competitive	Optimised	**
Gemfibrozil	CYP2C8	**Strong**	24.1	-	-	MBI	In Vitro	(2-4)
Gemfibrozil 1-O-β Glucuronide	CYP2C8	-	4.88	27.1*	6.5*	-	-	(2-6)
Trimethoprim	CYP2C8	Weak	8.47	-	-	Competitive	In Vitro	(4)
Amiodarone	CYP2C9	Moderate	0.425	0.028	0.6	MBI	In Vitro	(7, 8)
Mono-desethyl Amiodarone	CYP2C9	-	0.120	-	-	-	-	(7, 8)
Fluconazole	CYP2C9	**Moderate**	20.4	-	-	Competitive	In Vitro	(9)
Fluvoxamine	CYP2C9	Weak	0.126	-	-	Competitive	Optimised	**
Sulphaphenazole	CYP2C9	-	1.5*	-	-	Competitive	Optimised	(10)
Fluvoxamine	CYP2C19	**Strong**	0.006*	-	-	Competitive	Optimised	**
Fluoxetine	CYP2C19	Strong	9.39	2.69	2.16	MBI	In Vitro	(11)
Nor-Fluoxetine	CYP2C19	-	2.99	6*	3.27	MBI	Optimised	(11-13)
Ticlopidine	CYP2C19	Strong	-	0.432	4.43	MBI	In Vitro	(14)
Bupropion	CYP2D6	-	0.084*	-	-	Competitive	Optimised	(15)
OH-Bupropion	CYP2D6		0.053*	-	-	Competitive	Optimised	(15)
Cinacalcet	CYP2D6	Moderate	0.0006	-	-	Competitive	In Vitro	#
Fluoxetine	CYP2D6	**Strong**	0.012	-	-	Competitive	In Vitro	(16)
Fluvoxamine	CYP2D6	Weak	0.189*	-	-	Competitive	Optimised	**
Paroxetine	CYP2D6	**Strong**	0.46	0.137	10.2	MBI	In Vitro	(8, 17-24)
Quinidine	CYP2D6	Strong	0.0119	-	-	Competitive	In Vitro	(25)
Ritonavir	CYP2D6	Weak	0.04*	-	-	Competitive	Optimised	(26)
Amiodarone	CYP3A4	-	4.41	0.052	0.8	MBI	In Vitro	(7, 8)
Mono-desethyl Amiodarone	CYP3A4	-	0.482	-	-	-	-	(7, 8)
Aprepitant	CYP3A4	Moderate	1##	2.66##	6##	MBI	In Vitro	(27)
Atazanavir	CYP3A4/5	-	2.35	0.84	3	MBI	In Vitro	(28)
Cimetidine	CYP3A4	Weak	25*	-	-	Competitive	Optimised	(29)
Clarithromycin	CYP3A4	**Strong**	8.7	12	2.13	MBI	In Vitro	(30, 31)
Cyclosporine	CYP3A4	Moderate	0.89	-	-	Competitive	In Vitro	(32)
Diltiazem	CYP3A4/5	Moderate	36.1	4.75	0.70	MBI	In Vitro	(31, 33)

Desmethyl-Diltiazem	CYP3A4/5	-	2.43	1.74	1.09	-	-	(31, 33)
Erythromycin	CYP3A4	**Moderate**	29.8	17.64	2.25*	MBI	Optimised	(34)
Fluconazole	CYP3A4	**Moderate**	10.7	-	-	Competitive	In Vitro	(35)
Fluconazole	CYP3A5	**Moderate**	84.6	-	-	Competitive	In Vitro	(35)
Fluoxetine	CYP3A4	-	3.64	16	0.66	MBI	In Vitro	(11)
Fluvoxamine	CYP3A4	Moderate	0.789*	-	-	Competitive	Optimised	**
Fluvoxamine	CYP3A5	Moderate	5.82*	-	-	Competitive	Optimised	**
Itraconazole	CYP3A4	**Strong**	0.0023	-	-	Competitive	In Vitro	(36)
Ketoconazole	CYP3A4	Strong	0.0146	-	-	Competitive	In Vitro	(35)
Ketoconazole	CYP3A5	Strong	0.105	-	-	Competitive	In Vitro	(35)
Quinidine	CYP3A4	-	5.1	-	-	Competitive	In Vitro	(37)
Ritonavir	CYP3A4/5	Strong	0.0019	0.18	19.8	MBI	In Vitro	(38)
Verapamil	CYP3A4	**Moderate**	-	2.21	2	MBI	In Vitro	(31)
Verapamil	CYP3A5	**Moderate**	-	3.99	1.84	MBI	In Vitro	(39-41)
Norverapamil	CYP3A4	**Moderate**	-	10.3	18	MBI	In Vitro	(16)
Norverapamil	CYP3A5	**Moderate**	-	4.53	4.2	MBI	In Vitro	(16)

* Optimised value, # Measured Value, ##fu,mic was recalculated in the Simcyp Simulator

References

(1) Kim, M.-K., Nightingale, C.H. & Nicolau, D.P. Influence of sex on the pharmacokinetic interaction of fleroxacin and ciprofloxacin with caffeine. *Clinical pharmacokinetics* **42**, 985-96 (2003).

(2) Jenkins, S.M. *et al.* Studies to further investigate the inhibition of human liver microsomal CYP2C8 by the acyl-β-glucuronide of gemfibrozil. *Drug metabolism and disposition* **39**, 2421-30 (2011).

(3) Ogilvie, B.W. *et al.* Glucuronidation converts gemfibrozil to a potent, metabolism-dependent inhibitor of CYP2C8: implications for drug-drug interactions. *Drug metabolism and Disposition* **34**, 191-7 (2006).

(4) VandenBrink, B.M., Foti, R.S., Rock, D.A., Wienkers, L.C. & Wahlstrom, J.L. Evaluation of CYP2C8 inhibition in vitro: utility of montelukast as a selective CYP2C8 probe substrate. *Drug metabolism and disposition* **39**, 1546-54 (2011).

(5) Fujino, H., Yamada, I., Shimada, S., Hirano, M., Tsunenari, Y. & Kojima, J. Interaction between fibrates and statins-metabolic interactions with gemfibrozil. *Drug metabolism and drug interactions* **19**, 161-76 (2003).

(6) Parkinson, A., Kazmi, F., Buckley, D.B., Yerino, P., Ogilvie, B.W. & Paris, B.L. System-dependent outcomes during the evaluation of drug candidates as inhibitors of cytochrome P450 (CYP) and uridine diphosphate glucuronosyltransferase (UGT) enzymes: human hepatocytes versus liver microsomes versus recombinant enzymes. *Drug metabolism and pharmacokinetics* **25**, 16-27 (2010).

(7) McDonald, M.G., Au, N.T. & Rettie, A.E. P450-based drug-drug interactions of amiodarone and its metabolites: diversity of inhibitory mechanisms. *Drug Metabolism and Disposition* **43**, 1661-9 (2015).

(8) Rougée, L.R., Mohutsky, M.A., Bedwell, D.W., Ruterbories, K.J. & Hall, S.D. The impact of the hepatocyte-to-plasma pH gradient on the prediction of hepatic clearance and drug-drug interactions for CYP2C9 and CYP3A4 substrates. *Drug Metabolism and Disposition* **45**, 1008-18 (2017).

(9) Neal, J.M., Kunze, K.L., Levy, R.H., O'Reilly, R.A. & Trager, W.F. Kiiv, an in vivo parameter for predicting the magnitude of a drug interaction arising from competitive enzyme inhibition. *Drug metabolism and disposition* **31**, 1043-8 (2003).

(10) Veronese, M.E., Miners, J.O., Randles, D., Gregov, D. & Birkett, D.J. Validation of the tolbutamide metabolic ratio for population screening with use of sulfaphenazole to produce model phenotypic poor metabolizers. *Clinical Pharmacology & Therapeutics* **47**, 403-11 (1990).

(11) Lutz, J.D., VandenBrink, B.M., Babu, K.N., Nelson, W.L., Kunze, K.L. & Isoherranen, N. Stereoselective inhibition of CYP2C19 and CYP3A4 by fluoxetine and its metabolite: implications for risk assessment of multiple time-dependent inhibitor systems. *Drug Metabolism and Disposition* **41**, 2056-65 (2013).

(12) Stresser, D.M. *et al.* Differential time-and NADPH-dependent inhibition of CYP2C19 by enantiomers of fluoxetine. *Drug metabolism and disposition* **37**, 695-8 (2009).

(13) Vlase, L., Neag, M., Popa, A., Muntean, D. & Leucuta, S.E. Pharmacokinetic interaction between fluoxetine and omeprazole in healthy male volunteers: a prospective pilot study. *Current therapeutic research* **71**, 360-8 (2010).

(14) Nishiya, Y. *et al.* Comparison of mechanism-based inhibition of human cytochrome P450 2C19 by ticlopidine, clopidogrel, and prasugrel. *Xenobiotica* **39**, 836-43 (2009).

(15) Todor, I. *et al.* Evaluation of a potential metabolism-mediated drug-drug interaction between atomoxetine and bupropion in healthy volunteers. *Journal of Pharmacy & Pharmaceutical Sciences* **19**, 198-207 (2016).

(16) Nielsen, K.K., Flinois, J.P., Beaune, P. & Brøsen, K. The biotransformation of clomipramine in vitro, identification of the cytochrome P450s responsible for the separate metabolic pathways. *Journal of pharmacology and experimental therapeutics* **277**, 1659-64 (1996).

(17) Belpaire, F., Wijnant, P., Temmermann, A., Rasmussen, B. & Brøsen, K. The oxidative metabolism of metoprolol in human liver microsomes: inhibition by the selective serotonin reuptake inhibitors. *European journal of clinical pharmacology* **54**, 261-4 (1998).

(18) Crewe, H., Lennard, M., Tucker, G., Woods, F. & Haddock, R. The effect of selective serotonin re-uptake inhibitors on cytochrome P4502D6 (CYP2D6) activity in human liver microsomes. *British journal of clinical pharmacology* **34**, 262-5 (1992).

(19) Fogelman, S.M. *et al.* O-and N-demethylation of venlafaxine in vitro by human liver microsomes and by microsomes from cDNA-transfected cells: effect of metabolic inhibitors and SSRI antidepressants. *Neuropsychopharmacology* **20**, 480-90 (1999).

(20) Hemeryck, A., De Vriendt, C. & Belpaire, F.M. Effect of selective serotonin reuptake inhibitors on the oxidative metabolism of propafenone: in vitro studies using human liver microsomes. *Journal of clinical psychopharmacology* **20**, 428-34 (2000).

(21) Hemeryck, A., De Vriendt, C.A. & Belpaire, F.M. Metoprolol-paroxetine interaction in human liver microsomes: stereoselective aspects and prediction of the in vivo interaction. *Drug metabolism and disposition* **29**, 656-63 (2001).

(22) Jornil, J., Jensen, K.G., Larsen, F. & Linnet, K. Identification of cytochrome P450 isoforms involved in the metabolism of paroxetine and estimation of their importance for human paroxetine metabolism using a population-based simulator. *Drug Metabolism and Disposition* **38**, 376-85 (2010).

(23) Otton, S., Ball, S., Cheung, S., Inaba, T., Rudolph, R. & Sellers, E. Venlafaxine oxidation in vitro is catalysed by CYP2D6. *British journal of clinical pharmacology* **41**, 149-56 (1996).

(24) Skjelbo, E. & Brosen, K. Inhibitors of imipramine metabolism by human liver microsomes. *British journal of clinical pharmacology* **34**, 256-61 (1992).

(25) Abdel-Rahman, S.M., Marcucci, K., Boge, T., Gotschall, R.R., Kearns, G.L. & Leeder, J.S. Potent inhibition of cytochrome P-450 2D6-mediated dextromethorphan O-demethylation by terbinafine. *Drug metabolism and disposition* **27**, 770-5 (1999).

(26) Bertz, R., Cao, G., Cavanaugh, J., Hsu, A., Granneman, G. & Leonard, J. Effect of ritonavir on the pharmacokinetics of desipramine. *11th International Conference on AIDS*, 7-12 (1996).

(27) Prueksaritanont, T. *et al.* Drug–drug interaction studies: regulatory guidance and an industry perspective. *The AAPS journal* **15**, 629-45 (2013).

(28) CDER. Clinical Pharmacology and Biopharmaceutics Reviews *Application Number 21-567*.

(29) Martínez, C. *et al.* Comparative in vitro and in vivo inhibition of cytochrome P450 CYP1A2, CYP2D6, and CYP3A by H2-receptor antagonists. *Clinical pharmacology & therapeutics* **65**, 369-76 (1999).

(30) Jurima-Romet, M., Crawford, K., Cyr, T. & Inaba, T. Terfenadine metabolism in human liver. In vitro inhibition by macrolide antibiotics and azole antifungals. *Drug Metabolism and Disposition* **22**, 849-57 (1994).

(31) Yeo, K.R., Walsky, R., Jamei, M., Rostami-Hodjegan, A. & Tucker, G. Prediction of time-dependent CYP3A4 drug–drug interactions by physiologically based pharmacokinetic modelling: impact of inactivation parameters and enzyme turnover. *European journal of pharmaceutical sciences* **43**, 160-73 (2011).

(32) Amundsen, R., Åsberg, A., Ohm, I.K. & Christensen, H. Cyclosporine A-and tacrolimus-mediated inhibition of CYP3A4 and CYP3A5 in vitro. *Drug Metabolism and Disposition* **40**, 655-61 (2012).

(33) Zhang, X., Jones, D.R. & Hall, S.D. Prediction of the effect of erythromycin, diltiazem, and their metabolites, alone and in combination, on CYP3A4 inhibition. *Drug metabolism and disposition* **37**, 150-60 (2009).

(34) Olkkola, K.T. *et al.* A potentially hazardous interaction between erythromycin and midazolam. *Clinical Pharmacology & Therapeutics* **53**, 298-305 (1993).

(35) Gibbs, M.A., Thummel, K.E., Shen, D.D. & Kunze, K.L. Inhibition of cytochrome P-450 3A (CYP3A) in human intestinal and liver microsomes: comparison of K i values and impact of CYP3A5 expression. *Drug Metabolism and Disposition* **27**, 180-7 (1999).

(36) Isoherranen, N., Kunze, K.L., Allen, K.E., Nelson, W.L. & Thummel, K.E. Role of itraconazole metabolites in CYP3A4 inhibition. *Drug Metabolism and Disposition* **32**, 1121-31 (2004).

(37) Galetin, A., Clarke, S.E. & Houston, J.B. Quinidine and haloperidol as modifiers of CYP3A4 activity: multisite kinetic model approach. *Drug Metabolism and Disposition* **30**, 1512-22 (2002).

(38) Kirby, B.J. *et al.* Complex drug interactions of HIV protease inhibitors 2: in vivo induction and in vitro to in vivo correlation of induction of cytochrome P450 1A2, 2B6, and 2C9 by ritonavir or nelfinavir. *Drug metabolism and disposition* **39**, 2329-37 (2011).

(39) Obach, R.S., Walsky, R.L. & Venkatakrishnan, K. Mechanism-based inactivation of human cytochrome p450 enzymes and the prediction of drug-drug interactions. *Drug metabolism and disposition* **35**, 246-55 (2007).

(40) Wang, Y.-H., Jones, D.R. & Hall, S.D. Prediction of cytochrome P450 3A inhibition by verapamil enantiomers and their metabolites. *Drug Metabolism and Disposition* **32**, 259-66 (2004).

(41) Wang, Y.-H., Jones, D.R. & Hall, S.D. Differential mechanism-based inhibition of CYP3A4 and CYP3A5 by verapamil. *Drug metabolism and disposition* **33**, 664-71 (2005).

