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Accurate estimation of *in vivo* inhibition constants of inhibitors and fraction metabolized of substrates with physiologically-based pharmacokinetic drug-drug interaction models incorporating parent drugs and metabolites of substrates with cluster Newton method

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List of non-standard abbreviations:

CNM: Cluster Newton Method

DIDB: University of Washington Metabolism and Transport Drug Interaction Database

$SS_{log,Y}$: Sum of squares of log residuals for the objective function Y

$SS_{log,time}$: Sum of squares of log residuals

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Abstract

Accurate estimation of "in vivo" inhibition constants (K_i) of inhibitors and fraction metabolized (f_m) of substrates is of high importance for drug-drug interaction (DDI) prediction based on physiologically-based pharmacokinetic (PBPK) models. We hypothesized that analysis of the pharmacokinetic alterations of substrate metabolites in addition to parent drug would enable accurate estimation of in vivo K_i and f_m. Twenty four pharmacokinetic DDIs caused by CYP inhibition were analyzed with PBPK models using an emerging parameter estimation method, Cluster Newton Method, which enables efficient estimation of a large number of parameters to describe pharmacokinetics of parent and metabolized drugs. For each DDI, two analyses were conducted (with or without substrate metabolite data) and parameter estimates were compared to each other. In 17 out of 24 cases, inclusion of substrate metabolite information in PBPK analysis improved the reliability of both K_i and f_m. Importantly, estimated K_i for the same inhibitor from different DDI studies were generally consistent, suggesting that the estimated K_i from one study can be reliably used for the prediction of untested DDI cases with different victim drugs. Furthermore, large discrepancy was observed between reported in vitro K_i and in vitro estimates for some inhibitors, and the current in vivo K_i estimates might be used as reference values when optimizing IVIVE strategies. These results demonstrated that better utilization of substrate metabolite information in PBPK analysis of clinical DDI data can improve reliability of top-down parameter estimation and prediction of untested DDIs.

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Introduction

Pharmacokinetic drug-drug interactions (DDIs) alter the pharmacokinetics of substrate (victim) drugs, and consequently, leading to incidences of the adverse reactions including lethal events (Huang et al., 2008). For DDIs caused by the inhibition of drug metabolizing enzymes, magnitude of DDI depends on the inhibition constants (K_i) of the inhibitors against the enzymes, and the contribution of the enzyme inhibited to overall eliminations of the substrate drugs (f_m), as well as the exposure of the inhibitors at the enzyme active site (Ito et al., 1998; Brown et al., 2005; Obach et al., 2006; Houston and Galetin, 2008; Hisaka et al., 2010). Therefore, the reliability of these two parameters affects accuracy of the simulated results of the DDI cases.

Physiologically-based pharmacokinetic (PBPK) model has been employed for quantitative analysis of the clinically-reported DDIs (Rowland et al., 2011; Jones et al., 2015; Wagner et al., 2015; Luzon et al., 2016). With the aim of improving accuracy for PBPK model-based prediction of DDIs, Kato et al determined the in vivo K_i values of multiple inhibitor drugs for cytochrome P450 (CYP) enzymes by comprehensive analysis of the DDI data (Kato et al., 2008). They found that in vivo K_i estimates were smaller than in vitro K_i values for many inhibitors. They also found that in vivo K_i values showed up to 100-fold difference depending on clinical data. They suggested that the reproducibility of the clinical data from different study groups, and reliability of the fixed f_m values employed in PBPK analysis based on in vitro data are potential causes of such inconsistency. In fact, small variation of f_m in PBPK analysis can show a large impact on estimated K_i. The ratio of the area under the plasma concentration-time curve of a substrate drug in the presence of a perpetrator drug to that in its absence (AUCR) can be described as the following equation when a perpetrator drug (I_u: its plasma protein unbound concentration) competitively inhibits the specific drug metabolizing enzyme(s) (Khojasteh et al., 2011);

$$AUCR = \frac{1}{\frac{f_m}{1+\frac{I_u}{K_i}} + (1-f_m)} \qquad \text{(Eq. 1)}$$

For instance, a 3.3-fold increase in the area under the plasma concentration-time curve ratio (AUCR) of the substrates can be explained either by f_m of 70% with 99.6% inhibition of the enzyme, or f_m of 90% with 77% inhibition. The K_i values varied 70-fold in these two cases. In other words, K_i and f_m are

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susceptible to an identifiability problem.

To overcome such difficulty, we need to include additional information related to in vivo f_m or K_i. In this study, we focused on the concentration-time profiles of substrate drug metabolite. One can expect that less variety of metabolic enzymes are involved in the formation of metabolites compared to the overall elimination of parent drugs, since the elimination of parent drugs is often composed of the formation of multiple metabolic enzymes. Such specificity can be helpful in accurately determining the alteration in metabolic activity by each enzyme. Our hypothesis was that analysis of the pharmacokinetic alterations of the specific substrate metabolites in addition to substrate parent drug would enable accurate estimation of in vivo K_i and f_m. A technical difficulty of this approach is that conventional parameter estimation methods (e.g. Gauss-Newton algorithm) require an estimation of multiple sets of feasible initial parameters to obtain reliable fitted parameters; however, their preparation is laborious, and requires deep understanding of the pharmacokinetics not only for a substrate parent drug but also for substrate metabolites. It is challenging to estimate pharmacokinetic parameters of metabolites due to the paucity of clinical pharmacokinetic data in humans. Considering such situation, we introduced a new parameter estimation method referred to as Cluster Newton Method (CNM) (Yoshida et al., 2013; Aoki et al., 2014). CNM automatically prepares multiple initial parameter sets when the researchers just set the broad ranges of the initial parameters, and suggests multiple sets of fitted parameters as likely solution. In the present study, we performed PBPK analyses of various DDIs involving the inhibition of CYP enzymes to accurately estimate in vivo K_i and f_m by including substrate metabolite pharmacokinetic information.

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Materials and Methods

Definitions of pharmacokinetic parameters

The following pharmacokinetic parameters were used throughout this article: C, concentration of drugs; CL_{int}, hepatic intrinsic clearance with regard to unbound concentration; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; I, concentration of inhibitors; k_a, absorption rate constant; $k_{transit}$, transit rate constant in intestine; K_i, inhibition constant for unbound inhibitor concentrations; $K_{i,total}$, inhibition constant for total (bound + unbound) inhibitor concentrations; k_{LI}, transit rate constant to the large intestine; $K_{p,h}$, liver-to-blood concentration ratio; R_{MBI}, degree of inhibition with mechanism-based inhibitors; Q, blood flow rate; X, amount of drugs.

The following subscripts were used throughout the article: C, central compartment; H, liver compartment; LI, large intestine; Met, metabolite; Peri, peripheral compartment; Transit_intes, intestinal transit compartment.

Data source

University of Washington Metabolism and Transport Drug Interaction Database (DIDB: http://www.druginteractioninfo.org) was queried to retrieve in vivo pharmacokinetic interactions involving substrates of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A until March 2015. Twenty-four DDI cases that meet the following criteria were collected (Tables 1 and 2): (1) concentration-time profiles of parent substrates are available, (2) at least AUC and/or amount excreted into urine (A_e) of substrate metabolite are available, (3) information on the isoforms involved in the formation of the metabolites of interest was available in DIDB. For inhibitors, the following parameters for all metabolic enzymes were collected with DIDB: inhibition constant (K_i), inhibitor concentration at half the maximal inhibition potency (IC_{50}), maximal inactivation rate constant (k_{inact}), apparent inactivation constant ($K_{I,app}$).

PBPK model development

PBPK models were constructed to describe pharmacokinetics of substrates (Fig. 1b) and inhibitors (Fig. 1a). The models incorporated central, peripheral, liver, intestinal, and intestinal transit

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compartments. Formation of substrate metabolites occurs at a liver compartment and at an intestinal compartment. For the hepatic elimination of substrates, CYP enzyme-specific pathways and the inhibition by coadministered drugs were included in the model of a DDI case when the following criteria were met:

1. The enzyme was involved in the metabolism of the substrate, according to the in vitro metabolism data.
2. The inhibitor had predicted R_1 (competitive inhibition) or R_2 [mechanism-based inhibition (MBI)] value of more than 2 against the enzyme of interest (USFDA, 2012), using geometric mean of reported K_i, IC_{50}, k_{inact}, and $K_{I,app}$.

When R_2 criterion was met (clarithromycin on CYP3A, fluoxetine on CYP2C19 and CYP3A, or paroxetine on CYP2D6), the potency of mechanism-based inhibition by inhibitors was described with R_{MBI} parameter in PBPK model as described in the next section under an assumption that the inhibition potency by MBI at the steady-state is constant; otherwise K_i was used to describe the inhibition potencies of inhibitors on hepatic enzymes.

In addition, elimination pathways with other CYP enzymes or non-CYP enzymes were included to form “other pathways” in the model. CYP enzyme-specific pathways were further divided into the formation of each substrate metabolite, including “other metabolites” that were not quantified in reported DDIs. Similarly, CYP enzyme-specific and other elimination pathways were included in the eliminations of substrate metabolites.

Intestinal metabolism by CYP3A and its inhibition by coadministered drugs were considered when the following criteria were met:

1. CYP3A was involved in the metabolism of the substrate, according to the in vitro metabolism data.
2. The estimated F_aF_g of the substrate was less than 0.95 (Table 3).
3. The inhibitor used in a clinical study has predicted R_1 (using inhibitor concentration of dose/250mL) value of more than 11 against CYP3A (USFDA, 2012), using geometric mean of reported K_i and IC_{50}.

The potency of intestinal enzyme inhibition by inhibitors was described with R_{intes} parameter in our

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PBPK model as a constant value due to the following reasons/assumptions: (1) our PBPK model does not make an inference on effective intestinal inhibitor concentration, which makes estimation of K_i difficult, (2) the intestinal inhibition matters mainly at the early time points (before intestinal absorption of substrate drugs is finished) and time-dependent change in the magnitude of intestinal inhibition has less influence on substrate kinetics compared with inhibition of hepatic enzymes. This assumption is supported by the fact that estimated k_a of all of the substrates has geometric mean of ≥ 0.6 hr^{-1} (half-life of around 1 hour or less).

Elimination pathways with other CYP enzymes or non-CYP enzymes were included to form "other pathways" in the model.

Simulations with PBPK models

All the simulations were performed with PBPK models described in Figure 1 and in the following equations. First, hepatic or renal clearance and intestinal kinetic constants were calculated, where i, j, or k represent CYP enzymes, metabolites, or inhibitors, and apostrophe (') denotes the parameter values when inhibitor(s) were coadministered:

$$f_B CL_{CYP_A} = f_B CL_{int} \cdot \frac{CL_{CYP_A}/CL_{other}}{\sum_i (CL_{CYP_i}/CL_{other}) + 1}$$

$$f_B CL_{other} = f_B CL_{int} \cdot \frac{1}{\sum_i (CL_{CYP_i}/CL_{other}) + 1} \quad \text{(Eqs. 2 and 3)}$$

$$f_B CL_{CYP_A,Met_a} = f_B CL_{CYP_A} \cdot \frac{CL_{CYP_A,Met_a}/CL_{CYP_A,other}}{\sum_j (CL_{CYP_A,Met_j}/CL_{CYP_A,other}) + 1}$$

$$f_B CL_{other,Met_a} = f_B CL_{other} \cdot \frac{CL_{other,Met_a}/CL_{other,other}}{\sum_j (CL_{other,Met_j}/CL_{other,other}) + 1}$$

$$f_B CL_{other,other} = f_B CL_{other} \cdot \frac{1}{\sum_j (CL_{other,Met_j}/CL_{other,other}) + 1} \quad \text{(Eqs. 4-6)}$$

$$f_B CL'_{CYP_A,Met_a} = f_B CL_{CYP_A,Met_a} \cdot \frac{1}{1 + \sum_k (I_{H,k}/K_{i,total,k,CYP_A})} \cdot \frac{1}{\prod_k R_{MBI,k,CYP_A}} \quad \text{(Eq. 7)}$$

$$CL_R = \frac{Q_R CL_{R,int,app}}{Q_R + CL_{R,int,app}} \quad \text{(Eq. 8)}$$

$$k_{LI} = k_a \cdot \frac{1 - F_a F_g}{F_a F_g} \cdot \frac{1}{\sum_i (k_{CYP3A,CYP_i}/k_{LI}) + 1} \quad \text{(Eq. 9)}$$

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$$k_{CYP3A,Met_a} = k_a \cdot \frac{1 - F_a F_g}{F_a F_g} \cdot \frac{k_{CYP3A,CYP_A}/k_{LI}}{\sum_i (k_{CYP3A,CYP_i}/k_{LI}) + 1} \quad \text{(Eq. 10)}$$

$$k'_{CYP3A,Met_a} = k_{CYP3A,Met_a} \cdot \frac{1}{\prod_k R_{intes,CYP3A,k}} \quad \text{(Eq. 11)}$$

Using these parameters, the following ordinary differential equations were solved numerically:

Equations for substrates

Central compartment (C)

$$V_C \ \frac{dC_C}{dt} = Q_H \ \frac{C_H}{K_{P,H}} + k_{21} \ X_{Peri} - (Q_H + CL_{12} + CL_R) \ C_C \quad \text{(Eq. 12)}$$

Peripheral compartment (Peri)

$$\frac{dX_{Peri}}{dt} = -k_{21} \ X_{Peri} + CL_{12} \ C_C \quad \text{(Eq. 13)}$$

Intestinal and intestinal transit compartment (Transit_intes)

$$\frac{dX_{Transit\_intes}}{dt} = -k_{a,transit} \ X_{Transit\_intes}$$

$$\frac{dX_{Intestine}}{dt} = k_{a,transit} \ X_{Transit\_intes} - \left(k_a + k_{LI} + \sum_j k_{CYP3A,Met_j}\right) \ X_{Intestine} \quad \text{(Eqs. 14 and 15)}$$

Liver compartment (H)

$$V_H \ \frac{dC_H}{dt} = Q_H \cdot \left(C_C - \frac{C_H}{K_{P,H}}\right) - \sum_i \sum_j f_B CL_{CYP_i,Met_j} \cdot \frac{C_H}{K_{P,H}} \quad \text{(Eq. 16)}$$

Equations for metabolite (a) of substrates

Central compartment

$$V_C \ \frac{dC_C}{dt} = Q_H \ \frac{C_H}{K_{P,H}} + k_{21} \ X_{Peri} - (Q_H + CL_{12} + CL_R) \ C_C \quad \text{(Eq. 17)}$$

Peripheral compartment

$$\frac{dX_{Peri}}{dt} = -k_{21} \ X_{Peri} + CL_{12} \ C_C \quad \text{(Eq. 18)}$$

Intestinal compartment

$$\frac{dX_{Intestine}}{dt} = k_{CYP3A,Met_a,parent} \ X_{Transit\_intes,parent} - k_a \ X_{Intestine} \quad \text{(Eq. 19)}$$

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Liver compartment

$$V_H \frac{dC_H}{dt} = Q_H \cdot \left(C_C - \frac{C_H}{K_{P,H}} \right) - \sum_i f_B CL_{CYP_i} \cdot \frac{C_H}{K_{P,H}} + \sum_i f_B CL_{CYP_i, Met_a, parent} \cdot \frac{C_{H,parent}}{K_{P,H}}$$

(Eq. 20)

Equations for inhibitors

Central compartment

$$V_C \frac{dC_C}{dt} = Q_H \frac{C_H}{K_{P,H}} + k_{21} X_{Peri} - (Q_H + CL_{12} + CL_R) C_C$$

(Eq. 21)

Peripheral compartment

$$\frac{dX_{Peri}}{dt} = -k_{21} X_{Peri} + CL_{12} C_C$$

(Eq. 22)

Intestinal and intestinal transit compartment

$$\frac{dX_{Transit\_intes}}{dt} = -k_{a,transit} X_{Transit\_intes}$$
$$\frac{dX_{Intestine}}{dt} = k_{a,transit} X_{Transit\_intes} - \frac{k_a}{F_a F_g} X_{Intestine}$$

(Eqs. 23 and 24)

Liver compartment

$$V_H \frac{dC_H}{dt} = Q_H \cdot \left(C_C - \frac{C_H}{K_{P,H}} \right) - f_B CL_{int} \cdot \frac{C_H}{K_{P,H}}$$

(Eq. 25)

Parameter settings

The following physiological and pharmacokinetic parameters were fixed throughout the analyses: Q_H, V_H, dose, and F_aF_g. F_aF_g was calculated with the following equation (Table 3):

$$F_a F_g = F \cdot \frac{Q_H}{Q_H - (CL_{tot} - CL_R)}$$

(Eq. 26)

If calculated F_aF_g was larger than 0.95, we assumed F_aF_g was equal to 1. $K_{p,h}$ of inhibitors were fixed to predicted values by reported methods (Rodgers et al., 2005; Rodgers and Rowland, 2006) using clogP and pKa obtained with Scifinder Scholar (Chemical Abstracts Service, Columbus, OH). All the other parameters were estimated by CNM. The initial ranges of V_c were set as 0.0817–7.43 L/kg since the lower boundary was set to be 10% higher than the lowest limit of parameter value (blood volume =

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0.0743 L/kg) (Davies and Morris, 1993) to avoid the infinite transformed initial value (Eq. 27) when initial parameter is equal to its lower limit, except for fluoxetine, desipramine and imipramine (0.743–74.3 L/kg), where large elimination half-lives were observed. The initial ranges of k_a were set as 0.2–6 hr^{-1}, considering gastric emptying rate of 6 hr^{-1} (Ito K et al., 1998). The same ranges were used for $k_{transit}$. The initial ranges of f_BCL_{int} were set as 1/10–10-folds of the total body clearance of substrates (control group) or inhibitors. When the peripheral compartment is needed to reproduce observed concentration-time profiles, the same ranges were used for CL_{12} and k_{21} as f_BCL_{int}. For fluconazole, larger ranges of CL_{12} and k_{21} were needed to reproduce clinical observations. These ranges were also used for f_BCL_{int}, CL_{12}, k_{21}, and $CL_{R,int,app}$ (if renal clearance is unknown) of substrate metabolites, except for EXP3194 (a metabolite of losartan) and noroxycodone (a metabolite of oxycodone), where smaller ranges were needed to reproduce clinical observations. 0.03–30 were used as the initial ranges for the ratios of CYP enzyme-selective pathways to the other pathway in the overall eliminations of substrates, or the ratios of the substrate metabolites formations to the other pathway in the CYP enzyme-selective pathways, except for desipramine, flurbiprofen, imipramine, or oxycodone, where higher ranges (0.3–300) were needed to reproduce clinical observations. The initial ranges of $K_{p,h}$ were set as 0.03–30. The initial ranges of $K_{i,total}$ were set as 1000-folds containing maximum blood concentrations of inhibitors. The initial ranges of R_{MBI} - 1 were set as 1–100. The initial ranges of $R_{intes,3A}$ - 1 for quinidine or fluconazole were set as 0.03–30 or 0.1–100, respectively. Fixed parameter values and initial parameter ranges were summarized in Table 3 and Supplementary Tables S.01–S.17.

Transformations of parameters

In order to apply limitations in parameter values, the following parameter transformation was performed:

$$X = \ln\left(x - x_{limits,min}\right) \quad \text{(Eq. 27)}$$

where x, X, and $x_{limits,min}$ denote original parameters, transformed parameters, and lower limits of original parameters (different from minimum values in parameter ranges), respectively. After parameter optimization using transformed parameters, original parameter values and standard deviations were calculated using the following equations.

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$$x = \exp X + x_{limits,min} \quad \text{(Eq. 28)}$$

All parameters had $x_{limits,min}$ of 0, except for V_c (0.074 L/kg; blood volume (Davies and Morris, 1993)).

Parameter estimations with CNM

CNM was constructed previously (Yoshida et al., 2013; Aoki et al., 2014) and used in the parameter estimations in this study, using objective values summarized in Tables 1 and 2. Briefly, a group of initial parameter sets (1000 or 2000 virtual parameter sets for the analyses of inhibitor or substrate pharmacokinetics, respectively) was prepared with a random sampling from given parameter ranges. The linear approximations of the projections from one group of parameter sets ($\mathbf{X_b}$) into objective values generated the next group ($\mathbf{X_a}$). We calculated internally dividing point X_i with the ratio of dS:(1-dS), and applied the same inverse matrix to obtain new estimated parameters $\mathbf{X_a'}$. Value of dS was arbitrary set as 0.5. Parameter sets for the next iteration were obtained by randomly selecting $\mathbf{X_a}$ or $\mathbf{X_a'}$ for each virtual sample. 10 or 15 iterations of this process yielded a group of optimized parameter sets in the analyses of inhibitor or substrate pharmacokinetics, respectively. Sum of squares of log residuals for the objective function **Y** ($SS_{log,Y}$, AUC or A_e as summarized in Table 1 and 2) were calculated in each iteration to evaluate goodness of fit with the following equation:

$$SS_{log,Y} = \sum \left(\ln \frac{Y_{simulated}}{Y_{observed}} \right)^2 \quad \text{(Eq. 29)}$$

After completing the estimations of parameters, the concentration time-profiles were compared with the observed profiles, using sum of squares of log residuals ($SS_{log,time}$):

$$SS_{log,time} = \sum \left(\ln \frac{C_{simulated}}{C_{observed}} \right)^2 \quad \text{(Eq. 30)}$$

where $C_{simulated}$ and $C_{observed}$ represent the simulated or observed blood concentrations at each time point. Reliability of parameter estimates was assessed by summary statistics of 30 parameter sets reproducing concentration-time profiles with low $SS_{log,time}$.

Computations

Parameter estimations with CNM, including the use of ODE15S function to numerically solve

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ordinary differential equations, were performed under MATLAB software environments using a desktop computer (CPU: Core i7-870 2.93GHz ×1, OS: Windows 7 SP1 32 bit, RAM: 4GB, MATLAB version: 8.0.0) or a workstation (CPU: XeonE5-1620 3.60GHz ×1, OS: CentOS 6.4 64 bit, RAM: 16GB, MATLAB version: 8.1.0).

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Results

Collection of information of DDIs and metabolic enzymes that interact with substrates or inhibitors

Twenty-four cases of clinical DDIs from 17 reports with eight inhibitors (Tables 1 and 2), in which pharmacokinetic alterations of the substrate drug metabolites as well as substrate parent drugs were reported, were collected with DIDB (Hachad et al., 2010). AUCR of substrates ranged from 1.11 to 10.6 [mean: 2.49, median: 1.79]. Literature review for in vitro data using DIDB suggests formations of substrate metabolites quantified in the DDI reports were mediated by specific CYP enzyme, except for the formations of 7-hydroxy chlorpromazine (CYP1A2 and 2D6), 5-hydroxy lansoprazole (CYP2C19 and 3A), 5-hydroxy omeprazole (CYP2C19 and 3A), and EXP3194 (CYP2C9 and 3A). Evaluation of in vitro inhibition potency of inhibitors by static model with in vitro inhibition parameters and clinical inhibitor concentration in plasma (see Methods section) suggested that there were 4, 6, 8, and 13 DDI cases involving CYP2C9, CYP2C19, CYP2D6, and CYP3A as putative enzymes (Table 2). It also suggests that the inhibitor affects the activity of only one CYP enzyme in the liver in all the collected DDIs, except for the effect of fluconazole on losartan elimination (CYP2C9 and CYP3A) and fluoxetine on imipramine elimination (CYP2C19, CYP 2D6, and CYP3A). Effects of clarithromycin on CYP3A, fluoxetine on CYP2C19 and CYP3A, or paroxetine on CYP2D6 were reported to involve MBI, whereas others involve reversible inhibitions.

Analyses of inhibitor pharmacokinetic profiles with PBPK models

Blood concentration-time profiles of inhibitors in collected DDIs (Table 1) were analyzed using PBPK models (Fig. 1a). 1000 or 2000 parameter sets reproducing the objective values (minimizing $SS_{log,Y}$) were obtained with CNM. Among these, parameter sets that could reproduce time profiles of inhibitor blood concentrations (minimizing $SS_{log,time}$) were obtained (Supplementary Tables S.01–S.06, Supplementary Figs. S.01–S.06). Geometric coefficient of variation (CV) of $f_B CL_{int}$ was small (less than 20%) for all inhibitors after the parameter estimations using AUC_{inf} as an objective function, particularly when single dose pharmacokinetics were analyzed. In the case of fluconazole, for which time profiles after oral and intravenous administrations were simultaneously analyzed, geometric CV

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of F_aF_g was small (2.14 %). Geometric CV of most of the other parameters were large (>100% in many cases), suggesting that point estimates of these parameters were not possible only from clinical DDI data.

Analyses of the effects of inhibitors on pharmacokinetic profiles of substrates with PBPK models

Effects of inhibitors on blood concentration-time profiles of substrates in collected DDIs (Table 2) were analyzed using PBPK models (Fig. 1b), with or without including substrate metabolite pharmacokinetic profiles in the analyses. Urinary accumulation of substrate metabolites was analyzed when systemic exposure data were not available (hydroxyl metabolites of imipramine and desipramine). 1000 or 2000 parameter sets reproducing the objective values (minimizing $SS_{log,Y}$) were obtained with CNM. Among these, parameter sets that could reproduce time profiles of the blood concentrations of substrate parent drug and substrate metabolite (minimizing $SS_{log,time}$) were obtained (Fig. 2, Supplementary Tables S.07–S.17, Supplementary Figs. S.07–S.17). Geometric CV of f_BCL_{int} values were small for all cases of analyses (mostly around or less than 20%), regardless of whether or not the information of substrate metabolite was included in the analyses. In 17 out of 24 cases, inclusion of substrate metabolite information improved parameter estimation for K_i and f_m, as suggested by the smaller geometric CV of parameter estimates (Fig. 3). Conversely, inclusion of substrate metabolite information in the analyses had smaller effects on the accuracy of estimated f_m and K_i in DDIs between chlorpromazine and quinidine, lansoprazole and fluvoxamine, losartan and fluconazole, or omeprazole and fluvoxamine. Geometric CV of most of the other parameters were large (>100% in many cases), suggesting that point estimates of these parameters were not possible.

Cross-study comparison and comparison with in vitro estimates for f_m

Among the DDI cases for which inclusion of substrate metabolite information improved reliability of K_i or f_m estimates (Fig. 3a), f_m of substrates and K_i of inhibitors were compared to each other (Table 4, Fig. S18). Estimated values of f_m under multiple conditions (different inhibitors or doses of inhibitors) were consistent for desipramine, flurbiprofen, imipramine, and oxycodone (Table

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4, Fig. S18). On the other hand, f_m values of fentanyl estimated from two different DDI cases were not equivalent. Estimated f_m of one CYP enzyme in the overall eliminations of substrates were also in fair agreement with in vitro estimates, both for enzymes with large contribution (CYP2C9 for flurbiprofen (Yamazaki et al., 1998), CYP2D6 for desipramine (McGinnity et al., 2008), and CYP3A for oxycodone (Lalovic et al., 2004)) and with small–to–moderate contribution (CYP2D6 for hydrocodone (Hutchinson et al., 2004), imipramine (McGinnity et al., 2008), or oxycodone (Lalovic et al., 2004), and CYP3A for fentanyl (Guitton et al., 1997), lansoprazole (Naritomi et al., 2004), or ropivacaine (Ekstrom and Gunnarsson, 1996)).

Cross-study comparison and comparison with in vitro or previous PBPK estimates for K_i

Estimated in vivo K_i in the studies where metabolite information improved parameter estimates were compared to each other and to in vitro K_i. Since $K_{i,total}$ in the PBPK model was defined against hepatic total (bound + unbound) inhibitor concentration, in vivo unbound K_i was calculated for comparison as $f_B \times (K_{i,total}/K_{p,H})$, assuming that unbound inhibitor concentration in hepatocyte is the same as unbound hepatic blood concentration ($K_{p,H} = f_B/f_T$). As for the same combinations of inhibitors and CYP enzymes, estimated K_i by the analyses of different DDIs showed similar values among the different reports (Fig. S18, Table 4). In all cases where comparison was possible, inter-study variation of obtained K_i were narrower than the reported ranges obtained from the previous PBPK analyses of clinical DDIs (Kato et al., 2008). Obtained K_i of fluconazole for CYP2C9 or CYP3A and voriconazole for CYP3A were comparable to the median of collected in vitro K_i, while K_i of fluoxetine for CYP2D6, itraconazole on CYP3A, and quinidine for CYP2D6 were 100–1000-fold lower than in vitro K_i.

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Discussion

In this study, we aimed to accurately estimate two most important parameters, K_i and f_m, in determining the degrees of pharmacokinetic DDIs (Brown et al., 2005; Obach et al., 2006), based on clinical DDI data where pharmacokinetic alterations of not only substrates but also substrate metabolites has been analyzed. Since some substrate metabolites are produced by a specific enzyme, we hypothesized that utilization of substrate metabolite's pharmacokinetic profiles can improve an estimation of the effect of inhibitors on each CYP enzyme in vivo, leading to accurate estimations of above two parameters.

Firstly, the pharmacokinetic parameters for the inhibitors were determined. Then, using the fixed parameter sets, PBPK analyses of substrate parent drugs and/or substrate metabolites were performed. As shown in Figure 2, multiple solutions, *i.e*, parameter sets, could account for the time profiles of the blood concentration of substrates and their metabolites. Some parameters are convergent across the solutions, whereas additional information or constrain is necessary for the other parameters to be convergent.

f_BCL_{int} of both substrates and inhibitors were estimated with small geometric CV among these 30 parameter sets (Supplementary Figs. S.01–S.17). Since we estimated the parameters to reproduce AUC_{inf} or AUC with CNM, small geometric CV of f_BCL_{int} in all the substrates and inhibitors were reasonable. In contrast, estimated f_BCL_{int} of substrate metabolites showed large geometric CV (>100 % in most cases). Because exposure of substrate metabolites is determined not only by their elimination rate, but also by the formation rate, multiple solutions are allowed to account for the AUC of the substrate metabolites.

