Unravelling Pleiotropic Effects of Rifampicin by Utilising Physiologically Based Pharmacokinetic Modelling: Assessing the Induction Magnitude of P-glycoprotein-CYP3A4 Dual Substrates

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Supplemental Supporting Information

(A) Mathematical approach to model a P-gp inhibitory effect

(B) Mathematical steps to account for P-gp induction effect in the PBPK model

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Table S2 - Summary of clinical DDI studies with rifampicin

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Figure S3 – Simulated gut luminal concentrations for each intestinal segment of each drug

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Figure S5 – Simulated absorption rate and efflux rate for each intestinal segment of each drug

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Figure S7 – Simulated and observed plasma concentration-time profiles of bosutinib with and without co-administration of rifampicin.
A. **Mathematical approach to model competitive inhibition of P-gp:**

The overall inhibitory effect can be modelled using the same approach reported for metabolic interactions

\[
\text{CL}_{\text{int,T-inh}} = \frac{J_{\text{max}}}{K_m \left[1 + \sum_j \frac{(I_{u_j})}{K_{u_{inh}}}ight] + C_{t1}}
\]

- \(\text{CL}_{\text{int,T-inh}}\) is the transporter-mediated intrinsic clearance in the presence of an inhibitor,
- the “inh” suffix refers to the inhibited value,
- \(I_{u_j}\) is the unbound concentration of \(j^{th}\) inhibitor at the binding site of a transporter
- \(K_{u_{inh}}\) is the unbound concentration of \(j^{th}\) inhibitor that supports half maximal inhibition (corrected for nonspecific binding).

In the case of multiple inhibitors, it is assumed that all inhibitors are acting via the same mechanism (or the overall effect is similar) on a transporter. (Rostami-Hodjegan A, Tucker GT. 2004. ‘In silico’ simulations to assess the ‘in vivo’ consequences of ‘in vitro’ metabolic drug-drug interactions. Drug Discov Today Technol 1(4):441–448.)

Time-dependent inhibition (TDI) of metabolism was considered for verapamil. (Rowland Yeo K, Jamei M, Yang J, Tucker GT, Rostami-Hodjegan A. 2010. Physiologically based mechanistic modelling to predict complex drug-drug interactions involving simultaneous competitive and time-dependent enzyme inhibition by parent compound and its metabolite in both liver and gut—The effect of diltiazem on the time-course of exposure to triazolam. Eur J Pharm Sci 39(5):298–309.)
B. Mathematical steps to account for P-gp induction effect in the PBPK model

Three mathematical steps are followed when scaling the intestinal P-gp via a relative scaling approach:

**Step 1:** The in vitro apparent active permeability in a gut segment (i) is determined for each involved transporter (n) (\(P_{\text{app,trans,n,i}}\)). The REF algorithm first requires that the *in vitro* transporter based active Papp is calculated, in this case we give the example of an apical efflux (P-gp) transporter. We correct for surface area (so it is a Papp that’s calculated) and we have added the \(f_{\text{inc}}\) term (unbound fraction the *in vitro* incubation) to allow the incubation binding to be accounted for.

- \(J_{\text{max}}\) is the maximum rate of transport mediated efflux (pmol/min) from an *in vitro* cell model,
- the \(K_m\) is the Michaelis constant (\(\mu\)M),
- A is the insert growth area (cm\(^2\)) of the Transwell in which the cells were grown,
- \(f_{\text{gut}}\) is the unbound fraction of drug in the enterocyte,
- the \(C_{\text{ent,i}}\) is the total concentration of drug in the enterocyte of a given intestinal segment,
- REF is the relative expression factor (or could be a relative activity factor, as decided by the user).

**Step 2:** We then transfer the log \(P_{\text{app,trans,n}}\) into a regression equation to allow the \(P_{\text{efr}}\) to calculated for the active transporter where A is the slope and B the intercept so this is an empirical approach (Yang et al., 2007, Current Drug Metabolism 2007 Oct;8(7):676-84 https://pubmed.ncbi.nlm.nih.gov/17979655/).

**Step 3:** In the third step the *in vivo* segmental scale up occurs, using the segment surface area (S.A.\(i\)) of each individual. The permeability surface area product is the clearance, which is corrected for the relative segmental abundances for the transporter.

The relative abundance of a given transporter (n) in a given gut segment (i) is calculated thereby also with relevance to the phenotype (Jejunum I) assigned to that individual for a given transporter.
Mathematically, the REF (according to the equation discussed in Step 1) was in our simulations multiplied by the fold induction and two simulations were run: (A) substrate without inducer/inhibitor and (B) substrate with inducer/inhibitor and an altered REF for the substrate.