14.Appendix 5

Study Design and Results of DDI Simulations

Table A2. Enzyme mechanism, study design, predicted over observed AUC and Cmax ratios using Simcyp Simulator V19R1

ID	Enzyme	Comp Inh / MBI	Substrate Dose	Inhibitor Dose	Predicted /Observed		Reference
					Cmax Ratio	AUC Ratio	
1	CYP3A4/5	CI	Alfentanil 15 µg/kg single iv (3 hr after fluconazole)	Fluconazole, 100 mg (single oral)		1.06	Kharasch, E.D., Walker, A., Hoffer, C. & Sheffels, P. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimally invasive and noninvasive probes for hepatic and first-pass CYP3A activity. *The Journal of Clinical Pharmacology* 45, 1187-97 (2005).
2	CYP3A4/5	CI	Alfentanil 40 µg/kg single oral (3 hr after fluconazole)	Fluconazole, 100 mg (single oral)	1.22	0.84	Kharasch, E.D., Walker, A., Hoffer, C. & Sheffels, P. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimally invasive and noninvasive probes for hepatic and first-pass CYP3A activity. *The Journal of Clinical Pharmacology* 45, 1187-97 (2005).
3	CYP3A4/5	CI	Alfentanil 15 µg/kg single iv (3 hr after fluconazole)	Fluconazole, 200 mg (single oral)		0.94	Kharasch, E.D., Walker, A., Hoffer, C. & Sheffels, P. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimally invasive and noninvasive probes for hepatic and first-pass CYP3A activity. *The Journal of Clinical Pharmacology* 45, 1187-97 (2005).
4	CYP3A4/5	CI	Alfentanil 40 µg/kg single oral (3 hr after fluconazole)	Fluconazole, 200 mg (single oral)	1.14	0.73	Kharasch, E.D., Walker, A., Hoffer, C. & Sheffels, P. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimally invasive and noninvasive probes for hepatic and first-pass CYP3A activity. *The Journal of Clinical Pharmacology* 45, 1187-97 (2005).

5	CYP3A4/5	CI	Alfentanil 15 µg/kg single iv (3 hr after fluconazole)	Fluconazole, 400 mg (single oral)		0.88	Kharasch, E.D., Walker, A., Hoffer, C. & Sheffels, P. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimally invasive and noninvasive probes for hepatic and first-pass CYP3A activity. *The Journal of Clinical Pharmacology* 45, 1187-97 (2005).
6	CYP3A4/5	CI	Alfentanil 40 µg/kg single oral (3 hr after fluconazole)	Fluconazole, 400 mg (single oral)	1.28	0.65	Kharasch, E.D., Walker, A., Hoffer, C. & Sheffels, P. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimally invasive and noninvasive probes for hepatic and first-pass CYP3A activity. *The Journal of Clinical Pharmacology* 45, 1187-97 (2005).
7	CYP3A4/5	CI	unlabelled d0-Alfentanil 0.5 mg iv on day 4 (assuming at 7:00 AM)	Ketoconazole, 400 mg (oral) for 3 days at bedtime (3 doses, assuming at 11:30 PM)		0.82	Kharasch, E.D. *et al.* Concurrent assessment of hepatic and intestinal cytochrome P450 3A activities using deuterated alfentanil. *Clinical Pharmacology & Therapeutics* 89, 562-70 (2011).
8	CYP3A4/5	CI	deuterium-labelled d3-Alfentanil 1 mg oral on day 4 (assuming at 10:00 AM)	Ketoconazole, 400 mg (oral) for 3 days at bedtime (3 doses, assuming at 11:30 PM)	1.18	0.82	Kharasch, E.D. *et al.* Concurrent assessment of hepatic and intestinal cytochrome P450 3A activities using deuterated alfentanil. *Clinical Pharmacology & Therapeutics* 89, 562-70 (2011).
9	CYP3A4/5	CI	unlabelled d0-Alfentanil 0.5 mg iv on day 5 (assuming at 7:00 AM)	Ketoconazole, 400 mg (oral) for 4 days at bedtime (4 doses, assuming at 11:30 PM)		0.79	Kharasch, E.D. *et al.* Concurrent assessment of hepatic and intestinal cytochrome P450 3A activities using deuterated alfentanil. *Clinical Pharmacology & Therapeutics* 89, 562-70 (2011).
10	CYP3A4/5	CI	deuterium-labelled d3-Alfentanil 1 mg oral on day 5 (assuming at 7:00 AM)	Ketoconazole, 400 mg (oral) for 4 days at bedtime (4 doses, assuming at 11:30 PM)	0.74	0.85	Kharasch, E.D. *et al.* Concurrent assessment of hepatic and intestinal cytochrome P450 3A activities using deuterated alfentanil. *Clinical Pharmacology & Therapeutics* 89, 562-70 (2011).

11	CYP3A4/5	CI	Alprazolam, 1 mg SD (day 3), (1 h after 5th Ketoconazole dose)	Ketoconazole, 200 mg BID for 4 days (8 doses)	1.00	0.69	Greenblatt, D.J. *et al.* Ketoconazole inhibition of triazolam and alprazolam clearance: differential kinetic and dynamic consequences. *Clinical Pharmacology & Therapeutics* 64, 237-47 (1998).
12	CYP3A4/5	CI	Alprazolam, 0.5 mg SD (day 4)	Ketoconazole, 200 mg BID for 6 days (12 doses)	0.92	1.05	Boulenc, X. *et al.* CYP3A4-based drug–drug interaction: CYP3A4 substrates' pharmacokinetic properties and ketoconazole dose regimen effect. *European journal of drug metabolism and pharmacokinetics* 41, 45-54 (2016).
13	CYP3A4/5	CI	Alprazolam, 0.5 mg SD (day 4)	Ketoconazole, 200 mg QD for 6 days (6 doses)	0.93	1.00	Boulenc, X. *et al.* CYP3A4-based drug–drug interaction: CYP3A4 substrates' pharmacokinetic properties and ketoconazole dose regimen effect. *European journal of drug metabolism and pharmacokinetics* 41, 45-54 (2016).
14	CYP3A4/5	MBI	Alprazolam, 0.8 mg SD (day 8)	Erythromycin, 400 mg TID for 10 days (30 doses)	0.91	1.01	Yasui, N. *et al.* A kinetic and dynamic study of oral alprazolam with and without erythromycin in humans: in vivo evidence for the involvement of CYP3A4 in alprazolam metabolism. *Clinical Pharmacology & Therapeutics* 59, 514-9 (1996).
15	CYP3A4/5	MBI	Alprazolam, 1 mg SD (day 2) (At 8.30am)	Ritonavir, 200 mg 4 doses over 3 days at 4pm d1, 7.30am and 4.30pm D2, 9am D3	1.06	1.47	Greenblatt, D.J. *et al.* Alprazolam-ritonavir interaction: implications for product labeling. *Clinical Pharmacology & Therapeutics* 67, 335-41 (2000).
16	CYP3A4/5	MBI	Alprazolam, 1 mg SD on day 22	Fluoxetine, 20 mg QD for 24 days	0.94	0.94	Hall, J., Naranjo, C.A., Sproule, B.A. & Herrmann, N. Pharmacokinetic and pharmacodynamic evaluation of the inhibition of alprazolam by citalopram and fluoxetine. *Journal of clinical psychopharmacology* 23, 349-57 (2003).
17	CYP3A4/5	CI	Alprazolam, 0.5 mg TID for 7 days and morning dose on day 8	Cimetidine, 200 mg TID with 400 mg at night for 9 days	0.59	0.66	Pourbaix, S., Desager, J.-P., Hulhoven, R., Smith, R. & Harvengt, C. Pharmacokinetic consequences of long term coadministration of cimetidine and triazolobenzodiazepines, alprazolam and triazolam, in healthy subjects. *International journal of clinical pharmacology, therapy, and toxicology* 23, 447-51 (1985).
18	CYP3A4/5	CI	Aprepitant, 125 mg SD day 5	Ketoconazole, 400 mg QD for 10 days	0.97	0.89	CDER. Clinical Pharmacology and Biopharmaceutics Reviews Application. *Number 21-549*, (2003).

19	**CYP3A4/5**	**CI**	**Atazanavir, 400 mg QD for 13 days**	**Ketoconazole, 200 mg QD days 7 – 13**	**1.21**	**1.12**	**O'Mara, E., Cirincione, B., Mummaneni, V., Grasela, T. & Grasela, D. Population pharmacodynamic (PD) assessment of the safety and antiretroviral activity of atazanavir (BMS-232632). *41st Interscience Conference on Antimicrobial Agents and Chemotherapy*, (2001).**
20	**CYP3A4/5**	**MBI**	**Atazanavir, 400 mg QD for 10 days**	**Clarithromycin, 500mg BID days 7 – 10**	**1.03**	**0.86**	**Mummaneni V, R.D., Chabuel D, Gerlades M, O'Mara E. Steady state pharmacokinetic interactionstudy of atazanavir with clarithromycin in healthy subjects. 42nd Interscience Confereence on Antimicrobial Agents and Chemotherapy Sep 27-30, San Diego, (2002).**
21	**CYP3A4/5**	**MBI**	**Clarithromycin, 500 mg BID days 7 – 10**	**Atazanavir, 400 mg QD for 10 day**	**0.91**	**0.82**	**Mummaneni V, R.D., Chabuel D, Gerlades M, O'Mara E. Steady state pharmacokinetic interactionstudy of atazanavir with clarithromycin in healthy subjects. 42nd Interscience Confereence on Antimicrobial Agents and Chemotherapy Sep 27-30, San Diego, (2002).**
22	**CYP3A4/5**	**MBI**	**Clarithromycin, 500 mg BID for 4 days (8 doses)**	**Ritonavir, 200 mg TID for 4 days (12 doses)**	**1.14**	**1.59**	**Ouellet, D. *et al.* Pharmacokinetic interaction between ritonavir and clarithromycin. *Clinical Pharmacology & Therapeutics* 64, 355-62 (1998).**
23	**CYP3A4/5**	**CI**	**Dexamethasone, 4.5 mg Day 4 at 9am**	**Itraconazole, Capsule 200 mg QD at 7.30 am on day 1-3 and 8am on day 4**	**1.01**	**0.96**	**Varis, T., Kivistö, K.T., Backman, J.T. & Neuvonen, P.J. The cytochrome P450 3A4 inhibitor itraconazole markedly increases the plasma concentrations of dexamethasone and enhances its adrenal-suppressant effect. *Clinical Pharmacology & Therapeutics* 68, 487-94 (2000).**
24	**CYP3A4/5**	**MBI**	**Dexamethasone, 20 mg QD Day 1, 8 mg Day 2-5**	**Aprepitant, 125 mg QD day 1 (30 min before Dex), 80 mg QD day 2-5**	**0.76**	**0.61**	**McCrea, J.B. *et al.* Effects of the neurokinin1 receptor antagonist aprepitant on the pharmacokinetics of dexamethasone and methylprednisolone. *Clinical Pharmacology & Therapeutics* 74, 17-24 (2003).**
25	**CYP3A4/5**	**MBI**	**Dexamethasone, 20 mg QD Day 1, 8 mg Day 2-5**	**Aprepitant, 375 mg Day 1, 250 mg QD Day 2-5**	**0.77**	**0.51**	**NDA (2002, L.U. Clinical Pharmacology and Biopharmaceutics Review. https://wwwaccessdatafdagov/drugsatfda_docs/nda/2003/21-549_Emend_biopharmr.pdf, (Access 2002).**

26	CYP3A4/5	MBI	Dexamethasone, 12 mg QD Day 1, 4 mg Day 2-5	Aprepitant, 125 mg QD day 1, 80 mg QD Day 2-5	1.48	1.34	NDA (2002, L.U. Clinical Pharmacology and Biopharmaceutics Review. https://wwwaccessdatafdagov/drugsatfda_docs/nda/2003/21-549_Emend_biopharmr.pdf, (Access 2002).
27	CYP3A4/5	MBI	Dexamethasone, 20 mg QD Day 1, 8 mg Day 2-5	Aprepitant, 40 mg QD Day 1, 25 mg QD Day 2-5	0.95	0.90	NDA (2002, L.U. Clinical Pharmacology and Biopharmaceutics Review. https://wwwaccessdatafdagov/drugsatfda_docs/nda/2003/21-549_Emend_biopharmr.pdf, (Access 2002).
28	CYP3A4/5	CI	Ibrutinib, 120 mg on Day 4 at 10 am	Ketoconazole, 400 mg QD Days 1-6 at 9 am	0.73	1.11	de Jong, J. *et al.* Effect of CYP 3A perpetrators on ibrutinib exposure in healthy participants. *Pharmacology research & perspectives* 3, e00156 (2015).
29	CYP3A4/5	CI	Ibrutinib, 140 mg on Day 3 at 9 am (low fat fed)	Itraconazole, 200 mg BID Day 1 (8 am and 8 pm) and QD Days 2-3 (8 am)	1.16	1.38	Tapaninen, T. *et al.* Itraconazole Increases Ibrutinib Exposure 10-Fold and Reduces Interindividual Variation—A Potentially Beneficial Drug-Drug Interaction. *Clinical and translational science* 13, 345-51 (2020).
30	CYP3A4/5	CI	Midazolam, 7.5 mg SD (1 h after Fluconazole)	Fluconazole, 400 mg SD	0.83	0.81	Ahonen, J., Olkkola, K.T. & Neuvonen, P.J. Effect of route of administration of fluconazole on the interaction between fluconazole and midazolam. *European journal of clinical pharmacology* 51, 415-9 (1997).
31	CYP3A4/5	CI	Midazolam, 7.5 mg SD (day 4)	Itraconazole, 200 mg QD for 4 days (8 doses)	0.83	0.93	Olkkola, K.T., Backman, J.T. & Neuvonen, P.J. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clinical Pharmacology & Therapeutics* 55, 481-5 (1994).
32	CYP3A4/5	MBI	Midazolam, 15 mg SD (day 5)	Clarithromycin, 250 mg BID for 5 days (10 doses)	0.85	1.16	Yeates, R., Laufen, H. & Zimmermann, T. Interaction between midazolam and clarithromycin: comparison with azithromycin. *International journal of clinical pharmacology and therapeutics* 34, 400-5 (1996).
33	CYP3A4/5	MBI	Midazolam, 15 mg SD (day 2), (1 h after 4th Diltiazem dose)	Diltiazem, 60 mg TID for 2 days (5 doses)	0.76	0.54	Backman, J.T., Olkkola, K.T., Aranko, K., Himberg, J.-J. & Neuvonen, P.J. Dose of midazolam should be reduced during diltiazem and verapamil treatments. *British journal of clinical pharmacology* 37, 221-5 (1994).

34	CYP3A4/5	MBI	Midazolam, 15 mg SD (day 2), (1 h after 4th Verapamil dose)	Verapamil, 80 mg TID for 2 days Verapamil, 80 mg TID for 2 days	1.08	1.18	Backman, J.T., Olkkola, K.T., Aranko, K., Himberg, J.-J. & Neuvonen, P.J. Dose of midazolam should be reduced during diltiazem and verapamil treatments. *British journal of clinical pharmacology* 37, 221-5 (1994).
35	CYP3A4/5	CI	Midazolam, 6 mg SD (12 h after 1st Ketoconazole dose)	Ketoconazole, 200 mg BID for 2 days (3 doses)	0.84	0.79	Tsunoda, S.M., Velez, R.L., von Moltke, L.L. & Greenblatt, D.J. Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an in vivo probe: effect of ketoconazole. *Clinical pharmacology & therapeutics* 66, 461-71 (1999).
36	CYP3A4/5	CI	Midazolam, 2 mg IV SD (12 h after 1st Ketoconazole dose)	Ketoconazole, 200 mg BID for 2 days (3 doses)		0.92	Tsunoda, S.M., Velez, R.L., von Moltke, L.L. & Greenblatt, D.J. Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an in vivo probe: effect of ketoconazole. *Clinical pharmacology & therapeutics* 66, 461-71 (1999).
37	CYP3A4/5	CI	Midazolam, 7.5 mg SD (day 4)	Ketoconazole, 400 mg QD for 4 days (4 doses)	0.92	0.79	Olkkola, K.T., Backman, J.T. & Neuvonen, P.J. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clinical Pharmacology & Therapeutics* 55, 481-5 (1994).
38	CYP3A4/5	CI	Midazolam, 2 mg SD (day 5)	Ketoconazole, 400 mg QD for 5 days (5 doses)	0.69	0.87	Stoch, S. *et al.* Effect of different durations of ketoconazole dosing on the single-dose pharmacokinetics of midazolam: shortening the paradigm. *The Journal of Clinical Pharmacology* 49, 398-406 (2009).
39	CYP3A4/5	MBI	Midazolam, 2 mg SD oral coadministered with Aprepitant	Aprepitant, 250 mg SD	1.40	0.96	Report, A.P.A. Aprepitant. *Therapeutic Goods Administration*, (2012).
40	CYP3A4/5	MBI	Midazolam, 2 mg SD oral Aprepitant Day 8	Aprepitant, 250 mg SD Day 1	1.44	1.75	Report, A.P.A. Aprepitant. *Therapeutic Goods Administration*, (2012).
41	CYP3A4/5	MBI	Midazolam, 2 mg SD day 1 (1hr after Aprepitant)	Aprepitant, 125 mg day 1, 80 mg QD days 2-5	0.96	0.64	Majumdar, A.K. *et al.* Effects of aprepitant on cytochrome P450 3A4 activity using midazolam as a probe. *Clinical Pharmacology & Therapeutics* 74, 150-6 (2003).