As we hypothesized, estimated f_m and K_i values showed smaller geometric CVs for many (17 out of 24) studies when the pharmacokinetics of both parent drugs and metabolites of substrates were analyzed, compared with those estimated when only pharmacokinetics of parent substrates were analyzed (Fig. 3). These parameters were reliably estimated even from DDIs in which parent AUCR<1.5 (fentanyl-fluconazole or voriconazole, hydrocodone-quinidine, oxycodone-quinidine or paroxetine, ropivacaine-itraconazole), suggesting that the substrate metabolite can be a novel source of information for DDI analyses. K_i obtained from the analyses of multiple clinical DDIs showed small variation across the reports as far as the same combinations of inhibitors and target CYP enzymes are

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examined (Fig. 4, Table 4, Fig. S18). Moreover, similar K_i values of fluoxetine were obtained in the clinical studies where fluoxetine was given by single- and multiple-doses (Fig. 4). Such small inter-study variations in estimated f_m and K_i suggest the extrapolatability of these parameters to untested scenarios (different substrate/inhibitor/dosing regimen) and may guarantee the reasonable prediction of DDIs in future studies.

On the other hand, this approach has some limitations. For several DDIs, inclusions of substrate metabolite information in the analyses could not substantially contribute to reduce geometric CV in estimated f_m and K_i, such as DDIs between chlorpromazine and quinidine, lansoprazole and fluvoxamine, losartan and fluconazole, or omeprazole and fluvoxamine. The common characteristics of these DDIs are that multiple isoforms of CYP enzymes catalyze the formation of the substrate metabolites. Thus, uncertainty in the contribution of enzymes may inhibit convergence of f_m and K_i and additional information is needed, such as contribution of each CYP isoform to substrate metabolite formation.

The estimated K_i values were compared with those reported by Kato et al. (Kato et al., 2008), and those determined in vitro (Fig. 4). Inter-study variabilities in estimated K_i were narrower than those obtained by conventional PBPK analyses. When compared to in vitro K_i, estimated values for two inhibitors (fluconazole and voriconazole) were comparable to the median of collected in vitro K_i (Fig. 4). For fluoxetine, itraconazole, and quinidine, estimated K_i were much lower than the in vitro K_i (Fig. 4). For these inhibitors, predictions of DDIs using in vitro K_i would result in underestimations of the degree of DDIs, as pointed out previously (Isoherranen et al., 2004; Lutz and Isoherranen, 2012).

Kato et al. previously suggested that in vivo K_i were generally smaller than in vitro experimental K_i, whose discrepancies apparently depend on the lipophilicity of substrates. In our analyses, especially for highly-lipophilic itraconazole, our obtained K_i were closer to in vivo K_i reported by Kato et al. rather than in vitro K_i. Inconsistence of in vitro and in vivo K_i values may be attributable to the following mechanisms; additional contribution of P-gp inhibition (Benet et al., 2004), inaccurate estimations of blood unbound fractions of inhibitors, or incubation buffer for in vitro experiments (Thompson et al., 1988; Arredondo et al., 1999; Templeton et al., 2008), extensive accumulation of

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inhibitors into hepatocytes as seen in itraconazole (Yamano et al., 1999), or additional inhibition by inhibitor metabolites (Otton et al., 1993; Ching et al., 1995; Isoherranen et al., 2004; Templeton et al., 2008; Isoherranen et al., 2009; Lutz and Isoherranen, 2012). In the reports cited in this study, plasma concentrations of inhibitor metabolites were not measured, and the contribution of inhibitor metabolites was not taken into account in the PBPK analysis. Including such information may partly address the observed inconsistencies. Furthermore, due to limited number of available data for each inhibitor with different experimental conditions, we did not exclude in vitro studies that unbound fraction of inhibitors in the buffer was not measured, which can partly explain an inter-study variation of in vitro K_i values (Fig. 4). Interestingly, the lowest in vitro K_i of itraconazole (1 nM) was obtained in an experiment with low microsome concentration in the incubation buffer (0.025mg/ml) after correction with unbound fraction (Isoherranen et al., 2004). This result appears to support the importance of accurately estimating effective inhibitor concentration in the incubation buffer. For CYP3A, it is also possible that K_i of inhibitors sometimes depend on substrates tested (Fowler and Zhang, 2008). We will be able to partly bridge the gap between in vitro and in vivo K_i for more accurate predictions of clinical DDIs with carefully optimizing in vitro experimental approaches.

To predict the impact of DDIs on the pharmacokinetics of new investigational drugs, the contribution of enzymes and transporters must be evaluated accurately. Our results indicated that f_m values determined by our approach under multiple conditions were estimated to be consistent in most cases (4 out of 5 compounds with multiple DDI studies available for comparison, Table 4, Fig. S18), supporting the reliability of in vivo f_m values of substrates obtained by us. As for fentanyl whose estimated f_m values depended on the reported DDI cases, the original DDI study reported similar magnitude of change in parent and metabolite AUC between the voriconazole and fluconazole, while concentration-time profiles of norfentanyl appeared to be different (Saari et al., 2008). This inconsistency might have led to different estimates of f_m in two DDI cases.

We must be careful about quantitatively extrapolating in vitro observations into in vivo parameters for new investigational drugs. There are two major difficulties in such extrapolations. The first one is that contribution of each CYP enzyme in vitro were estimated by investigating the formation of one or a few metabolite(s), but not the disappearance of substrates, in most of these

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studies. Theoretically, to accurately estimate overall f_m from metabolite formation data, one has to measure formation of all the metabolites. In particular, when using liver microsomes, contribution of enzymes located in cytosolic fraction or those whose enzymatic reaction requires certain cofactor(s) (e.g. phase II enzymes) cannot be considered, causing over-estimation of the contribution of CYP enzymes. Another difficulty is that the activity of each CYP enzyme can be highly dependent on study design, such as selection of microsomes (pooled batch, individual batch) and selection of medium including phosphate concentration (Crespi, 1998).

These aspects reinforce the importance of reliably establishing in vivo f_m for developing optimal in vitro experimental conditions. In an ideal scenario of drug development, it is also important to consider the outcome of mass-balance study for realizing a definitive in vivo f_m estimates, but only a few data are available at least in the public domain at this moment.

Our findings suggest an additional factor to consider for selecting probe substrates for clinical DDI studies to improve PBPK model-based parameter estimation. We showed that specificity of enzymes involved in the formation of the metabolites was important in improving reliability of f_m and K_i estimates. Current regulatory guidance/guidelines on pharmacokinetic drug interaction recommend the conduct of clinical DDI studies with in vivo probe substrates with predominant contribution of a single enzyme to their overall elimination (USFDA, 2012; EMA, 2013; PMDA, 2014). In order to accurately determine an interaction potential of a new investigational drug as an inhibitor, selection of substrates with metabolites formed by the specific enzyme of interest (e.g. substrates listed in Fig. 3a) can be important, in addition to other considerations such as likelihood of co-medication.

In conclusion, this study demonstrated the importance of considering pharmacokinetic alterations of substrate metabolites as well as substrate parent drugs in accurate determinations of in vivo K_i and f_m using PBPK modeling. The obtained K_i values are expected to increase the accuracy of the predicted degrees of DDIs for untested combinations of substrates and inhibitors.

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Author Contributions

Wrote Manuscript: Yoshida, Maeda, Kusuhara

Designed Research: Yoshida, Maeda

Performed Research: Yoshida, Maeda

Analyzed Data: Yoshida, Maeda, Kusuhara

Contributed New Reagents/Analytical Tools: Konagaya

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Conflict of Interest

The authors have no conflict of interest to declare.

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Figure Legends

Fig. 1 PBPK models for the simulations of blood concentration-time profiles of inhibitors (a) or substrates and their metabolites (b).

CL_{int}, hepatic intrinsic clearance; CL_R, renal clearance; CL_{12}, transport clearance from central to peripheral compartment; D_{IV}, intravenous dose; D_{PO}, oral dose; f_B, protein unbound fraction in blood; k_a, absorption rate constant; $K_{p,h}$, liver-to-blood concentration ratio; k_{LI}, transit rate constant to the large intestine; $k_{transit}$, transit rate constant to the small intestine; k_{21}, transport rate constant from peripheral to central compartment; Q_H, hepatic blood flow rate.

Fig. 2 Simulated and reported blood concentration-time profiles (a, b) and estimated parameter distributions (c, d) after the analyses of DDI between flurbiprofen and fluconazole [Hanley et al, 2013], with (a, c) or without (b, d) including substrate metabolites' pharmacokinetic alterations as a typical example of the results of the analyses, and list of parameters estimated by CNM (e).

(a, b) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing objective functions (AUC or Ae as summarized in Tables 1 and 2) and top three parameter sets best reproducing concentration-time profiles, respectively (see "Parameter estimations with CNM" in the Methods section). Orange circles represents observed time profiles. (c, d) Blue and yellow lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively. (e) List of parameters estimated by CNM with corresponding ID numbers (ID1: parameters for analysis with substrate metabolite, ID2: parameters for analysis without substrate metabolite). Parent: flurbiprofen, Metabolite 1: 4'-hydroxy flurbiprofen, Inhibitor: fluconazole. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, renal apparent intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; D_{IV}, intravenous dose; D_{PO}, oral dose; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver-to-blood concentration ratio; k_{LI}, rate constant to the large intestine; $k_{transit}$, transit rate constant to the small intestine; k_{21}, rate constant from peripheral to central compartment; PBPK, physiologically-based pharmacokinetic; Q_H, hepatic blood flow rate.

Fig. 3 Comparison of the coefficient of variation for f_m and $K_{i,total}$ / R_{MBI} estimates, with or without including substrate metabolites' pharmacokinetic profiles in the PBPK analyses.

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(a) DDI cases for which inclusion of substrate metabolite information reduced geometric CV of these parameters by at least 2-fold, (b) DDI cases for which inclusion of substrate metabolite information did not reduce geometric CV. Closed circles and open triangles represent the geometric CV of f_m or $K_{i,total}/R_{MBI}$, estimated with and without including substrate metabolites' pharmacokinetic profiles, respectively. ID of studies analyzed corresponds to those listed in Table 2. CV, coefficient of variation; f_m, fraction metabolized by corresponding CYP isoforms; $K_{i,total}$, inhibition constant for total (bound + unbound) inhibitor concentration; R_{MBI}, degree of inhibition with mechanism-based inhibitors.

Fig. 4 Comparison of the estimated $K_{i,unbound}$ with reported values obtained with in vitro experiments or with conventional PBPK analyses of drug-drug interactions without substrate metabolites' pharmacokinetic information.

Circles represent $K_{i,unbound}$ for estimated parameter sets with 30 lowest $SS_{log,time}$ values with PBPK models including substrate metabolites' pharmacokinetic profiles. Each square represents reported in vitro $K_{i,unbound}$ in a report collected from the University of Washington Metabolism and Transport Drug Interaction Database (DIDB). Bars represent maximum, geometric mean, and minimum values of estimated $K_{i,unbound}$ by analyzing drug-drug interactions with PBPK models in the previous report by Kato et al. (Kato et al., 2008). $K_{i,unbound}$, inhibition constant with regard to unbound inhibitor concentration

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Table 1 List of inhibitors analyzed in this study

Drug	Administration	Objective function	Fig/Table#	Reference (PubMed ID)
clarithromycin[1]	-	-	-	-
fluconazole	Single intravenous / oral	AUC_{inf}	S.01	2540363
fluoxetine	Single / Multiple oral	AUC_{inf}	S.02.1 / S.02.2	1544284
fluvoxamine	Single oral	AUC_{inf}	S.03	8499580
itraconazole	Multiple oral	AUC_{inf}	S.04	2848442
paroxetine[1]	-	-	-	-
quinidine	Single oral	AUC_{inf}	S.05	7693389
voriconazole	Multiple oral	AUC_{inf}	S.06	16291712

Initial parameter settings and the results of the analyses are summarized in corresponding Figures and Tables.

[1] Plasma concentration-time profiles were not considered in the analyses of DDI and were not analyzed with PBPK models, since the inhibition of CYP enzymes involve mechanism-based inhibition. AUC, area under the plasma concentration-time curve; AUC_{inf}, AUC from time zero to infinity.

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Table 2 List of drug-drug interactions analyzed in this study

Substrates	Metabolites	Inhibitors	Putative enzyme(s) involved	AUCR	ID#	Fig/Table#	Objective function: Parent	Objective function: Metabolite	Reference (PubMed ID)
chlorpromazine	7-hydroxy chlorpromazine	quinidine (166 mg)	CYP2D6, CYP3A	1.40	1	S.07	AUC_{inf}	AUC_{inf}	8739822
desipramine	2-hydroxy desipramine	fluoxetine (60 mg)	CYP2D6	2.58 [a]	2	S.08.1	AUC_{inf}	A_e	1544284
				10.6 [b]	3	S.08.2			
fentanyl	norfentanyl	fluconazole (200 mg)	CYP3A	1.26	4	S.09.1	AUC_{inf}	AUC_{inf}	17987285
		voriconazole (200 mg)	CYP3A	1.39	5	S.09.2			
flurbiprofen	4'-hydroxy flurbiprofen	fluconazole (200 mg)	CYP2C9	1.75	6	2 / S.10.1	AUC	AUC	22943633
				2.02	7	S.10.2			23047652
hydrocodone	hydromorphone	quinidine (83 mg)	CYP2D6	1.21	8	S.11	AUC_{inf}	AUC_{inf}	7693389
imipramine	2-hydroxy imipramine, desipramine	fluoxetine (60 mg)	CYP2D6, CYP2C19, CYP3A	2.08 [a]	9	S.12.1	AUC_{inf}	AUC_{inf} / A_e	1544284
				3.56 [b]	10	S.12.2			
lansoprazole	5-hydroxy lansoprazole, lansoprazole sulfone	fluvoxamine (25 mg)	CYP2C19	4.00 [c]	11	S.13.1	AUC_{inf}	AUC_{inf}	15496639
				2.50 [d]	12	S.13.2			
		clarithromycin (400 mg)	CYP3A	1.38 [c]	13	S.13.3			15752376
				1.76 [d]	14	S.13.4			
				1.81 [e]	15	S.13.5			
losartan	EXP-3174	fluconazole (200 mg)	CYP2C9, CYP3A	1.69	16	S.14.1	AUC_{inf}	AUC_{inf}	9357393
				1.27	17	S.14.2			9551703
omeprazole	5-hydroxy omeprazole, omeprazole sulfone	fluvoxamine (25 mg)	CYP2C19	5.62 [c]	18	S.15.1	AUC_{inf}	AUC / AUC_{inf}	15025747
				2.38 [d]	19	S.15.2			
oxycodone	noroxycodone, oxymorphone	quinidine (166 mg)	CYP2D6	1.13	20	S.16.1	AUC_{inf}	AUC / AUC_{inf}	9871425
		voriconazole (200 mg)	CYP3A	3.57	21	S.16.2			18836708
		itraconazole (200 mg)	CYP3A	2.43	22	S.16.3			20076952
		paroxetine (20 mg)	CYP2D6	1.11	23	S.16.4			20642550
ropivacaine	(S)-2',6'-Pipecoloxylidide	itraconazole (200 mg)	CYP3A	1.23	24	S.17	AUC_{inf}	AUC	11322176

Initial parameter settings and the results of the analyses are summarized in corresponding Figures and Tables.

References after 51 can be found in Supplementary Data S2.

AUC, area under the plasma concentration-time curve; AUC_{inf}, AUC from time zero to infinity; A_e, accumulated amount excreted into urine. [a]Fluoxetine single dose, [b]Fluoxetine multiple dose, [c]CYP2C19 extensive metabolizer (EM), [d]CYP2C19 intermediate metabolizer (IM), [e]CYP2C19 poor metabolizer (PM).

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Table 3 Summary of calculated pharmacokinetic parameters fixed in PBPK analyses

Substrates	$K_{p,h}$[1]	F_aF_g	R_B	f_B	References (PubMed ID)
chlorpromazine	-[2]	0.685	0.78	-	10534321, [6]
desipramine	-[2]	1.03[3]	0.89	-	3365915
fentanyl	-[2]	-	1[4]	-	6121896
flurbiprofen	-[2]	>1[3]	0.56	-	[7]
hydrocodone	-[2]	1[4]	1[4]	-	
imipramine	-[2]	0.97	1.1	-	6429693, 10534321
lansoprazole	-[2]	1.97[3]	0.56	-	8803522, 20056146
losartan	-[2]	0.896	0.6[5]	-	8529329
omeprazole	-[2]	1.83[3]	0.58	-	3858978
oxycodone	-[2]	1.05[3]	1.3	-	19417618, 22798176
ropivacaine	-[2]	-	0.69	-	11322176
Inhibitors					
fluconazole	0.647	-[2]	1	0.89	18483837
fluoxetine	13.6	0.722	0.96	0.063	18483837
fluvoxamine	12.1	0.971	1	0.23	18483837
itraconazole	6.38	0.885	0.58	0.062	18483837, 17495874
quinidine	11.6	0.869	0.92	0.14	18483837
voriconazole	0.562	1	1[4]	0.42	

If not indicated, pharmacokinetic parameters were derived from the University of Washington Metabolism and Transport Drug Interaction Database (DIDB). [1]Predicted with the reported in silico methods [15, 16]. [2]Estimated in the following PBPK analysis. [3]F_aF_g calculated to be >1 was fixed as 1 for PBPK analysis. [4]Assumed to be equal to 1. [5]Assumed to be equal to 0.6. [6] Thummel K et al. Design and Optimization of Dosage Regimens: Pharmacokinetic Data. In*: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th ed*, (McGraw-Hill, 2010). [7] Tohno M et al. Pharmacokinetic and metabolic studies of LFP83 in man after single and repeated intravenous administration. Clin Rep (26), 83 (1992). $K_{Pp,h}$, liver-to-blood concentration ratio; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; R_B, blood-to-plasma concentration ratio.

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Table 4 Estimated parameter values for f_m and K_i for DDI cases

(a) f_m

Substrates	Inhibitors	Isoforms for parameters	ID#	Geometric mean	Geometric CV [%]
desipramine	fluoxetine	CYP2D6	2 [a]	0.995	0.578
			3 [b]	0.959	0.543
fentanyl	fluconazole	CYP3A	4	0.472	9.59
	voriconazole	CYP3A	5	0.732	12.8
flurbiprofen	fluconazole	CYP2C9	6	0.945	7.99
			7	0.964	5.91
hydrocodone	quinidine	CYP2D6	8	0.512	27.7
imipramine	fluoxetine	CYP2D6	9 [a]	0.697	3.26
			10 [b]	0.752	1.32
lansoprazole	clarithromycin	CYP3A	13 [c]	0.318	13.6
			14 [d]	0.483	8.46
			15 [e]	0.662	23.4
oxycodone	quinidine	CYP2D6	20	0.253	6.44
	voriconazole	CYP3A	21	0.826	1.23
	itraconazole	CYP3A	22	0.845	4.35
	paroxetine	CYP2D6	23	0.184	21.5
ropivacaine	itraconazole	CYP3A	24	0.338	6.16

(b) K_i

Inhibitors	Substrates	Isoforms for parameters	ID#	Geometric mean [μM]	Geometric CV [%]
fluconazole	fentanyl	CYP3A	4	7.73	14.9
	flurbiprofen	CYP2C9	6	17	4.64
			7	19.9	5.51
fluoxetine	desipramine	CYP2D6	2 [a]	0.000195	14.5
			3 [b]	0.000127	5.09
	imipramine		9 [a]	0.000177	7.9
			10 [b]	0.000149	4.17
itraconazole	oxycodone	CYP3A	22	0.000115	7.67
	ropivacaine		24	0.000103	3.45
quinidine	hydrocodone	CYP2D6	8	0.0000977	37.4
	oxycodone		20	0.000552	41.8
voriconazole	oxycodone	CYP3A	5	1.71	47.1
			21	0.535	17

The table only includes DDI cases for which inclusion of substrate metabolite information improved geometric CV (Fig 3a). Parameter estimates represent summary statistics of 30 parameter sets reproducing concentration-time profiles (low $SS_{log,time}$). Refer to Table 2 for details of DDI information with corresponding ID#. CV, coefficient of variation. [a]Fluoxetine single dose, [b]Fluoxetine multiple dose, [c]CYP2C19 extensive metabolizer (EM), [d]CYP2C19 intermediate metabolizer (IM), [e]CYP2C19 poor metabolizer (PM).

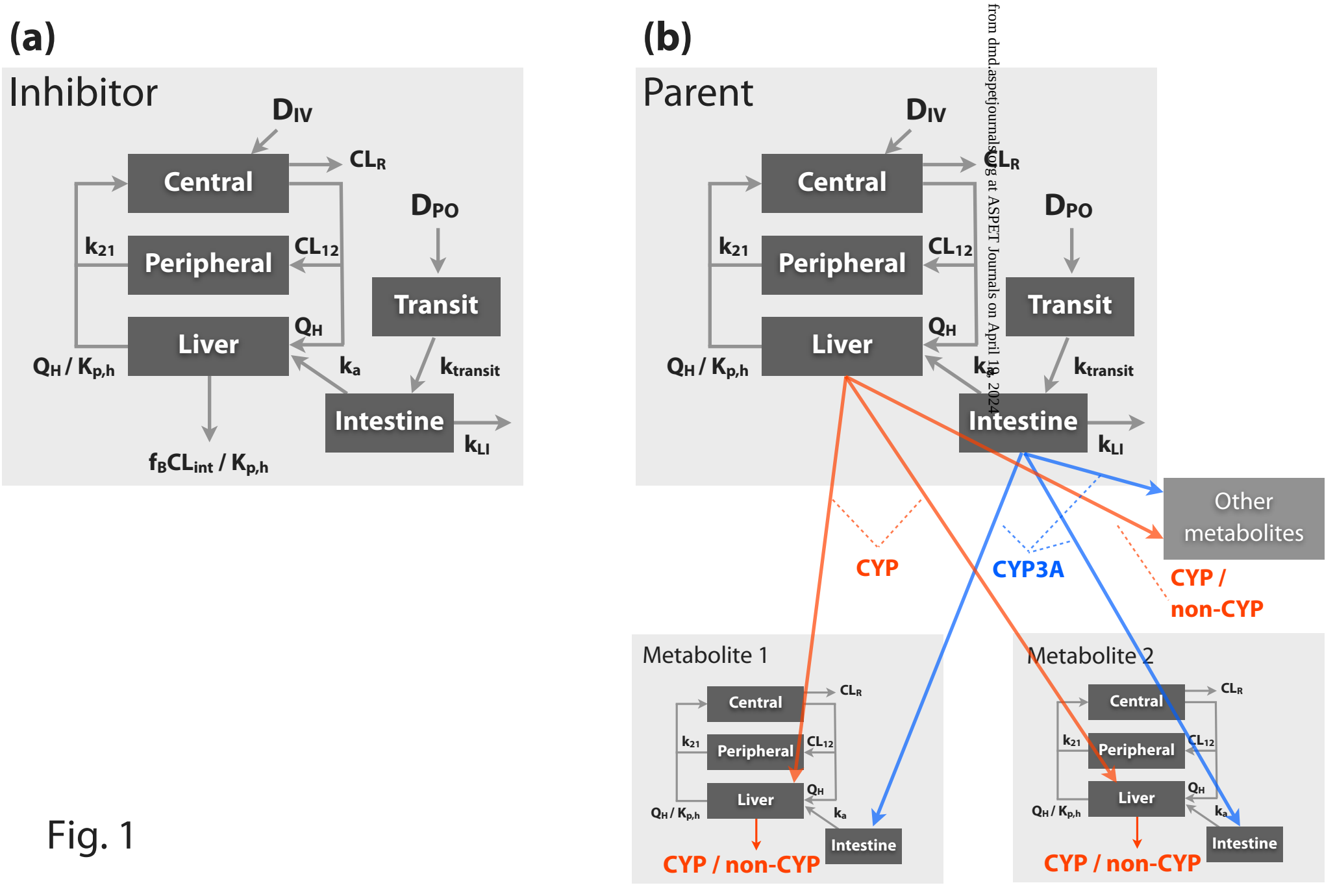
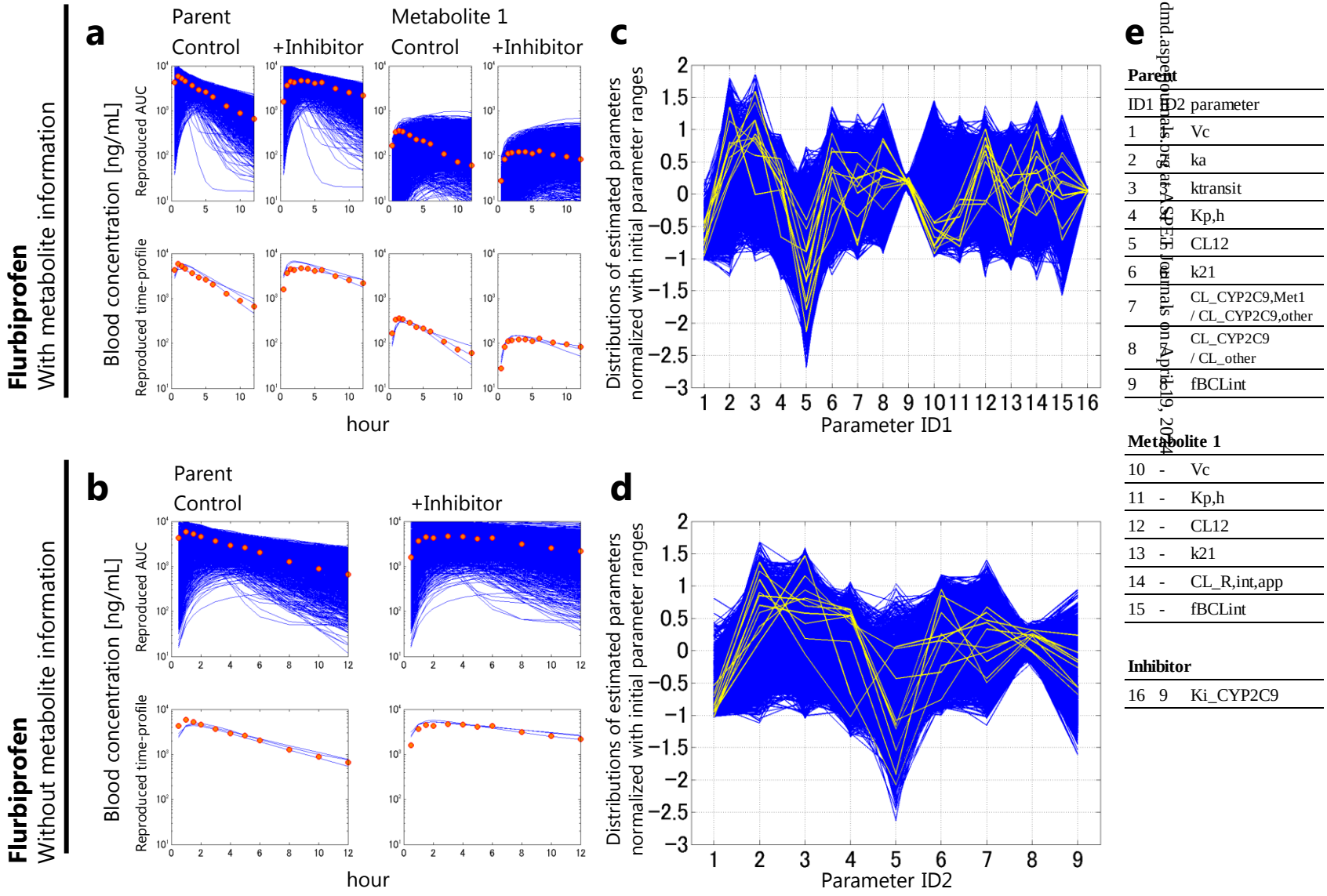


Fig. 1



e

Parent

ID1	ID2	parameter
1		Vc
2		ka
3		ktransit
4		Kp,h
5		CL12
6		k21
7		CL_CYP2C9,Met1 / CL_CYP2C9,other
8		CL_CYP2C9 / CL_other
9		fBCLint

Metabolite 1

10	-	Vc
11	-	Kp,h
12	-	CL12
13	-	k21
14	-	CL_R,int,app
15	-	fBCLint

Inhibitor

16	9	Ki_CYP2C9

Fig. 2

	Substrates	Inhibitors	Isoforms for parameters	ID#
(a)	desipramine	fluoxetine	CYP2D6	2
				3
	fentanyl	fluconazole	CYP3A	4
		voriconazole	CYP3A	5
	flurbiprofen	fluconazole	CYP2C9	6
				7
	hydrocodone	quinidine	CYP2D6	8
	imipramine	fluoxetine	CYP2D6	9
				10
	lansoprazole	clarithromycin	CYP3A	13
				14
				15
	oxycodone	quinidine	CYP2D6	20
		voriconazole	CYP3A	21
		itraconazole	CYP3A	22
		paroxetine	CYP2D6	23
	ropivacaine	itraconazole	CYP3A	24
(b)	chlorpromazine	quinidine	CYP2D6	1
	lansoprazole	fluvoxamine	CYP2C19	11
				12
	losartan	fluconazole	CYP2C9	16
			CYP3A	
			CYP2C9	17
			CYP3A	
	omeprazole	fluvoxamine	CYP2C19	18
				19