Then the AUC and \( C_{\text{max}} \) ratios were calculated by comparing the interaction profiles from simulation B with the baseline profiles of the substrate alone of simulation A.
Table S1. Input parameters of PBPK model

| Parameter                      | Value                  | Method/Reference |
|-------------------------------|------------------------|------------------|
| **Abemaciclib**               |                        |                  |
| Adopted from Posada et al (2020) (1) and refined to include advanced dissolution, absorption and metabolism (ADAM) model and intestinal P-gp efflux transport (2). |
| Absorption Model              | ADAM Model             |                  |
| fu_gut                        | 1                      | Assumed          |
| Peff,man (10^-4 cm/s)         | 2.46                   | Predicted        |
| Permeability Assay            | Physiochemical         |                  |
| PSA (Å²)                      | 71.4                   | (1)              |
| HBD                           | 1                      | (1)              |
| Formulation                   | Immediate Release - Diffusion Layer Model with solubility-pH profile | (3) |
| Transporter (gut)             | ABCB1 (P-gp/MDR1)      |                  |
| Jmax (pmol/min/cm²)           | 20                     | Optimised to recover the clinically reported T_max (4) |
| Km,u (µM)                     | 0.57                   | Assuming the same as in vitro IC50 value against P-gp (4) |
| RAF/REF                       | 1                      |                  |

| **Acalabrutinib**             |                        |                  |
| Adopted from Zhou et al (2019) (5) and refined to include ADAM absorption model and intestinal P-gp efflux transport. |
| Absorption Model              | ADAM                   |                  |
| fu_gut                        | 0.026                  | (5)              |
| Peff,man (10^-4 cm/s)         | 7.72                   | Predicted        |
| Permeability Method           | Mechanistic Model      |                  |
| P_trans,0 (10^-6 cm/s)        | 640.7                  | Predicted based on LogP |
**Formulation** | Immediate Release - Diffusion Layer Model
---|---
**Intrinsic solubility (mg/mL)** | 0.12 | Optimised
**Transporter (gut)** | ABCB1 (P-gp/MDR1)
**CL\text{int,T} (\mu L/min)** | 27.3 | Estimated by SIVA (three-compartment model) using experimental data in Caco-2 cells (in house data)
**RAF/REF** | 1

**Bosutinib** | Adopted from Yamazaki et al (2018) (6) and refined for P-gp efflux kinetic parameters.

| Parameter | Value | Method/Reference |
|---|---|---|
| Transporter (gut) | ABCB1 (P-gp/MDR1) | |
| J\text{max} (pmol/min/cm}\text{\textsuperscript{2}} | 67.4 | Estimated by SIVA (three-compartment model) using experimental data in Caco-2 cells (in house data) |
| K\text{m,u} (\mu M) | 0.58 | |
| RAF/REF | 1 | |

**Crizotinib** | Simcyp library compound with refined P-gp efflux kinetic parameters

| Parameter | Value | Method/Reference |
|---|---|---|
| Molecular weight (g/mol) | 450.34 | (7) |
| log P | 4.28 | (7) |
| Compound type | Diprotic Base | (7) |
| pKa | 9.4, 5.6 | (7) |
| B/P | 1.1 | (7) |
| fu | 0.093 | (7) |
| Main plasma binding protein | Human serum albumin | (7) |
| Absorption | ADAM | |
| fu\text{gut} | 0.093 | Assumed the same as fu |
| P\text{eff,man} (10\textsuperscript{-4} cm/s) | 0.578 | Predicted |
| Permeability Assay | Physiochemical | |
| **PSA (Å²)** | 78 | **ACD/Percepta14.0.0 (Build 2254), accessed on 2019/06/20** |
| **HBD** | 3 |
| **Formulation** | Immediate Release – Diffusion Layer Model |
| **Intrinsic solubility (mg/mL)** | 0.00047 | Predicted by Simcyp based on MW, LogP and melting point of 245.07 °C (EPI Suite) |
| **Transporter (gut)** | ABCB1 (P-gp/MDR1) |
| **K_{m,u} (μM)** | 3.8 | Estimated by SIVA (three-compartment model) using experimental data in Caco-2 cells (in-house data) |
| **J_{max} (pmol/min/cm²)** | 165.4 |
| **RAF/REF** | 1 |
| **Distribution Model** | Full PBPK Model |
| **V_{SS} (L/kg)** | 22.26 | Predicted - Method 2 |
| **Kp scalar** | 3.4 | Optimised to recover the clinically reported volume of distribution (8) |
| **Enzyme** | CYP3A4 |
| **K_{m,u} (μM)** | 3.65 | 1/2 of IC_{50} value obtained from in vitro crizotinib inhibition study on testosterone activity (7) |
| **V_{max} (pmol/min/pmol of isoform)** | 4.6 | 70% of total CL_{int} back-calculated from in vivo systemic clearance (8) was assigned to CYP3A4 in order to recover the clinically reported interaction between crizotinib and ketoconazole (7). V_{max} of CYP3A4 was calculated by CL_{int, CYP3A4} multiplying K_{m, CYP3A4}. |
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| Parameter | Value | Method/Reference |
|-----------|-------|------------------|
| CL\textsubscript{int} (HLM) (μL/min/mg protein) | 68.36 | 30% of the total CL\textsubscript{int} retrograde from systemic clearance (8) |
| CL\textsubscript{R} (L/h) | 2.51 | (7) |
| Interaction | | |
| Enzyme | CYP3A4 | |
| Ki (μM) | 3.88 | (7) |
| K\textsubscript{app} (μM) | 1.25 | (9, 10) |
| k\textsubscript{inact} (1/h) | 1.11 | (9, 10) |
| Ind\textsubscript{max} | 20.5 | (7) |
| IndC\textsubscript{50} (μM) | 1.45 | (7) |
| Naldemedine | Adopted from NDA review (11) and refined |