42	CYP3A4/5	MBI	Midazolam, 2 mg SD day 1 (1hr after Aprepitant) Day 5	Aprepitant, 125 mg day 1, 80 mg QD days 2-5	0.83	0.60	Majumdar, A.K. *et al.* Effects of aprepitant on cytochrome P450 3A4 activity using midazolam as a probe. *Clinical Pharmacology & Therapeutics* 74, 150-6 (2003).
43	CYP3A4/5	MBI	Midazolam, Midazolam 1.96 mg iv SD, 1hr after Aprepitant	Aprepitant, 125 mg SD		0.70	Majumdar, A.K. *et al.* Effect of aprepitant on the pharmacokinetics of intravenous midazolam. *The Journal of Clinical Pharmacology* 47, 744-50 (2007).
44	CYP3A4/5	MBI	Midazolam, Midazolam 2 mg iv SD day 4	Aprepitant, 125 mg day 1, 80 mg QD days 2-3	1.26	0.98	Shadle, C.R. *et al.* Evaluation of potential inductive effects of aprepitant on cytochrome P450 3A4 and 2C9 activity. *The Journal of Clinical Pharmacology* 44, 215-23 (2004).
45	CYP3A4/5	MBI	Midazolam, Midazolam 2 mg iv SD day 8	Aprepitant, 125 mg day 1, 80 mg QD days 2-3	0.93	1.35	Shadle, C.R. *et al.* Evaluation of potential inductive effects of aprepitant on cytochrome P450 3A4 and 2C9 activity. *The Journal of Clinical Pharmacology* 44, 215-23 (2004).
46	CYP3A4/5	CI	Midazolam, 15 mg SD (Day 2) (30min after cimetidine)	Cimetidine, 400 mg BID (3 doses)		1.01	Fee, J., Collier, P., Howard, P. & Dundee, J. Cimetidine and ranitidine increase midazolam bioavailability. *Clinical Pharmacology & Therapeutics* 41, 80-4 (1987).
47	CYP3A4/5	CI	Midazolam, 15 mg SD (2hrs after cimetidine)	Cimetidine, 400 mg SD	0.88	0.91	Salonen, M., Aantaa, E., Aaltonen, L. & Kanto, J. Importance of the interaction of midazolam and cimetidine. *Acta pharmacologica et toxicologica* 58, 91-5 (1986).
48	CYP3A4/5	CI	Midazolam, 15 mg SD (Day 2) (2.5hr after cimetidine)	Cimetidine, 200 mg TID with 400 mg at night on day 1. 200 mg on day 2	0.46	0.54	Elliott, P., Dundee, J., Elwood, R. & Collier, P. The influence of H2 receptor antagonists on the plasma concentrations of midazolam and temazepam. *European journal of anaesthesiology* 1, 245-51 (1984).
49	CYP3A4/5	MBI	Midazolam, 15 mg Day 6	Erythromycin, 500 mg TID 7 days	1.02	1.72	Olkkola, K.T. *et al.* A potentially hazardous interaction between erythromycin and midazolam. *Clinical Pharmacology & Therapeutics* 53, 298-305 (1993).

50	CYP3A4/5	CI	Midazolam, 10 mg SD on day 12 (1h after fluvoxamine)	Fluvoxamine, 50 mg BID days 1-6, 100 mg (73.3 mg free base) BID days 7-12	0.91	0.99	Lam, Y.F., Alfaro, C.L., Ereshefsky, L. & Miller, M. Pharmacokinetic and pharmacodynamic interactions of oral midazolam with ketoconazole, fluoxetine, fluvoxamine, and nefazodone. *The Journal of Clinical Pharmacology* 43, 1274-82 (2003).
51	CYP3A4/5	CI	Midazolam, 7.5 mg SD day 4	Itraconazole, 100 mg QD for 4 days	0.93	0.93	Ahonen, J., Olkkola, K.T. & Neuvonen, P.J. Effect of itraconazole and terbinafine on the pharmacokinetics and pharmacodynamics of midazolam in healthy volunteers. *British journal of clinical pharmacology* 40, 270-2 (1995).
52	CYP3A4/5	CI	Midazolam, 7.5 mg SD day 4	Itraconazole, 200 mg QD for 4 days	0.97	1.39	Backman, J.T., Kivistö, K.T., Olkkola, K.T. & Neuvonen, P.J. The area under the plasma concentration–time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. *European journal of clinical pharmacology* 54, 53-8 (1998).
53	CYP3A4/5	CI	Midazolam, 2 mg SD	Ketoconazole, 400 mg SD	0.75	1.16	Stoch, S. *et al.* Effect of different durations of ketoconazole dosing on the single-dose pharmacokinetics of midazolam: shortening the paradigm. *The Journal of Clinical Pharmacology* 49, 398-406 (2009).
54	CYP3A4/5	CI	Midazolam, 2 mg SD	Ketoconazole, 200 mg SD 2 hrs before MDZ	1.25	1.31	McCrea, J. *et al.* Concurrent administration of the erythromycin breath test (EBT) and oral midazolam as in vivo probes for CYP3A activity. *The Journal of Clinical Pharmacology* 39, 1212-20 (1999).
55	CYP3A4/5	CI	Midazolam, 75 ug SD Day 3	Ketoconazole, 200 mg BID 2 days	0.70	0.80	Eap, C.B. *et al.* Oral administration of a low dose of midazolam (75 µg) as an in vivo probe for CYP3A activity. *European journal of clinical pharmacology* 60, 237-46 (2004).
56	CYP3A4/5	MBI	Midazolam, 3 mg day 4	Ritonavir, 30 mg for 5 days		1.18	Eichbaum, C., Cortese, M., Blank, A., Burhenne, J. & Mikus, G. Concentration effect relationship of CYP3A inhibition by ritonavir in humans. *European journal of clinical pharmacology* 69, 1795-800 (2013).
57	CYP3A4/5	MBI	Midazolam, 3 mg day 4	Ritonavir, 100 mg for 5 days		1.07	Eichbaum, C., Cortese, M., Blank, A., Burhenne, J. & Mikus, G. Concentration effect relationship of CYP3A inhibition by ritonavir in humans. *European journal of clinical pharmacology* 69, 1795-800 (2013).
58	CYP3A4/5	MBI	Midazolam, 3 mg day 4	Ritonavir, 300 mg for 5 days		0.83	Eichbaum, C., Cortese, M., Blank, A., Burhenne, J. & Mikus, G. Concentration effect relationship of CYP3A inhibition by

							ritonavir in humans. *European journal of clinical pharmacology* 69, 1795-800 (2013).
59	CYP3A4/5	MBI	Midazolam, 1 mg IV Dose	Ritonavir, 200 mg for 11 days		0.98	Mathias, A., West, S., Hui, J. & Kearney, B. Dose–response of ritonavir on hepatic CYP3A activity and elvitegravir oral exposure. *Clinical Pharmacology & Therapeutics* 85, 64-70 (2009).
60	CYP3A4/5	MBI	Midazolam, 3 mg SD on Day 2 (8 am)	Ritonavir, 100 mg at 6 pm day 1 and 7.30 am and 6 pm Day 2 (2 doses)	0.85	0.65	Greenblatt, D.J. *et al.* Inhibition of oral midazolam clearance by boosting doses of ritonavir, and by 4, 4-dimethyl-benziso-(2H)-selenazine (ALT-2074), an experimental catalytic mimic of glutathione oxidase. *British journal of clinical pharmacology* 68, 920-7 (2009).
61	CYP3A4/5	MBI	Midazolam, 0.1 mg SD	Ritonavir, 100 mg BID	0.87	0.79	Ieiri, I. *et al.* Mechanisms of pharmacokinetic enhancement between ritonavir and saquinavir; micro/small dosing tests using midazolam (CYP3A4), fexofenadine (P-glycoprotein), and pravastatin (OATP1B1) as probe drugs. *The Journal of Clinical Pharmacology* 53, 654-61 (2013).
62	CYP3A4/5	MBI	Midazolam, 5 mg SD	Ritonavir, 100 mg BID for 14 days	0.85	0.79	Mathias, A. *et al.* Pharmacokinetics and pharmacodynamics of GS-9350: a novel pharmacokinetic enhancer without anti-HIV activity. *Clinical Pharmacology & Therapeutics* 87, 322-9 (2010).
63	CYP3A4/5	MBI	Midazolam, 2 mg on Day 16	Ritonavir, 200 mg TID day 1, 300 mg Day 2-7, 400 mg BID days 8-15		1.81	Kirby, B.J. *et al.* Complex drug interactions of HIV protease inhibitors 2: in vivo induction and in vitro to in vivo correlation of induction of cytochrome P450 1A2, 2B6, and 2C9 by ritonavir or nelfinavir. *Drug metabolism and disposition* 39, 2329-37 (2011).
64	CYP3A4/5	CI	Nifedipine, 10 mg TID for 4 days, 1 dose day 5	Cimetidine, 800 mg QD for 5 days	0.60	0.81	Khan, A., Langley, S., Mullins, F., Dixon, J. & Toon, S. The pharmacokinetics and pharmacodynamics of nifedipine at steady state during concomitant administration of cimetidine or high dose ranitidine. *British journal of clinical pharmacology* 32, 519-22 (1991).
65	CYP3A4/5	CI	Nifedipine, 10 mg QID for 6 days, 1 dose day 7	Cimetidine, 200 mg TID with 400 mg at night for 7 days	0.57	0.76	Kirch, W., Rämsch, K., Janisch, H. & Ohnhaus, E. The influence of two histamine H 2-receptor antagonists, cimetidine and ranitidine, on the plasma levels and clinical effect of nifedipine and metoprolol. In: *Disease, Metabolism and Reproduction in*

							the Toxic Response to Drugs and Other Chemicals **256-9 (Springer, 1984).**
66	**CYP3A4/5**	**CI**	**Nifedipine, 20 mg SD day 2 (1hr after first cimetidine dose)**	**Cimetidine, 200 mg TID with 400 mg at night for 3 days**		**0.93**	**Schellens, J., Van der Wart, J., Brugman, M. & Breimer, D. Influence of enzyme induction and inhibition on the oxidation of nifedipine, sparteine, mephenytoin and antipyrine in humans as assessed by a" cocktail" study design. *Journal of Pharmacology and Experimental Therapeutics* 249, 638-45 (1989).**
67	**CYP3A4/5**	**CI**	**Nifedipine, 20 mg SD day 7**	**Cimetidine, 300 mg QID for 7 days**	**0.85**	**0.86**	**Schwartz, J.B., Upton, R.A., Lin, E.T., Williams, R.L. & Benet, L.Z. Effect of cimetidine or ranitidine administration on nifedipine pharmacokinetics and pharmacodynamics. *Clinical Pharmacology & Therapeutics* 43, 673-80 (1988).**
68	**CYP3A4/5**	**CI**	**Nifedipine, 20 mg SD day 5 (1hr after cimetidine)**	**Cimetidine, 800 mg QD for 5 days**	**0.91**	**0.94**	**Smith, S., Kendall, M., Lobo, J., Beerahee, A., Jack, D. & Wilkins, M. Ranitidine and cimetidine; drug interactions with single dose and steady-state nifedipine administration. *British journal of clinical pharmacology* 23, 311-5 (1987).**
69	**CYP3A4/5**	**MBI**	**Nifedipine, 20 mg SD**	**Diltiazem, 60 mg 1 hour before nifedipine**		**0.83**	**Ohashi, K. *et al.* The influence of pretreatment periods with diltiazem on nifedipine kinetics. *The Journal of Clinical Pharmacology* 33, 222-5 (1993).**
70	**CYP3A4/5**	**MBI**	**Nifedipine, 20 mg SD**	**Diltiazem, 60 mg TDS for 3 days and a final dose 1 hour before nifedipine**		**0.98**	**Ohashi, K. *et al.* The influence of pretreatment periods with diltiazem on nifedipine kinetics. *The Journal of Clinical Pharmacology* 33, 222-5 (1993).**
71	**CYP3A4/5**	**MBI**	**Nifedipine, 20 mg SD**	**Diltiazem, 60 mg TDS for 6 days and a final dose 1 hour before nifedipine**		**0.85**	**Ohashi, K. *et al.* The influence of pretreatment periods with diltiazem on nifedipine kinetics. *The Journal of Clinical Pharmacology* 33, 222-5 (1993).**
72	**CYP3A4/5**	**MBI**	**Nifedipine, 20 mg SD**	**Diltiazem, 30 mg TDS for 3 days and**	**0.70**	**0.82**	**Tateishi, T. *et al.* Dose dependent effect of diltiazem on the pharmacokinetics of nifedipine. *The Journal of Clinical Pharmacology* 29, 994-7 (1989).**

				a final dose 1 hour before nifedipine			
73	CYP3A4/5	MBI	Nifedipine, 20 mg SD	Diltiazem, 90 mg TDS for 3 days and a final dose 1 hour before nifedipine	1.08	1.08	Tateishi, T. *et al.* Dose dependent effect of diltiazem on the pharmacokinetics of nifedipine. *The Journal of Clinical Pharmacology* 29, 994-7 (1989).
74	CYP3A4/5	MBI	Quinidine, 332 mg SD (day 4)	Verapamil, 80 mg TID for 4 days (12 doses)		1.40	Edwards, D.J., Lavoie, R., Beckman, H., Blevins, R. & Rubenfire, M. The effect of coadministration of verapamil on the pharmacokinetics and metabolism of quinidine. *Clinical Pharmacology & Therapeutics* 41, 68-73 (1987).
75	CYP3A4/5	MBI	Quinidine, 332 mg SD (day 4)	Verapamil, 120 mg TID for 4 days (12 doses)		1.57	Edwards, D.J., Lavoie, R., Beckman, H., Blevins, R. & Rubenfire, M. The effect of coadministration of verapamil on the pharmacokinetics and metabolism of quinidine. *Clinical Pharmacology & Therapeutics* 41, 68-73 (1987).
76	CYP3A4/5	MBI	Quinidine, 200 mg SD (day 3), (1.5 h after last Diltiazem dose)	Diltiazem, 90 mg BID for 3 days (5 doses)	1.08	1.13	Laganière, S. *et al.* Pharmacokinetic and pharmacodynamic interactions between diltiazem and quinidine. *Clinical Pharmacology & Therapeutics* 60, 255-64 (1996).
77	CYP3A4/5	CI	Quinidine, 330 mg (free base) SD on day 6	Cimetidine, 300 mg QID for 7 days	0.89	0.73	Hardy, B.G., Zador, I.T., Golden, L., Lalka, D. & Schentag, J.J. Effect of cimetidine on the pharmacokinetics and pharmacodynamics of quinidine. *The American journal of cardiology* 52, 172-5 (1983).
78	CYP3A4/5	CI	Quinidine, 330 mg (free base) SD on day 4	Cimetidine, 300 mg QID for 3 days, morning dose day 4	1.06	0.86	Kolb, K.W., Garnett, W.R., Small, R.E., Vetrovec, G.W., Kline, B.J. & Fox, T. Effect of cimetidine on quinidine clearance. *Therapeutic drug monitoring* 6, 306-12 (1984).
79	CYP3A4/5	CI	Quinidine, 166 mg free base, single dose, oral	Itraconazole, 100 mg, QD, 7 days, oral	1.02	0.89	Damkier, P., Hansen, L.L. & Brøsen, K. Effect of diclofenac, disulfiram, itraconazole, grapefruit juice and erythromycin on the pharmacokinetics of quinidine. *British journal of clinical pharmacology* 48, 829-38 (1999).