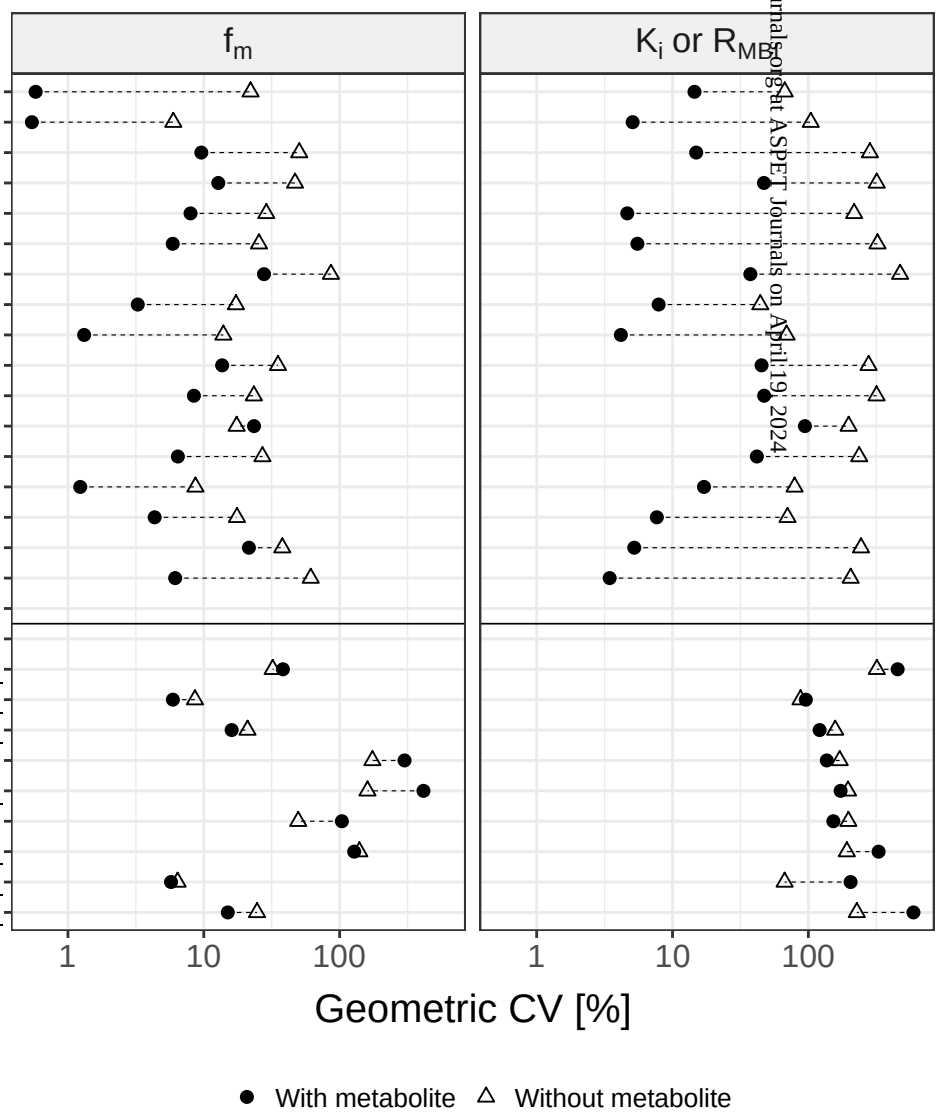


Fig. 3

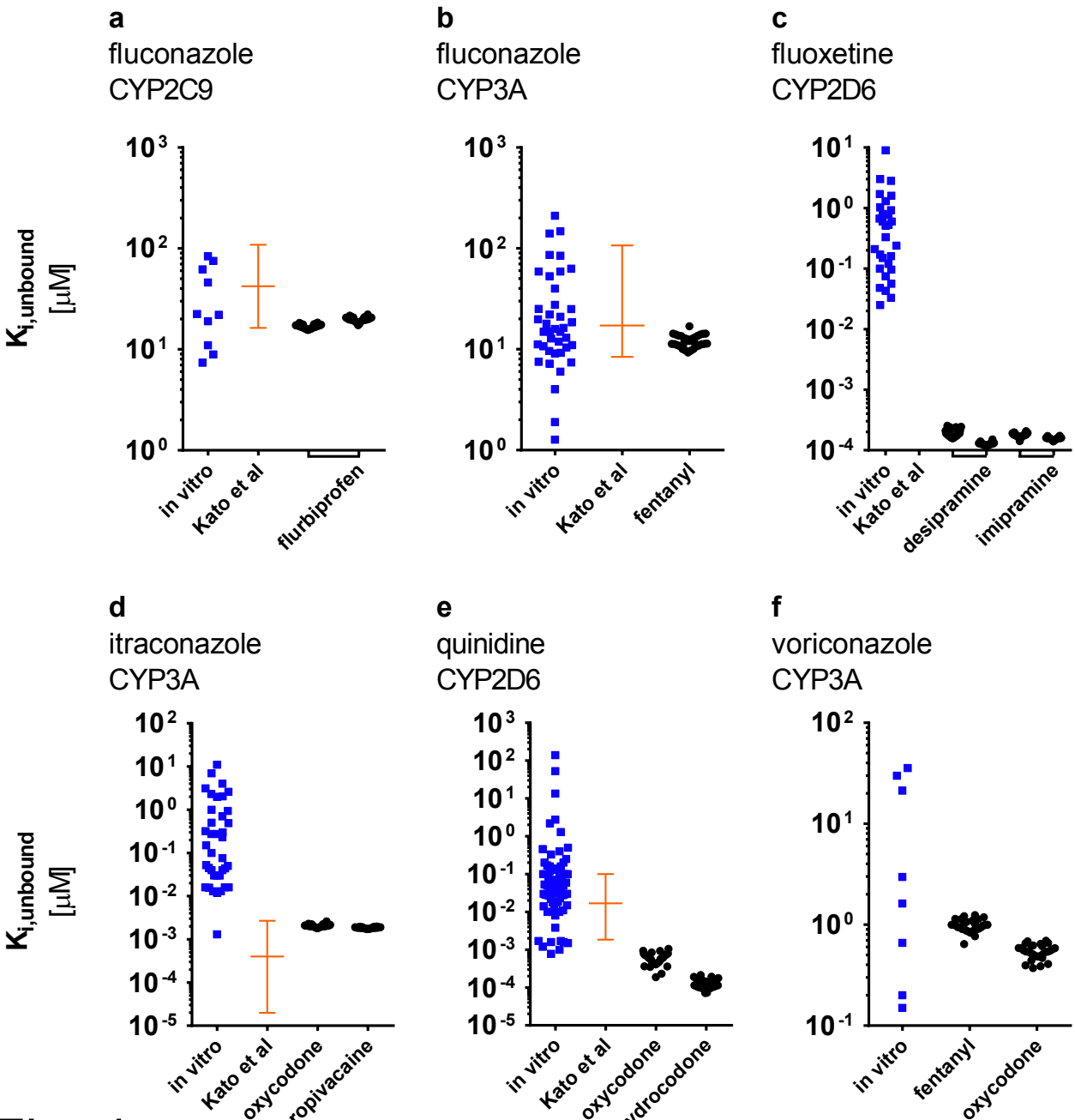

a
fluconazole
CYP2C9
b
fluconazole
CYP3A
c
fluoxetine
CYP2D6
d
itraconazole
CYP3A
e
quinidine
CYP2D6
f
voriconazole
CYP3A
Ki,unbound [μM]
in vitro
Kato et al
flurbiprofen
fentanyl
desipramine
imipramine
oxycodone
ropivacaine
hydrocodone

Fig. 4

Supplemental Data

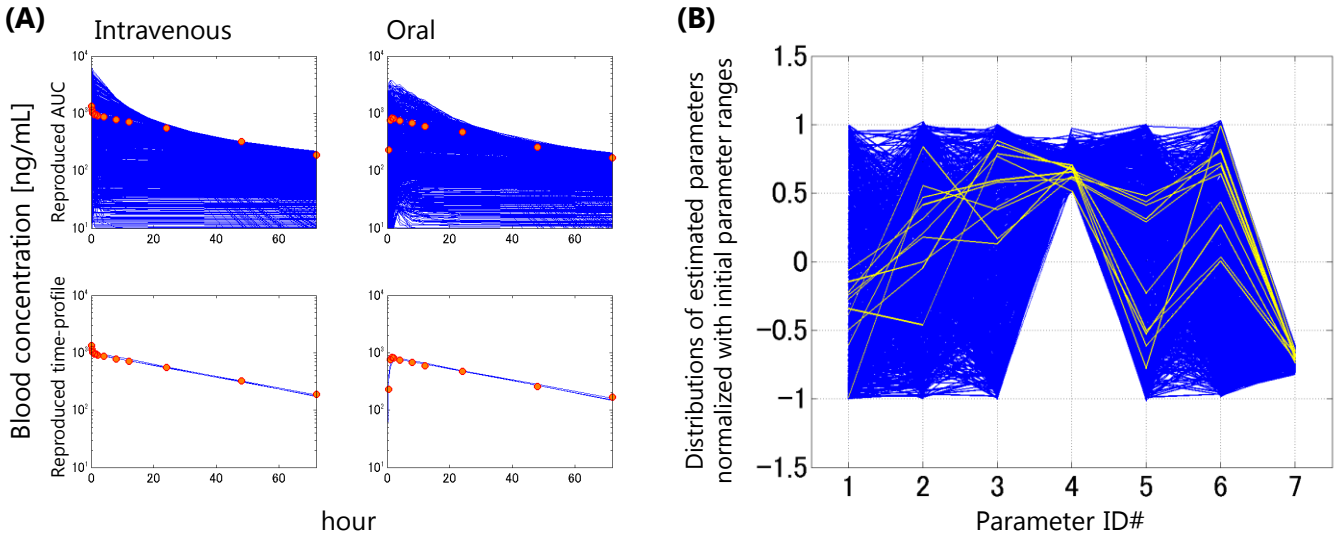
Kenta Yoshida, Kazuya Maeda, Akihiko Konagaya, and Hiroyuki Kusuhara, Accurate estimation of *in vivo* inhibition constants and fraction metabolized with physiologically-based pharmacokinetic models incorporating parent drugs and metabolites of substrates with cluster Newton method, Drug Metabolism and Disposition

Table S.01 Initial and estimated parameter values for fluconazole

			Parameter values			Final estimates	
			Fixed/	Free parameters		Geometric	Geometric
ID	parameter	unit		min	max	mean	CV [%]
1	Vc	L/kg		0.0817	7.43	0.318	73.3
2	ka	/hr		0.200	6.00	2.14	84.9
3	ktransit	/hr		0.200	6.00	1.91	77.8
4	FaFg	-		0.500	0.950	0.850	2.14
	Kp,h	-	0.647				
5	CL12	L/hr/kg		0.0601	6.01	0.442	240
6	k21	/hr		0.0601	6.01	1.674	140
	CL_R	L/hr/kg	0.0123				
7	fBCLint	L/hr/kg		0.00200	0.200	0.00404	5.54
	Dose	μg/kg	683				

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; CL_R, renal clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; V_c, distribution volume of central compartment.

Fig S.01 Simulated and reported blood concentration-time profiles (A) and estimated parameter distributions (B) after the analyses of intravenous and oral dose pharmacokinetics of fluconazole



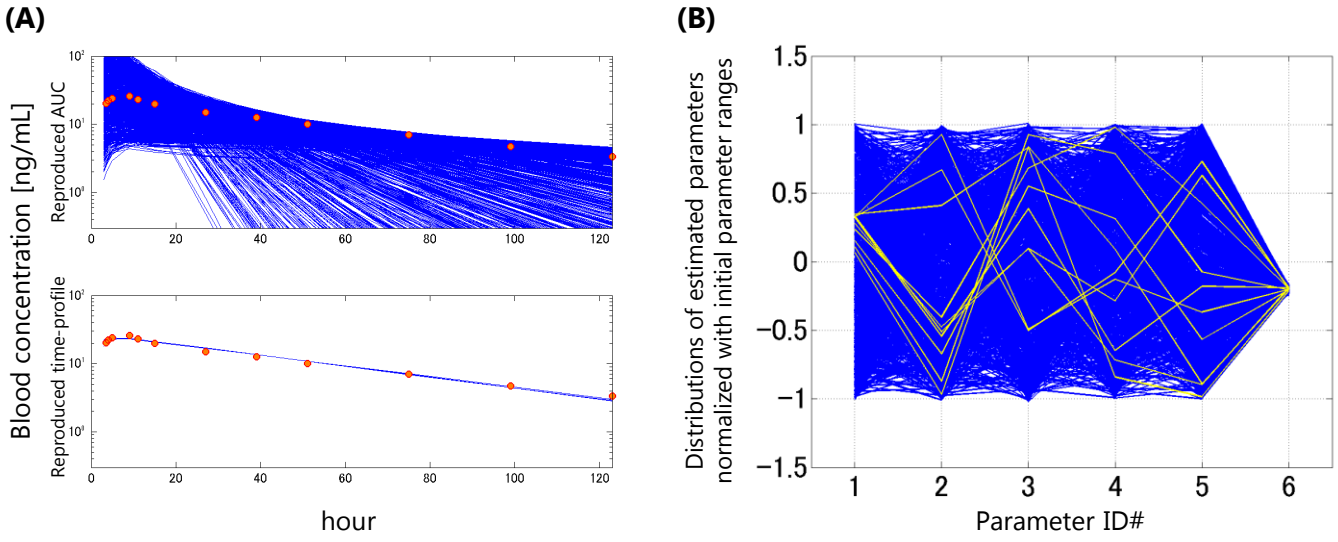
(A) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (B) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.02.1 Initial and estimated parameter values for fluoxetine after a single oral administration

			Parameter values			Final estimates	
			Fixed/	Free parameters		Geometric	Geometric
ID	parameter	unit		min	max	mean	CV [%]
1	Vc	L/kg		0.743	74.3	13.2	22.4
2	ka	/hr		0.200	6.00	1.09	127
3	ktransit	/hr		0.200	6.00	1.34	139
	FaFg	-	0.722				
	Kp,h	-	13.6				
4	CL12	L/hr/kg		0.0163	1.63	0.138	184
5	k21	/hr		0.0163	1.63	0.225	144
	CL_R	L/hr/kg	0.00771				
6	fBCLint	L/hr/kg		0.0163	1.63	0.140	3.59
	Dose	μg/kg	785				

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; CL_R, renal clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; V_c, distribution volume of central compartment.

Fig S.02.1 Simulated and reported blood concentration-time profiles (A) and estimated parameter distributions (B) after the analyses of single-dose pharmacokinetics of fluoxetine



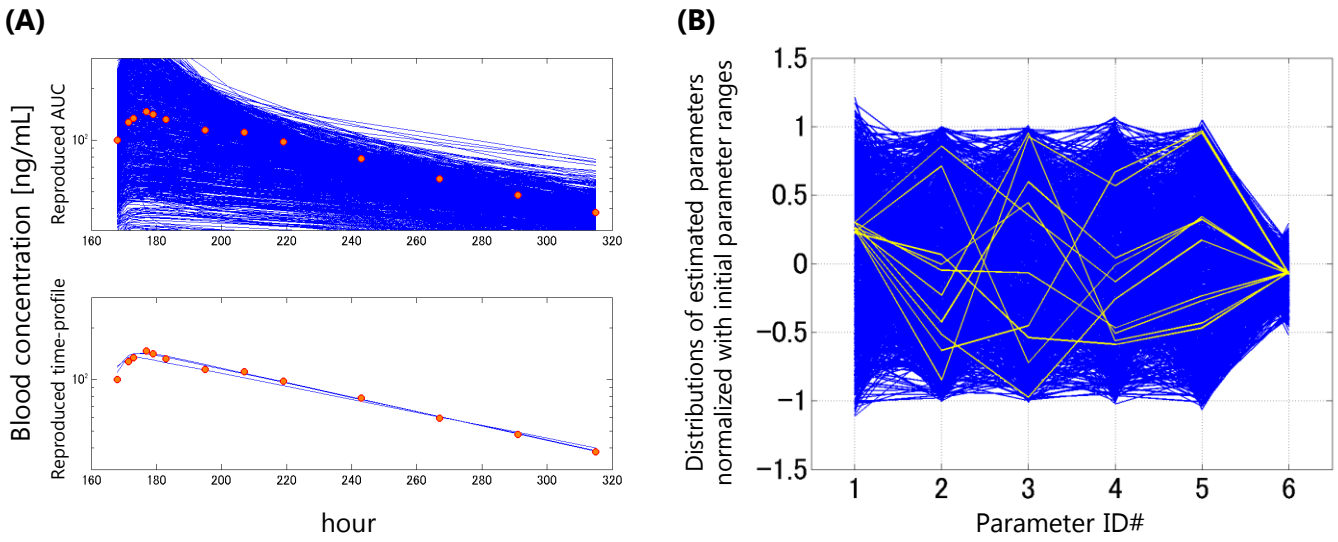
(A) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (B) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.02.2 Initial and estimated parameter values for fluoxetine after multiple oral administrations

			Parameter values			Final estimates	
			Fixed/	Free parameters		Geometric	Geometric
ID	parameter	unit		min	max	mean	CV [%]
1	Vc	L/kg		0.743	74.3	13.1	63.4
2	ka	/hr		0.200	6.00	1.39	149
3	ktransit	/hr		0.200	6.00	1.68	89.7
	FaFg	-	0.722				
	Kp,h	-	13.6				
4	CL12	L/hr/kg		0.0591	5.91	0.543	173
5	k21	/hr		0.0591	5.91	0.56	217
	CL_R	L/hr/kg	0.00771				
6	fBCLint	L/hr/kg		0.0591	5.91	0.371	2.63
	Dose	μg/kg	785				

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; CL_R, renal clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; V_c, distribution volume of central compartment.

Fig S.02.2 Simulated and reported blood concentration-time profiles (A) and estimated parameter distributions (B) after the analyses of multiple-dose pharmacokinetics of fluoxetine



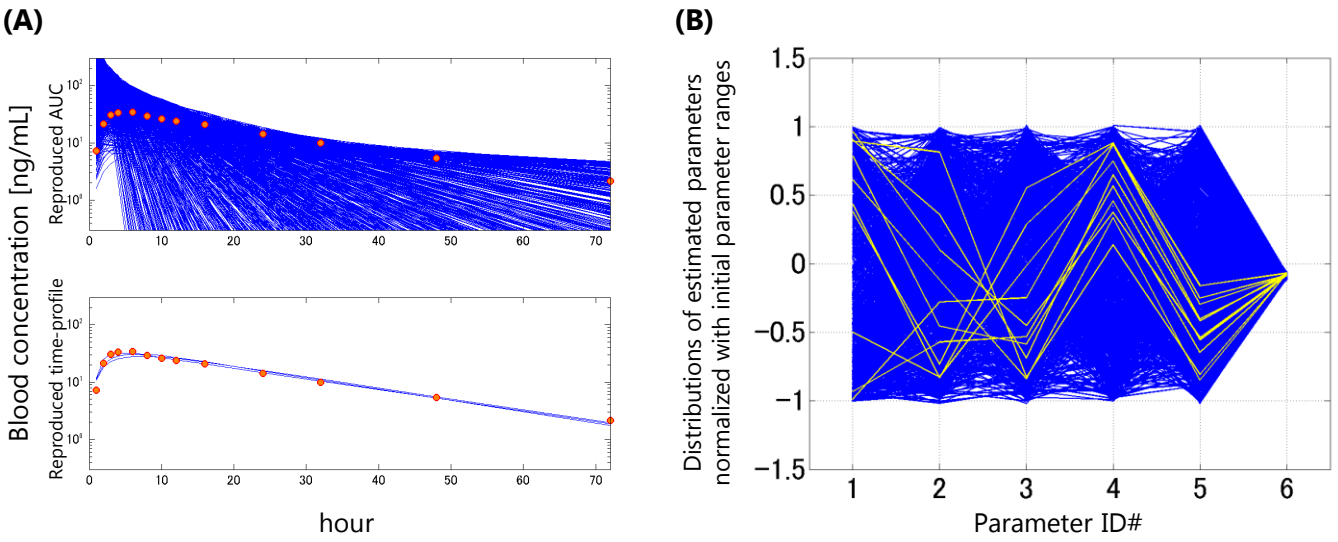
(A) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (B) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.03 Initial and estimated parameter values for fluvoxamine

			Parameter values			Final estimates	
			Fixed/	Free parameters		Geometric	Geometric
ID	parameter	unit		min	max	mean	CV [%]
1	Vc	L/kg		0.0817	7.43	0.745	433
2	ka	/hr		0.200	6.00	0.62	97.7
3	ktransit	/hr		0.200	6.00	0.62	84.6
	FaFg	-	0.971				
	Kp,h	-	12.1				
4	CL12	L/hr/kg		0.180	18.0	7.01	73.5
5	k21	/hr		0.180	18.0	0.575	57.6
	CL_R	L/hr/kg	0.000853				
6	fBCLint	L/hr/kg		0.180	18.0	1.50	1.46
	Dose	μg/kg	1370				

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; CL_R, renal clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; V_c, distribution volume of central compartment.

Fig S.03 Simulated and reported blood concentration-time profiles (A) and estimated parameter distributions (B) after the analyses of single-dose pharmacokinetics of fluvoxamine



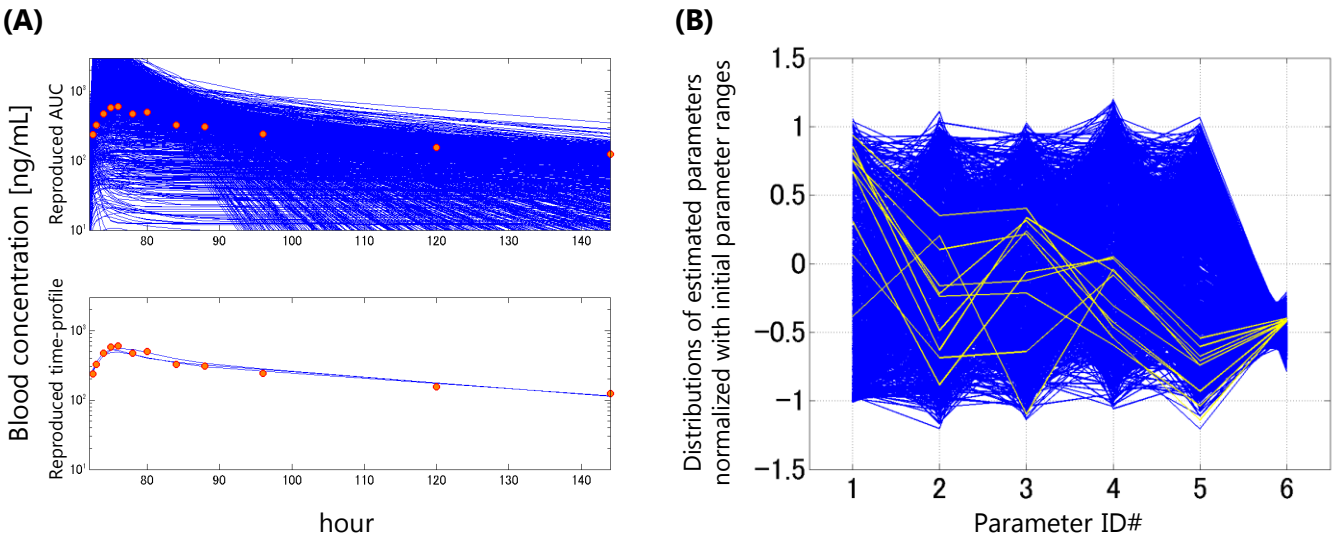
(A) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (B) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.04 Initial and estimated parameter values for itraconazole

			Parameter values			Final estimates	
			Fixed/	Free parameters		Geometric	Geometric
ID	parameter	unit		min	max	mean	CV [%]
1	Vc	L/kg		0.0817	7.43	1.591	210
2	ka	/hr		0.200	6.00	0.776	113
3	ktransit	/hr		0.200	6.00	0.807	95.5
	FaFg	-	0.957				
	Kp,h	-	6.38				
4	CL12	L/hr/kg		0.0348	34.8	1.544	291
5	k21	/hr		0.0348	34.8	0.2029	316
	CL_R	L/hr/kg					
6	fBCLint	L/hr/kg		0.0348	34.8	0.265	4.71
	Dose	μg/kg	3072				

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; CL_R, renal clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; V_c, distribution volume of central compartment.

Fig S.04 Simulated and reported blood concentration-time profiles (A) and estimated parameter distributions (B) after the analyses of multiple-dose pharmacokinetics of itraconazole



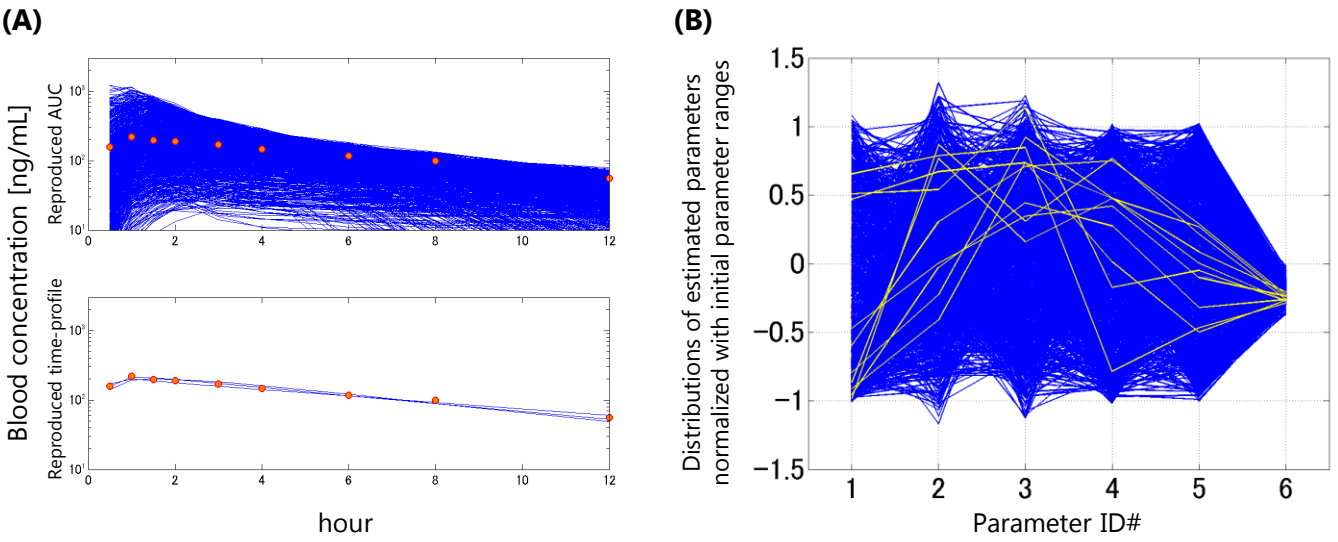
(A) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (B) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.05 Initial and estimated parameter values for quinidine

			Parameter values			Final estimates	
			Fixed/	Free parameters		Geometric	Geometric
ID	parameter	unit		min	max	mean	CV [%]
1	Vc	L/kg		0.0817	7.43	0.570	252
2	ka	/hr		0.200	6.00	1.92	104
3	ktransit	/hr		0.200	6.00	3.21	52.9
	FaFg	-	0.869				
	Kp,h	-	11.6				
4	CL12	L/hr/kg		0.100	10.0	1.06	212
5	k21	/hr		0.100	10.0	0.95	124
	CL_R	L/hr/kg	0.0519				
6	fBCLint	L/hr/kg		0.100	10.0	0.563	6.55
	Dose	μg/kg	1429				

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; CL_R, renal clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; V_c, distribution volume of central compartment.

Fig S.05 Simulated and reported blood concentration-time profiles (A) and estimated parameter distributions (B) after the analyses of single-dose pharmacokinetics of quinidine



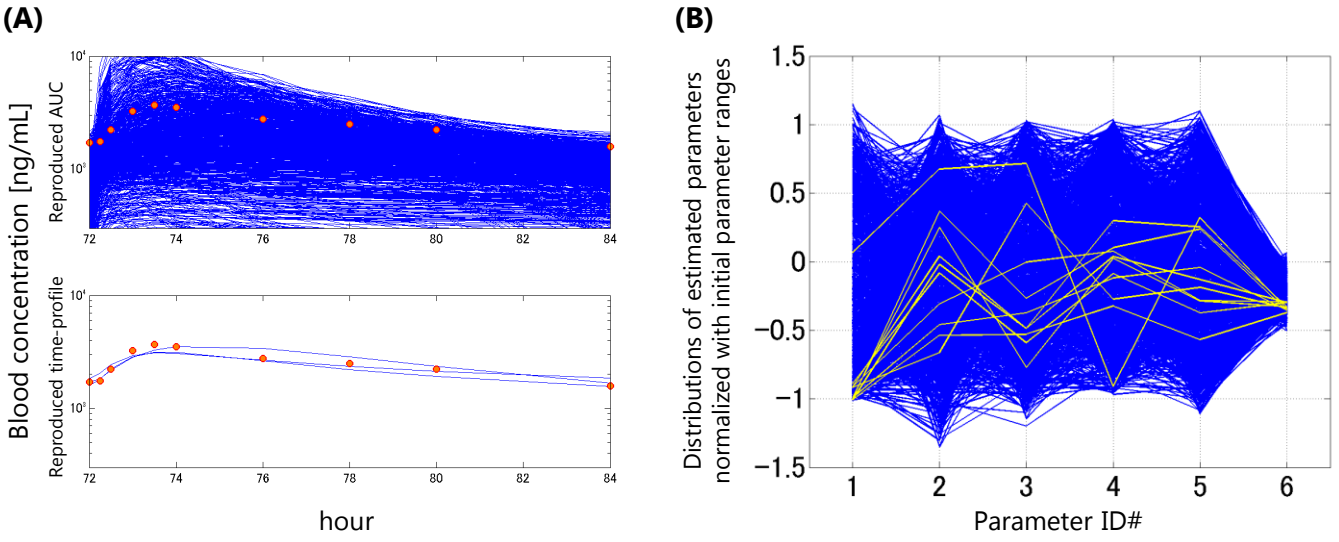
(A) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (B) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.06 Initial and estimated parameter values for voriconazole

			Parameter values			Final estimates	
			Fixed/	Free parameters		Geometric	Geometric
ID	parameter	unit		min	max	mean	CV [%]
1	Vc	L/kg		0.0817	7.43	0.1256	77.9
2	ka	/hr		0.200	6.00	0.882	157
3	ktransit	/hr		0.200	6.00	1.217	145
	FaFg	-	1.00				
	Kp,h	-	0.562				
4	CL12	L/hr/kg		0.00961	9.61	0.198	200
5	k21	/hr		0.00961	9.61	0.276	216
	CL_R	L/hr/kg					
6	fBCLint	L/hr/kg		0.00961	9.61	0.0994	11.3
	Dose	μg/kg	2857				

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; CL_R, renal clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; V_c, distribution volume of central compartment.