| Parameter | Value | Method/Reference |
|-----------|-------|------------------|
| Absorption Model | ADAM Model | |
| fu\textsubscript{gut} | 0.063 | Assumed the same as fu |
| P\textsubscript{eff,man} (10\textsuperscript{-4} cm/s) | 3.16 | Predicted |
| Permeability Method | Mechanistic Model | |
| P\textsubscript{trans,0} (10\textsuperscript{-6} cm/s) | 512.9 | Predicted based on LogP |
| Formulation | Solution | |
| Transporter (gut) | ABCB1 (P-gp/MDR1) | Estimated by SIVA (three-compartment model) using experimental data in Caco-2 cells (11) |
| CL\textsubscript{int,T} (μL/min) | 12 | |
| RAF/REF | 1 | |
| Distribution Model | Full PBPK Model | |
| V\textsubscript{ss} (L/kg) | 0.74 | Predicted - Method 2 |
| Kp scalar | 0.5 | Optimised to recover the clinically reported V\textsubscript{ss} (11) |
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| Enzyme         | CYP3A4 | Pathway         | Pathway 1 | Optimised to recover PK profile following single oral dose (12) as in vitro obtained CYP3A4 kinetics under-predicted the elimination |
|----------------|--------|-----------------|-----------|-------------------------------------------------------------------|
| CL\text{int} (μL/min/pmol) | 0.15   |                 |           | Optimised to recover benzamidine amount reported in mass balance study (13) |
| Gut Lumen metabolism (μl/h/g of total luminal content) | 150    |                 |           |                                                                   |
| CL\text{R} (L/h) | 1.6    |                 |           | (12)                                                              |
| Permeability limited liver model |        |                 |           |                                                                   |
| CL\text{PD} (μL/min/million hepatocytes) | 1.1    |                 |           |                                                                   |
| Transporter (liver) | ABCB1 (P-gp/MDR1) |                 |           |                                                                   |
| CL\text{int,T} (μL/min/million hepatocytes) | 12     |                 |           | Assumption P-gp in 1 million hepatocytes has the same P-gp activity as the P-gp available in 1 cm² of Caco-2 cells in the Transwell system |
| RAF/REF | 0.75   |                 |           | Scaled based on P-gp expression level differences in gut and liver |

### Olaparib
Adopted from Pilla Reddy et al (2019) (14) and refined to include intestinal P-gp efflux transport.

| Parameter                  | Value | Method/Reference                                                                 |
|----------------------------|-------|----------------------------------------------------------------------------------|
| Transporter (gut)          | ABCB1 (P-gp/MDR1) |                                                                   |
| \(J_{\text{max}}\) (pmol/min/cm²) | 241   | Estimated by SIVA (three-compartment model) using experimental data in Caco-2 cells (in house data). |
| \(K_{\text{m,u}}\) (μM)     | 28.8  |                                                                   |
| RAF/REF                    | 1     |                                                                   |
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### Quinidine

| Parameter                  | Value                      | Method/Reference                      |
|----------------------------|----------------------------|---------------------------------------|
| Absorption Model           | ADAM Model                 |                                       |
| $f_{ugut}$                 | 1                          | Assumed                               |
| $P_{eff,man}$ ($10^{-4} \text{cm/s}$) | 3.47                       | Predicted                             |
| Passive Permeability Assay | Caco-2                     | Apical pH to Basolateral pH (7.4:7.4) |
| Passive Permeability ($10^{-6} \text{cm/s}$) | 112                        | (16)                                  |
| Reference Compounds        | Atenolol, Propranolol, Metoprolol | (16)                                  |
| Transporter (gut)          | ABCB1 (P-gp/MDR1)          |                                       |
| $J_{\text{max}}$ (pmol/min/cm$^2$) | 11.3                       | Estimated by SIVA (three-compartment model) using experimental data in Caco-2 cells (17). |
| $K_{m,u}$ ($\mu$M)         | 0.278                      |                                       |
| RAF/REF                    | 1                          |                                       |