80	CYP3A4/5	CI	Quinidine, 83 mg free base, single dose, day 4	Itraconazole, 200 mg QD, oral, 4 days	0.86	1.17	Kaukonen, K.M., Olkkola, K.T. & Neuvonen, P.J. Itraconazole increases plasma concentrations of quinidine. *Clinical pharmacology & therapeutics* 62, 510-7 (1997).
81	CYP3A4/5	MBI	Quinidine, 166 mg free base, single dose, oral	Erythromycin, 250 mg, QID, 7 days, oral	0.92	2.18	Damkier, P., Hansen, L.L. & Brøsen, K. Effect of diclofenac, disulfiram, itraconazole, grapefruit juice and erythromycin on the pharmacokinetics of quinidine. *British journal of clinical pharmacology* 48, 829-38 (1999).
82	CYP3A4/5	CI	Quinidine, 166 mg free base, single dose, oral, day 5	Fluvoxamine, 100 mg, QD, oral, 6 days	0.82	0.87	Damkier, P., Hansen, L. & Brøsen, K. Effect of fluvoxamine on the pharmacokinetics of quinidine. *European journal of clinical pharmacology* 55, 451-6 (1999).
83	CYP3A4/5	CI	Repaglinide, 0.25 mg on day 3 (1 h after Itraconazole)	Itraconazole, 200 mg (1st dose) 100 mg BID for 3 days (7 doses)	1.00	1.13	Niemi, M., Backman, J.T., Neuvonen, M. & Neuvonen, P.J. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide: potentially hazardous interaction between gemfibrozil and repaglinide. *Diabetologia* 46, 347-51 (2003).
84 (86)	CYP3A4/5	CI	Repaglinide, 0.25 mg SD (1h after 2nd Cyclosporine dose)	Cyclosporine, 100 mg BID (2 doses, 8 pm and 8 am)	0.91	0.61	Kajosaari, L.I., Niemi, M., Neuvonen, M., Laitila, J., Neuvonen, P.J. & Backman, J.T. Cyclosporine markedly raises the plasma concentrations of repaglinide. *Clinical Pharmacology & Therapeutics* 78, 388-99 (2005).
85	CYP3A4/5	MBI	Repaglinide, 0.25 mg day 5 given 1 hour after clarithromycin	Clarithromycin, 250 mg oral every 12 h for 4 days	0.83	1.04	Niemi, M., Neuvonen, P.J. & Kivistö, K.T. The cytochrome P4503A4 inhibitor clarithromycin increases the plasma concentrations and effects of repaglinide. *Clinical Pharmacology & Therapeutics* 70, 58-65 (2001).
87	CYP3A4/5	CI	Rifabutin, 300 mg QD for 14 days	Fluconazole, 200 mg QD for 14 days	0.69	0.90	FDA. Mycobutin Capsules (rifabutin capsules, USP). *Pharmacia and Upjohn* LAB-0217-3.0, (2007).
88	CYP3A4/5	CI	Rifabutin, 300 mg QD from day 15	Fluconazole, 200 mg QD for 28 days	0.76	0.92	Trapnell, C.B., Narang, P.K., Li, R. & Lavelle, J.P. Increased plasma rifabutin levels with concomitant fluconazole therapy in HIV-infected patients. *Annals of internal medicine* 124, 573-6 (1996).
89	CYP3A4/5	MBI	Rifabutin, 300 mg QD for 10 days	Ritonavir, 500 mg BD for 10 days	1.13	0.87	FDA. Mycobutin Capsules (rifabutin capsules, USP). *Pharmacia and Upjohn* LAB-0217-3.0, (2007).

90	CYP3A4/5	MBI	Rifabutin, 300 mg QD for 42 days	Clarithromycin, 500 mg BD from day 15	0.86	0.98	Hafner, R. *et al.* Tolerance and pharmacokinetic interactions of rifabutin and clarithromycin in human immunodeficiency virus-infected volunteers. *Antimicrobial agents and chemotherapy* 42, 631-9 (1998).
91	CYP3A4/5	MBI	Sildenafil, 50 mg SD (2 h after Clarithromycin dose)	Clarithromycin, 500 mg SD	0.60	0.74	Hedaya, M.A., El-Afify, D.R. & El-Maghraby, G.M. The effect of ciprofloxacin and clarithromycin on sildenafil oral bioavailability in human volunteers. *Biopharmaceutics & drug disposition* 27, 103-10 (2006).
92	CYP3A4/5	MBI	Sildenafil, 100 mg (day 5), (1 h after 10th Erythromycin dose)	Erythromycin, 500 BID for 5 days (10 doses)	1.02	1.69	Muirhead, G.J., Faulkner, S., Harness, J.A. & Taubel, J. The effects of steady-state erythromycin and azithromycin on the pharmacokinetics of sildenafil in healthy volunteers. *British journal of clinical pharmacology* 53, 37S-43S (2002).
93	CYP3A4/5	MBI	Sildenafil, 100 mg SD (day 6)	Ritonavir, 300 mg BID Day 1, 400 mg BID Day 2, 500 mg BID Days 3-7, (14 doses)	0.72	1.02	Muirhead, G.J., Wulff, M.B., Fielding, A., Kleinermans, D. & Buss, N. Pharmacokinetic interactions between sildenafil and saquinavir/ritonavir. *British journal of clinical pharmacology* 50, 99-107 (2000).
94	CYP3A4/5	CI	Sildenafil, 50 mg SD day 3 (2hrs after cimetidine)	Cimetidine, 800 mg QD for 4 days	0.88	0.91	Wilner, K., Laboy, L. & LeBel, M. The effects of cimetidine and antacid on the pharmacokinetic profile of sildenafil citrate in healthy male volunteers. *British journal of clinical pharmacology* 53, 31S-6S (2002).
95	CYP3A4/5	MBI	Simvastatin, 20 mg SD (day 15), (12 h after last Diltiazem dose)	Diltiazem, 120 mg BID for 14 days (28 doses)	1.25	1.29	Mousa, O., Brater, D.C., Sundblad, K.J. & Hall, S.D. The interaction of diltiazem with simvastatin. *Clinical Pharmacology & Therapeutics* 67, 267-74 (2000).
96	CYP3A4/5	MBI	Simvastatin, 40 mg QD on Day 4	Amiodarone, 40 mg Days 1-4	0.85	0.93	Becquemont, L. *et al.* Amiodarone interacts with simvastatin but not with pravastatin disposition kinetics. *Clinical Pharmacology & Therapeutics* 81, 679-84 (2007).
97	CYP3A4/5	MBI	Simvastatin, 40 mg SD on Day 2	Erythromycin, 500 mg t.i.d. for 2 days	2.03	1.64	Kantola, T., Kivistö, K.T. & Neuvonen, P.J. Erythromycin and verapamil considerably increase serum simvastatin and

							simvastatin acid concentrations. *Clinical Pharmacology & Therapeutics* 64, 177-82 (1998).
98	CYP3A4/5	MBI	Simvastatin, 40 mg SD on Day 2	Verapamil, 80mg t.i.d. for 2 days	2.74	1.97	Kantola, T., Kivistö, K.T. & Neuvonen, P.J. Erythromycin and verapamil considerably increase serum simvastatin and simvastatin acid concentrations. *Clinical Pharmacology & Therapeutics* 64, 177-82 (1998).
99	CYP3A4/5	CI	Simvastatin, 40 mg SD on Day 6. 2hrs before Ketoconazole	Ketoconazole, 400 mg QD for 10 days	0.60	0.82	Chung, E., Nafziger, A.N., Kazierad, D.J. & Bertino Jr, J.S. Comparison of midazolam and simvastatin as cytochrome P450 3A probes. *Clinical Pharmacology & Therapeutics* 79, 350-61 (2006).
100	CYP3A4/5	MBI	Simvastatin, 40 mg QD for 8 days	Clarithromycin, 500 mg bid for 8 days	1.57	1.94	Jacobson, T.A. Comparative pharmacokinetic interaction profiles of pravastatin, simvastatin, and atorvastatin when coadministered with cytochrome P450 inhibitors. *The American journal of cardiology* 94, 1140-6 (2004).
101	CYP3A4/5	MBI	Triazolam, 0.25 mg (day 2), (1h after 5th Diltiazem dose)	Diltiazem, 60 mg TID for 2 days (5 doses)	0.78	0.72	Varhe, A., Olkkola, K.T. & Neuvonen, P.J. Diltiazem enhances the effects of triazolam by inhibiting its metabolism. *Clinical Pharmacology & Therapeutics* 59, 369-75 (1996).
102 (112)	CYP3A4/5	CI	Triazolam, 0.25 mg SD (day 4 @3pm)	Fluconazole, 100 mg QD for 4 days (@2pm)	0.85	0.93	Varhe, A., Olkkola, K.T. & Neuvonen, P.J. Effect of fluconazole dose on the extent of fluconazole-triazolam interaction. *British journal of clinical pharmacology* 42, 465-70 (1996).
103 (113)	CYP3A4/5	CI	Triazolam, 0.25 mg SD on day 4 (1 hour after Fluconazole)	Fluconazole, 50 mg QD for 4 days (4 doses)	1.19	1.01	Varhe, A., Olkkola, K.T. & Neuvonen, P.J. Effect of fluconazole dose on the extent of fluconazole-triazolam interaction. *British journal of clinical pharmacology* 42, 465-70 (1996).
104	CYP3A4/5	CI	Triazolam, 0.25mg SD on day 4 (1 hour after Fluconazole)	Fluconazole, 100 mg QD for 4 days (4 doses)	1.02	0.98	Varhe, A., Olkkola, K.T. & Neuvonen, P.J. Fluconazole, but not terbinafine, enhances the effects of triazolam by inhibiting its metabolism. *British journal of clinical pharmacology* 41, 319 (1996).
105	CYP3A4/5	CI	Triazolam, 0.25 mg SD on day 4 (1 hour after fluconazole)	Fluconazole, 400 mg Day 1, then 200	0.72	0.68	Varhe, A., Olkkola, K.T. & Neuvonen, P.J. Fluconazole, but not terbinafine, enhances the effects of triazolam by inhibiting its metabolism. *British journal of clinical pharmacology* 41, 319 (1996).

				mg QD for 3 days (4 doses)			
106	CYP3A4/5	CI	Triazolam, 0.25 mg SD (day 3), (1 h after Ketoconazole dose)	Ketoconazole, 200 mg BID for 4 days (8 doses)	1.06	1.02	Greenblatt, D.J. *et al.* Ketoconazole inhibition of triazolam and alprazolam clearance: differential kinetic and dynamic consequences. *Clinical Pharmacology & Therapeutics* 64, 237-47 (1998).
107	CYP3A4/5	MBI	Triazolam, 0.125 mg SD (day 2), (1h after 3rd Clarithromycin dose)	Clarithromycin, 500 mg given at 8 am and 4 pm Day 1 and 8 am and 5 pm on Day 2, (4 doses)	0.87	0.79	Greenblatt, D.J. *et al.* Inhibition of triazolam clearance by macrolide antimicrobial agents: in vitro correlates and dynamic consequences. *Clinical Pharmacology & Therapeutics* 64, 278-85 (1998).
108	CYP3A4/5	CI	Triazolam, 0.5 mg SD (Day 2)	Cimetidine, 300 mg QID for 2 days	0.85	0.92	Friedman, H., Greenblatt, D.J., Burstein, E.S., Scavone, J.M., Harmatz, J.S. & Shader, R.I. Triazolam kinetics: interaction with cimetidine, propranolol, and the combination. *The Journal of Clinical Pharmacology* 28, 228-33 (1988).
109	CYP3A4/5	CI	Triazolam, 0.5 mg QD at night for 7 days	Cimetidine, 200 mg TID with, 400 mg at night for 9 days	0.81	0.59	Pourbaix, S., Desager, J.-P., Hulhoven, R., Smith, R. & Harvengt, C. Pharmacokinetic consequences of long term coadministration of cimetidine and triazolobenzodiazepines, alprazolam and triazolam, in healthy subjects. *International journal of clinical pharmacology, therapy, and toxicology* 23, 447-51 (1985).
110	CYP3A4/5	CI	Triazolam, 0.5 mg SD (1 hr after 3rd cimetidine dose)	Cimetidine, 300 mg QID (4 doses)	0.86	0.77	Cox, S.R., Kroboth, P.D., Anderson, P.H. & Smith, R.B. Mechanism for the interaction between triazolam and cimetidine. *Biopharmaceutics & drug disposition* 7, 567-75 (1986).
111	CYP3A4/5	MBI	Triazolam, 0.125 mg Day 2 at 3 pm	Erythromycin, 500 mg BID 2 days	0.94	1.07	Greenblatt, D.J. *et al.* Inhibition of triazolam clearance by macrolide antimicrobial agents: in vitro correlates and dynamic consequences. *Clinical Pharmacology & Therapeutics* 64, 278-85 (1998).

114	CYP3A4/5	CI	Zolpidem, 10 mg (8.04mg free base) SD day 4	Itraconazole (Fed capsule), 200 mg QD for 4 days	1.07	1.32	Luurila, H., Kivistö, K.T. & Neuvonen, P.J. Effect of itraconazole on the pharmacokinetics and pharmacodynamics of zolpidem. *European journal of clinical pharmacology* 54, 163-6 (1998).
115	CYP2C9	CI	S-Warfarin, 0.375 mg/kg SD day 7 (1hr before Fluconazole)	Fluconazole, 400 mg QD for 14 days	-	0.94	Black, D.J. *et al.* Warfarin-fluconazole. II. A metabolically based drug interaction: in vivo studies. *Drug metabolism and disposition* 24, 422-8 (1996).
116	CYP2C9	CI	S-Warfarin, 8.75 mg SD day 14	Fluconazole, 400 mg QD for 20 days	0.96	1.38	Bavisotto, L.M., Ellis, D.J., Milner, P.G., Combs, D.L., Irwin, I. & Canafax, D.M. Tecarfarin, a novel vitamin K reductase antagonist, is not affected by CYP2C9 and CYP3A4 inhibition following concomitant administration of fluconazole in healthy participants. *The Journal of Clinical Pharmacology* 51, 561-74 (2011).
117	CYP2C9	CI	S-Warfarin, 8.75 mg SD day 14	Fluconazole, 400 mg QD for 20 days	0.96	1.38	Bavisotto, L.M., Ellis, D.J., Milner, P.G., Combs, D.L., Irwin, I. & Canafax, D.M. Tecarfarin, a novel vitamin K reductase antagonist, is not affected by CYP2C9 and CYP3A4 inhibition following concomitant administration of fluconazole in healthy participants. *The Journal of Clinical Pharmacology* 51, 561-74 (2011).
118	CYP2C9	CI	Tolbutamide, 500 mg SD, (2hrs after Fluconazole)	Fluconazole, 150 mg SD	0.89	0.69	Lazar, J.D. & Wilner, K.D. Drug interactions with fluconazole. *Reviews of infectious diseases* 12, S327-S33 (1990).
119	CYP2C9	CI	Tolbutamide, 500 mg SD Day 8 (2hrs after fluconazole)	Fluconazole, 150 mg QD, day 1; 100 mg QD days 2 - 8	0.87	0.72	Lazar, J.D. & Wilner, K.D. Drug interactions with fluconazole. *Reviews of infectious diseases* 12, S327-S33 (1990).
120	CYP2C9	CI	Phenytoin, 250 mg SD day 5 (8AM)	Fluconazole, 400 mg QD, 6 days (8AM)	-	1.05	Touchette, M., Chandrasekar, P., Milad, M. & Edwards, D. Contrasting effects of fluconazole and ketoconazole on phenytoin and testosterone disposition in man. *British journal of clinical pharmacology* 34, 75-8 (1992).