Fig S.06 Simulated and reported blood concentration-time profiles (A) and estimated parameter distributions (B) after the analyses of multiple-dose pharmacokinetics of voriconazole



(A) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (B) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.07 Parameters for analyzing a DDI between chlorpromazine and quinidine

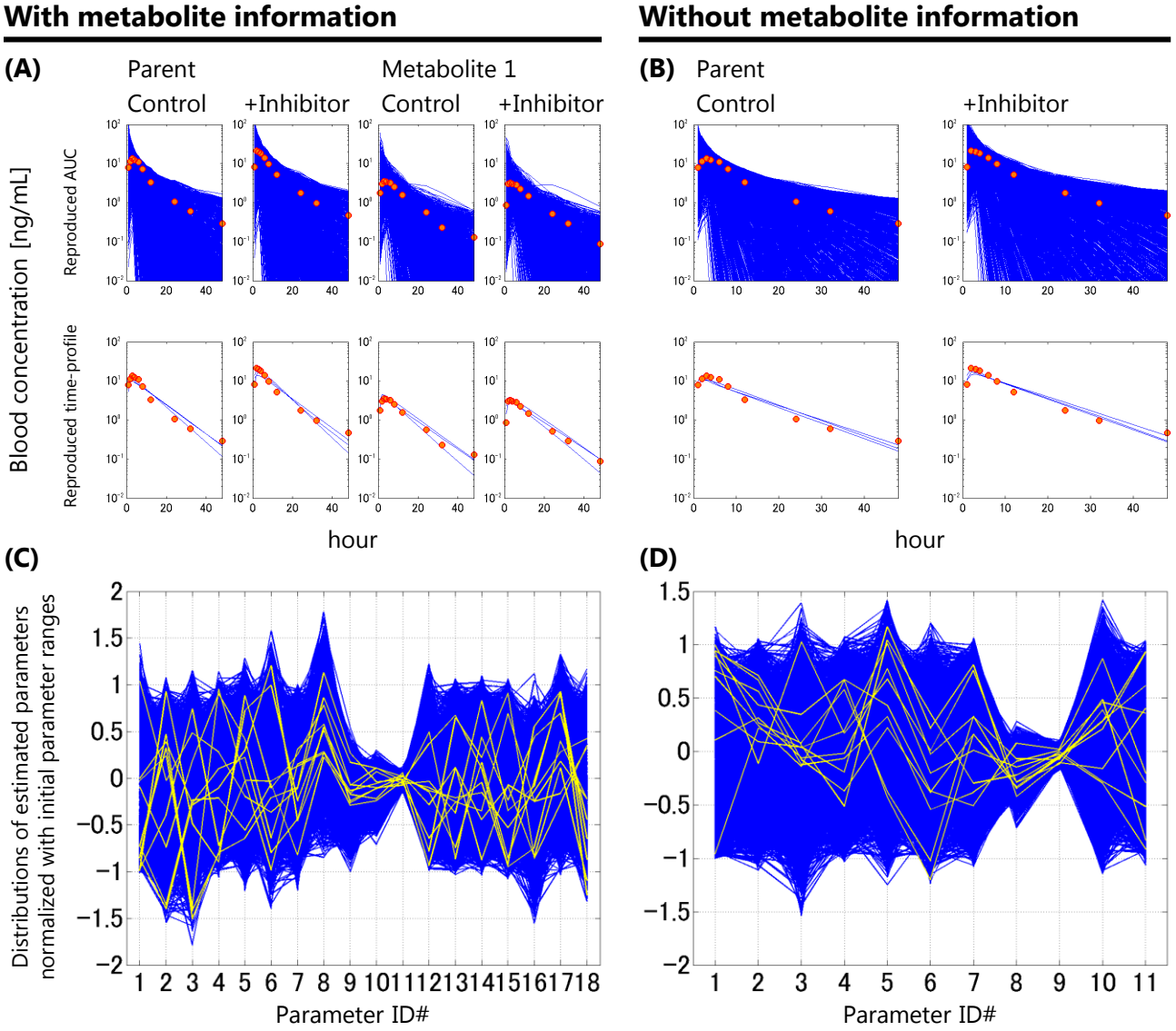
Parent: chlorpromazine, Metabolite 1: 7-hydroxy chlorpromazine, Metabolite 2: NA, Inhibitor: quinidine.
CYPa: CYP2D6, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	1.135	541	1.277	416
2	2	ka	/hr		0.200	6.000	1.039	111	1.754	60
3	3	ktransit	/hr		0.200	6.000	0.788	174	1.185	117
		FaFg	-	0.685						
4	4	Kp,h	-		0.030	30.000	0.671	589	1.275	697
5	5	CL12	L/hr/kg		0.673	67.310	7.199	226	19.067	157
6	6	k21	/hr		0.673	67.310	3.902	254	3.218	136
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
7	7	k_3A,other / kLI	-		0.030	30.000	0.991	405	0.719	542
8		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	4.760	360		
		CL_CYPb,Met1 / CL_CYP2,other	-							
9		CL_other,Met1 / CL_other,other	-		0.030	30.000	0.545	106		
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
10	8	CL_CYPa / CL_other	-		0.030	30.000	0.620	64	0.509	51
		CL_CYPb / CL_other	-							
11	9	fBCLint	L/hr/kg		0.673	67.310	6.256	12.0	6.324	11.3
		Dose	µg/kg	1105						
Metabolite 1										
12		Vc	L/kg		0.082	7.429	0.547	184		
13		Kp,h	-		0.030	30.000	1.111	644		
14		CL12	L/hr/kg		0.673	67.310	9.750	132		
15		k21	/hr		0.673	67.310	3.165	157		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
16		fBCLint	L/hr/kg		0.673	67.310	3.720	175		
		MW corr	-	1.050						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
17	10	Ki_CYP1	µg/L		0.300	300.0	27.237	454	23.171	319
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
18	11	R_intes,3A - 1	-		0.030	30.000	0.311	916	1.028	1148
		Dose	µg/kg	2041						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.07 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between chlorpromazine and quinidine, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: chlorpromazine, Metabolite 1: 7-hydroxy chlorpromazine, Metabolite 2: NA, Inhibitor: quinidine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.08.1 Parameters for analyzing a DDI between desipramine and single-dose fluoxetine

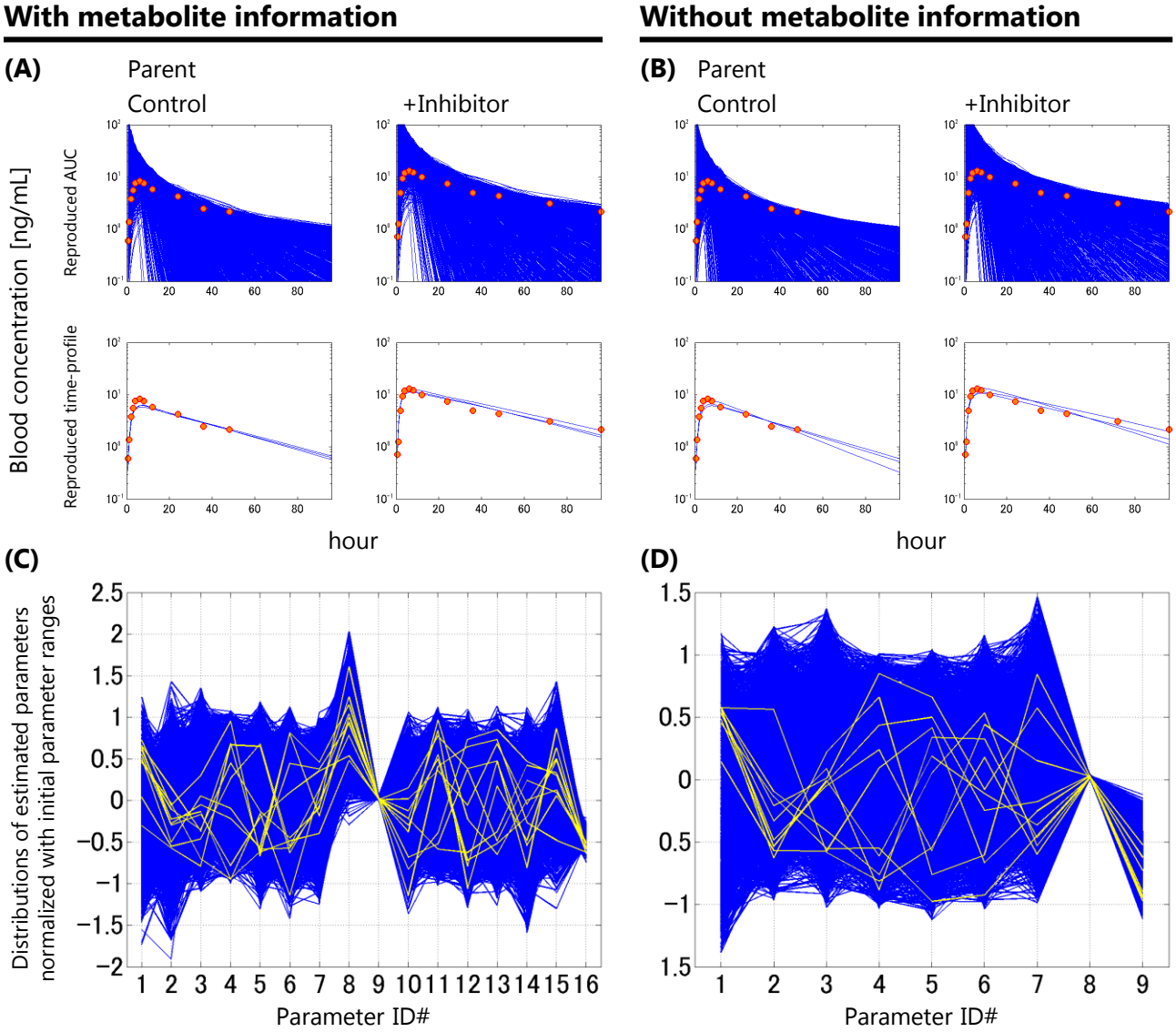
Parent: desipramine, Metabolite 1: 2-hydroxy desipramine, Metabolite 2: NA, Inhibitor: fluoxetine.
CYPa: CYP2D6, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.743	74.286	20.372	108	24.728	28
2	2	ka	/hr		0.200	6.000	0.687	83	0.676	69
3	3	ktransit	/hr		0.200	6.000	0.851	79	0.845	62
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	1.432	659	0.762	620
5	5	CL12	L/hr/kg		0.225	22.523	1.667	207	1.991	203
6	6	k21	/hr		0.225	22.523	1.783	218	2.522	210
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.300	300.000	7.137	464		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
8	7	CL_CYPa / CL_other	-		0.300	300.000	328.628	128	11.713	536
		CL_CYPb / CL_other	-							
9	8	fBCLint	L/hr/kg		0.225	22.523	2.351	2.7	2.375	1.4
		Dose	μg/kg	640						
Metabolite 1										
10		Vc	L/kg		0.743	74.286	3.270	127		
11		Kp,h	-		0.030	30.000	1.362	483		
12		CL12	L/hr/kg		0.225	22.523	2.009	225		
13		k21	/hr		0.225	22.523	1.863	223		
14		CL_R,int,app	L/hr/kg		0.225	22.523	1.844	171		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
15		fBCLint	L/hr/kg		0.225	22.523	3.866	104		
		MW corr	-	1.060						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
16	9	Ki_CYP1	μg/L		3.000	3000.0	13.105	15	6.553	67
		Ki_CYP2	μg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	μg/kg	768						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.08.1 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between desipramine and single-dose fluoxetine, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: desipramine, Metabolite 1: 2-hydroxy desipramine, Metabolite 2: NA, Inhibitor: fluoxetine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.08.2 Parameters for analyzing a DDI between desipramine and multiple-dose fluoxetine

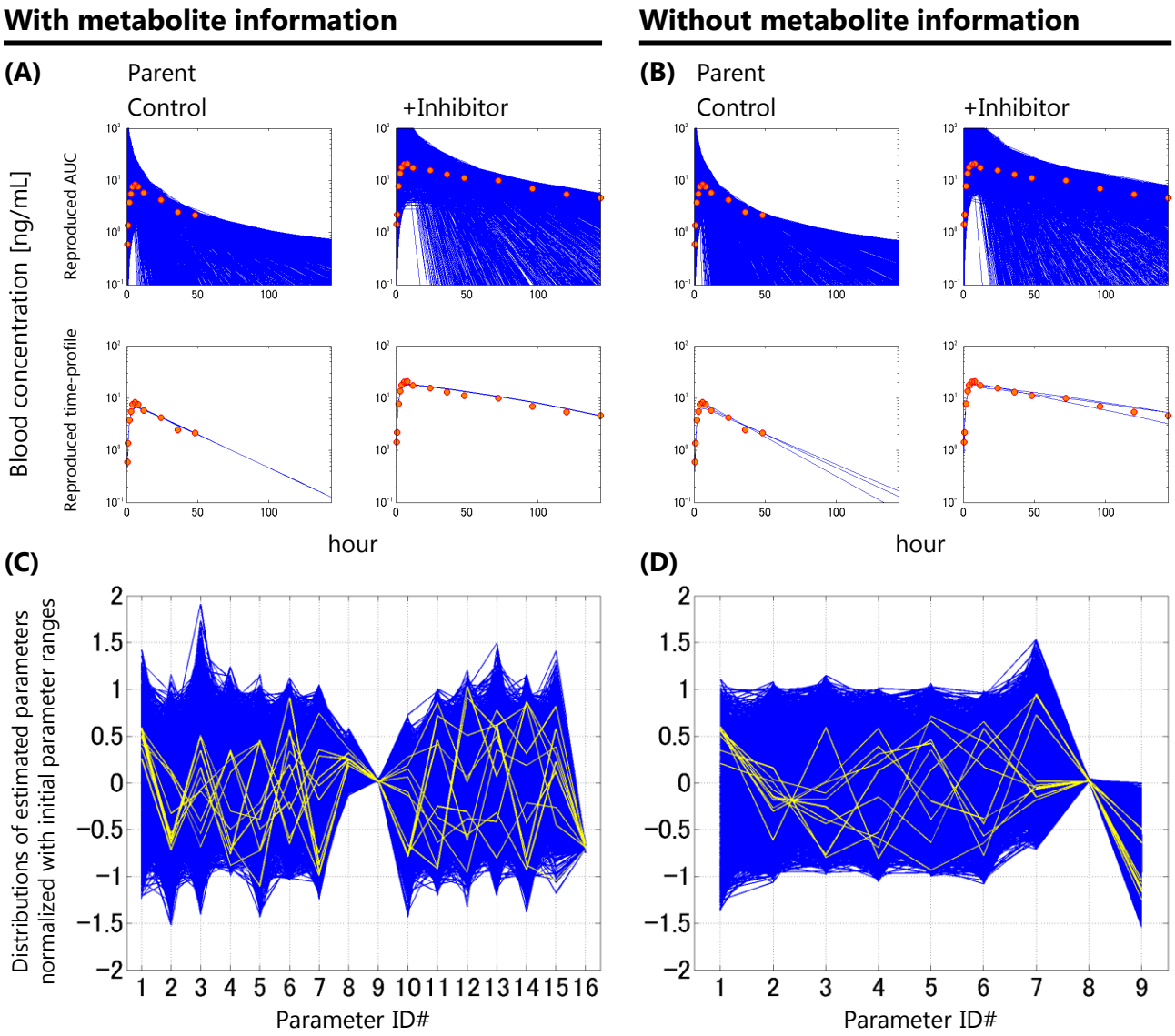
Parent: desipramine, Metabolite 1: 2-hydroxy desipramine, Metabolite 2: NA, Inhibitor: fluoxetine.
CYPa: CYP2D6, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.743	74.286	19.735	49	20.609	59
2	2	ka	/hr		0.200	6.000	0.676	90	0.751	75
3	3	ktransit	/hr		0.200	6.000	0.931	78	0.832	82
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	1.462	487	0.895	1073
5	5	CL12	L/hr/kg		0.225	22.523	2.455	205	2.942	152
6	6	k21	/hr		0.225	22.523	1.931	234	1.789	229
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.300	300.000	6.347	998		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
8	7	CL_CYPa / CL_other	-		0.300	300.000	23.612	14	21.634	214
		CL_CYPb / CL_other	-							
9	8	fBCLint	L/hr/kg		0.225	22.523	2.346	1.3	2.355	1.4
		Dose	µg/kg	640						
Metabolite 1										
10		Vc	L/kg		0.743	74.286	3.456	104		
11		Kp,h	-		0.030	30.000	0.593	1106		
12		CL12	L/hr/kg		0.225	22.523	1.507	248		
13		k21	/hr		0.225	22.523	3.068	190		
14		CL_R,int,app	L/hr/kg		0.225	22.523	2.099	311		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
15		fBCLint	L/hr/kg		0.225	22.523	2.642	122		
		MW corr	-	1.060						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
16	9	Ki_CYP1	µg/L		3.000	3000.0	8.557	5.1	3.805	104
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	768						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.08.2 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between desipramine and multiple-dose fluoxetine, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: desipramine, Metabolite 1: 2-hydroxy desipramine, Metabolite 2: NA, Inhibitor: fluoxetine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.09.1 Parameters for analyzing a DDI between fentanyl and fluconazole

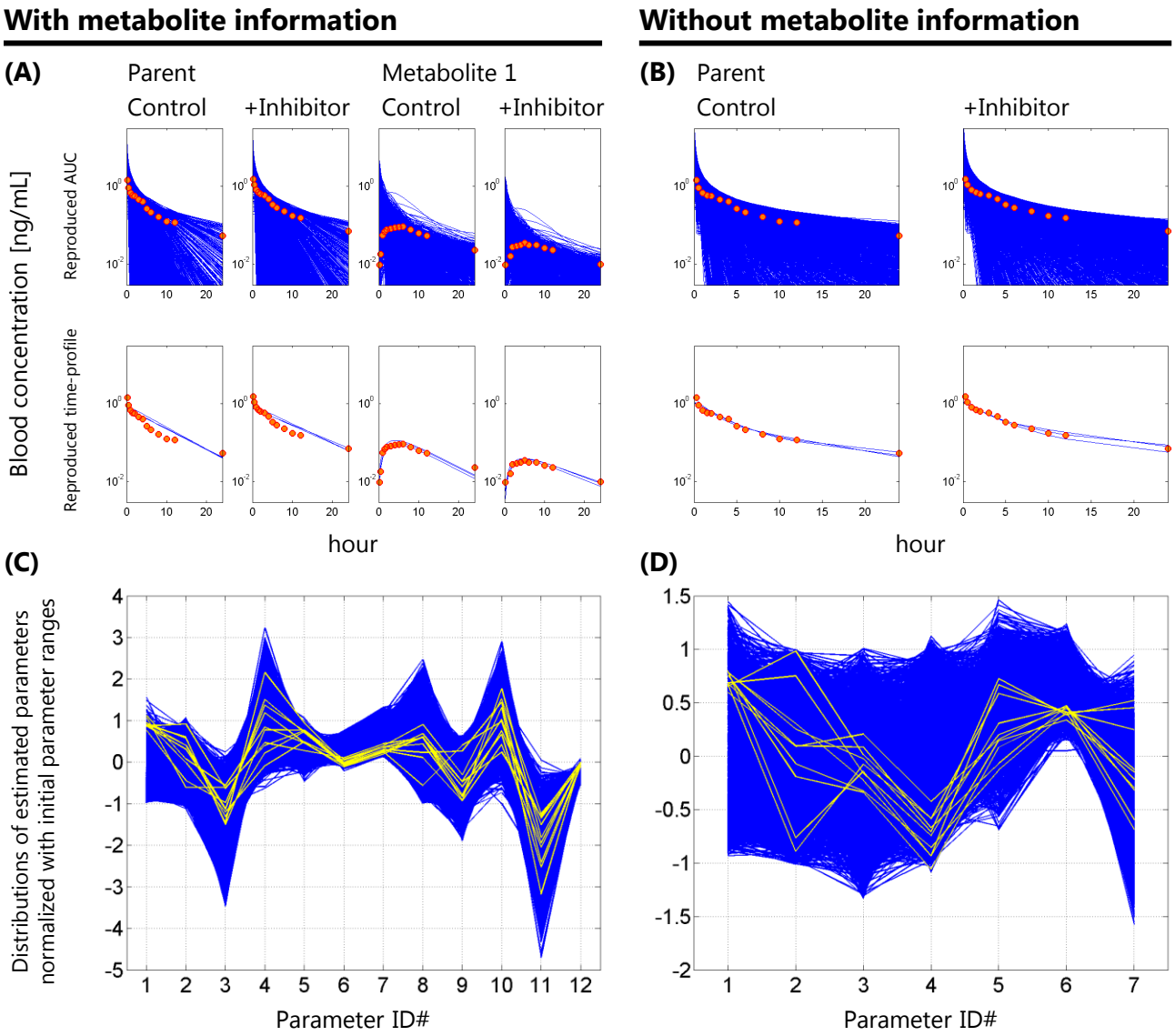
Parent: fentanyl, Metabolite 1: norfentanyl, Metabolite 2: NA, Inhibitor: fluconazole.
CYPa: CYP3A, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	5.926	28	3.377	27
		ka	/hr							
		ktransit	/hr							
		FaFg	-	1.000						
2	2	Kp,h	-		0.030	30.000	2.359	404	0.952	1317
3	3	CL12	L/hr/kg		0.084	8.400	0.323	210	1.024	83
4	4	k21	/hr		0.084	8.400	5.589	380	0.223	79
		CL_R,int,app,cont	L/hr/kg	0.050						
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
5		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	3.130	201		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
6	5	CL_CYPa / CL_other	-		0.030	30.000	0.901	18	1.632	190
		CL_CYPb / CL_other	-							
7	6	fBCLint	L/hr/kg		0.084	8.400	2.015	15.8	2.396	17.0
		Dose	µg/kg	5						
Metabolite 1										
8		Vc	L/kg		0.082	7.429	0.994	136		
9		Kp,h	-		0.030	30.000	0.741	575		
10		CL12	L/hr/kg		0.084	8.400	0.116	446		
11		k21	/hr		0.084	8.400	6.161	154		
12		CL_R,int,app	L/hr/kg		0.084	8.400	0.347	238		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
13		fBCLint	L/hr/kg		0.084	8.400	0.150	160		
		MW corr	-	0.690						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
14	7	Ki_CYP1	µg/L		100.0	100000	1724.657	15	1190.173	284
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	2857						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.09.1 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between fentanyl and fluconazole, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: fentanyl, Metabolite 1: norfentanyl, Metabolite 2: NA, Inhibitor: fluconazole.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.09.2 Parameters for analyzing a DDI between fentanyl and voriconazole

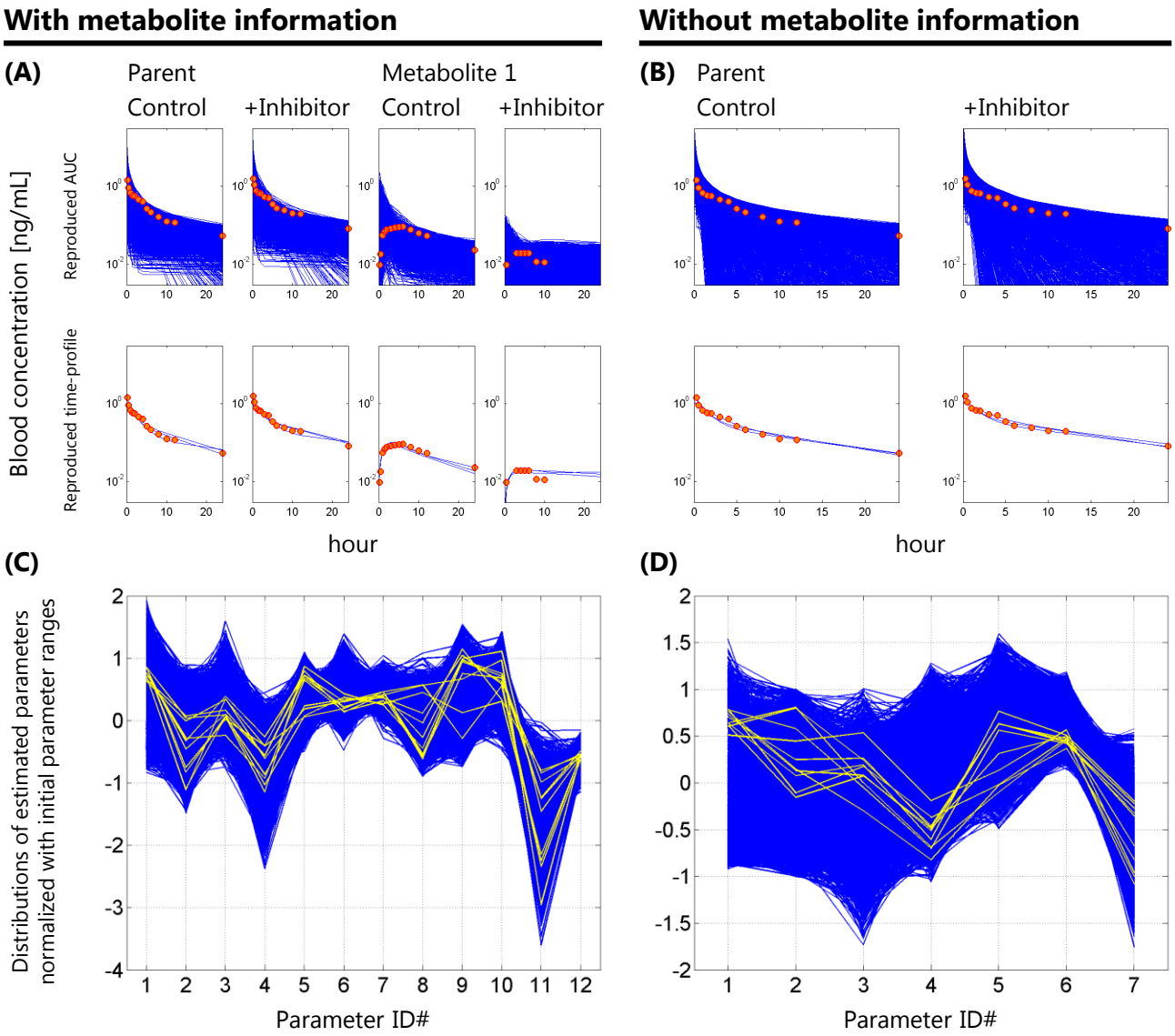
Parent: fentanyl, Metabolite 1: norfentanyl, Metabolite 2: NA, Inhibitor: voriconazole.
CYPa: CYP3A, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	3.309	35	3.591	30
		ka	/hr							
		ktransit	/hr							
		FaFg	-	1.000						
2	2	Kp,h	-		0.030	30.000	0.404	460	0.689	827
3	3	CL12	L/hr/kg		0.084	8.400	1.392	72	1.234	63
4	4	k21	/hr		0.084	8.400	0.344	113	0.269	66
		CL_R,int,app,cont	L/hr/kg	0.050						
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
5		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	1.302	217		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
6	5	CL_CYPa / CL_other	-		0.030	30.000	2.956	51	6.934	291
		CL_CYPb / CL_other	-							
7	6	fBCLint	L/hr/kg		0.084	8.400	1.832	19.7	2.581	13.0
		Dose	µg/kg	5						
Metabolite 1										
8		Vc	L/kg		0.082	7.429	1.890	81		
9		Kp,h	-		0.030	30.000	2.083	1525		
10		CL12	L/hr/kg		0.084	8.400	0.338	97		
11		k21	/hr		0.084	8.400	0.194	140		
12		CL_R,int,app	L/hr/kg		0.084	8.400	0.635	236		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
13		fBCLint	L/hr/kg		0.084	8.400	0.272	111		
		MW corr	-	0.690						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
14	7	Ki_CYP1	µg/L		100.000	100000	797.135	47	788.283	319
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	2857						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.09.2 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between fentanyl and voriconazole, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: fentanyl, Metabolite 1: norfentanyl, Metabolite 2: NA, Inhibitor: voriconazole.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.10.1 Parameters for analyzing a DDI between flurbiprofen and fluconazole [Hanley et al, 2013]