121	CYP2C9	CI	S-Warfarin, 0.375 mg/kg SD day 7 (2h before Fluconazole)	Fluconazole, 100 mg QD for 18 days	-	1.12	Neal, J.M., Kunze, K.L., Levy, R.H., O'Reilly, R.A. & Trager, W.F. Kiiv, an in vivo parameter for predicting the magnitude of a drug interaction arising from competitive enzyme inhibition. *Drug metabolism and disposition* 31, 1043-8 (2003).
122	CYP2C9	CI	S-Warfarin, 0.375 mg/kg SD day 7 (2h before fluconazole)	Fluconazole, 200 mg QD for 18 days	-	1.07	Neal, J.M., Kunze, K.L., Levy, R.H., O'Reilly, R.A. & Trager, W.F. Kiiv, an in vivo parameter for predicting the magnitude of a drug interaction arising from competitive enzyme inhibition. *Drug metabolism and disposition* 31, 1043-8 (2003).
123	CYP2C9	CI	S-Warfarin, 0.375 mg/kg SD day 7 (2h before fluconazole)	Fluconazole, 300 mg QD for 18 days	-	1.23	Neal, J.M., Kunze, K.L., Levy, R.H., O'Reilly, R.A. & Trager, W.F. Kiiv, an in vivo parameter for predicting the magnitude of a drug interaction arising from competitive enzyme inhibition. *Drug metabolism and disposition* 31, 1043-8 (2003).
124	CYP2C9	CI	Celecoxib, 200 mg SD day 7	Fluconazole, 200 mg QD for 7 days	0.84	0.96	FDA. NDA #20998 Clinical pharmacology/biopharmaceutics review section Celecoxib. (1998).
125	CYP2C9	CI	Tolbutamide, 500 mg SD on day 5 (8.00 am)	Fluvoxamine, 75 mg (54.98 mg free base) QD for 5 days (dosed at 8.00 pm)	-	1.11	Madsen, H., Enggaard, T.P., Hansen, L.L., Klitgaard, N.A. & Brøsen, K. Fluvoxamine inhibits the CYP2C9 catalyzed biotransformation of tolbutamide. *Clinical Pharmacology & Therapeutics* 69, 41-7 (2001).
126	CYP2C9	CI	Tolbutamide, 500 mg SD on day 5 (8.00 am)	Fluvoxamine, 150 mg (109.95 mg free base) QD for 5 days (dosed at 8.00 pm)	-	0.88	Madsen, H., Enggaard, T.P., Hansen, L.L., Klitgaard, N.A. & Brøsen, K. Fluvoxamine inhibits the CYP2C9 catalyzed biotransformation of tolbutamide. *Clinical Pharmacology & Therapeutics* 69, 41-7 (2001).
127	CYP2C9	CI	Phenytoin, 100 mg IV SD day 7	Sulphaphenazole, 200 mg QD for 7 days	-	1.25	Hansen, J.M. *et al.* The effect of different sulfonamides on phenytoin metabolism in man. *Acta Medica Scandinavica* 205, 106-10 (1979).
128	CYP2C9	CI	Flurbiprofen, 100 mg SD (0.5 hrs after last dose Fluconazole)	Fluconazole, 200 mg 2 doses, 12 hours apart	0.88	0.94	Greenblatt, D.J., von Moltke, L.L., Perloff, E.S., Luo, Y., Harmatz, J.S. & Zinny, M.A. Interaction of flurbiprofen with cranberry juice, grape juice, tea, and fluconazole: in vitro and clinical studies. *Clinical Pharmacology & Therapeutics* 79, 125-33 (2006).

129	CYP2C9	CI	Flurbiprofen, 100 mg SD (0.5 hrs after last dose Fluconazole)	Fluconazole, 200 mg 2 doses, 14.5 hours apart	0.95	1.06	Hanley, M.J. *et al.* Effect of blueberry juice on clearance of buspirone and flurbiprofen in human volunteers. *British journal of clinical pharmacology* 75, 1041-52 (2013).
130	CYP2C9	CI	Flurbiprofen, 100 mg SD (0.5 hrs after last dose Fluconazole)	Fluconazole, 200 mg 2 doses, 14.5 hours apart	0.74	0.85	Hanley, M.J., Masse, G., Harmatz, J.S., Court, M.H. & Greenblatt, D.J. Pomegranate juice and pomegranate extract do not impair oral clearance of flurbiprofen in human volunteers: divergence from in vitro results. *Clinical Pharmacology & Therapeutics* 92, 651-7 (2012).
131	CYP2C9	MBI	S-Warfarin, 0.375 mg/kg day 3	Amiodarone, 300 mg QD duration of study	-	1.14	Heimark, L.D. *et al.* The mechanism of the interaction between amiodarone and warfarin in humans. *Clinical Pharmacology & Therapeutics* 51, 398-407 (1992).
132	CYP2C9	MBI	S-Warfarin, 0.75 mg/kg day 4	Amiodarone, 200 mg QD for duration of study	-	1.02	O'Reilly, R.A., Trager, W.F., Rettie, A.E. & Goulart, D.A. Interaction of amiodarone with racemic warfarin and its separated enantiomorphs in humans. *Clinical Pharmacology & Therapeutics* 42, 290-4 (1987).
133	CYP2C9	MBI	Phenytoin, 5 mg/kg IV infusion (30 min) (At SS of Amiodarone)	Amiodarone, 200 mg QD for duration of study	-	1.00	Nolan Jr, P.E., Marcus, F.I., Hoyer, G.L., Bliss, M. & Gear, K. Pharmacokinetic interaction between intravenous phenytoin and amiodarone in healthy volunteers. *Clinical Pharmacology & Therapeutics* 46, 43-9 (1989).
134	CYP2C9	MBI	Phenytoin, 2 to 4 mg/kg QD for 14 days (start at SS of Amiodarone)	Amiodarone, 200 mg QD for duration of study	0.92	1.03	Nolan Jr, P.E., Marcus, F.I., Hoyer, G.L., Bliss, M. & Gear, K. Pharmacokinetic interaction between intravenous phenytoin and amiodarone in healthy volunteers. *Clinical Pharmacology & Therapeutics* 46, 43-9 (1989).
XX01	CYP2C9	CI	500 mg IV 30 sec bolus day 4 @10am	500 mg BID for 4 days @ 9am and 9pm, 7 doses	-	1.22	Back, D.J., Tjia, J., Mönig, H., Ohnhaus, E.E., Park B.K. Selective inhibition of drug oxidation after simultaneous administration of two probe drugs, antipyrine and tolbutamide. Eur J Clin Pharmacol. 1988;34(2):157-63. doi: 10.1007/BF00614553.

135	**CYP2D6**	**CI**	**Atomoxetine, 25 mg Day 6**	**Fluvoxamine, 50 mg (36.7 mg free base) QD days 1-3, 100 mg (73.3 mg free base) QD days 4-6**	**1.02**	**1.10**	**Todor, I. *et al.* Evaluation of the potential pharmacokinetic interaction between atomoxetine and fluvoxamine in healthy volunteers. *Pharmacology* 99, 84-8 (2017).**
136	**CYP2D6**	**MBI**	**Atomoxetine, 25 mg Day 6**	**Paroxetine, 20 mg BID Days 1-2, 20 mg QD days 3-6**	**0.95**	**0.97**	**Todor, I. *et al.* The influence of paroxetine on the pharmacokinetics of atomoxetine and its main metabolite. *Clujul Medical* 88, 513 (2015).**
137	**CYP2D6**	**MBI**	**Atomoxetine, 20 mg BID from day 12 (11 doses)**	**Paroxetine, 20 mg QD for 17 days**	**0.92**	**0.81**	**Belle, D.J., Ernest, C.S., Sauer, J.M., Smith, B.P., Thomasson, H.R. & Witcher, J.W. Effect of potent CYP2D6 inhibition by paroxetine on atomoxetine pharmacokinetics. *The Journal of Clinical Pharmacology* 42, 1219-27 (2002).**
138	**CYP2D6**	**MBI**	**Desipramine HCL, 50 mg on Day 6**	**Paroxetine, 20 mg QD for 9 days**	**1.02**	**0.99**	**Nichols, A.I. *et al.* The effects of desvenlafaxine and paroxetine on the pharmacokinetics of the cytochrome P450 2D6 substrate desipramine in healthy adults. *The Journal of Clinical Pharmacology* 49, 219-28 (2009).**
139	**CYP2D6**	**MBI**	**Desipramine HCL, 100 mg on Day 11 (8 am)**	**Paroxetine, 20 mg QD for 20 days (8 am)**	**0.84**	**0.99**	**Brøsen, K., Hansen, J., Nielsen, K.K., Sindrup, S. & Gram, L. Inhibition by paroxetine of desipramine metabolism in extensive but not in poor metabolizers of sparteine. *European journal of clinical pharmacology* 44, 349-55 (1993).**
140	**CYP2D6**	**MBI**	**Desipramine HCL, 100 mg on Day 11 (8 am)**	**Paroxetine, 20 mg QD for 20 days (8 am)**	**1.10**	**1.09**	**Brøsen, K., Hansen, J., Nielsen, K.K., Sindrup, S. & Gram, L. Inhibition by paroxetine of desipramine metabolism in extensive but not in poor metabolizers of sparteine. *European journal of clinical pharmacology* 44, 349-55 (1993).**
141	**CYP2D6**	**MBI**	**Desipramine HCL, 50 mg QD for 20 days**	**Paroxetine 20 mg QD days 8-17, 30 mg QD days 18-20**	**1.13**	**1.16**	**Alderman, J. *et al.* Desipramine pharmacokinetics when coadministered with paroxetine or sertraline in extensive metabolizers. *Journal of clinical psychopharmacology* 17, 284-91 (1997).**
142	**CYP2D6**	**CI**	**Desipramine HCL, 50 mg (3 hours after Fluoxetine)**	**Fluoxetine, 60 mg Day 1**	**0.96**	**1.06**	**Bergstrom, R.F., Peyton, A.L. & Lemberger, L. Quantification and mechanism of the fluoxetine and tricyclic antidepressant interaction. *Clinical Pharmacology & Therapeutics* 51, 239-48 (1992).**

143	CYP2D6	CI	Desipramine HCL, 50 mg on Day 8 (3 hours after Fluoxetine)	Fluoxetine, 60 mg QD for 8 Days	0.82	0.71	Bergstrom, R.F., Peyton, A.L. & Lemberger, L. Quantification and mechanism of the fluoxetine and tricyclic antidepressant interaction. *Clinical Pharmacology & Therapeutics* 51, 239-48 (1992).
144	CYP2D6	CI	Desipramine HCL, 50 mg QD for 28 Days	Fluoxetine, 20 mg QD Days 8-28	1.00	0.96	Preskorn, S.H., Alderman, J., Chung, M., Harrison, W., Messig, M. & Harris, S. Pharmacokinetics of desipramine coadministered with sertraline or fluoxetine. *Journal of clinical psychopharmacology*, (1994).
145	CYP2D6	CI	Desipramine HCL, 50 mg on Day 5	Cinacalcet, 90 mg QD for 7 days	1.14	0.97	Harris, R.Z., Salfi, M., Posvar, E., Hoelscher, D. & Padhi, D. Pharmacokinetics of desipramine HCl when administered with cinacalcet HCl. *European journal of clinical pharmacology* 63, 159-63 (2007).
146	CYP2D6	CI	Desipramine HCL, 50 mg on Day 15	Bupropion, 15 mg QD days 1-3, 150 mg BID days 4-14, 150 mg SD Day 15	1.13	0.95	Reese, M.J., Wurm, R.M., Muir, K.T., Generaux, G.T., John-Williams, L.S. & Mcconn, D.J. An in vitro mechanistic study to elucidate the desipramine/bupropion clinical drug-drug interaction. *Drug metabolism and disposition* 36, 1198-201 (2008).
147	CYP2D6	CI	Desipramine HCL, 50 mg on Day 17	Ritonavir, 100 mg BID for 20 days	1.08	1.04	Aarnoutse, R.E. *et al.* Effect of low-dose ritonavir (100 mg twice daily) on the activity of cytochrome P450 2D6 in healthy volunteers. *Clinical Pharmacology & Therapeutics* 78, 664-74 (2005).
148	CYP2D6	CI	Dextromethorphan, 30 mg SD	Quinidine, 50 mg (1 hour before dextromethorphan)	1.00	0.80	Manap, A. The antitussive effect of dextromethorphan in relation to CYP2D6 activity. *British journal of clinical pharmacology* 48, 382-7 (1999).
149	CYP2D6	CI	Dextromethorphan, 30 mg SD	Quinidine, 50 mg (1 hour before dextromethorphan)	0.69	0.88	Capon, D.A., Bochner, F., Kerry, N., Mikus, G., Danz, C. & Somogyi, A.A. The influence of CYP2D6 polymorphism and quinidine on the disposition and antitussive effect of dextromethorphan in humans. *Clinical Pharmacology & Therapeutics* 60, 295-307 (1996).

150	CYP2D6	CI	Dextromethorphan, 30 mg on Day 12	Fluoxetine, 20 mg SD Day 1, then 60mg QD days 2-14 (1 hour before Dextromethorphan)	-	0.51	Sager, J.E., Lutz, J.D., Foti, R.S., Davis, C., Kunze, K.L. & Isoherranen, N. Fluoxetine-and Norfluoxetine-Mediated Complex Drug–Drug Interactions: In Vitro to In Vivo Correlation of Effects on CYP2D6, CYP2C19, and CYP3A4. *Clinical Pharmacology & Therapeutics* 95, 653-62 (2014).
151	CYP2D6	MBI	Metoprolol Tartrate, 100 mg BID on Day 7	Paroxetine, 20 mg QD for 6 days	1.07	0.93	Parker, R.B. & Soberman, J.E. Effects of paroxetine on the pharmacokinetics and pharmacodynamics of immediate-release and extended-release metoprolol. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* 31, 630-41 (2011).
152	CYP2D6	MBI	Metoprolol Tartrate, 100 mg on Day 7	Paroxetine, 10 mg every 12 hours for 7 days (13 doses)	0.92	0.79	Hemeryck, A., Lefebvre, R.A., De Vriendt, C. & Belpaire, F.M. Paroxetine affects metoprolol pharmacokinetics and pharmacodynamics in healthy volunteers. *Clinical Pharmacology & Therapeutics* 67, 283-91 (2000).
153	CYP2D6	CI	Metoprolol Tartrate, 200 mg on Day 4 (2 hours after Quinidine)	Quinidine, 100 mg QD for 5 days	-	0.94	Johnson, J.A. & Burlew, B.S. Metoprolol metabolism via cytochrome P4502D6 in ethnic populations. *Drug metabolism and disposition* 24, 350-5 (1996).
154	CYP2D6	CI	Metoprolol Tartrate, 20mg IV infusion	Quinidine, 50 mg SD (12 hours before metoprolol)	-	0.61	Leemann, T., Devi, K. & Dayer, P. Similar effect of oxidation deficiency (debrisoquine polymorphism) and quinidine on the apparent volume of distribution of (±)-metoprolol. *European journal of clinical pharmacology* 45, 65-71 (1993).
155	CYP2D6	MBI	Nebivolol, 5 mg on Day 8	Paroxetine, 20 mg BID Days 1-2, 20mg QD days 3-7	1.56	1.05	Briciu, C. *et al.* A pharmacokinetic drug interaction study between nebivolol and paroxetine in healthy volunteers. *Journal of clinical pharmacy and therapeutics* 39, 535-40 (2014).
156	CYP2D6	CI	Nebivolol, 5 mg on Day 8	Bupropion, 150 mg BID days 1-3, 300 mg days 4-7	1.96	1.23	Gheldiu, A.-M. *et al.* Assessment of a potential pharmacokinetic interaction between nebivolol and bupropion in healthy volunteers. *Pharmacology* 98, 190-8 (2016).
157	CYP2D6	CI	Nebivolol, 5 mg on Day 8	Fluvoxamine, 50 mg QD days 1-3, 100 mg QD days 3-7	1.27	1.03	Gheldiu, A.-M. *et al.* Investigation of a potential pharmacokinetic interaction between nebivolol and fluvoxamine in healthy volunteers. *Journal of Pharmacy & Pharmaceutical Sciences* 20, 68-80 (2017).

158	CYP2D6	CI	Nebivolol, 10 mg SD on day 21	Fluoxetine, 20 mg QD days 1-21	1.41	0.84	Lindamood, C., Ortiz, S., Shaw, A., Rackley, R. & Gorski, J.C. Effects of commonly administered agents and genetics on nebivolol pharmacokinetics: drug-drug interaction studies. *The Journal of Clinical Pharmacology* 51, 575-85 (2011).
159	CYP2D6	CI	Tolterodine, 2.36 mg BID days 22-24 (5 doses)	Fluoxetine, 20 mg QD for 24 days	1.05	1.37	Brynne, N., Svanström, C., Åberg-Wistedt, A., Hallen, B. & Bertilsson, L. Fluoxetine inhibits the metabolism of tolterodine—pharmacokinetic implications and proposed clinical relevance. *British journal of clinical pharmacology* 48, 553 (1999).
160	CYP2D6	CI	Tolterodine, 2.36 mg BID days 22-24 (5 doses)	Fluoxetine, 20 mg QD for 24 days	0.77	0.90	Brynne, N., Svanström, C., Åberg-Wistedt, A., Hallen, B. & Bertilsson, L. Fluoxetine inhibits the metabolism of tolterodine—pharmacokinetic implications and proposed clinical relevance. *British journal of clinical pharmacology* 48, 553 (1999).
161	CYP2C8	CI	Repaglinide, Oral 0.25 mg on day 3 at 9 AM	Trimethoprim, Oral 160 mg every 12 hours for 3 days at 8 AM and 8 PM	0.89	0.86	Niemi, M., Kajosaari, L.I., Neuvonen, M., Backman, J.T. & Neuvonen, P.J. The CYP2C8 inhibitor trimethoprim increases the plasma concentrations of repaglinide in healthy subjects. *British journal of clinical pharmacology* 57, 441-7 (2004).
162	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 3 (1hr after Gemfibrozil dosing)	Gemfibrozil, Oral 600 mg BID for 3 days (5 doses)	1.13	0.98	Backman, J.T. *et al.* CYP2C8 activity recovers within 96 hours after gemfibrozil dosing: estimation of CYP2C8 half-life using repaglinide as an in vivo probe. *Drug metabolism and disposition* 37, 2359-66 (2009).
163	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 3 (concomitant after Gemfibrozil dosing)	Gemfibrozil, Oral 600 mg BID for 3 days	1.11	0.92	Tornio, A. *et al.* The effect of gemfibrozil on repaglinide pharmacokinetics persists for at least 12 h after the dose: evidence for mechanism-based inhibition of CYP2C8 in vivo. *Clinical Pharmacology & Therapeutics* 84, 403-11 (2008).
164	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 3 (1hr after Gemfibrozil dosing)	Gemfibrozil, Oral 600 mg BID for 3 days (5 doses)	1.17	0.94	Kalliokoski, A., Backman, J.T., Kurkinen, K., Neuvonen, P.J. & Niemi, M. Effects of gemfibrozil and atorvastatin on the pharmacokinetics of repaglinide in relation to SLCO1B1 polymorphism. *Clinical Pharmacology & Therapeutics* 84, 488-96 (2008).

165	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 3 (1hr after Gemfibrozil dosing)	Gemfibrozil, Oral 600 mg BID for 3 days	1.24	1.02	Kalliokoski, A., Backman, J.T., Kurkinen, K., Neuvonen, P.J. & Niemi, M. Effects of gemfibrozil and atorvastatin on the pharmacokinetics of repaglinide in relation to SLCO1B1 polymorphism. *Clinical Pharmacology & Therapeutics* 84, 488-96 (2008).
166	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 3 (1hr after Gemfibrozil dosing)	Gemfibrozil, Oral 600 mg BID for 3 days	1.17	0.91	Kalliokoski, A., Backman, J.T., Kurkinen, K., Neuvonen, P.J. & Niemi, M. Effects of gemfibrozil and atorvastatin on the pharmacokinetics of repaglinide in relation to SLCO1B1 polymorphism. *Clinical Pharmacology & Therapeutics* 84, 488-96 (2008).
167	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 5 (1hr after Gemfibrozil dosing)	Gemfibrozil, Oral 600 mg BID for 5 days (9 doses)	1.45	0.92	Honkalammi, J., Niemi, M., Neuvonen, P. & Backman, J. Gemfibrozil is a strong inactivator of CYP2C8 in very small multiple doses. *Clinical Pharmacology & Therapeutics* 91, 846-55 (2012).
169	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 3 (1 hr after Gemfibrozil)	Gemfibrozil, Oral 600 mg BID for 3 days (5 doses)	1.23	1.08	Niemi, M., Backman, J.T., Neuvonen, M. & Neuvonen, P.J. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide: potentially hazardous interaction between gemfibrozil and repaglinide. *Diabetologia* 46, 347-51 (2003).
170	CYP2C8	CI	Rosiglitazone, Oral 4 mg on day 3 at 9 AM	Trimethoprim, Oral 160 mg every 12 hours for 4 days (at 8 AM and 8 PM on day 1, 2, 1834; at 8 AM and 9 PM on day 3)	0.95	1.02	Niemi, M., Backman, J.T. & Neuvonen, P.J. Effects of trimethoprim and rifampin on the pharmacokinetics of the cytochrome P450 2C8 substrate rosiglitazone. *Clinical Pharmacology & Therapeutics* 76, 239-49 (2004).
171	CYP2C8	CI	Rosiglitazone, Oral 8 mg on day 4 at 8 AM	Trimethoprim, Oral 200 mg every 12 hours for 5 days (at 7:30 AM and 7:30 PM), (9 doses)	1.25	1.13	Hruska, M., Amico, J., Langaee, T., Ferrell, R., Fitzgerald, S. & Frye, R. The effect of trimethoprim on CYP2C8 mediated rosiglitazone metabolism in human liver microsomes and healthy subjects. *British journal of clinical pharmacology* 59, 70-9 (2005).

172	CYP2C8	MBI	Rosiglitazone, Oral 4 mg at 9 AM on day 3	Gemfibrozil, Oral 600 mg every 12 hours at 8 AM and 8 PM for 7 doses	0.96	1.04	Niemi, M., Backman,J. T. , Granfors, M., Laitila,J. , Neuvonen, M., Neuvonen, P. J. Gemfibrozil considerably increases the plasma concentrations of rosiglitazone. Diabetologia (2003) 46:1319–1323
New 001	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 5 (1hr after Gemfibrozil dosing)	Gemfibrozil, Oral 100 mg BID for 5 days (9 doses)	0.96	0.45	Honkalammi, J., Niemi, M., Neuvonen, P. & Backman, J. Gemfibrozil is a strong inactivator of CYP2C8 in very small multiple doses. *Clinical Pharmacology & Therapeutics* 91, 846-55 (2012).
New 002	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 5 (1hr after Gemfibrozil dosing)	Gemfibrozil, Oral 30 mg BID for 5 days (9 doses)	0.90	0.44	Honkalammi, J., Niemi, M., Neuvonen, P. & Backman, J. Gemfibrozil is a strong inactivator of CYP2C8 in very small multiple doses. *Clinical Pharmacology & Therapeutics* 91, 846-55 (2012).
New 003	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 3 (3 h after Gemfibrozil dosing)	Gemfibrozil, Oral 600 mg BID for 3 days (5 doses)	1.48	0.91	Tornio, A. *et al.* The effect of gemfibrozil on repaglinide pharmacokinetics persists for at least 12 h after the dose: evidence for mechanism-based inhibition of CYP2C8 in vivo. *Clinical Pharmacology & Therapeutics* 84, 403-11 (2008).
New 004	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 3 (6 h after Gemfibrozil dosing)	Gemfibrozil, Oral 600 mg BID for 3 days (5 doses)	0.96	0.56	Tornio, A. *et al.* The effect of gemfibrozil on repaglinide pharmacokinetics persists for at least 12 h after the dose: evidence for mechanism-based inhibition of CYP2C8 in vivo. *Clinical Pharmacology & Therapeutics* 84, 403-11 (2008).
New 005	CYP2C8	MBI	Repaglinide, Oral 0.25 mg on day 3 (12 h after Gemfibrozil dosing)	Gemfibrozil, Oral 600 mg BID for 3 days (5 doses)	0.69	0.39	Tornio, A. *et al.* The effect of gemfibrozil on repaglinide pharmacokinetics persists for at least 12 h after the dose: evidence for mechanism-based inhibition of CYP2C8 in vivo. *Clinical Pharmacology & Therapeutics* 84, 403-11 (2008).
173	CYP1A2	CI	Caffeine, 3 mg/kg SD (Day 4 @9:00 AM)	Ciprofloxacin, 100 mg BID for 4 days (7 doses)	1.05	1.08	Harder et al., 1988, 35:651-656. Eur J Clin Pharmacol. 4-Quinolones inhibit biotransformation of caffeine.

174	CYP1A2	CI	Caffeine, 3 mg/kg SD (Day 4)	Ciprofloxacin, 250 mg BID for 4 days (7 doses)	1.08	0.95	Harder et al., 1988, 35:651-656. Eur J Clin Pharmacol. 4-Quinolones inhibit biotransformation of caffeine.
175	CYP1A2	CI	Caffeine, 3 mg/kg SD (Day 4)	Ciprofloxacin, 500 mg BID for 3 days (6 doses) + 500 mg morning dose	1.05	1.10	Harder et al., 1988, 35:651-656. Eur J Clin Pharmacol. 4-Quinolones inhibit biotransformation of caffeine.
176	CYP1A2	CI	Caffeine, 100 mg TID for 3 days (7 doses)	Ciprofloxacin, 500 mg BID for 3 days (5 doses)	1.07	1.07	Kim, M.-K., Nightingale, C.H. & Nicolau, D.P. Influence of sex on the pharmacokinetic interaction of fleroxacin and ciprofloxacin with caffeine. *Clinical pharmacokinetics* 42, 985-96 (2003).
177	CYP1A2	CI	Caffeine, 100 mg TID for 3 days (7 doses)	Ciprofloxacin, 500 mg BID for 3 days (5 doses)	0.91	0.98	Kim, M.-K., Nightingale, C.H. & Nicolau, D.P. Influence of sex on the pharmacokinetic interaction of fleroxacin and ciprofloxacin with caffeine. *Clinical pharmacokinetics* 42, 985-96 (2003).
178	CYP1A2	CI	Caffeine, 183 mg QD for 5 days	Ciprofloxacin, 750 mg BID for 7 days (14 doses)	1.28	0.98	Mahr, G. *et al.* Effects of temafloxacin and ciprofloxacin on the pharmacokinetics of caffeine. *Clinical pharmacokinetics* 22, 90-7 (1992).
179	CYP1A2	CI	Caffeine, 250 mg SD (Day 2)	Fluvoxamine, 100 mg (73.3 mg free base) BID for 2 days (4 doses)	0.96	0.90	Culm-Merdek, K.E., Von Moltke, L.L., Harmatz, J.S. & Greenblatt, D.J. Fluvoxamine impairs single-dose caffeine clearance without altering caffeine pharmacodynamics. *British journal of clinical pharmacology* 60, 486-93 (2005).
180	CYP1A2	CI	Caffeine, 100 mg SD (Day 6)	Fluvoxamine, 25 mg (18.3 mg free base) BID for 6 days (12 doses)	0.46	0.73	Christensen, M. *et al.* Low daily 10-mg and 20-mg doses of fluvoxamine inhibit the metabolism of both caffeine (cytochrome P4501A2) and omeprazole (cytochrome P4502C19). *Clinical Pharmacology & Therapeutics* 71, 141-52 (2002).
181	CYP1A2	CI	Theophylline, 250 mg SD (Day 8 @8 AM)	Fluvoxamine, 25 mg (18.3 mg free base)	1.06	1.68	Yao, C. *et al.* Fluvoxamine-theophylline interaction: gap between in vitro and in vivo inhibition constants toward cytochrome P4501A2. *Clinical Pharmacology & Therapeutics* 70, 415-24 (2001).

				QD for 9 days (9 doses)			
182	CYP1A2	CI	Theophylline, 250 mg SD (Day 8 @8 AM)	Fluvoxamine, 50 mg (36.7 mg free base) QD on day 1, 75 mg (55 mg free base) QD days 2-9 (dosed at 4 PM)	0.90	1.41	Yao, C. *et al.* Fluvoxamine-theophylline interaction: gap between in vitro and in vivo inhibition constants toward cytochrome P4501A2. *Clinical Pharmacology & Therapeutics* 70, 415-24 (2001).
183	CYP1A2	CI	Theophylline, 4 mg/kg SD (Day 6)	Fluvoxamine, 50 mg QD days 1-2, 50 mg (36.7 mg free base) BID days 3-7	0.97	1.51	Orlando, R., Padrini, R., Perazzi, M., De Martin, S., Piccoli, P. & Palatini, P. Liver dysfunction markedly decreases the inhibition of cytochrome P450 1A2–mediated theophylline metabolism by fluvoxamine. *Clinical Pharmacology & Therapeutics* 79, 489-99 (2006).
184	CYP1A2	CI	Theophylline, 3.4 mg/kg SD (Day 4 @9.00 AM)	Ciprofloxacin, 500 mg BID for 5 days (Day 1 @9.00 PM to Day 5 @9.00 PM) (9 doses)	-	1.27	Batty, K., Davis, T., Ilett, K., Dusci, L. & Langton, S. The effect of ciprofloxacin on theophylline pharmacokinetics in healthy subjects. *British journal of clinical pharmacology* 39, 305-11 (1995).
185	CYP1A2	CI	Theophylline, 5 mg/kg SD IV infusion (30 minutes) (Day 5 @9 AM)	Ciprofloxacin, 500 mg BID for 7 days (Day 1 @9 AM to Day 7 @9 AM) (13 doses)	0.92	0.97	Prince, R.A., Casabar, E., Adair, C.G., Wexler, D.B., Lettieri, J. & Kasik, J.E. Effect of quinolone antimicrobials on theophylline pharmacokinetics. *The Journal of Clinical Pharmacology* 29, 650-4 (1989).
186	CYP1A2	CI	Theophylline, 125 mg oral dose TID for 7 days (Day 1 @8 AM to Day 7 @8 AM)	Ciprofloxacin, 500 mg BID for 7 days (Day 1 @8 AM to Day 7 @8 AM)	-	1.18	Robson, R., Begg, E., Atkinson, H., Saunders, D. & Frampton, C. Comparative effects of ciprofloxacin and lomefloxacin on the oxidative metabolism of theophylline. *British journal of clinical pharmacology* 29, 491-3 (1990).

187	**CYP1A2**	**CI**	**Theophylline, 5 mg/kg SD IV infusion (30 minutes) (Day 5 @9 AM)**	**Ciprofloxacin, 500 mg BID for 8 days (16 doses)**	**-**	**1.04**	**Loi, C.-M., Parker, B.M., Cusack, B.J. & Vestal, R.E. Aging and drug interactions. III. Individual and combined effects of cimetidine and ciprofloxacin on theophylline metabolism in healthy male and female nonsmokers. *Journal of Pharmacology and Experimental Therapeutics* 280, 627-37 (1997).**
188	**CYP1A2**	**CI**	**Theophylline, 5 mg/kg SD IV infusion (30 minutes) (Day 5 @9 AM)**	**Ciprofloxacin, 500 mg BID for 8 days (16 doses)**	**-**	**1.04**	**Loi, C.-M., Parker, B.M., Cusack, B.J. & Vestal, R.E. Aging and drug interactions. III. Individual and combined effects of cimetidine and ciprofloxacin on theophylline metabolism in healthy male and female nonsmokers. *Journal of Pharmacology and Experimental Therapeutics* 280, 627-37 (1997).**
189	**CYP1A2**	**CI**	**Theophylline, 5 mg/kg SD IV infusion (30 minutes) (Day 5 @9 AM)**	**Ciprofloxacin, 500 mg BID for 7 days (Day 1 @7 AM to Day 7 @7 PM) (14 doses)**	**-**	**1.02**	**Loi, C., Parker, B., Cusack, B. & Vestal, R. Individual and combined effects of cimetidine and ciprofloxacin on theophylline metabolism in male nonsmokers. *British journal of clinical pharmacology* 36, 195-200 (1993).**
190	**CYP1A2**	**CI**	**Caffeine, 100 mg SD (Day 2 @9 AM)**	**Ciprofloxacin, 750 mg BID for 2 days (3 doses)**	**1.15**	**1.21**	**Healy, D., Polk, R., Kanawati, L., Rock, D. & Mooney, M. Interaction between oral ciprofloxacin and caffeine in normal volunteers. *Antimicrobial agents and chemotherapy* 33, 474-8 (1989).**
191	**CYP1A2**	**CI**	**Tizanidine, 4 mg SD (Day 4, 1h after Fluvoxamine)**	**Fluvoxamine, 100 mg (73.3 mg free base) QD for 4 days**	**0.81**	**0.99**	**Granfors, M.T., Backman, J.T., Neuvonen, M., Ahonen, J. & Neuvonen, P.J. Fluvoxamine drastically increases concentrations and effects of tizanidine: a potentially hazardous interaction. *Clinical Pharmacology & Therapeutics* 75, 331-41 (2004).**
192	**CYP1A2**	**CI**	**Tizanidine, 4 mg SD (Day 3, 1h after morning Ciprofloxacin dose)**	**Ciprofloxacin, 500 mg BID for 3 days (6 doses)**	**0.46**	**0.35**	**Granfors, M.T., Backman, J.T., Neuvonen, M. & Neuvonen, P.J. Ciprofloxacin greatly increases concentrations and cypotensive effect of tizanidine by inhibiting its cytochrome P450 1A2–mediated presystemic metabolism. *Clinical Pharmacology & Therapeutics* 76, 598-606 (2004).**