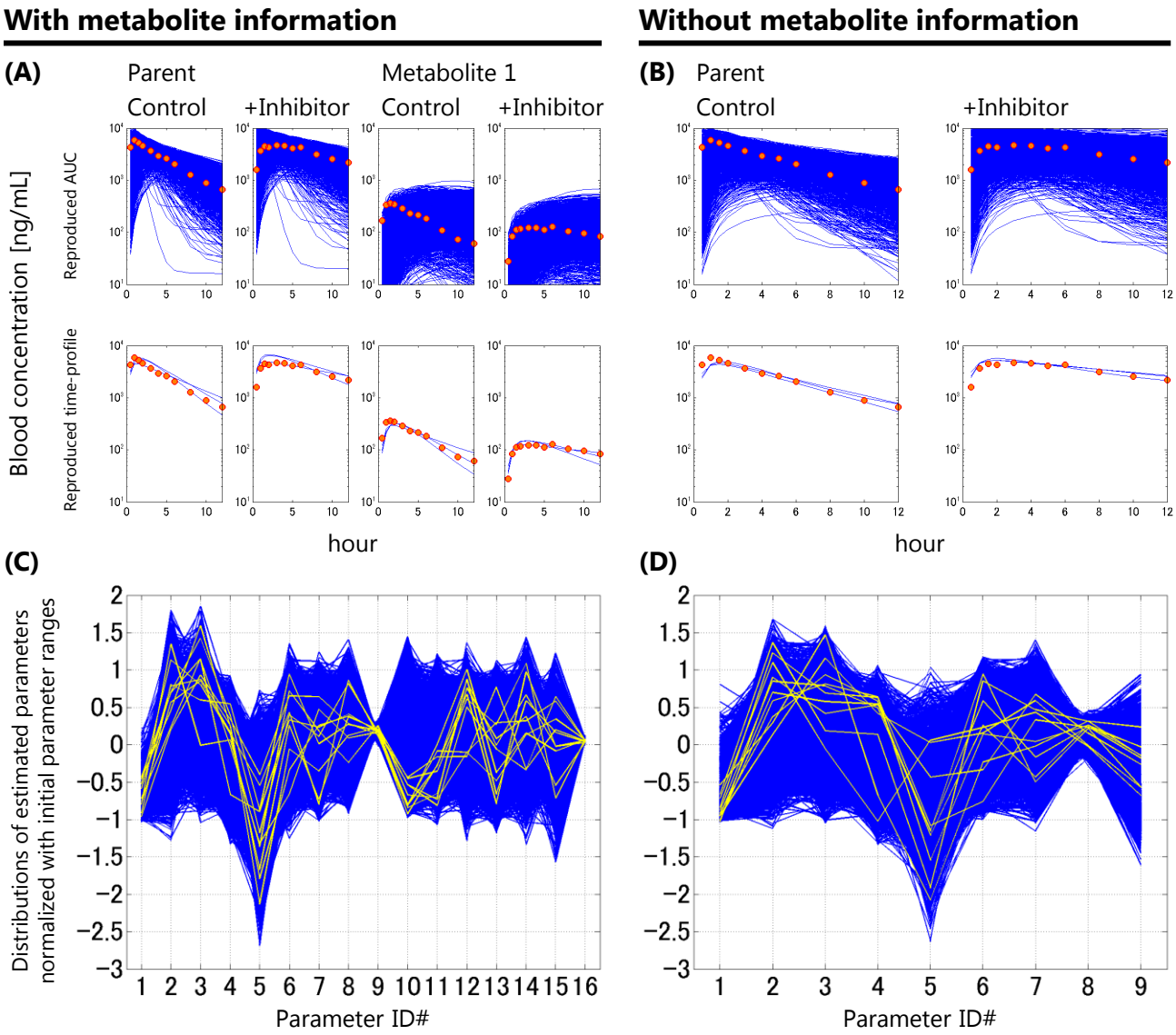
Parent: flurbiprofen, Metabolite 1: 4'-hydroxy flurbiprofen, Metabolite 2: NA, Inhibitor: fluconazole.
CYPa: CYP2C9, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.171	40	0.140	39
2	2	ka	/hr		0.200	6.000	3.900	69	3.846	76
3	3	ktransit	/hr		0.200	6.000	5.173	72	3.095	69
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.823	341	0.800	1021
5	5	CL12	L/hr/kg		0.00246	0.246	0.003	190	0.004	363
6	6	k21	/hr		0.00246	0.246	0.029	214	0.039	208
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.300	300.000	13.435	330		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
8	7	CL_CYPa / CL_other	-		0.300	300.000	36.373	237	12.215	400
		CL_CYPb / CL_other	-							
9	8	fBCLint	L/hr/kg		0.002	0.246	0.036	13.0	0.035	28.5
		Dose	µg/kg	1429						
Metabolite 1										
10		Vc	L/kg		0.082	7.429	0.205	60		
11		Kp,h	-		0.030	30.000	0.285	124		
12		CL12	L/hr/kg		0.00246	0.246	0.053	210		
13		k21	/hr		0.00246	0.246	0.020	173		
14		CL_R,int,app	L/hr/kg		0.00246	0.246	0.056	154		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
15		fBCLint	L/hr/kg		0.00246	0.246	0.032	111		
		MW corr	-	1.066						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
16	9	Ki_CYP1	µg/L		100.000	100000	3775.180	5	1287.829	217
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	2857						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.10.1 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between flurbiprofen and fluconazole [Hanley et al, 2013], with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: flurbiprofen, Metabolite 1: 4'-hydroxy flurbiprofen, Metabolite 2: NA, Inhibitor: fluconazole.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.10.2 Parameters for analyzing a DDI between flurbiprofen and fluconazole [Hanley et al, 2012]

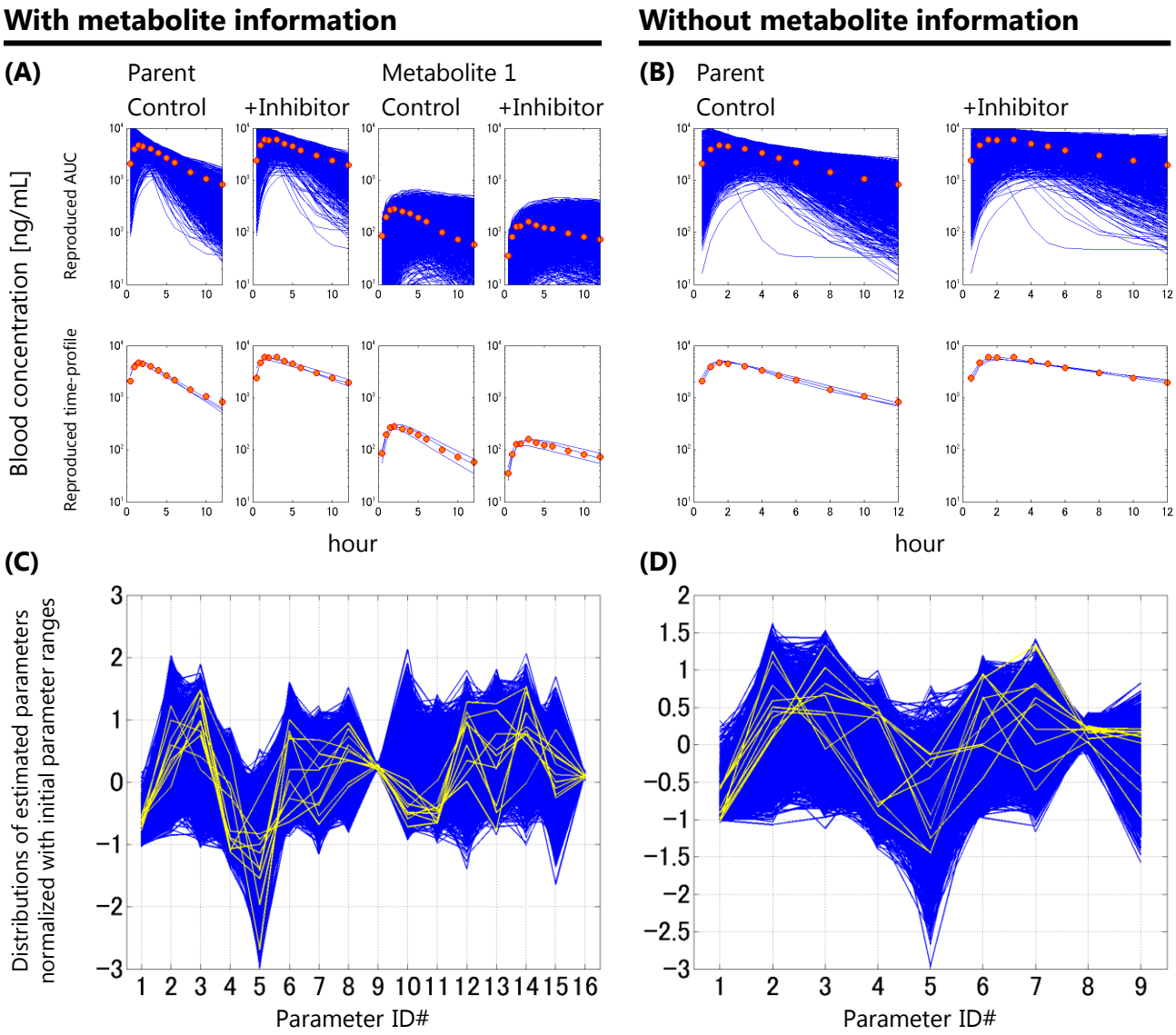
Parent: flurbiprofen, Metabolite 1: 4'-hydroxy flurbiprofen, Metabolite 2: NA, Inhibitor: fluconazole.
CYPa: CYP2C9, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.164	33	0.129	45
2	2	ka	/hr		0.200	6.000	3.629	98	2.709	92
3	3	ktransit	/hr		0.200	6.000	3.774	93	2.638	80
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.317	611	0.673	881
5	5	CL12	L/hr/kg		0.00263	0.263	0.001	249	0.005	297
6	6	k21	/hr		0.00263	0.263	0.070	215	0.055	186
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.300	300.000	14.140	389		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
8	7	CL_CYPa / CL_other	-		0.300	300.000	67.069	235	37.741	1016
		CL_CYPb / CL_other	-							
9	8	fBCLint	L/hr/kg		0.003	0.263	0.044	7.8	0.037	20.7
		Dose	µg/kg	1429						
Metabolite 1										
10		Vc	L/kg		0.082	7.429	0.340	50		
11		Kp,h	-		0.030	30.000	0.431	165		
12		CL12	L/hr/kg		0.00263	0.263	0.098	208		
13		k21	/hr		0.00263	0.263	0.040	209		
14		CL_R,int,app	L/hr/kg		0.00263	0.263	0.235	149		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
15		fBCLint	L/hr/kg		0.00263	0.263	0.050	125		
		MW corr	-	1.066						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
16	9	Ki_CYP1	µg/L		100.000	100000	4430.411	6	1542.979	323
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	2857						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.10.2 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between flurbiprofen and fluconazole [Hanley et al, 2012], with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: flurbiprofen, Metabolite 1: 4'-hydroxy flurbiprofen, Metabolite 2: NA, Inhibitor: fluconazole.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.11 Parameters for analyzing a DDI between hydrocodone and quinidine

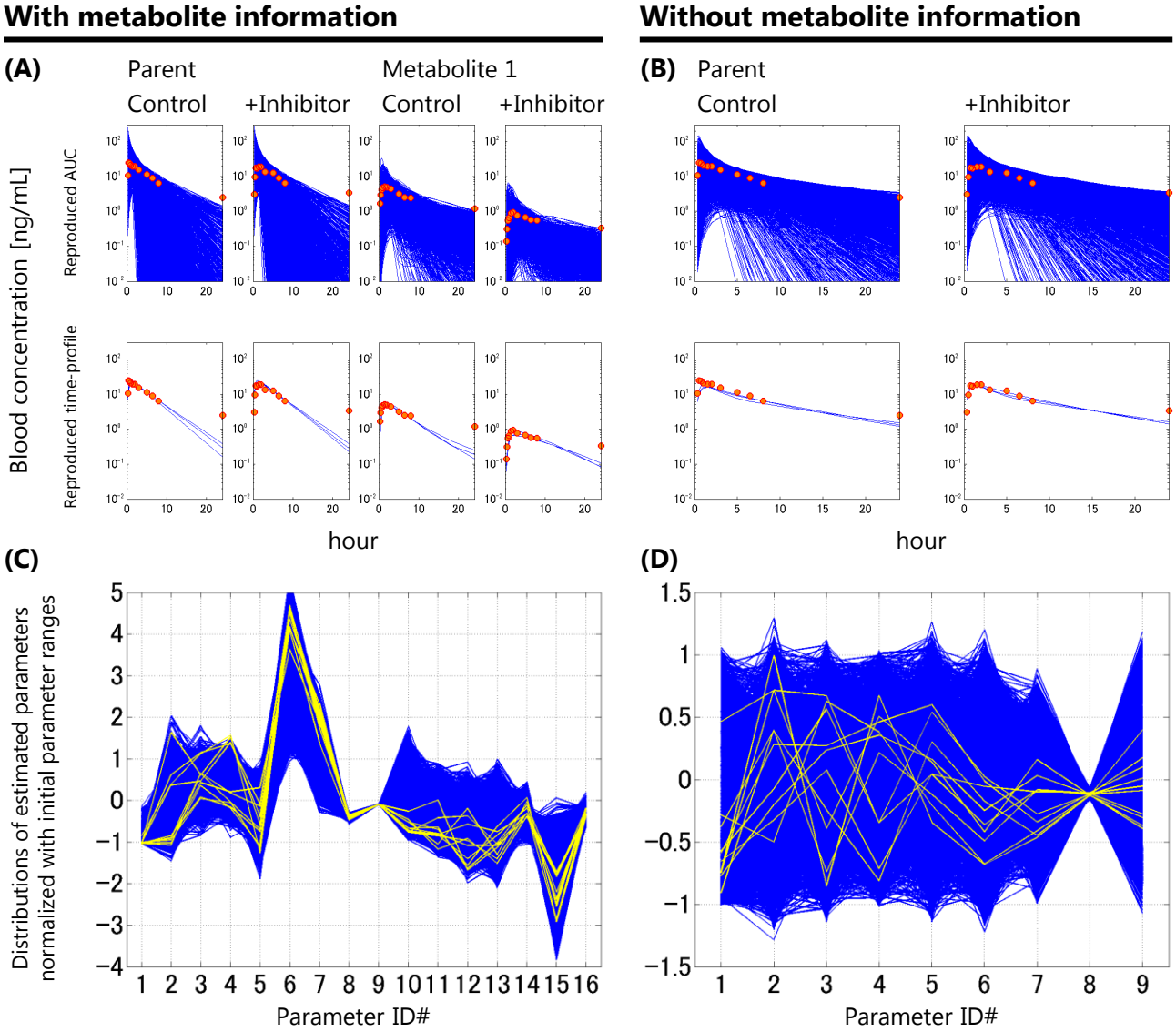
Parent: hydrocodone, Metabolite 1: hydromorphone, Metabolite 2: NA, Inhibitor: quinidine.
CYPa: CYP2D6, CYPb: NA.

				Parameter values			Final estimates with metabolite		without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	2.577	37	0.164	94
2	2	ka	/hr		0.200	6.000	35.607	136	1.022	142
3	3	ktransit	/hr		0.200	6.000	24.247	184	0.785	143
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.777	663	0.616	682
5	5	CL12	L/hr/kg		0.0664	6.640	0.286	35	0.362	47
6	6	k21	/hr		0.0022	0.221	0.007	72	0.065	89
		CL_R,int,app,cont	L/hr/kg	0.057						
		CL_R,int,app,inhi	L/hr/kg	0.084						
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	6.889	308		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
8	7	CL_CYPa / CL_other	-		0.030	30.000	1.144	65	0.870	276
		CL_CYPb / CL_other	-							
9	8	fBCLint	L/hr/kg		0.0664	6.640	0.400	27.7	0.461	9.9
		Dose	µg/kg	84						
Metabolite 1										
10		Vc	L/kg		0.082	7.429	0.978	160		
11		Kp,h	-		0.030	30.000	1.981	569		
12		CL12	L/hr/kg		0.0664	6.640	0.109	201		
13		k21	/hr		0.0664	6.640	1.778	212		
14		CL_R,int,app	L/hr/kg		0.0664	6.640	0.342	104		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
15		fBCLint	L/hr/kg		0.0664	6.640	0.088	147		
		MW corr	-	0.953						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
16	9	Ki_CYP1	µg/L		0.300	300.0	2.604	37	8.480	474
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	1153						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.11 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between hydrocodone and quinidine, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: hydrocodone, Metabolite 1: hydromorphone, Metabolite 2: NA, Inhibitor: quinidine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.12.1 Parameters for analyzing a DDI between imipramine and single-dose fluoxetine

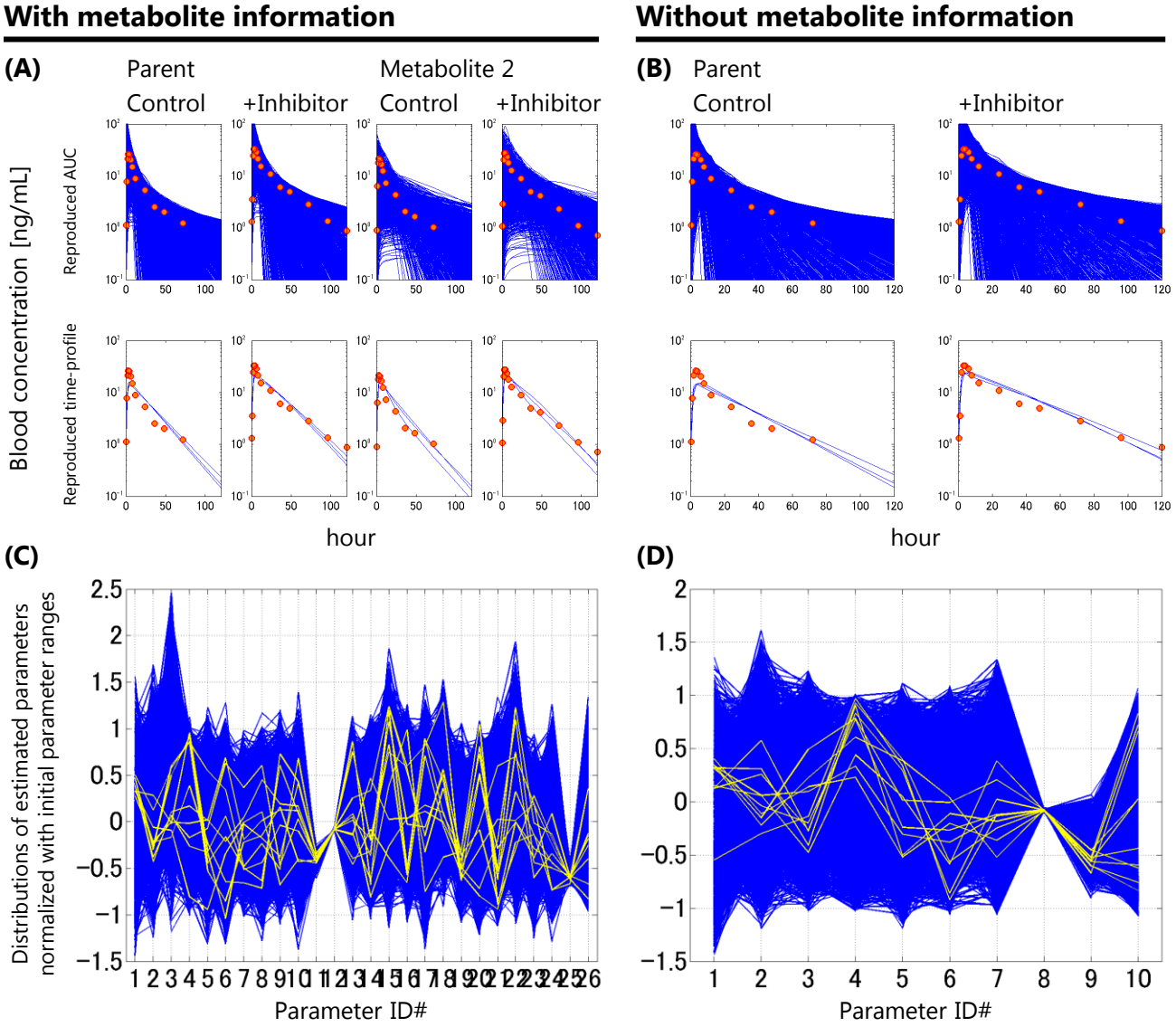
Parent: imipramine, Metabolite 1: 2-hydroxy imipramine, Metabolite 2: desipramine, Inhibitor: fluoxetine. CYPa: CYP2D6, CYPb: CYP2C19 and 3A.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.743	74.286	14.278	59	12.852	60
2	2	ka	/hr		0.200	6.000	0.936	76	1.089	82
3	3	ktransit	/hr		0.200	6.000	1.593	80	1.192	75
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	2.074	894	4.708	388
5	5	CL12	L/hr/kg		0.180	18.009	1.451	144	1.528	186
6	6	k21	/hr		0.180	18.009	1.237	191	1.145	206
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.300	300.000	6.977	239		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
8		CL_CYPa,Met2 / CL_CYP1,other	-		0.030	30.000	0.947	284		
9		CL_CYPb,Met2 / CL_CYP2,other	-		0.030	30.000	1.042	387		
10		CL_other,Met2 / CL_other,other	-		0.030	30.000	1.590	209		
11	7	CL_CYPa / CL_other	-		0.300	300.000	2.310	11	12.513	309
		CL_CYPb / CL_other	-							
12	8	fBCLint	L/hr/kg		0.180	18.009	1.493	1.3	1.499	1.3
		Dose	µg/kg	670						
Metabolite 1										
13		Vc	L/kg		0.743	74.286	7.419	150		
14		Kp,h	-		0.030	30.000	0.407	554		
15		CL12	L/hr/kg		0.180	18.009	5.166	191		
16		k21	/hr		0.180	18.009	1.761	264		
17		CL_R,int,app	L/hr/kg		0.180	18.009	2.347	163		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
18		fBCLint	L/hr/kg		0.180	18.009	3.277	111		
		MW corr	-	1.057						
Metabolite 2										
19		Vc	L/kg		0.743	74.286	2.744	40		
20		Kp,h	-		0.030	30.000	1.376	1154		
21		CL12	L/hr/kg		0.180	18.009	0.898	186		
22		k21	/hr		0.180	18.009	5.275	182		
		CL_R,int,app	L/hr/kg							
23		CL_CYPa / CL_other	-		0.030	30.000	0.535	168		
		CL_CYPb / CL_other	-							
24		fBCLint	L/hr/kg		0.180	18.009	0.918	91		
		MW corr	-							
Inhibitor										
25	9	Ki_CYP1	µg/L		3.000	3000.0	11.901	8	17.433	44
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
26	10	R_MBI_CYP2 - 1	-		1.000	100.000	8.130	180	9.049	220
		R_intes,3A - 1	-							
		Dose	µg/kg	804						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.12.1 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between imipramine and single-dose fluoxetine, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: imipramine, Metabolite 1: 2-hydroxy imipramine, Metabolite 2: desipramine, Inhibitor: fluoxetine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.12.2 Parameters for analyzing a DDI between imipramine and multiple-dose fluoxetine

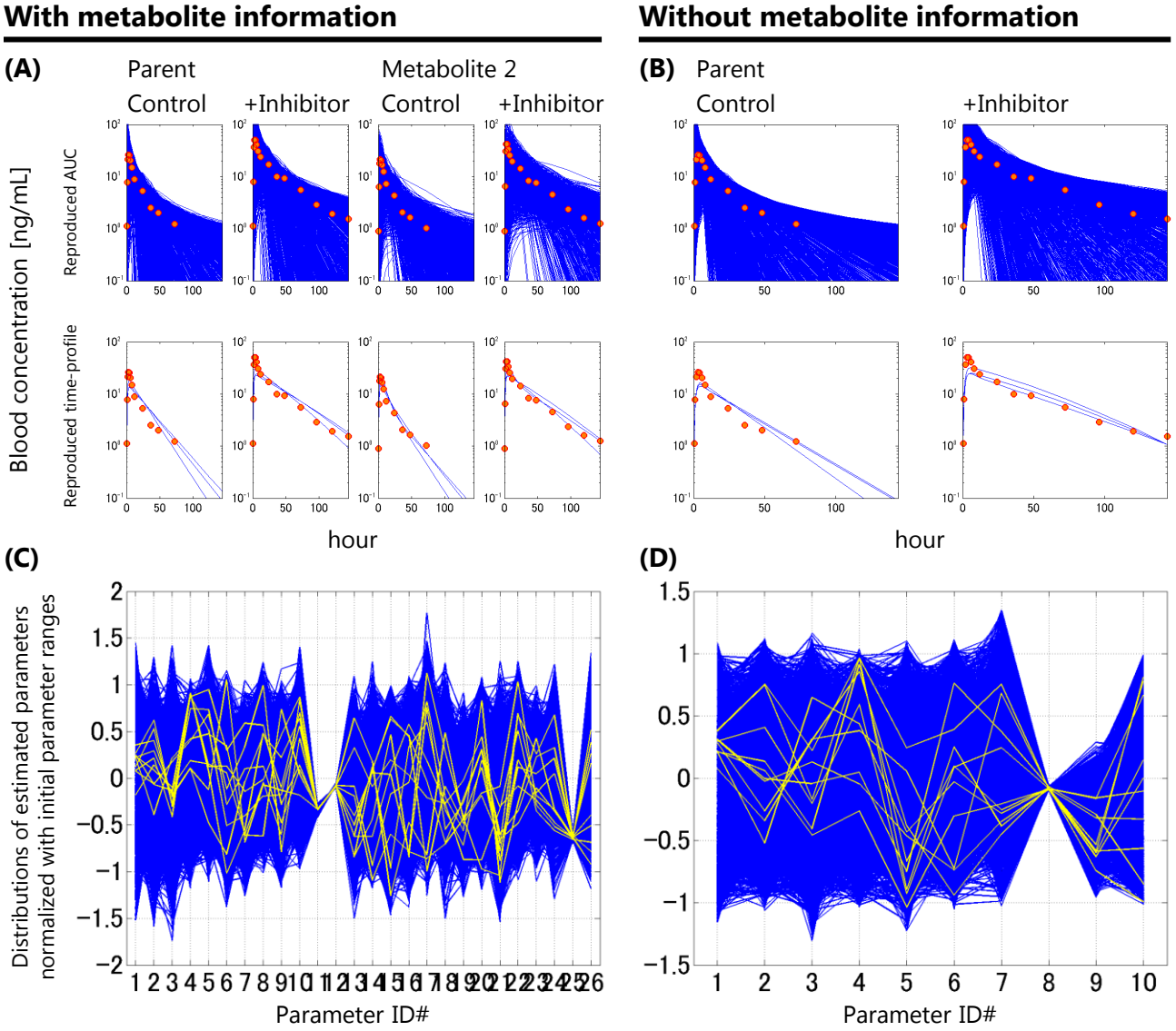
Parent: imipramine, Metabolite 1: 2-hydroxy imipramine, Metabolite 2: desipramine, Inhibitor: fluoxetine.
CYPa: CYP2D6, CYPb: CYP2C19 and 3A.

				Parameter values			Final estimates with metabolite		without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.743	74.286	11.026	60	12.473	46
2	2	ka	/hr		0.200	6.000	2.445	81	1.228	90
3	3	ktransit	/hr		0.200	6.000	0.571	67	0.939	57
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	2.685	532	4.976	344
5	5	CL12	L/hr/kg		0.180	18.009	3.778	233	1.149	253
6	6	k21	/hr		0.180	18.009	1.696	285	1.926	210
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.300	300.000	19.152	278		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
8		CL_CYPa,Met2 / CL_CYP1,other	-		0.030	30.000	3.050	277		
9		CL_CYPb,Met2 / CL_CYP2,other	-		0.030	30.000	0.566	496		
10		CL_other,Met2 / CL_other,other	-		0.030	30.000	1.935	91		
11	7	CL_CYPa / CL_other	-		0.300	300.000	3.039	5	13.713	231
		CL_CYPb / CL_other	-							
12	8	fBCLint	L/hr/kg		0.180	18.009	1.487	1.1	1.496	1.3
		Dose	µg/kg	670						
Metabolite 1										
13		Vc	L/kg		0.743	74.286	2.189	113		
14		Kp,h	-		0.030	30.000	0.898	503		
15		CL12	L/hr/kg		0.180	18.009	1.068	192		
16		k21	/hr		0.180	18.009	1.884	195		
17		CL_R,int,app	L/hr/kg		0.180	18.009	4.515	160		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
18		fBCLint	L/hr/kg		0.180	18.009	1.752	124		
		MW corr	-	1.057						
Metabolite 2										
19		Vc	L/kg		0.743	74.286	1.928	47		
20		Kp,h	-		0.030	30.000	0.918	437		
21		CL12	L/hr/kg		0.180	18.009	3.071	145		
22		k21	/hr		0.180	18.009	2.829	131		
		CL_R,int,app	L/hr/kg							
23		CL_CYPa / CL_other	-		0.030	30.000	0.898	122		
		CL_CYPb / CL_other	-							
24		fBCLint	L/hr/kg		0.180	18.009	0.738	78		
		MW corr	-							
Inhibitor										
25	9	Ki_CYP1	µg/L		3.000	3000.0	9.999	4	23.203	69
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
26	10	R_MBI_CYP2 - 1	-		1.000	100.000	54.349	224	11.916	255
		R_intes,3A - 1	-							
		Dose	µg/kg	804						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.12.2 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between imipramine and multiple-dose fluoxetine, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: imipramine, Metabolite 1: 2-hydroxy imipramine, Metabolite 2: desipramine, Inhibitor: fluoxetine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.13.1 Parameters for analyzing a DDI between lansoprazole and fluvoxamine in CYP2C19 EM subjects

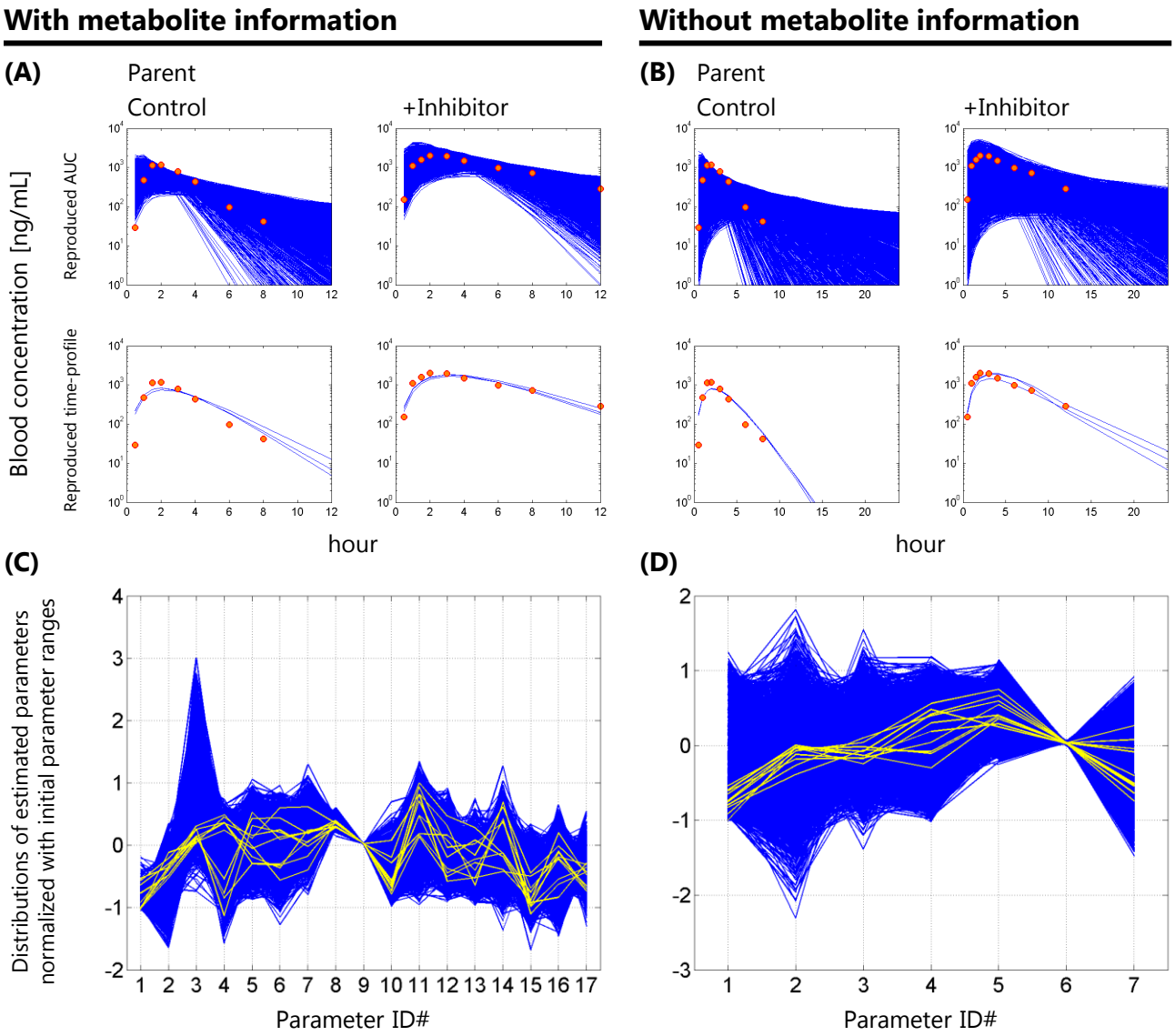
Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine. CYPa: CYP2C19, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.126	32	0.145	36
2	2	ka	/hr		0.200	6.000	1.139	38	0.965	37
3	3	ktransit	/hr		0.200	6.000	0.682	31	0.939	46
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.817	273	0.746	294
5	5	CL12	L/hr/kg		0.020	1.966	0.023	71	0.054	55
6	6	k21	/hr		0.020	1.966	0.600	179	0.776	236
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	0.562	65		
		CL_CYPb,Met1 / CL_CYP2,other	-							
8		CL_other,Met1 / CL_other,other	-		0.030	30.000	1.509	143		
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
9		CL_other,Met2 / CL_other,other	-		0.030	30.000	1.279	161		
10	7	CL_CYPa / CL_other	-		0.030	30.000	3.057	23	7.725	87
		CL_CYPb / CL_other	-							
11	8	fBCLint	L/hr/kg		0.020	1.966	0.209	2.2	0.216	5.9
		Dose	µg/kg	702						
Metabolite 1										
12		Vc	L/kg		0.082	7.429	0.222	82		
13		Kp,h	-		0.030	30.000	0.617	422		
14		CL12	L/hr/kg		0.020	1.966	0.268	151		
15		k21	/hr		0.020	1.966	0.255	107		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
16		fBCLint	L/hr/kg		0.020	1.966	0.111	88		
		MW corr	-	1.043						
Metabolite 2										
17		Vc	L/kg		0.082	7.429	0.459	101		
18		Kp,h	-		0.030	30.000	1.435	395		
19		CL12	L/hr/kg		0.020	1.966	0.145	107		
20		k21	/hr		0.020	1.966	0.474	160		
		CL_R,int,app	L/hr/kg							
21		CL_CYPa / CL_other	-		0.030	30.000	0.139	134		
		CL_CYPb / CL_other	-							
22		fBCLint	L/hr/kg		0.020	1.966	0.094	98		
		MW corr	-	1.043						
Inhibitor										
23	9	Ki_CYP1	µg/L		0.300	300.0	0.399	96	2.170	88
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	439						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.13.1 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between lansoprazole and fluvoxamine in CYP2C19 EM subjects, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.13.2 Parameters for analyzing a DDI between lansoprazole and fluvoxamine in CYP2C19 IM subjects