193	**CYP2C19**	**CI**	**S-Mephenytoin, 100 mg SD on day 9 (8 AM)**	**Fluvoxamine, 37.5 mg (27.5 mg free base) QD for 11 days (dosed at 4 PM)**	**1.01**	**1.16**	**Yao, C., Kunze, K.L., Trager, W.F., Kharasch, E.D. & Levy, R.H. Comparison of in vitro and in vivo inhibition potencies of fluvoxamine toward CYP2C19. *Drug metabolism and disposition* 31, 565-71 (2003).**
194	**CYP2C19**	**CI**	**S-Mephenytoin, 100 mg SD on day 9 (8 AM)**	**Fluvoxamine, 62.5 mg (45.8mg free base) QD for 11 days (dosed at 4 PM)**	**1.02**	**1.21**	**Yao, C., Kunze, K.L., Trager, W.F., Kharasch, E.D. & Levy, R.H. Comparison of in vitro and in vivo inhibition potencies of fluvoxamine toward CYP2C19. *Drug metabolism and disposition* 31, 565-71 (2003).**
195	**CYP2C19**	**CI**	**S-Mephenytoin, 100 mg SD on day 9 (8 AM)**	**Fluvoxamine, 50 mg (36.7mg free base) QD (days 1-2), 87.5 mg (64.1 mg free base) QD (days 3-11) (dosed at 4 PM)**	**1.09**	**1.08**	**Yao, C., Kunze, K.L., Trager, W.F., Kharasch, E.D. & Levy, R.H. Comparison of in vitro and in vivo inhibition potencies of fluvoxamine toward CYP2C19. *Drug metabolism and disposition* 31, 565-71 (2003).**
196 - EM	**CYP2C19**	**CI**	**Omeprazole, 20 mg SD day7**	**Fluvoxamine, 25 mg (18.3 mg free base) BID for 7 days**	**-**	**0.92**	**Christensen, M. *et al.* Low daily 10-mg and 20-mg doses of fluvoxamine inhibit the metabolism of both caffeine (cytochrome P4501A2) and omeprazole (cytochrome P4502C19). *Clinical Pharmacology & Therapeutics* 71, 141-52 (2002).**
196 - PM	**CYP2C19**	**CI**	**Omeprazole, 20 mg SD day7**	**Fluvoxamine, 25 mg (18.3 mg free base) BID for 7 days**	**-**	**1.15**	**Christensen, M. *et al.* Low daily 10-mg and 20-mg doses of fluvoxamine inhibit the metabolism of both caffeine (cytochrome P4501A2) and omeprazole (cytochrome P4502C19). *Clinical Pharmacology & Therapeutics* 71, 141-52 (2002).**
196 - ALL	**CYP2C19**	**CI**	**Omeprazole, 20 mg SD day7**	**Fluvoxamine, 25 mg (18.3 mg free base) BID for 7 days**	**-**	**0.85**	**Christensen, M. *et al.* Low daily 10-mg and 20-mg doses of fluvoxamine inhibit the metabolism of both caffeine (cytochrome P4501A2) and omeprazole (cytochrome P4502C19). *Clinical Pharmacology & Therapeutics* 71, 141-52 (2002).**

197	**CYP2C19**	**MBI**	**Omeprazole, 20 mg SD day 12 (1 h after Fluoxetine)**	**Fluoxetine, 20 mg SD day 1, 60 mg QD days 2-14**	**0.66**	**0.89**	**Sager, J.E., Lutz, J.D., Foti, R.S., Davis, C., Kunze, K.L. & Isoherranen, N. Fluoxetine-and Norfluoxetine-Mediated Complex Drug–Drug Interactions: In Vitro to In Vivo Correlation of Effects on CYP2D6, CYP2C19, and CYP3A4. *Clinical Pharmacology & Therapeutics* 95, 653-62 (2014).**
198	**CYP2C19**	**MBI**	**Imipramine, 44.26 mg SD (3hrs after Fluoxetine)**	**Fluoxetine, 60 mg SD**	**1.02**	**0.80**	**Bergstrom, R.F., Peyton, A.L. & Lemberger, L. Quantification and mechanism of the fluoxetine and tricyclic antidepressant interaction. *Clinical Pharmacology & Therapeutics* 51, 239-48 (1992).**
199	**CYP2C19**	**MBI**	**Imipramine, 44.26 mg SD on day 8 (3hrs after Fluoxetine)**	**Fluoxetine, 60 mg QD for 8 days**	**1.03**	**0.98**	**Bergstrom, R.F., Peyton, A.L. & Lemberger, L. Quantification and mechanism of the fluoxetine and tricyclic antidepressant interaction. *Clinical Pharmacology & Therapeutics* 51, 239-48 (1992).**
200	**CYP2C19**	**MBI**	**Omeprazole, 20 mg SD (on day 8) 1h after Ticlopidine**	**Ticlopidine, 200 mg ticlopidine HCL (175.65 mg free base) once a day at 8 AM (8 doses)**	**0.74**	**0.84**	**Ieiri, I., Kimura, M., Irie, S., Urae, A., Otsubo, K. & Ishizaki, T. Interaction magnitude, pharmacokinetics and pharmacodynamics of ticlopidine in relation to CYP2C19 genotypic status. *Pharmacogenetics and genomics* 15, 851-9 (2005).**
201	**CYP2C19**	**MBI**	**Omeprazole, 40 mg SD on day 7**	**Ticlopidine, 100 mg of ticlopidine HCL (87.83 mg free base) TID (19 doses)**	**1.15**	**1.77**	**Tateishi, T., Kumai, T., Watanabe, M., Nakura, H., Tanaka, M. & Kobayashi, S. Ticlopidine decreases the in vivo activity of CYP2C19 as measured by omeprazole metabolism. *British journal of clinical pharmacology* 47, 454-7 (1999).**

15. Appendix 6

Published Analysis – Mechanism/Enzyme level data

Data from the original published analysis based on enzymes and inhibitory mechanism are presented below.[12] The prediction accuracy of the updated (n=210) *versus* original dataset (n=201) were similar; AFE values were 0.92 *versus* 0.94 for competitive inhibition and 1.03 *versus* 0.99 for MBI, respectively.

Predicted *versus* observed changes in AUC and C_{max} across each of the CYP enzymes investigated are shown in Figure A3 for competitive inhibition (n=123 DDIs) and in Figure A4 for mechanism-based inhibition (n=78 DDIs).

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 CYP3A4/5 (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 3/125 falling outside 2-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 1 DDI investigated falling outside of 2-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 2 out of the 62 studies falling outside of 2-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 3 predictions fell outside 2-fold of the observed AUC ratio for omeprazole (CYP2C19 substrate), quinidine and simvastatin (CYP3A4 substrates).

Data from the original analysis based on enzymes and substrates are presented below for C_{max} ratios (Figure A5) and AUC ratios (Figure A6).[12]