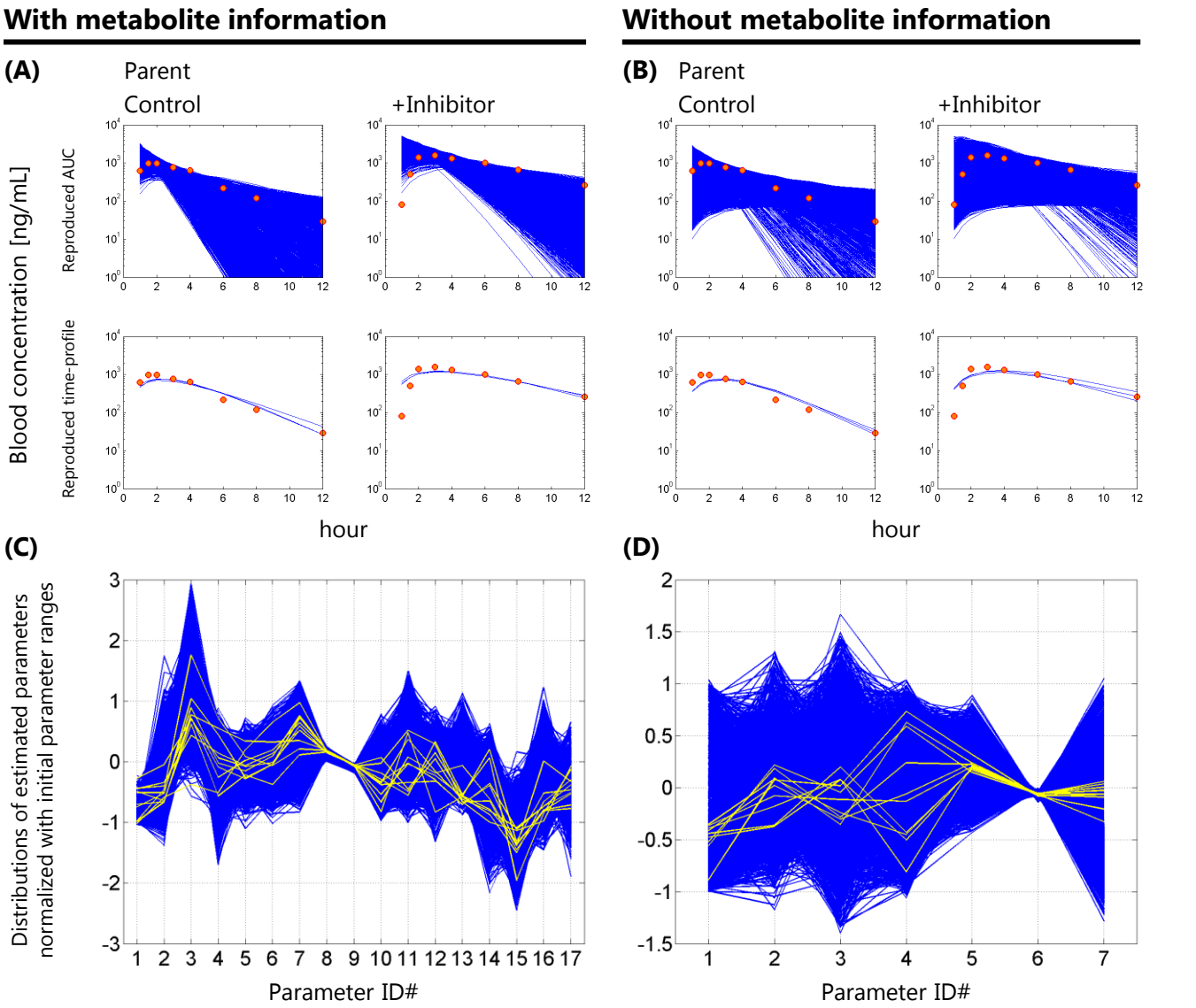
Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine. CYPa: CYP2C19, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.290	43	0.186	50
2	2	ka	/hr		0.200	6.000	0.878	79	1.204	62
3	3	ktransit	/hr		0.200	6.000	1.465	57	0.739	38
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.786	959	1.533	471
5	5	CL12	L/hr/kg		0.014	1.407	0.007	136	0.041	93
6	6	k21	/hr		0.014	1.407	0.347	177	0.333	263
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	1.160	232		
		CL_CYPb,Met1 / CL_CYP2,other	-							
8		CL_other,Met1 / CL_other,other	-		0.030	30.000	0.361	380		
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
9		CL_other,Met2 / CL_other,other	-		0.030	30.000	8.893	236		
10	7	CL_CYPa / CL_other	-		0.030	30.000	3.099	63	3.355	121
		CL_CYPb / CL_other	-							
11	8	fBCLint	L/hr/kg		0.014	1.407	0.193	3.0	0.185	12.3
		Dose	μg/kg	755						
Metabolite 1										
12		Vc	L/kg		0.082	7.429	0.237	95		
13		Kp,h	-		0.030	30.000	0.172	369		
14		CL12	L/hr/kg		0.014	1.407	0.095	123		
15		k21	/hr		0.014	1.407	0.335	157		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
16		fBCLint	L/hr/kg		0.014	1.407	0.124	121		
		MW corr	-	1.043						
Metabolite 2										
17		Vc	L/kg		0.082	7.429	0.291	72		
18		Kp,h	-		0.030	30.000	0.416	262		
19		CL12	L/hr/kg		0.014	1.407	0.052	152		
20		k21	/hr		0.014	1.407	0.318	194		
		CL_R,int,app	L/hr/kg							
21		CL_CYPa / CL_other	-		0.030	30.000	0.009	59		
		CL_CYPb / CL_other	-							
22		fBCLint	L/hr/kg		0.014	1.407	0.050	70		
		MW corr	-	1.043						
Inhibitor										
23	9	Ki_CYP1	μg/L		0.300	300.0	4.126	121	3.094	158
		Ki_CYP2	μg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	μg/kg	472						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.13.2 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between lansoprazole and fluvoxamine in CYP2C19 IM subjects, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.13.3 Parameters for analyzing a DDI between lansoprazole and clarithromycin in CYP2C19 EM subjects

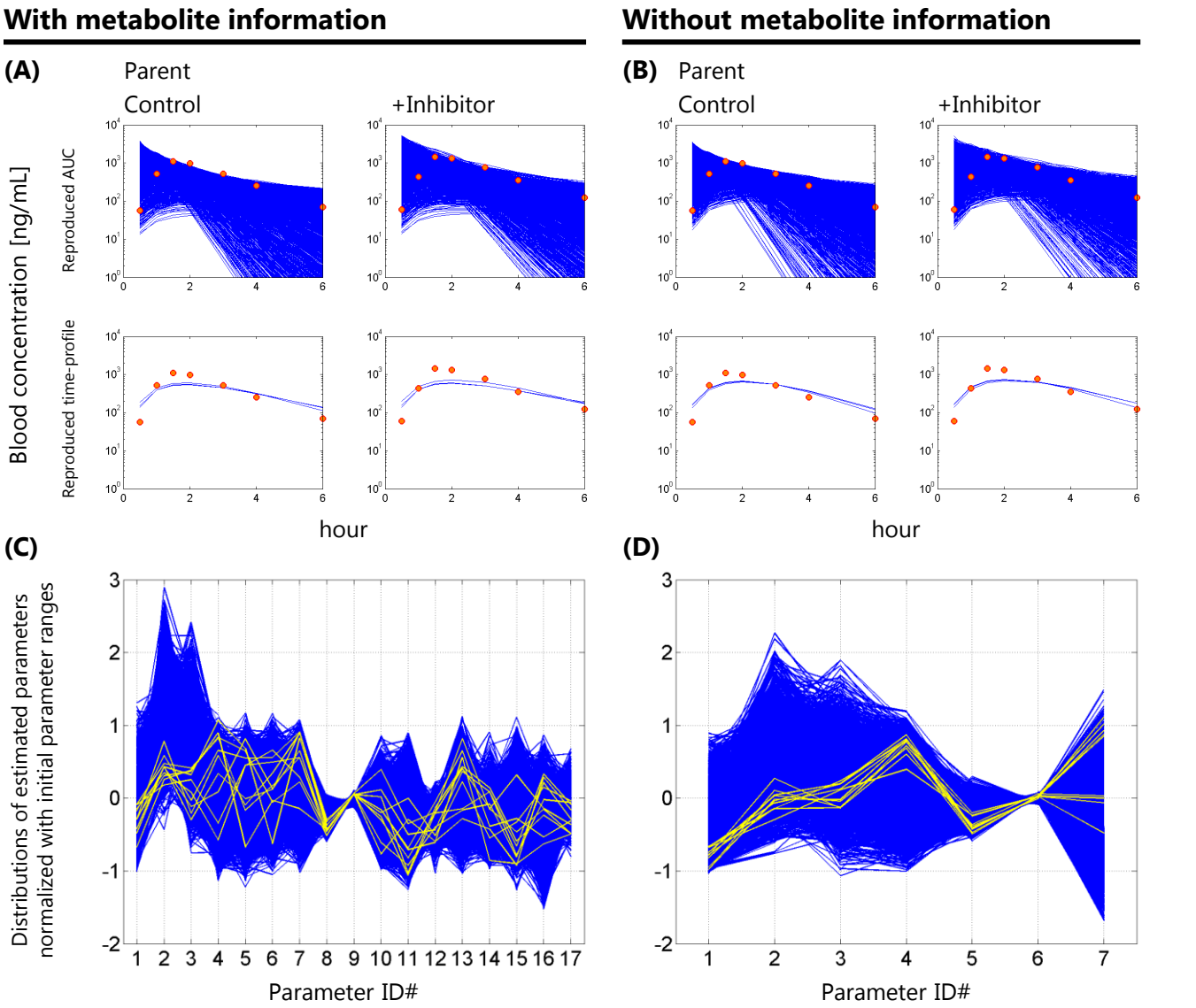
Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine. CYPa: NA, CYPb: CYP3A.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.221	56	0.168	71
2	2	ka	/hr		0.200	6.000	1.051	54	1.172	48
3	3	ktransit	/hr		0.200	6.000	1.319	37	1.028	46
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	3.994	351	3.360	471
5	5	CL12	L/hr/kg		0.037	3.715	0.071	94	0.088	54
6	6	k21	/hr		0.037	3.715	0.146	186	0.142	226
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
		CL_CYPa,Met1 / CL_CYP1,other	-							
7		CL_CYPb,Met1 / CL_CYP2,other	-		0.030	30.000	2.736	137		
8		CL_other,Met1 / CL_other,other	-		0.030	30.000	1.369	140		
		CL_CYPa,Met2 / CL_CYP1,other	-							
9		CL_CYPb,Met2 / CL_CYP2,other	-		0.030	30.000	0.896	189		
		CL_other,Met2 / CL_other,other	-							
		CL_CYPa / CL_other	-							
10	7	CL_CYPb / CL_other	-		0.030	30.000	0.468	20	0.564	58
11	8	fBCLint	L/hr/kg		0.037	3.715	0.415	16.8	0.413	18.4
		Dose	μg/kg	968						
Metabolite 1										
12		Vc	L/kg		0.082	7.429	0.218	88		
13		Kp,h	-		0.030	30.000	0.574	605		
14		CL12	L/hr/kg		0.037	3.715	0.444	132		
15		k21	/hr		0.037	3.715	0.173	168		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
16		CL_CYPb / CL_other	-		0.030	30.000	0.693	85		
17		fBCLint	L/hr/kg		0.037	3.715	0.323	106		
		MW corr	-	1.043						
Metabolite 2										
18		Vc	L/kg		0.082	7.429	0.528	138		
19		Kp,h	-		0.030	30.000	0.689	925		
20		CL12	L/hr/kg		0.037	3.715	0.309	164		
21		k21	/hr		0.037	3.715	0.257	238		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
22		fBCLint	L/hr/kg		0.037	3.715	0.151	136		
		MW corr	-	1.043						
Inhibitor										
		Ki_CYP1	μg/L							
		Ki_CYP2	μg/L							
		R_MBI_CYP1 - 1	-							
23	9	R_MBI_CYP2 - 1	-		1.000	100.000	8.314	45	6.595	278
		R_intes,3A - 1	-							
		Dose	μg/kg							

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.13.3 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between lansoprazole and clarithromycin in CYP2C19 EM subjects, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.13.4 Parameters for analyzing a DDI between lansoprazole and clarithromycin in CYP2C19 IM subjects

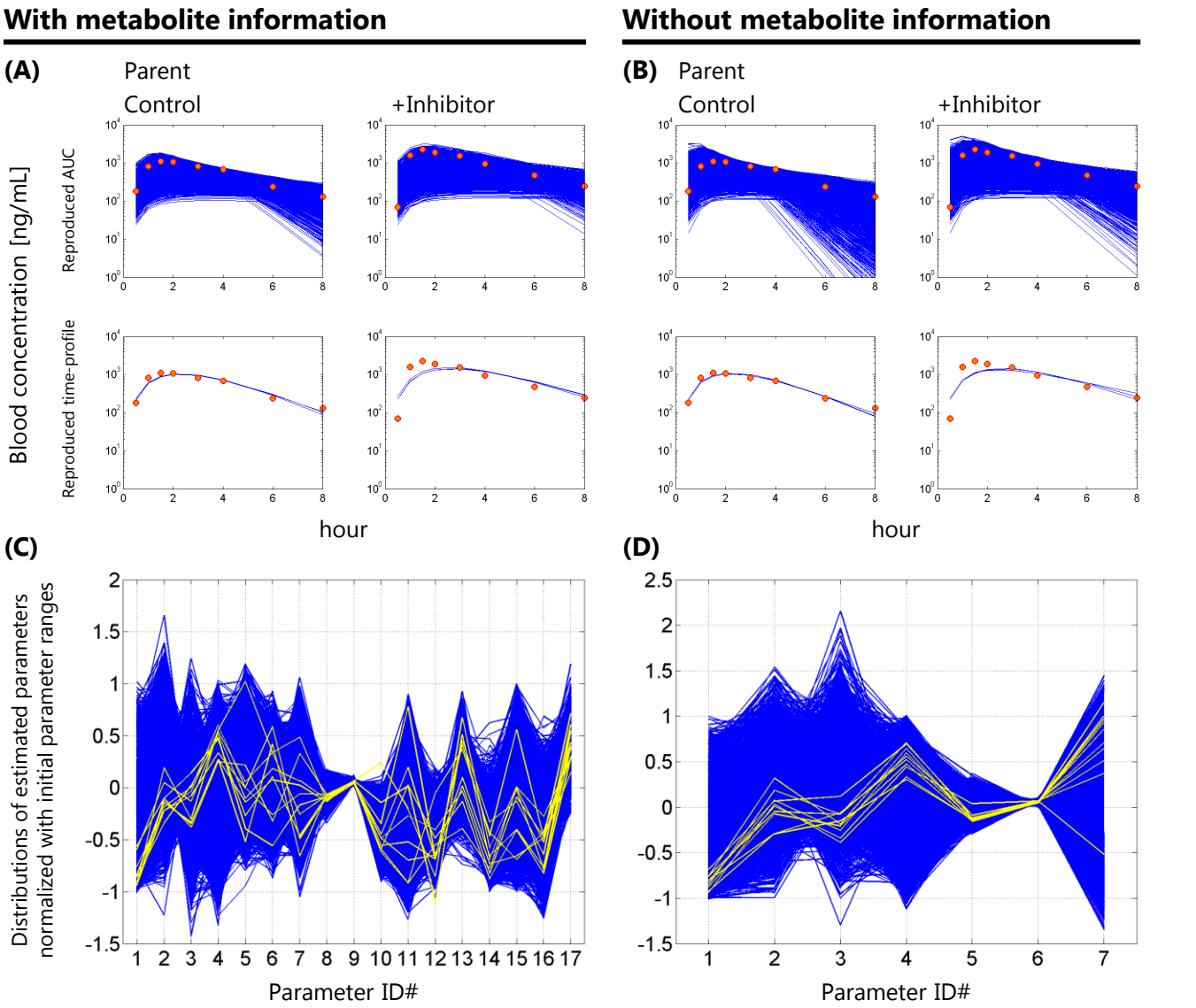
Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine. CYPa: NA, CYPb: CYP3A.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.201	45	0.177	55
2	2	ka	/hr		0.200	6.000	1.074	46	0.991	41
3	3	ktransit	/hr		0.200	6.000	1.134	43	1.014	55
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	1.807	424	1.057	427
5	5	CL12	L/hr/kg		0.021	2.082	0.033	57	0.044	51
6	6	k21	/hr		0.021	2.082	0.068	128	0.148	132
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
		CL_CYPa,Met1 / CL_CYP1,other	-							
7		CL_CYPb,Met1 / CL_CYP2,other	-		0.030	30.000	4.894	162		
8		CL_other,Met1 / CL_other,other	-		0.030	30.000	0.932	212		
		CL_CYPa,Met2 / CL_CYP1,other	-							
9		CL_CYPb,Met2 / CL_CYP2,other	-		0.030	30.000	1.450	150		
		CL_other,Met2 / CL_other,other	-							
		CL_CYPa / CL_other	-							
10	7	CL_CYPb / CL_other	-		0.030	30.000	0.942	17	1.127	53
11	8	fBCLint	L/hr/kg		0.021	2.082	0.225	8.2	0.227	13.1
		Dose	μg/kg	1053						
Metabolite 1										
12		Vc	L/kg		0.082	7.429	0.423	134		
13		Kp,h	-		0.030	30.000	1.131	423		
14		CL12	L/hr/kg		0.021	2.082	0.825	180		
15		k21	/hr		0.021	2.082	0.311	139		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
16		CL_CYPb / CL_other	-		0.030	30.000	0.471	158		
17		fBCLint	L/hr/kg		0.021	2.082	0.496	111		
		MW corr	-	1.043						
Metabolite 2										
18		Vc	L/kg		0.082	7.429	0.444	92		
19		Kp,h	-		0.030	30.000	1.457	285		
20		CL12	L/hr/kg		0.021	2.082	0.234	159		
21		k21	/hr		0.021	2.082	0.291	155		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
22		fBCLint	L/hr/kg		0.021	2.082	0.103	122		
		MW corr	-	1.043						
Inhibitor										
		Ki_CYP1	μg/L							
		Ki_CYP2	μg/L							
		R_MBI_CYP1 - 1	-							
23	9	R_MBI_CYP2 - 1	-		1.000	100.000	15.905	47	11.686	318
		R_intes,3A - 1	-							
		Dose	μg/kg							

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.13.4 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between lansoprazole and clarithromycin in CYP2C19 IM subjects, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.13.5 Parameters for analyzing a DDI between lansoprazole and clarithromycin in CYP2C19 PM subjects

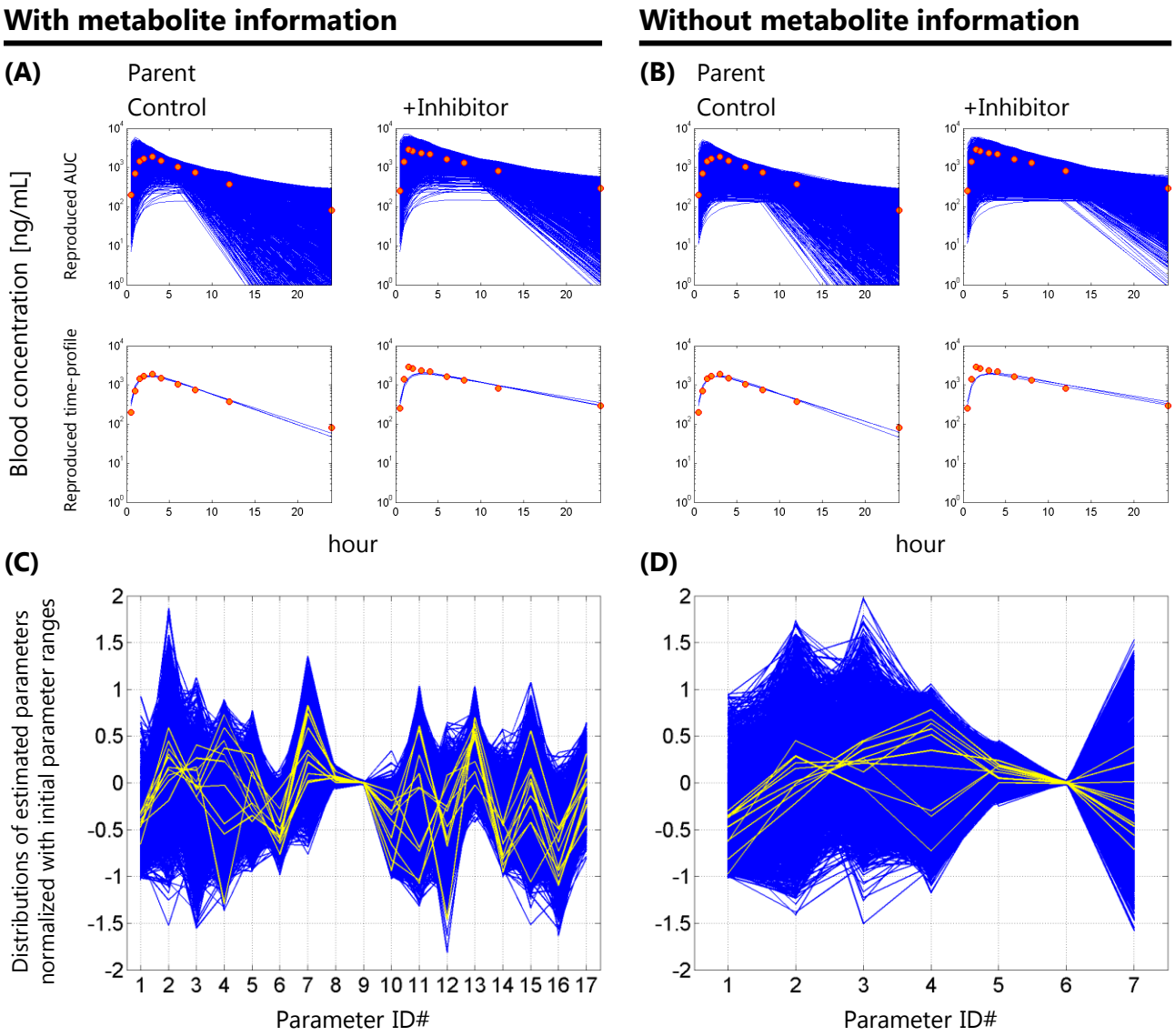
Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine. CYPa: NA, CYPb: CYP3A.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.111	47	0.139	47
2	2	ka	/hr		0.200	6.000	0.858	78	0.927	101
3	3	ktransit	/hr		0.200	6.000	0.954	70	0.929	70
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	1.241	373	0.405	606
5	5	CL12	L/hr/kg		0.007	0.683	0.029	53	0.020	69
6	6	k21	/hr		0.007	0.683	0.059	133	0.083	88
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
		CL_CYPa,Met1 / CL_CYP1,other	-							
7		CL_CYPb,Met1 / CL_CYP2,other	-		0.030	30.000	0.806	148		
8		CL_other,Met1 / CL_other,other	-		0.030	30.000	0.100	226		
		CL_CYPa,Met2 / CL_CYP1,other	-							
9		CL_CYPb,Met2 / CL_CYP2,other	-		0.030	30.000	2.984	186		
		CL_other,Met2 / CL_other,other	-							
		CL_CYPa / CL_other	-							
10	7	CL_CYPb / CL_other	-		0.030	30.000	2.386	98	1.381	48
11	8	fBCLint	L/hr/kg		0.007	0.683	0.052	31.8	0.062	17.7
		Dose	μg/kg	984						
Metabolite 1										
12		Vc	L/kg		0.082	7.429	0.234	99		
13		Kp,h	-		0.030	30.000	0.995	538		
14		CL12	L/hr/kg		0.007	0.683	0.314	81		
15		k21	/hr		0.007	0.683	0.102	83		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
16		CL_CYPb / CL_other	-		0.030	30.000	0.410	156		
17		fBCLint	L/hr/kg		0.007	0.683	0.099	129		
		MW corr	-	1.043						
Metabolite 2										
18		Vc	L/kg		0.082	7.429	0.455	65		
19		Kp,h	-		0.030	30.000	0.445	210		
20		CL12	L/hr/kg		0.007	0.683	0.012	107		
21		k21	/hr		0.007	0.683	0.053	199		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
22		fBCLint	L/hr/kg		0.007	0.683	0.026	81		
		MW corr	-	1.043						
Inhibitor										
		Ki_CYP1	μg/L							
		Ki_CYP2	μg/L							
		R_MBI_CYP1 - 1	-							
23	9	R_MBI_CYP2 - 1	-		1.000	100.000	15.761	94	24.746	198
		R_intes,3A - 1	-							
		Dose	μg/kg							

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.13.5 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between lansoprazole and clarithromycin in CYP2C19 PM subjects, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: lansoprazole, Metabolite 1: 5-hydroxy lansoprazole, Metabolite 2: lansoprazole sulfone, Inhibitor: fluvoxamine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.14.1 Parameters for analyzing a DDI between losartan and fluconazole [Kazierad et al, 1997]

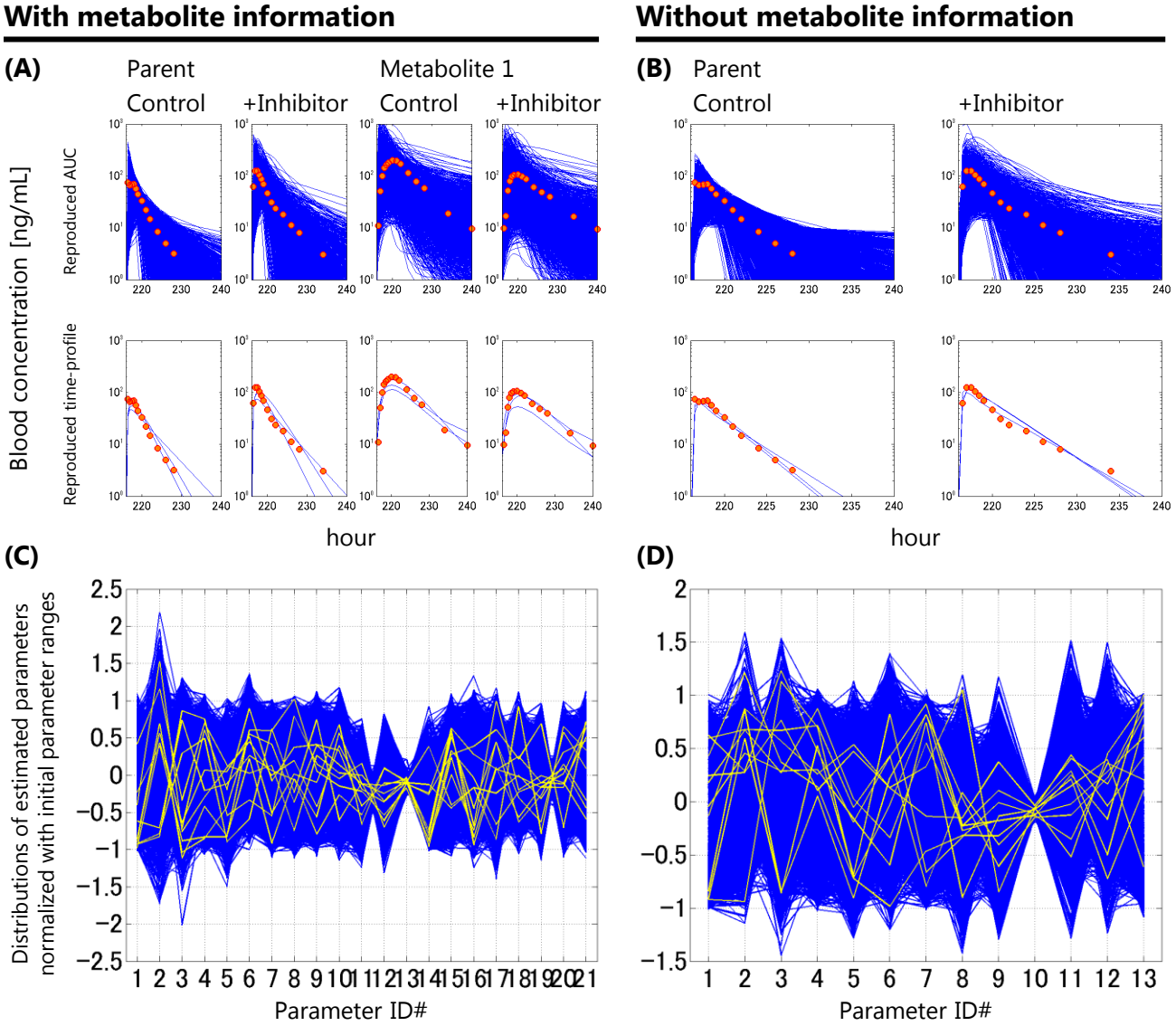
Parent: losartan, Metabolite 1: EXP-3174, Metabolite 2: NA, Inhibitor: fluconazole.
CYPa: CYP2C9, CYPb: CYP3A.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.142	58	0.676	231
2	2	ka	/hr		0.200	6.000	0.636	113	2.576	100
3	3	ktransit	/hr		0.200	6.000	0.775	97	4.701	109
		FaFg	-	0.896						
4	4	Kp,h	-		0.030	30.000	0.765	418	2.032	346
5	5	CL12	L/hr/kg		0.429	42.883	1.146	142	4.206	100
6	6	k21	/hr		0.429	42.883	6.432	182	2.758	124
		CL_R,int,app,cont	L/hr/kg	0.081						
		CL_R,int,app,inhi	L/hr/kg							
7	7	k_3A,Met1 / kLI	-		0.030	30.000	0.928	405	0.000	NaN
8	8	k_3A,other / kLI	-		0.030	30.000	0.759	1102	1.107	796
9		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	1.794	350		
10		CL_CYPb,Met1 / CL_CYP2,other	-		0.030	30.000	1.083	296		
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
11	9	CL_CYPa / CL_other	-		0.030	30.000	0.382	336	0.582	245
12	10	CL_CYPb / CL_other	-		0.030	30.000	0.301	336	0.483	233
13	11	fBCLint	L/hr/kg		0.429	42.883	3.441	4.0	3.178	6.6
		Dose	µg/kg	1245						
Metabolite 1										
14		Vc	L/kg		0.082	7.429	0.408	96		
15		Kp,h	-		0.030	30.000	0.876	807		
16		CL12	L/hr/kg		0.004	0.429	0.040	151		
17		k21	/hr		0.004	0.429	0.026	147		
		CL_R,int,app	L/hr/kg	0.02142						
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
18		fBCLint	L/hr/kg		0.004	0.429	0.071	144		
		MW corr	-	1.033						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
19	12	Ki_CYP1	µg/L		100	100000	2959.487	137	6092.130	170
20	13	Ki_CYP2	µg/L		100	100000	3419.134	173	3194.170	196
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
21	14	R_intes,3A - 1	-		0.100	100.000	3.145	491	4.350	962
		Dose	µg/kg	2491						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.14.1 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between losartan and fluconazole [Kazierad et al, 1997], with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: losartan, Metabolite 1: EXP-3174, Metabolite 2: NA, Inhibitor: fluconazole.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.14.2 Parameters for analyzing a DDI between losartan and fluconazole [Kaukonen et al, 1998]

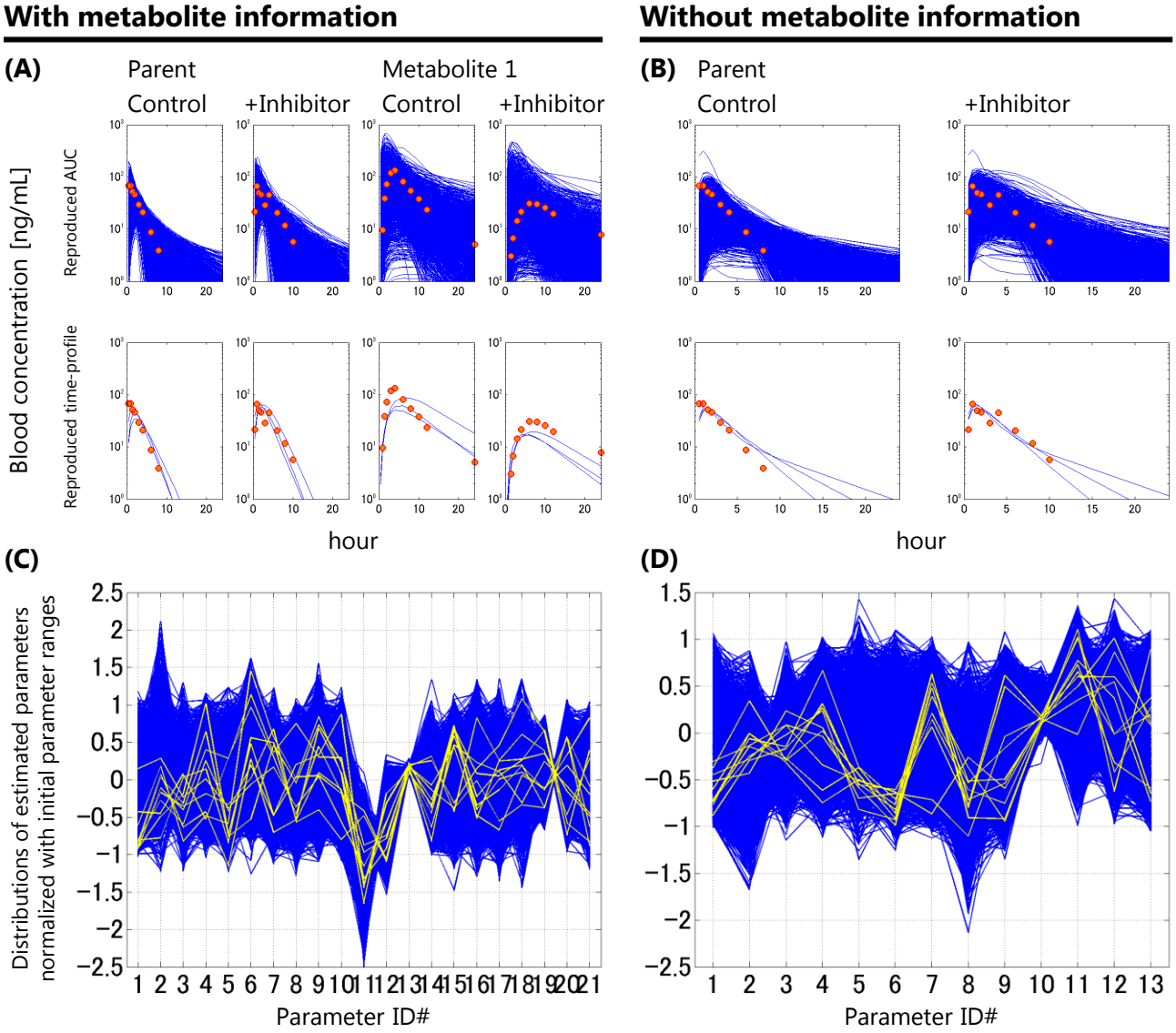
Parent: losartan, Metabolite 1: EXP-3174, Metabolite 2: NA, Inhibitor: fluconazole.
CYPa: CYP2C9, CYPb: CYP3A.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.191	51	0.154	67
2	2	ka	/hr		0.200	6.000	1.067	48	0.763	57
3	3	ktransit	/hr		0.200	6.000	0.290	33	1.089	57
		FaFg	-	0.896						
4	4	Kp,h	-		0.030	30.000	0.441	354	0.393	312
5	5	CL12	L/hr/kg		0.199	19.900	0.930	83	0.934	69
6	6	k21	/hr		0.199	19.900	4.325	161	0.719	191
		CL_R,int,app,cont	L/hr/kg	0.081						
		CL_R,int,app,inhi	L/hr/kg							
7	7	k_3A,Met1 / kLI	-		0.030	30.000	1.126	477		
8	8	k_3A,other / kLI	-		0.030	30.000	1.392	548	1.703	398
9		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	1.205	181		
10		CL_CYPb,Met1 / CL_CYP2,other	-		0.030	30.000	0.808	394		
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
		CL_other,Met2 / CL_other,other	-							
11	9	CL_CYPa / CL_other	-		0.300	30.000	0.239	118	1.470	162
12	10	CL_CYPb / CL_other	-		0.030	30.000	0.140	118	0.108	136
13	11	fBCLint	L/hr/kg		0.199	19.900	2.978	16.0	2.569	15.0
		Dose	µg/kg	746						
Metabolite 1										
14		Vc	L/kg		0.082	7.429	0.654	107		
15		Kp,h	-		0.030	30.000	0.595	354		
16		CL12	L/hr/kg		0.002	0.199	0.029	123		
17		k21	/hr		0.002	0.199	0.020	216		
		CL_R,int,app	L/hr/kg	0.02142						
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
18		fBCLint	L/hr/kg		0.002	0.199	0.046	135		
		MW corr	-	1.033						
Metabolite 2										
		Vc	L/kg							
		Kp,h	-							
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-							
Inhibitor										
19	12	Ki_CYP1	µg/L		100	100000	1726.210	153	18533.924	197
20	13	Ki_CYP2	µg/L		100	100000	6878.937	329	16092.858	192
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
21	14	R_intes,3A - 1	-		0.100	100.000	2.094	204	5.714	511
		Dose	µg/kg	2985						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.14.2 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between losartan and fluconazole [Kaukonen et al, 1998], with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: losartan, Metabolite 1: EXP-3174, Metabolite 2: NA, Inhibitor: fluconazole.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.15.1 Parameters for analyzing a DDI between omeprazole and fluvoxamine in CYP2C19 EM subjects

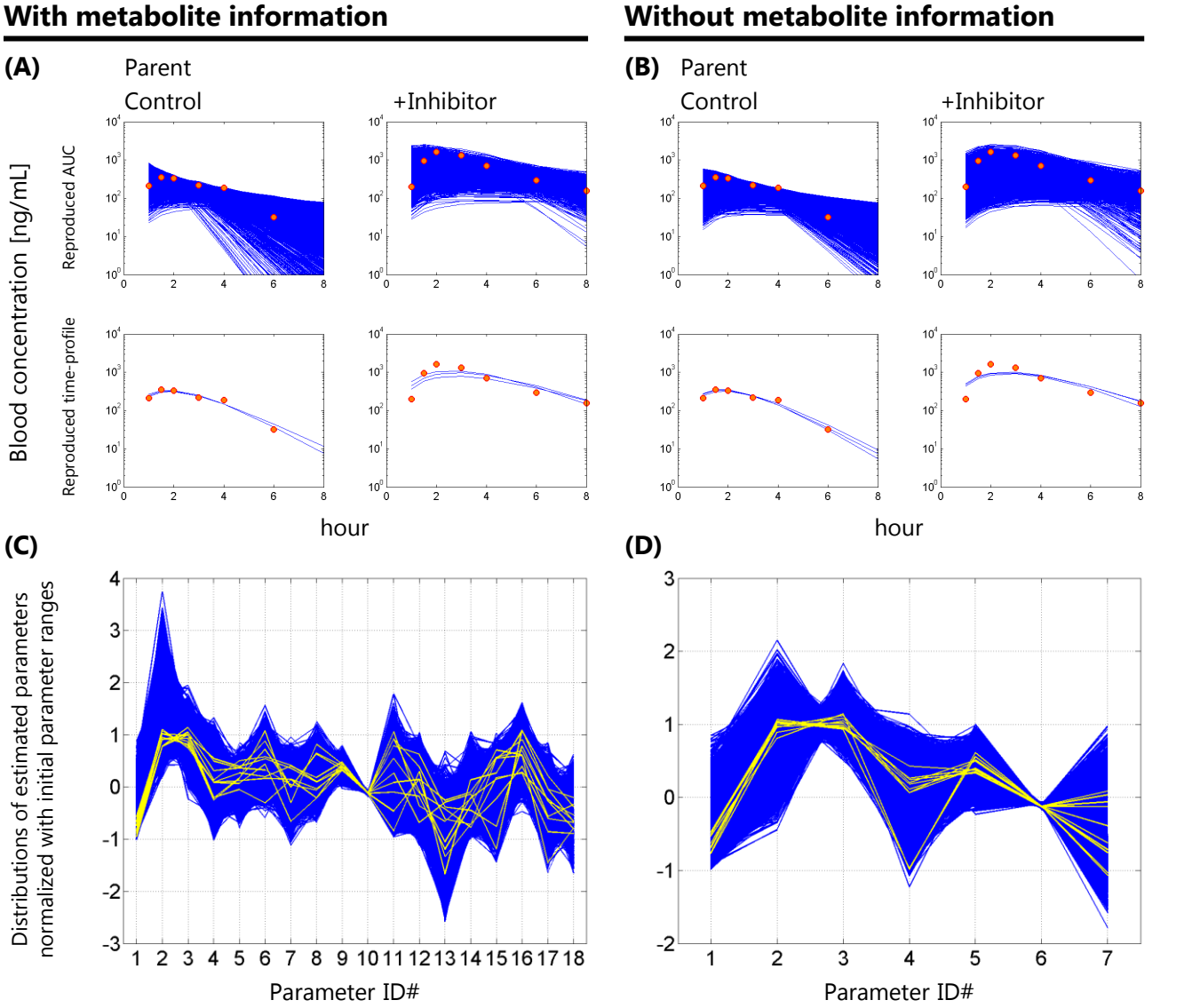
Parent: omeprazole, Metabolite 1: 5-hydroxy omeprazole, Metabolite 2: omeprazole sulfone, Inhibitor: fluvoxamine. CYPa: CYP2C19, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.157	62	0.212	38
2	2	ka	/hr		0.200	6.000	0.876	46	0.993	34
3	3	ktransit	/hr		0.200	6.000	1.056	61	0.997	56
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.489	474	0.586	307
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
5		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	1.495	111		
		CL_CYPb,Met1 / CL_CYP2,other	-							
6		CL_other,Met1 / CL_other,other	-		0.030	30.000	4.742	190		
7		CL_CYPa,Met2 / CL_CYP1,other	-		0.030	30.000	0.393	242		
		CL_CYPb,Met2 / CL_CYP2,other	-							
8		CL_other,Met2 / CL_other,other	-		0.030	30.000	1.844	185		
9	5	CL_CYPa / CL_other	-		0.030	30.000	5.572	36	5.815	47
		CL_CYPb / CL_other	-							
10	6	fBCLint	L/hr/kg		0.070	7.016	0.544	2.3	0.548	1.9
		Dose	µg/kg	606						
Metabolite 1										
11		Vc	L/kg		0.082	7.429	0.621	222		
12		Kp,h	-		0.030	30.000	1.316	492		
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg	0.22693						
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
13		fBCLint	L/hr/kg		0.070	7.016	0.115	127		
		MW corr	-	1.043						
Metabolite 2										
14		Vc	L/kg		0.082	7.429	0.640	137		
15		Kp,h	-		0.030	30.000	0.129	251		
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
16		CL_CYPa / CL_other	-		0.030	30.000	11.899	229		
		CL_CYPb / CL_other	-							
17		fBCLint	L/hr/kg		0.070	7.016	0.206	145		
		MW corr	-	1.0433						
Inhibitor										
18	7	Ki_CYP1	µg/L		0.300	300.0	1.081	205	2.460	67
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	379						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.15.1 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between omeprazole and fluvoxamine in CYP2C19 EM subjects, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: omeprazole, Metabolite 1: 5-hydroxy omeprazole, Metabolite 2: omeprazole sulfone, Inhibitor: fluvoxamine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.15.2 Parameters for analyzing a DDI between omeprazole and fluvoxamine in CYP2C19 IM subjects

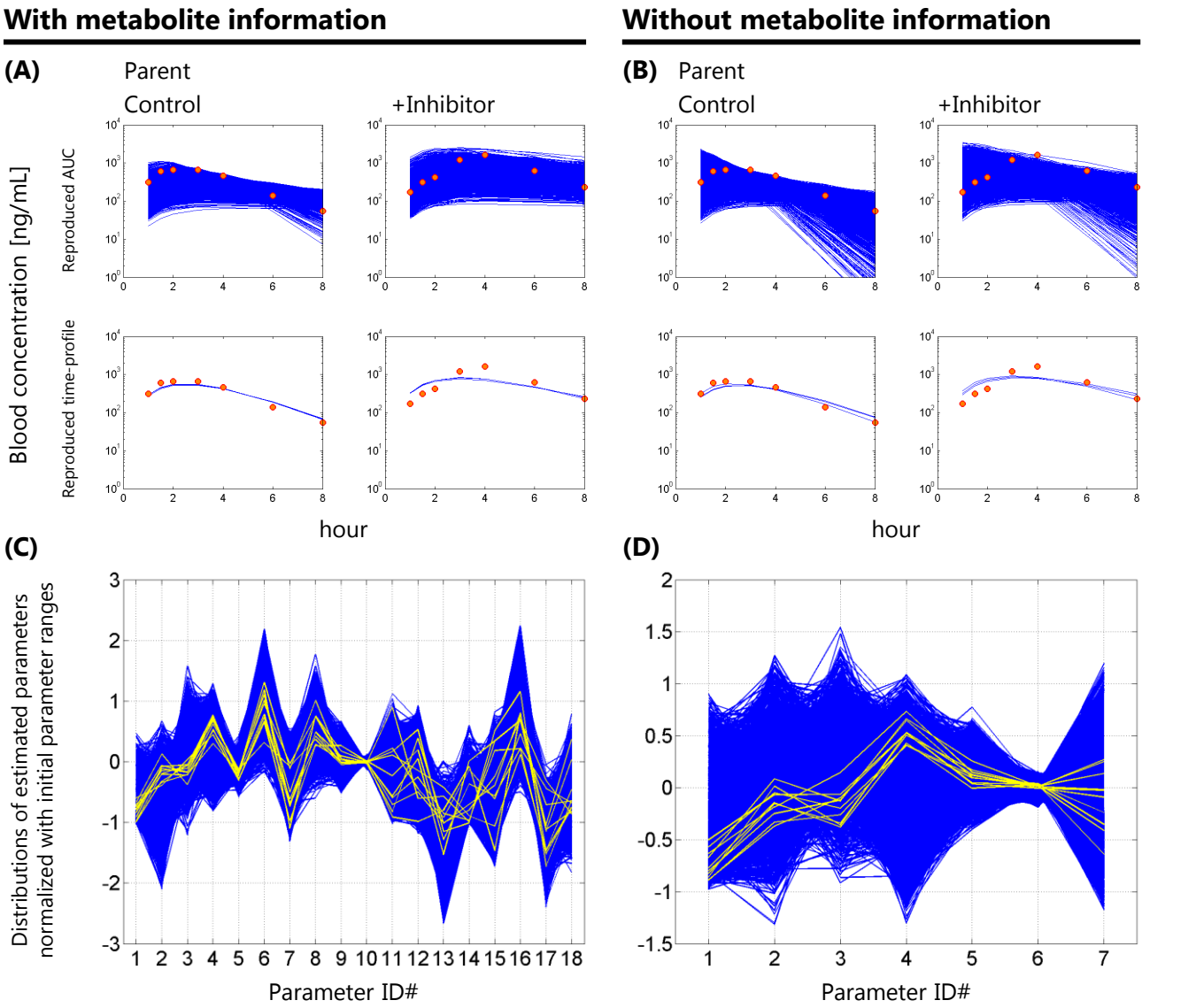
Parent: omeprazole, Metabolite 1: 5-hydroxy omeprazole, Metabolite 2: omeprazole sulfone, Inhibitor: fluvoxamine. CYPa: CYP2C19, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.574	25	0.151	49
2	2	ka	/hr		0.200	6.000	1.654	53	1.069	37
3	3	ktransit	/hr		0.200	6.000	0.812	45	0.789	35
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.127	432	4.574	340
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
5		CL_CYPa,Met1 / CL_CYP1,other	-		0.030	30.000	0.662	59		
		CL_CYPb,Met1 / CL_CYP2,other	-							
6		CL_other,Met1 / CL_other,other	-		0.030	30.000	143.735	365		
7		CL_CYPa,Met2 / CL_CYP1,other	-		0.030	30.000	0.706	113		
		CL_CYPb,Met2 / CL_CYP2,other	-							
8		CL_other,Met2 / CL_other,other	-		0.030	30.000	9.864	310		
9	5	CL_CYPa / CL_other	-		0.030	30.000	0.908	29	2.813	97
		CL_CYPb / CL_other	-							
10	6	fBCLint	L/hr/kg		0.026	2.552	0.227	6.7	0.246	1.7
		Dose	µg/kg	656						
Metabolite 1										
11		Vc	L/kg		0.082	7.429	1.953	33		
12		Kp,h	-		0.030	30.000	0.320	185		
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg	0.22693						
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
13		fBCLint	L/hr/kg		0.026	2.552	0.001	336		
		MW corr	-	1.043						
Metabolite 2										
14		Vc	L/kg		0.082	7.429	0.099	56		
15		Kp,h	-		0.030	30.000	0.008	283		
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
16		CL_CYPa / CL_other	-		0.03	30.00	112.642	334		
		CL_CYPb / CL_other	-							
17		fBCLint	L/hr/kg		0.026	2.552	0.014	103		
		MW corr	-	1.043						
Inhibitor										
18	7	Ki_CYP1	µg/L		0.300	300.0	0.000	594	5.563	227
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	410						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.15.2 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between omeprazole and fluvoxamine in CYP2C19 IM subjects, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: omeprazole, Metabolite 1: 5-hydroxy omeprazole, Metabolite 2: omeprazole sulfone, Inhibitor: fluvoxamine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.16.1 Parameters for analyzing a DDI between oxycodone and quinidine

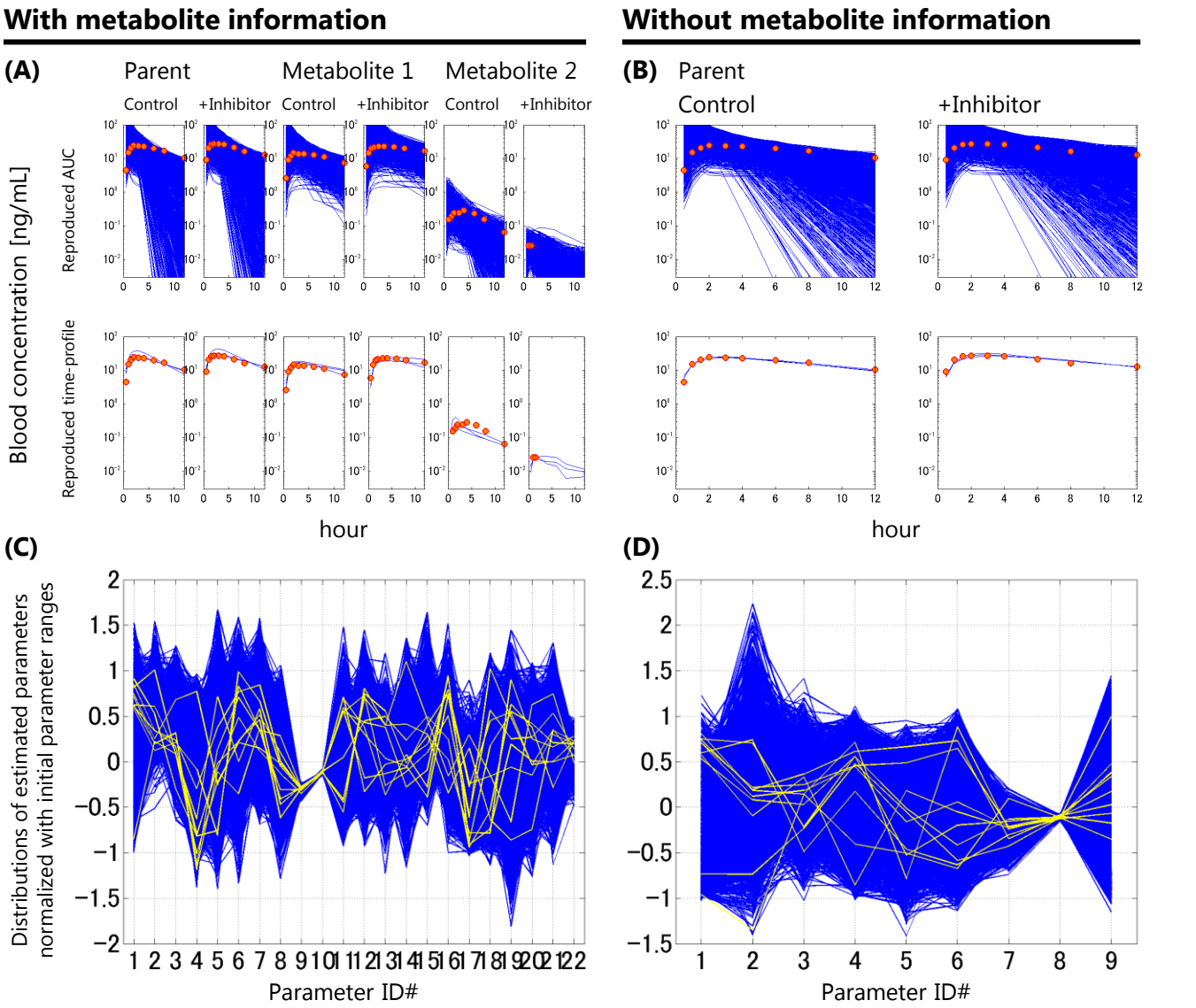
Parent: oxycodone, Metabolite 1: noroxycodone, Metabolite 2: oxymorphone, Inhibitor: quinidine.
CYPa: NA, CYPb: CYP2D6.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	2.618	166	1.597	206
2	2	ka	/hr		0.200	6.000	1.964	57	1.001	146
3	3	ktransit	/hr		0.200	6.000	1.735	71	1.371	68
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.227	746	1.450	560
5	5	CL12	L/hr/kg		0.110	11.021	1.475	160	1.219	132
6	6	k21	/hr		0.110	11.021	2.771	155	1.075	178
		CL_R,int,app,cont	L/hr/kg	0.056						
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
		CL_CYPa,Met1 / CL_CYP1,other	-							
		CL_CYPb,Met1 / CL_CYP2,other	-							
7		CL_other,Met1 / CL_other,other	-		0.030	30.000	4.847	248		
		CL_CYPa,Met2 / CL_CYP1,other	-							
8		CL_CYPb,Met2 / CL_CYP2,other	-		0.030	30.000	0.823	252		
		CL_other,Met2 / CL_other,other	-							
		CL_CYPa / CL_other	-							
9	7	CL_CYPb / CL_other	-		0.030	30.000	0.339	9	0.491	42
10	8	fBCLint	L/hr/kg		0.110	11.021	0.854	2.2	0.858	2.2
		Dose	μg/kg	285						
Metabolite 1										
11		Vc	L/kg		0.082	7.429	1.155	134		
12		Kp,h	-		0.030	30.000	3.988	173		
13		CL12	L/hr/kg		0.011	1.102	0.147	84		
14		k21	/hr		0.011	1.102	0.153	124		
		CL_R,int,app	L/hr/kg	0.27195						
		CL_CYPa / CL_other	-							
15		CL_CYPb / CL_other	-		0.030	30.000	2.048	153		
16		fBCLint	L/hr/kg		0.011	1.102	0.277	145		
		MW corr	-	0.956						
Metabolite 2										
17		Vc	L/kg		0.082	7.429	0.303	135		
18		Kp,h	-		0.030	30.000	0.683	355		
19		CL12	L/hr/kg		0.110	11.021	1.505	182		
20		k21	/hr		0.110	11.021	1.754	108		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
21		fBCLint	L/hr/kg		0.110	11.021	2.443	110		
		MW corr	-	0.956						
Inhibitor										
		Ki_CYP1	μg/L							
22	9	Ki_CYP2	μg/L		0.300	300.0	14.737	42	26.217	237
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	μg/kg	2361						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.16.1 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between oxycodone and quinidine, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: oxycodone, Metabolite 1: noroxycodone, Metabolite 2: oxymorphone, Inhibitor: quinidine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.16.2 Parameters for analyzing a DDI between oxycodone and voriconazole