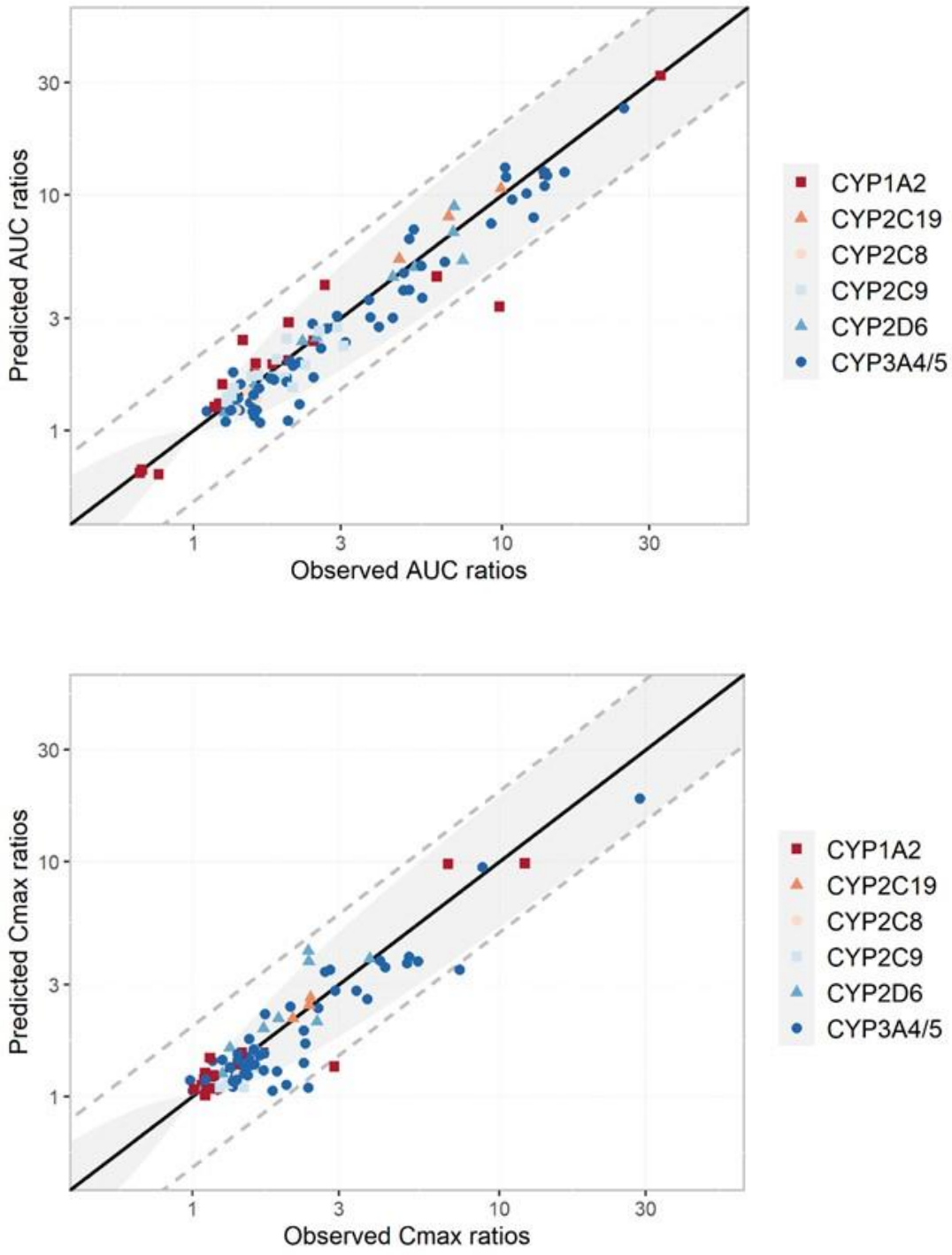


Figure A3. Predicted *versus* observed DDIs involving competitive inhibition

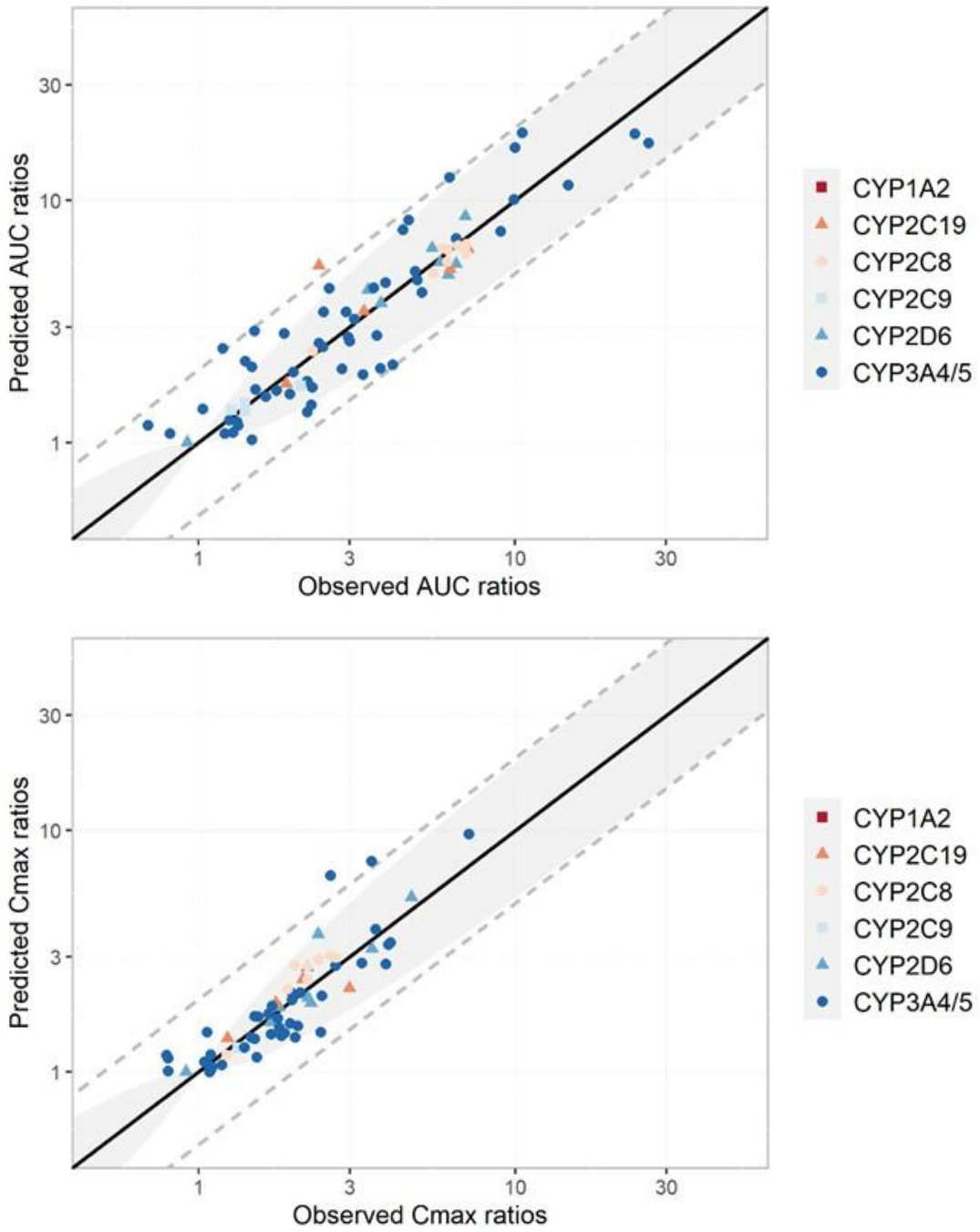


Figure A4. Predicted *versus* observed DDIs involving mechanism-based inhibition

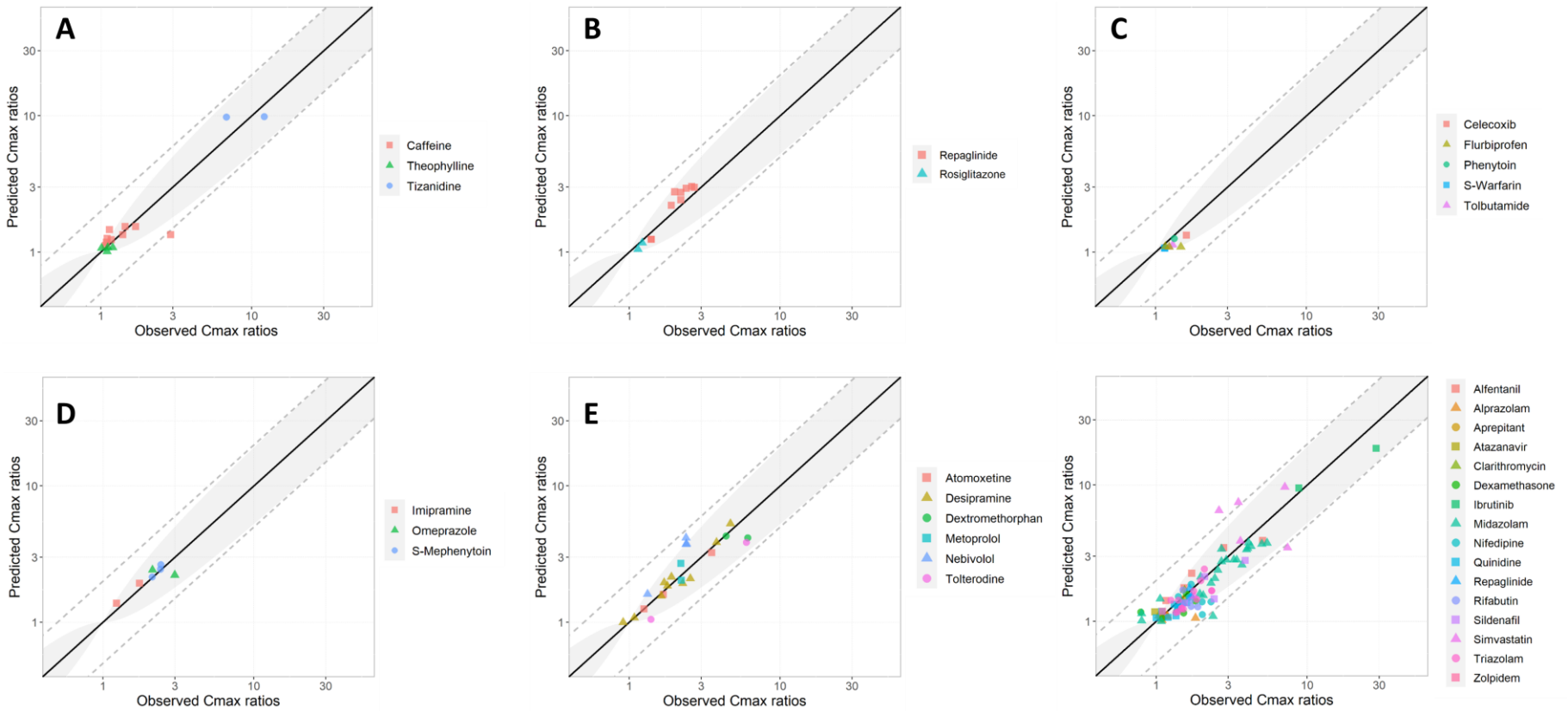


Figure A5. Predicted and observed C_{max} ratios for the qualification of CYP1A2 (A), CYP2C8 (B), CYP2C9 (C), CYP2C19 (D), CYP2D6 (E) and CYP3A4/5 (F) mediated competitive and mechanism based inhibition using the Simcyp Simulator (V19 R1).

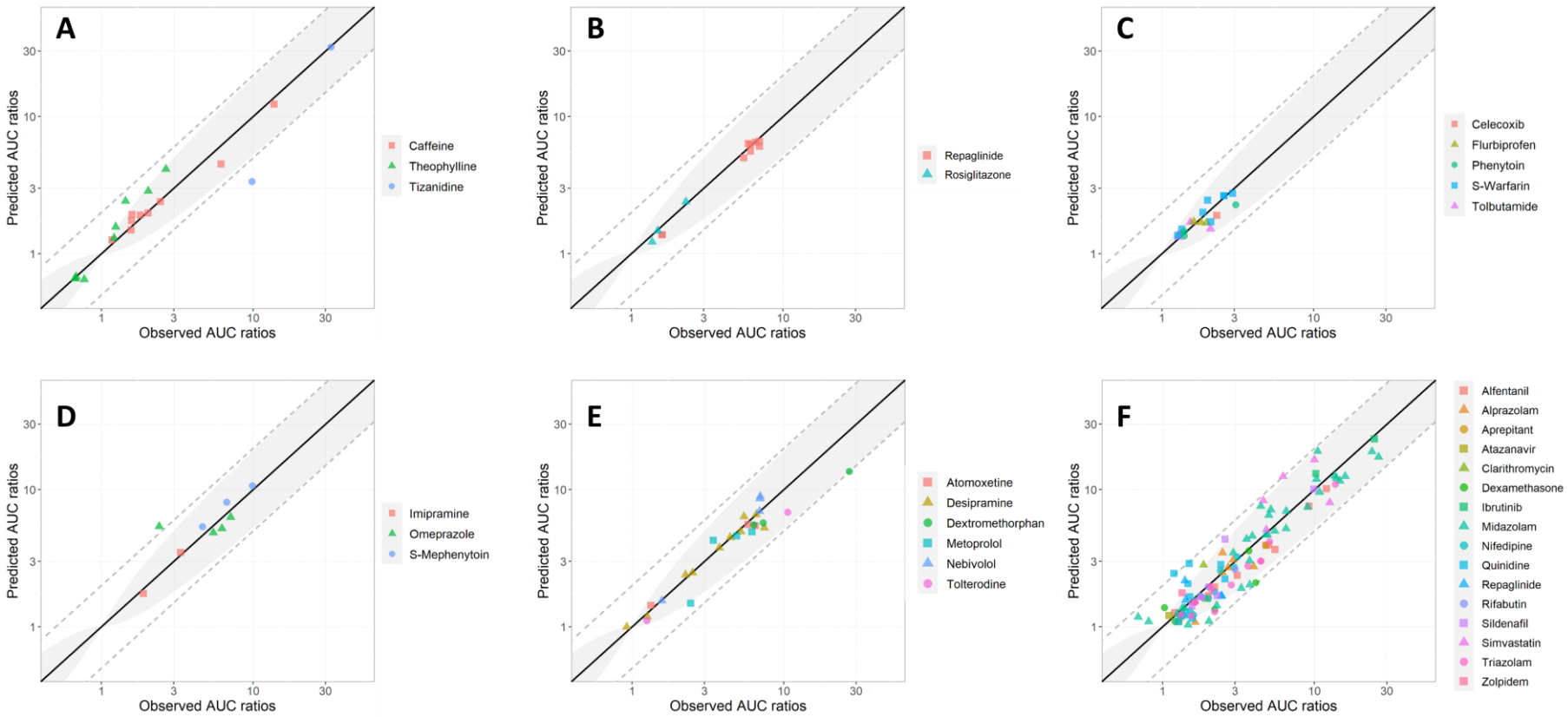


Figure A6. Predicted and observed AUC ratios for the qualification of CYP1A2 (A), CYP2C8 (B), CYP2C9 (C), CYP2C19 (D), CYP2D6 (E) and CYP3A4/5 (F) mediated competitive and mechanism based inhibition using the Simcyp Simulator (V19 R1).

16.Appendix 7

Compound file comparison – V19/V20/V21

Two sets of comparisons are typically performed for version-to-version comparisons.

Compound files developed in a new version e.g. V20 files in the V20 simulator would be compared against V20 files in the V19 simulator – this comparison gives an indication of the impact of the changes in compound file parameters.

In addition, V20 files in the V20 simulator would be compared against V19 files in the V19 simulator. This comparison would reflect changes in system parameters as well as changes in compound file parameters.

The former has been provided for V19/V20 and the latter for V20/V21. The changes in compound file parameters which are shown in Appendix 7 do not lead to significant differences in PK between versions as can be seen by the graphs shown in the attachments below.

V20R1_V21R1_Versi
onComparison_64bi

V19R1_V20R1_Versi
onComparison_64bi

17.Appendix 8

Version Comparisons – V19/V20/V21

Changes to input parameters were made for some compound files in going from V19 to V20 to V21 of the Simcyp Simulator. These are indicated below. The impact of these changes, which is minimal in terms of the predicted PK, are indicated in Appendix 6, where correlations of simulations for each compound are shown for V19 *versus* V20 *versus* V21.

Changes in the V20 input data for inhibitors and substrates used in the CYP-qualification in Simcyp V19:

Compound – Inhibitor file	**Parameter**	**V19**	**V20**	**Comments**
Amiodarone				
Aprepitant				
Atazanavir				
Bupropion				
Cimetidine				
Cinacalcet	Released in V20			
Ciprofloxacin				
Clarithromycin				
Cyclosporine				
Desmethyl-Diltiazem				
Diltiazem				
Erythromycin - EC	CYP3A4 K_i (μM)	82	32.8	Refined based on Meta-Analysis
	CYP3A Kapp (μM)	23.2	17.64	
	CYP3A4 K_{inact} (1/h)	2.25	0.8	
Erythromycin	fa	1	0.60	Where Erythromycin is not dosed as EC formulation alternative file can be used with refined absorption parameters
	Ka (1/h)	3.58	0.52	
Fluconazole				
Fluoxetine				
Fluvoxamine				
Gemfibrozil				
Gemfibrozil 1-O-β Glucuronide				
Itraconazole				

Ketoconazole				
Mono-desethyl Amiodarone				
Nor-Fluoxetine				
Norverapamil				
OH-Bupropion				
Paroxetine				
Quinidine				
Ritonavir	fa	-	0.5	Option to select FO file
	Ka (1/h)	-	0.45	Option to select FO file, optimised to recover profile
	fu_{gut}	-	0.015	Same as fup
Sulphaphenazole				
Ticlopidine				
Trimethoprim				
Verapamil				

Compound – Substrate file	**Parameter**	**V19**	**V20**	**Comments**
Alfentanil				
Alprazolam				
Atazanavir				
Atomoxetine		SV Atomoxetine	SV Atomoxetine	V21 2D6 Vmax increased due to phenotype changes
Caffeine				
Clarithromycin				
Desipramine				
Dexamethasone	Released in V20			
Dextromethorphan				
Flurbiprofen	Released in V21			
Ibrutinib	Released in V21			
Imipramine	Ka	0.45	0.8	Optimised to capture clinical profiles
	T_{max}	0.45	0.8	Revised to capture T_{max}
	V_{ss} CV (%)	30	20	Revised to capture variability
	2-OH CYP1A2 V_{max} (pmol/min/pmol)	2.6	1.6	Elimination parameters were determined using a retrograde

	2-OH CYP2C19 V_{max} (pmol/min/pmol)	56.8	237.2	approach and then refined based on fm data available for formation of desipramine. The retrograde CL_{int} was then used to back calculate V_{max} values using *in vitro* Km values.
	2-OH CYP2D6 V_{max} (pmol/min/pmol)	22.6	5.6	
	N-Desmethyl CYP1A2 V_{max} (pmol/min/pmol)	16.9	105.8	
	N-Desmethyl CYP2C19 V_{max} (pmol/min/pmol)	119.2	175	
	N-Desmethyl CYP2D6 V_{max} (pmol/min/pmol)	13.3	204.8	
	UGT1A4 V_{max} (pmol/min/mg)	292.5	136.78	
Metoprolol				
Midazolam				
Nebivolol	Released in V20			
Nifedipine				
Omeprazole				
Phenytoin				
Quinidine				
Repaglinide				
Rifabutin				
Rosiglitazone				
Sildenafil				
Simvastatin				
S-Mephenytoin				
S-Warfarin				
Theophylline				
Tizanidine	Not released so far			
Tolbutamide				
Tolterodine				
Triazolam				
Zolpidem				

Summary of changes to existing compound files in Version 20

Compound	Parameter	Value		Comments
		V19.1	V20	
SV-Ritonavir	fa	-	0.5	Option to select FO file
	Ka (1/h)	-	0.45	Option to select FO file, optimised to recover profile

	fu_{gut}	-	0.015	Same as fup
SV-Imipramine	ka	0.45	0.8	Optimised to capture clinical profiles
	T_{max}	0.45	0.8	Revised to capture T_{max}
	V_{ss} CV (%)	30	20	Revised to capture variability
	2-OH CYP1A2 V_{max} (pmol/min/pmol)	2.6	1.6	Elimination parameters were determined using a retrograde approach and then refined based on fm data available for formation of desipramine. The retrograde CL_{int} was then used to back calculate V_{max} values using *in vitro* Km values.
	2-OH CYP2C19 V_{max} (pmol/min/pmol)	56.8	237.2	
	2-OH CYP2D6 V_{max} (pmol/min/pmol)	22.6	5.6	
	N-Desmethyl CYP1A2 V_{max} (pmol/min/pmol)	16.9	105.8	
	N-Desmethyl CYP2C19 V_{max} (pmol/min/pmol)	119.2	175	
	N-Desmethyl CYP2D6 V_{max} (pmol/min/pmol)	13.3	204.8	
	UGT1A4 V_{max} (pmol/min/mg)	292.5	136.78	
SV-Erythromycin-EC	CYP3A4 K_i (µM)	82	32.8	Refined based on Meta-Analysis
	CYP3A Kapp (µM)	23.2	17.64	
	CYP3A4 K_{inact} (1/h)	2.25	0.8	
SV-Erythromycin	fa	1	0.60	Where Erythromycin is not dosed as EC formulation alternative file can be used with refined absorption parameters
	Ka (1/h)	3.58	0.52	

Summary of changes to existing compound files in Version 21

Compound	Parameter	Value		Comments
		V20	V21	
SV-Itraconazole (Fasted Soltn and Fed Capsule)	Intestinal P-gp Ki (µM)	-	0.0939	Ki scaled from *in vitro* data
	Liver P-gp Ki (µM)	-	0.0939	Ki scaled from *in vitro* data
SV- OH-Itraconazole	Intestinal P-gp Ki (µM)	-	0.0939	Ki scaled from *in vitro* data
	Liver P-gp Ki (µM)	-	0.0939	Ki scaled from *in vitro* data
SV-Trimethoprim	Kidney OCT2 Ki (µM)	6.97	56.0	Revised to capture DDI with Metformin after updated to EGD model
	Kidney MATE Ki (µM)	0.32	15.0	
SV-3-MethoxyMorphinian	V_{ss} (L/kg)	14.3	5.46	Updated to predicted V_{ss} using Method 3 to match approach used for parent
SV-Atomoxetine	CYP2D6 V_{max} (pmol/min/mg)	476.51	857.7	Updated to reflect changes in population with addition of IM phenotype
SV-Dextromethorphan	File name change	-	-	Updated to indicate that file should be used with peripheral sampling
	Ka (1/h)	2.6	0.45	Updated for full PBPK
	Distribution	Min	Full	Updated to full PBPK

	V_{ss}	14.3	17.95	Predicted using Method 3 & Olive Oil partition coefficient
	CYP2D6 CL_{int} O-demethylation (µl/min/mg)	250.85	678	Updated to reflect changes in population with addition of IM phenotype
	CYP2D6 CL_{int} N-demethylation (µl/min/mg)	2.15	6.08	
SV-Efavirenz	CYP1A2 CL_{int} (µl/min/pmol)	0.03	0.02028	CL_{int} was refitted using the updated populations with CYP genotype and phenotype frequencies
	CYP3A4 CL_{int} (µl/min/pmol)	0.01	0.00775	
	CYP2B6 CL_{int} (µl/min/pmol)	1.36	1.024	
	CYP2A6 CL_{int} (µl/min/pmol)	0.47	0.2387	
	Add HLM CL_{int} (µl/min/mg)	0.694	2.39	
Sim-Bufuralol	File name	Sim	SV	Updated to reflect changes in population with addition of IM phenotype
	CYP2D6 V_{max} 1-OH (pmol/min/pmol)	27.7	45.09	
	CYP2D6 V_{max} 6-OH (pmol/min/pmol)	1.5	2.44	
SV-Fluvoxamine	CYP2C19 Ki (µM)	0.006	0.0087	Updated to reflect changes in abundance and frequency of CYP2C19 in population
SV-Bupropion_SR	CYP2B6 CL_{int} (µl/min/pmol)	9.1	7.24	Updated to reflect changes in abundance and frequency of CYP2B6 in population
	CYP3A4 CL_{int} (µl/min/pmol)	0.041	0.036	
	CYP2C19 CL_{int} (µl/min/pmol)	0.406	1.565	
	CYP2C19 Pathway	OH	Pathway 2	
	Add HLM CL_{int} (µl/min/mg)	105.6	80.8	
SV-Nebivolol	CYP2D6 CL_{int} (µl/min/pmol)	433.6	607	Updated to reflect changes in population with addition of IM phenotype
SV-S-Mephenytoin	CYP2C19 CL_{int} (µl/min/pmol)	12.16	9.10	Updated to reflect changes in abundance and frequency of CYP2C19 in population
SV-Atorvastatin	rUGT Scalar – Liver	1	0.250	Updated to reflect changes in abundance and frequency of UGT1A3 in population
	rUGT Scalar – Intestine	1	0.250	
	rUGT Scalar - Kidney	1	0.250	