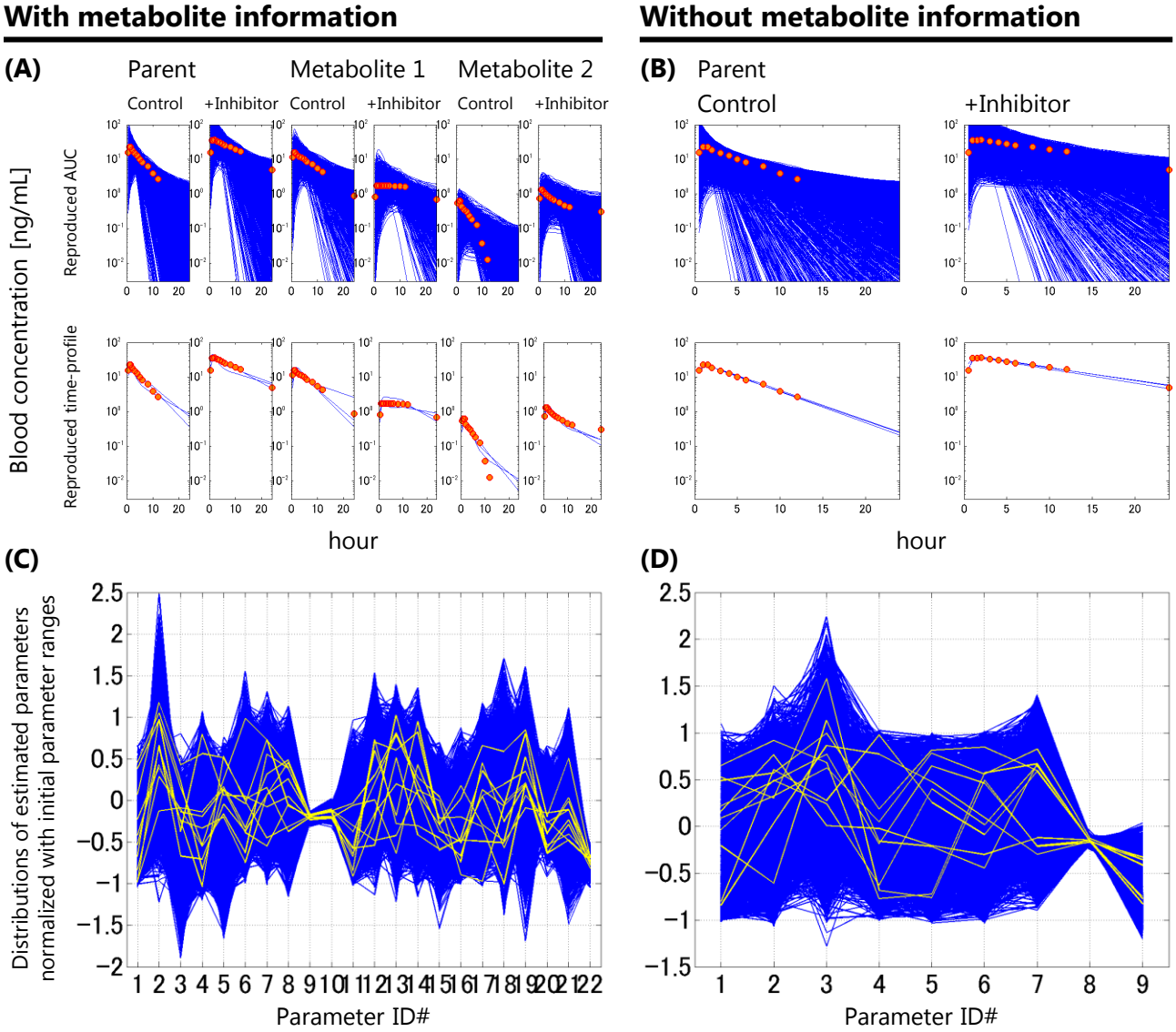
Parent: oxycodone, Metabolite 1: noroxycodone, Metabolite 2: oxymorphone, Inhibitor: voriconazole.
CYPa: CYP3A, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.291	150	0.862	179
2	2	ka	/hr		0.200	6.000	3.899	82	2.123	117
3	3	ktransit	/hr		0.200	6.000	0.833	52	3.682	93
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.280	414	0.615	579
5	5	CL12	L/hr/kg		0.136	13.567	1.257	68	1.533	220
6	6	k21	/hr		0.136	13.567	0.651	98	1.763	138
		CL_R,int,app,cont	L/hr/kg	0.056						
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.300	300.000	19.448	357		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
8		CL_other,Met2 / CL_other,other	-		0.030	30.000	1.094	190		
9	7	CL_CYPa / CL_other	-		0.300	300.000	4.764	7	24.860	282
		CL_CYPb / CL_other	-							
10	8	fBCLint	L/hr/kg		0.136	13.567	0.954	8.1	0.950	3.1
		Dose	µg/kg	138						
Metabolite 1										
11		Vc	L/kg		0.082	7.429	0.259	100		
12		Kp,h	-		0.100	10.000	1.844	157		
13		CL12	L/hr/kg		0.014	1.357	0.224	171		
14		k21	/hr		0.014	1.357	0.273	153		
		CL_R,int,app	L/hr/kg	0.27195						
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
15		fBCLint	L/hr/kg		0.014	1.357	0.070	81		
		MW corr	-	0.956						
Metabolite 2										
16		Vc	L/kg		0.082	7.429	0.390	96		
17		Kp,h	-		0.030	30.000	0.770	431		
18		CL12	L/hr/kg		0.136	13.567	1.177	149		
19		k21	/hr		0.136	13.567	2.991	159		
		CL_R,int,app	L/hr/kg							
20		CL_CYPa / CL_other	-		0.030	30.000	0.312	77		
		CL_CYPb / CL_other	-							
21		fBCLint	L/hr/kg		0.136	13.567	1.035	115		
		MW corr	-	0.956						
Inhibitor										
22	9	Ki_CYP1	µg/L		100.000	100000	249.846	17	468.557	79
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	µg/kg	2759						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.16.2 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between oxycodone and voriconazole, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: oxycodone, Metabolite 1: noroxycodone, Metabolite 2: oxymorphone, Inhibitor: voriconazole.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.16.3 Parameters for analyzing a DDI between oxycodone and itraconazole

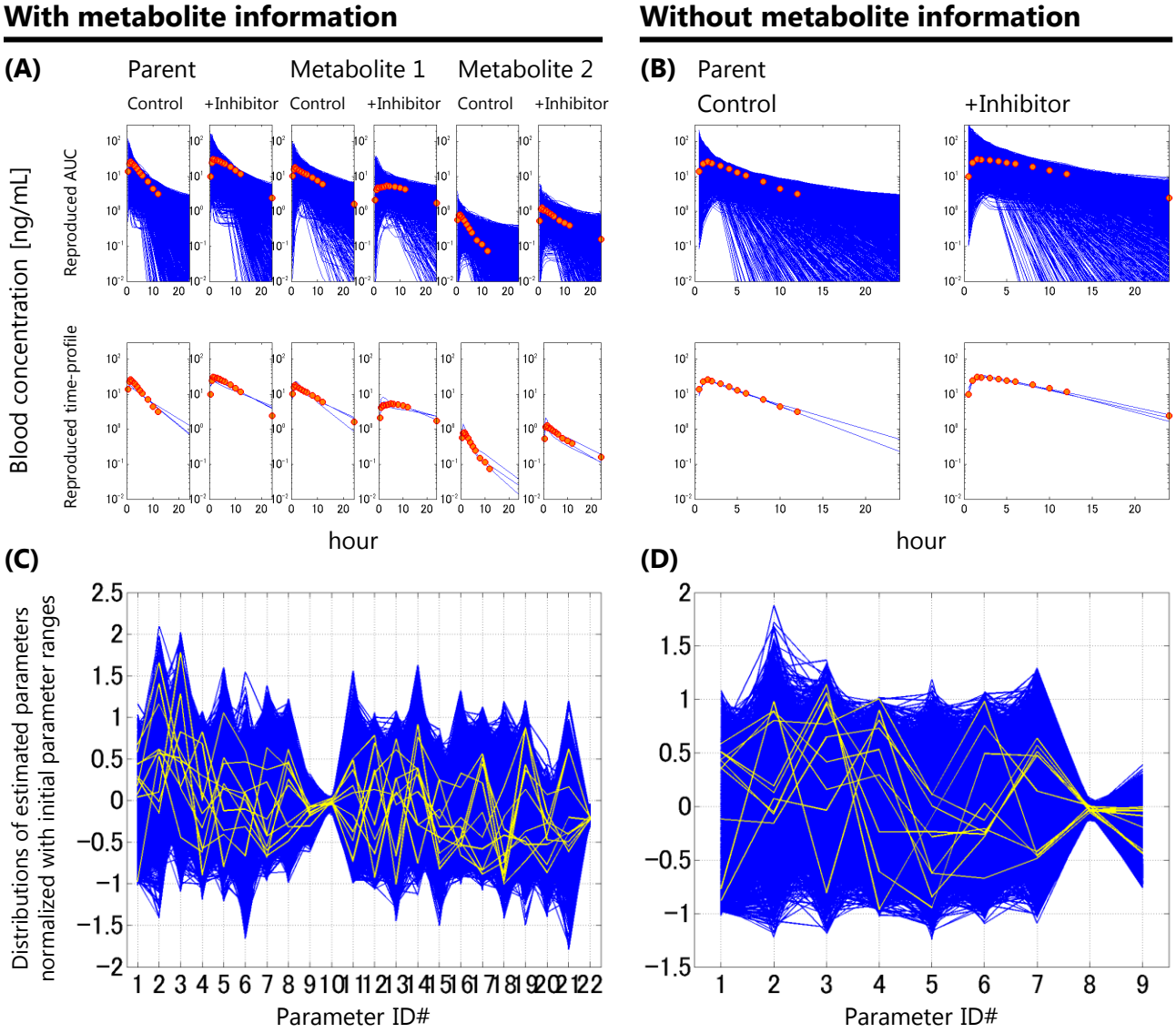
Parent: oxycodone, Metabolite 1: noroxycodone, Metabolite 2: oxymorphone, Inhibitor: itraconazole.
CYPa: CYP3A, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.771	196	0.864	201
2	2	ka	/hr		0.200	6.000	3.209	98	2.439	76
3	3	ktransit	/hr		0.200	6.000	2.566	112	1.993	116
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	0.571	647	0.903	1221
5	5	CL12	L/hr/kg		0.078	7.790	1.284	199	0.557	220
6	6	k21	/hr		0.078	7.790	0.886	129	1.028	137
		CL_R,int,app,cont	L/hr/kg	0.056						
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
7		CL_CYPa,Met1 / CL_CYP1,other	-		0.300	300.000	11.163	574		
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
		CL_CYPb,Met2 / CL_CYP2,other	-							
8		CL_other,Met2 / CL_other,other	-		0.030	30.000	1.509	248		
9	7	CL_CYPa / CL_other	-		0.300	300.000	5.636	27	12.831	350
		CL_CYPb / CL_other	-							
10	8	fBCLint	L/hr/kg		0.078	7.790	0.752	6.1	0.730	3.9
		Dose	μg/kg	127						
Metabolite 1										
11		Vc	L/kg		0.082	7.429	0.614	144		
12		Kp,h	-		0.030	30.000	1.464	841		
13		CL12	L/hr/kg		0.008	0.779	0.079	237		
14		k21	/hr		0.008	0.779	0.096	279		
		CL_R,int,app	L/hr/kg	0.27195						
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
15		fBCLint	L/hr/kg		0.008	0.779	0.043	134		
		MW corr	-	0.956						
Metabolite 2										
16		Vc	L/kg		0.082	7.429	0.380	77		
17		Kp,h	-		0.030	30.000	1.056	676		
18		CL12	L/hr/kg		0.078	7.790	0.288	136		
19		k21	/hr		0.078	7.790	0.718	148		
		CL_R,int,app	L/hr/kg							
20		CL_CYPa / CL_other	-		0.030	30.000	0.224	106		
		CL_CYPb / CL_other	-							
21		fBCLint	L/hr/kg		0.078	7.790	0.535	139		
		MW corr	-	0.956						
Inhibitor										
22	9	Ki_CYP1	μg/L		10.000	10000	149.865	8	149.288	70
		Ki_CYP2	μg/L							
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	μg/kg	2532						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.16.3 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between oxycodone and itraconazole, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: oxycodone, Metabolite 1: noroxycodone, Metabolite 2: oxymorphone, Inhibitor: itraconazole.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.16.4 Parameters for analyzing a DDI between oxycodone and paroxetine

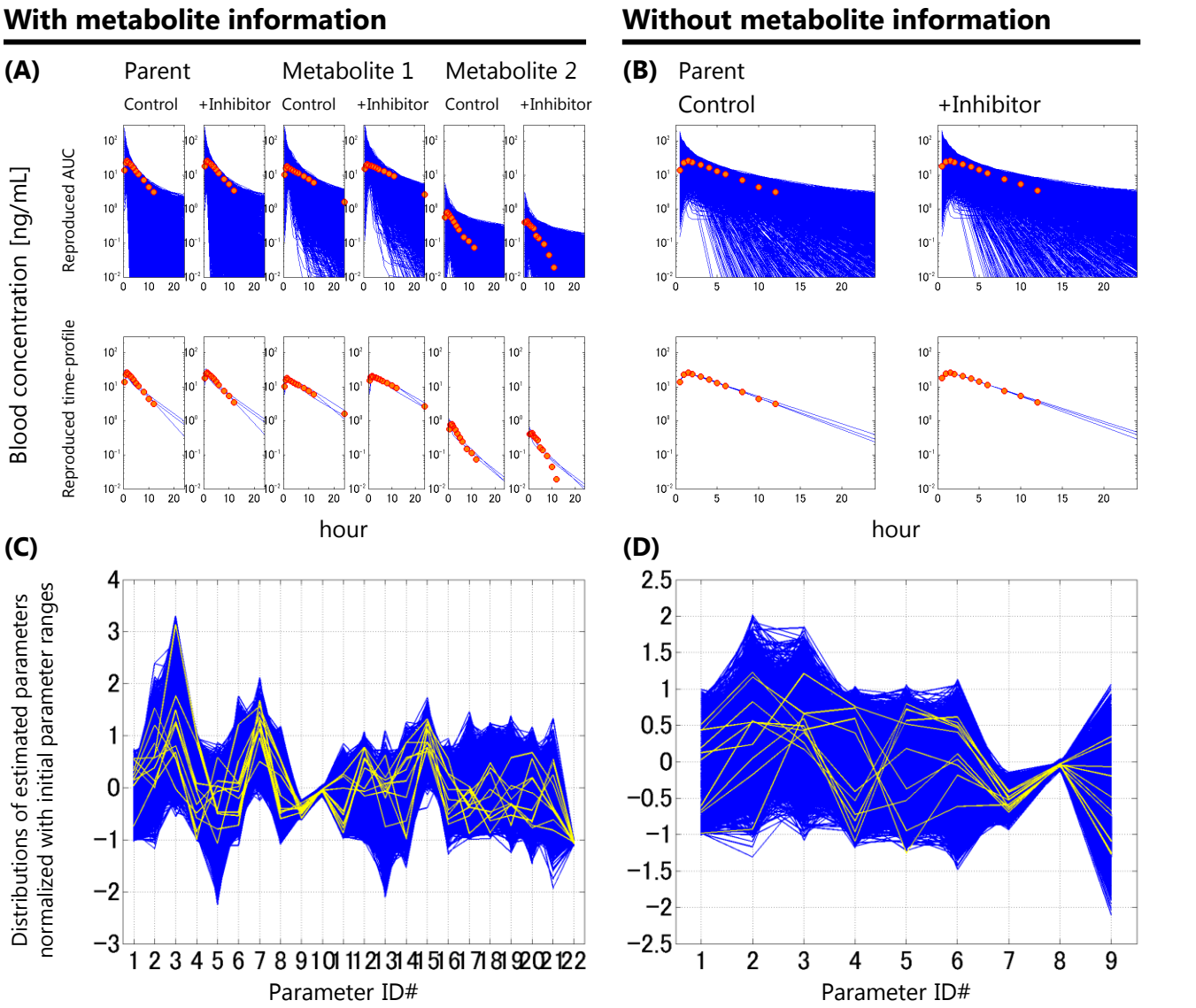
Parent: oxycodone, Metabolite 1: noroxycodone, Metabolite 2: oxymorphone, Inhibitor: paroxetine.
CYPa: NA, CYPb: CYP2D6.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	1.088	133	0.469	234
2	2	ka	/hr		0.200	6.000	2.753	124	2.121	141
3	3	ktransit	/hr		0.200	6.000	8.598	157	2.020	105
		FaFg	-	1.000						
4	4	Kp,h	-		0.030	30.000	1.004	1265	0.315	681
5	5	CL12	L/hr/kg		0.078	7.790	0.417	244	0.604	263
6	6	k21	/hr		0.078	7.790	0.952	166	1.111	110
		CL_R,int,app,cont	L/hr/kg	0.056						
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
		CL_CYPa,Met1 / CL_CYP1,other	-							
		CL_CYPb,Met1 / CL_CYP2,other	-							
7		CL_other,Met1 / CL_other,other	-		0.030	30.000	34.559	250		
		CL_CYPa,Met2 / CL_CYP1,other	-							
8		CL_CYPb,Met2 / CL_CYP2,other	-		0.030	30.000	1.262	224		
		CL_other,Met2 / CL_other,other	-							
		CL_CYPa / CL_other	-							
9	7	CL_CYPb / CL_other	-		0.030	30.000	0.227	27	0.138	44
10	8	fBCLint	L/hr/kg		0.078	7.790	0.720	6.2	0.713	4.8
		Dose	µg/kg	127						
Metabolite 1										
11		Vc	L/kg		0.082	7.429	0.427	153		
12		Kp,h	-		0.030	30.000	1.739	500		
13		CL12	L/hr/kg		0.008	0.779	0.038	198		
14		k21	/hr		0.008	0.779	0.059	297		
		CL_R,int,app	L/hr/kg	0.27195						
		CL_CYPa / CL_other	-							
15		CL_CYPb / CL_other	-		0.030	30.000	25.634	176		
16		fBCLint	L/hr/kg		0.008	0.779	0.066	160		
		MW corr	-	0.956						
Metabolite 2										
17		Vc	L/kg		0.082	7.429	0.312	85		
18		Kp,h	-		0.030	30.000	1.436	421		
19		CL12	L/hr/kg		0.078	7.790	0.421	122		
20		k21	/hr		0.078	7.790	0.644	192		
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
21		fBCLint	L/hr/kg		0.078	7.790	0.514	133		
		MW corr	-	0.956						
Inhibitor										
		Ki_CYP1	µg/L							
		Ki_CYP2	µg/L							
		R_MBI_CYP1 - 1	-							
22	9	R_MBI_CYP2 - 1	-		1.000	100.000	0.933	5	3.598	244
		R_intes,3A - 1	-							
		Dose	µg/kg							

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.16.4 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between oxycodone and paroxetine, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: oxycodone, Metabolite 1: noroxycodone, Metabolite 2: oxymorphone, Inhibitor: paroxetine.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

Table S.17 Parameters for analyzing a DDI between ropivacaine and itraconazole

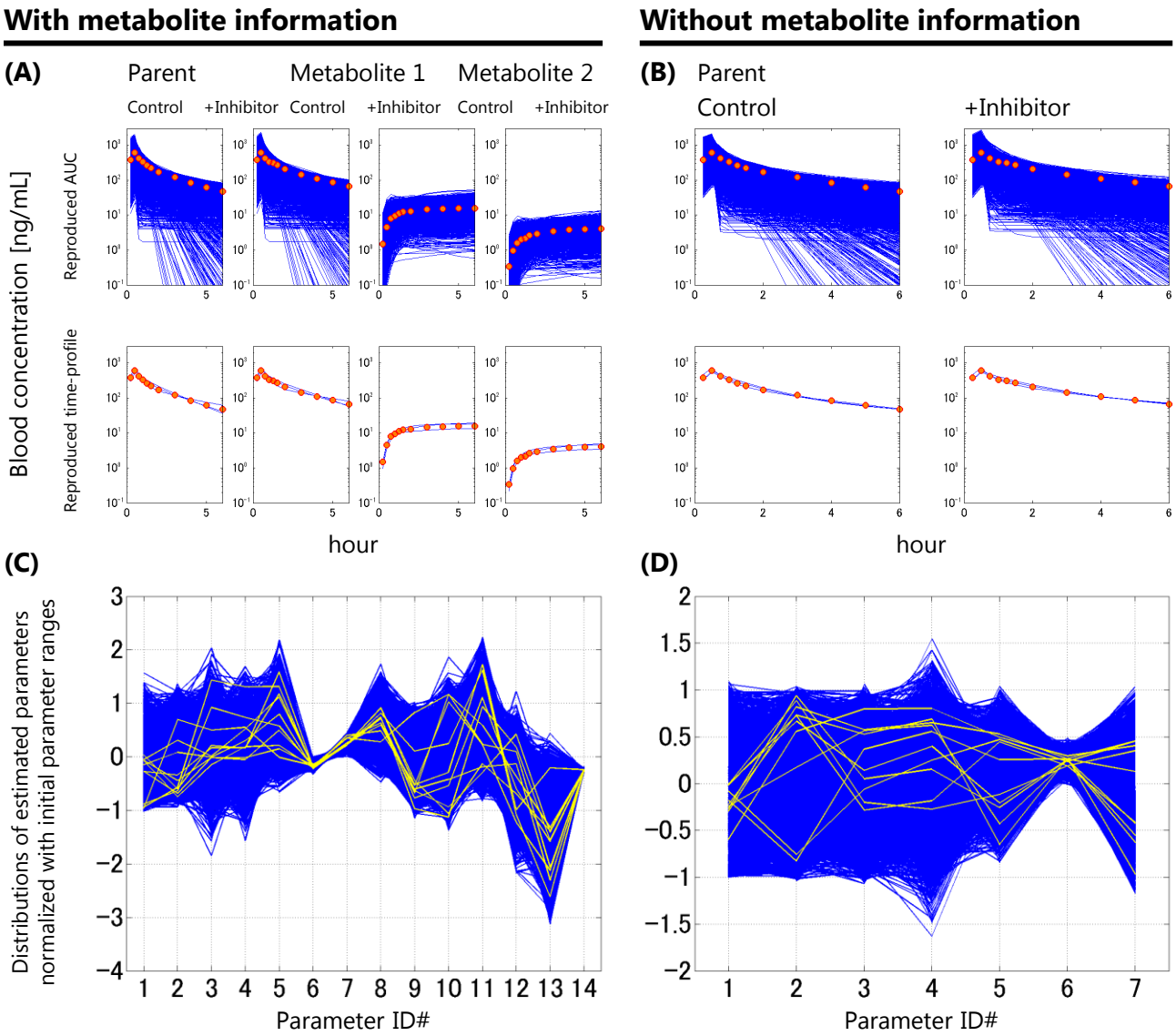
Parent: ropivacaine, Metabolite 1: (S)-2',6'-Pipecoloxylidide, Metabolite 2: NA, Inhibitor: itraconazole.
CYPa: CYP3A, CYPb: NA.

				Parameter values			Final estimates with metabolite		Final estimates without metabolite	
Parent				Fixed/	Free parameters		Geometric	Geometric	Geometric	Geometric
ID1	ID2	parameter	unit		min	max	mean	CV [%]	mean	CV [%]
1	1	Vc	L/kg		0.082	7.429	0.504	102	0.481	63
		ka	/hr							
		ktransit	/hr							
		FaFg	-	1.000						
2	2	Kp,h	-		0.030	30.000	0.445	490	1.522	1091
3	3	CL12	L/hr/kg		0.047	4.718	0.629	185	0.926	95
4	4	k21	/hr		0.047	4.718	1.092	141	1.134	99
		CL_R,int,app,cont	L/hr/kg							
		CL_R,int,app,inhi	L/hr/kg							
		k_3A,Met1 / kLI	-							
		k_3A,other / kLI	-							
		CL_CYPa,Met1 / CL_CYP1,other	-							
		CL_CYPb,Met1 / CL_CYP2,other	-							
		CL_other,Met1 / CL_other,other	-							
		CL_CYPa,Met2 / CL_CYP1,other	-							
5		CL_CYPb,Met2 / CL_CYP2,other	-		0.030	30.000	11.071	394		
		CL_other,Met2 / CL_other,other	-							
		CL_CYPa / CL_other	-							
6	5	CL_CYPb / CL_other	-		0.030	30.000	0.512	9	0.891	141
7	6	fBCLint	L/hr/kg		0.047	4.718	0.858	12.7	0.837	8.2
		Dose	μg/kg	600						
Metabolite 1										
		Vc	L/kg	1						
		Kp,h	-	1						
		CL12	L/hr/kg							
		k21	/hr							
		CL_R,int,app	L/hr/kg							
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
		fBCLint	L/hr/kg							
		MW corr	-	1.058						
Metabolite 2										
8		Vc	L/kg		0.082	7.429	3.426	72		
9		Kp,h	-		0.030	30.000	0.301	651		
10		CL12	L/hr/kg		0.047	4.718	0.569	586		
11		k21	/hr		0.047	4.718	2.316	251		
12		CL_R,int,app	L/hr/kg		0.047	4.718	0.155	274		
		CL_CYPa / CL_other	-							
		CL_CYPb / CL_other	-							
13		fBCLint	L/hr/kg		0.047	4.718	0.018	328		
		MW corr	-	0.847						
Inhibitor										
		Ki_CYP1	μg/L							
14	7	Ki_CYP2	μg/L		10.0	10000	134.581	3	158.657	205
		R_MBI_CYP1 - 1	-							
		R_MBI_CYP2 - 1	-							
		R_intes,3A - 1	-							
		Dose	μg/kg	2632						

Final estimates represent summary statistics of estimated values for 30 parameter sets reproducing concentration-time profiles. CL_{int}, hepatic intrinsic clearance; $CL_{R,int,app}$, apparent renal intrinsic clearance; CL_{12}, transport clearance from central to peripheral compartment; F_aF_g, intestinal availability; f_B, protein unbound fraction in blood; k_a, absorption rate constant; K_i, inhibition constant; $K_{p,h}$, liver to blood concentration ratio; $k_{transit}$, transit rate constant in the intestine; k_{21}, kinetic constant from peripheral to central compartment; R_{MBI}, ratio of inhibition with mechanism-based inhibitors; $R_{intes,3A}$, ratio of inhibition for intestinal CYP3A activity; V_c, distribution volume of central compartment.

Fig S.17 Simulated and reported blood concentration-time profiles (A,B) and estimated parameter distributions (C,D) after the analyses of a DDI between ropivacaine and itraconazole, with (A,C) or without (B,D) including metabolites' pharmacokinetic alterations

Parent: ropivacaine, Metabolite 1: (S)-2',6'-Pipecoloxylidide, Metabolite 2: NA, Inhibitor: itraconazole.



(A,B) Lines in upper and lower panels represent simulated blood concentration-time profiles with all the parameter sets reproducing AUCs and three parameter sets reproducing concentration-time profiles, respectively. Orange circles represents observed time profiles. (C,D) Dark and light lines represent estimated parameter values for all the parameter sets reproducing AUCs and ten parameter sets reproducing concentration-time profiles, respectively.

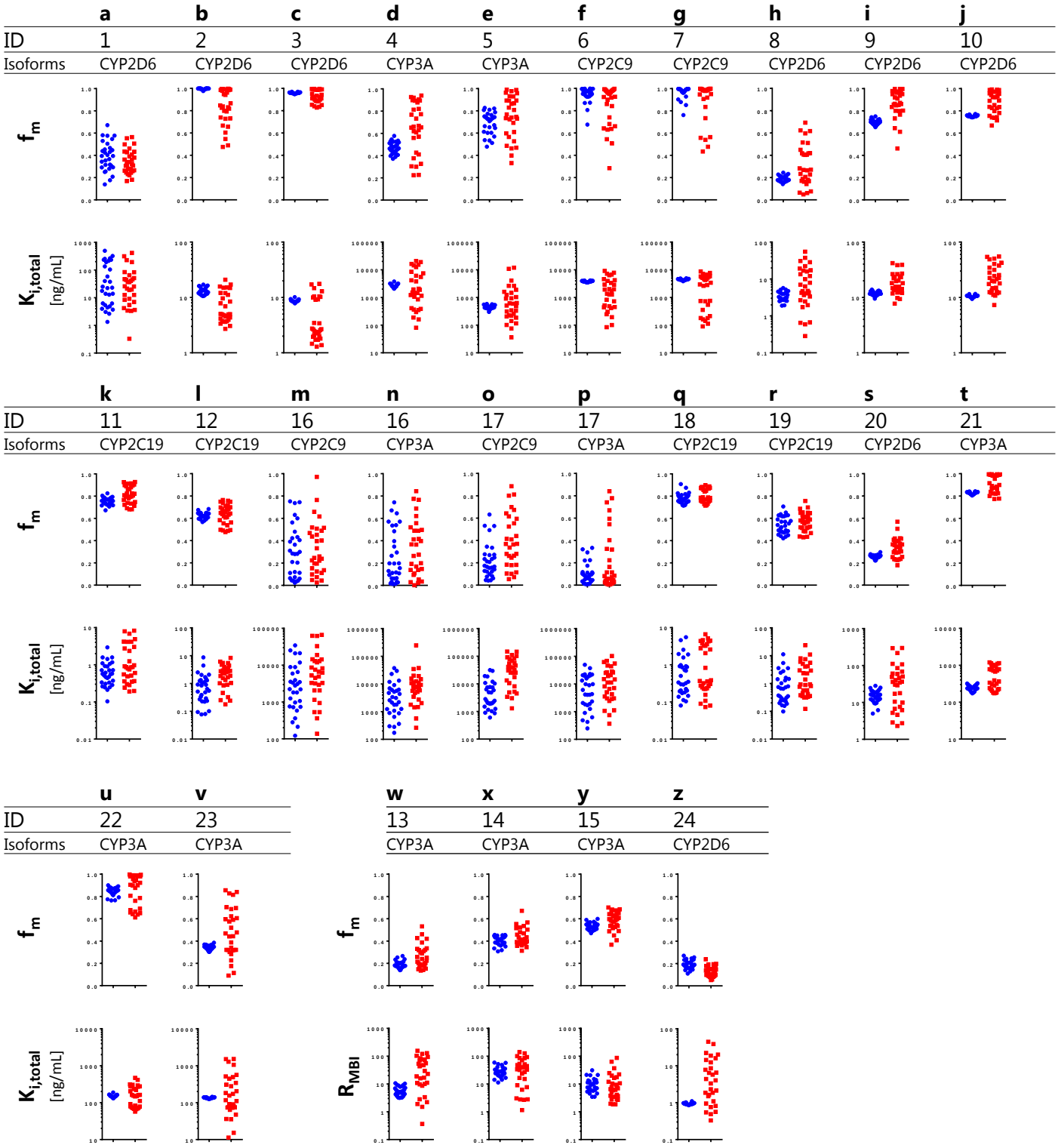


Fig. S18 Comparison of the estimated f_m and $K_{i,total}$ / R_{MBI} values, with or without including substrate metabolites' pharmacokinetic profiles in the PBPK analyses.

Blue circles (right) and red squares (left) represent the values of f_m or $K_{i,total}/R_{MBI}$ for estimated parameter sets from each DDI study with 30 lowest $SS_{log,time}$ values with and without including substrate metabolites' pharmacokinetic profiles, respectively. IDs of studies analyzed correspond to those listed in Table 2. f_m, fraction metabolized by corresponding CYP isoforms; $K_{i,total}$, inhibition constant for total (bound + unbound) inhibitor concentration; R_{MBI}, degree of inhibition with mechanism-based inhibitors.