### Rifampicin (default model)

| Parameter                  | Value                      | Method/Reference                      |
|----------------------------|----------------------------|---------------------------------------|
| Interaction                |                            |                                       |
| Enzyme                     | CYP1A2                     | Optimised to recover clinically reported interaction with theophylline (19) |
| $\text{Ind}_{\text{max}}$  | 2.7                        |                                       |
| $\text{IndC}_{50}$ ($\mu$M) | 0.1                        | (20)                                  |
| Enzyme                     | CYP2B6                     |                                       |
| $\text{Ind}_{\text{max}}$  | 5.04                       | In house data and (21)               |
| $\text{IndC}_{50}$ ($\mu$M) | 0.07                       | In house data                         |
| Enzyme                     | CYP2C8                     |                                       |
| $K_{i}$ ($\mu$M)           | 24.5                       | (22)                                  |
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| Enzyme   | Ind\textsubscript{max} | IndC\textsubscript{50} (µM) | Description                                                                 |
|----------|-------------------------|-----------------------------|-----------------------------------------------------------------------------|
| CYP2C9   | 6                       | 0.3                         | Derived from the in vitro induction data in mRNA expression level using human hepatocytes (20). |
| CYP2C19  | 5.5                     | 0.45                        | Optimised to recover clinically reported interaction with tolbutamide (23)    |
| UGT1A1   | 3.16                    | 0.39                        | (24)                                                                        |

| Enzyme   | Ind\textsubscript{max} | IndC\textsubscript{50} (µM) | Description                                                                 |
|----------|-------------------------|-----------------------------|-----------------------------------------------------------------------------|
| Verapamil| Simcyp library compound |                             |                                                                             |

**Parameter** | **Value** | **Method/Reference** |
|---------------|-----------|----------------------|
| Molecular weight (g/mol) | 454.6 | Pubchem               |
| log P         | 4.46     | (26-29)               |
| Compound type | Monoprotic Base |                      |
| pKa           | 8.78     | (30-32)               |
| B/P           | 0.709    | (33, 34)              |
| fu            | 0.09     | Meta-analysis (35-42) |
| Main plasma binding protein | Human serum albumin | (43)                 |
| Absorption Model | ADAM Model |                      |
| fu\textsubscript{gut} | 1 | Assumed               |
| P\textsubscript{eff,man} (10⁻⁴ cm/s) | 6.08 | Predicted            |
| Passive Permeability Assay | Caco-2 | Apical pH to Basolateral pH (7.4:7.4) |
| Passive Permeability (10⁻⁶ cm/s) | 149.6 | (44)                 |
| Reference Compound | Propranolol | (45)                 |
| Transporter (gut) | ABCB1 (P-gp/MDR1) |                      |
### Unravelling Pleiotropic Effects of Rifampicin by Utilising Physiologically Based Pharmacokinetic Modelling: Assessing the Induction Magnitude of P-glycoprotein-CYP3A4 Dual Substrates

| Pathway | RMTEE | V<sub>max</sub> (pmol/min/pmol isoform) | Km (μM) | Enzyme | Pathway | V<sub>max</sub> (pmol/min/pmol isoform) | Km (μM) | Enzyme | Pathway |
|---------|-------|--------------------------------------|---------|--------|---------|--------------------------------------|---------|--------|---------|
| Norverapamil | CYP2C8 | 221.2 | 140.5 | CYP3A4 | Norverapamil | 154.3 | 122 | CYP3A5 | Norverapamil |
| Norverapamil | CYP3A4 | 159.3 | 87.5 | CYP2C8 | D-617 | 218.9 | 156 | CYP3A4 | D-617 |
| Norverapamil | D-617 | 218.9 | 156 | CYP3A4 | D-617 | 218.9 | 156 | CYP3A4 | D-617 |

**Table Notes:**
- **J<sub>max</sub> (pmol/min/cm<sup>2</sup>):** 2.814
- **K<sub>m,u</sub> (μM):** 0.734
- **RAF/REF:** 0.608
- **Transporter (gut):** ABCC2 (MRP2)
- **CL<sub>int,T</sub> (μL/min):** 18
- **RAF/REF:** 0.608

**A retrograde approach was used to calculate CL<sub>int</sub> for CYP3A4/5 and CYP2C8 using in vivo CL<sub>iv</sub> from meta-analysis (41, 42, 48, 49), then converted to V<sub>max</sub> using reported Km (50).**

**Km and J<sub>max</sub> was estimated by SIVA (three-compartment model) using experimental data (46).**

**Calculated based on the relative expression of P-gp (46, 47).**

**Fitted to single oral dose PK profile (43).**

**Predicted - Method 2.**

**Distribution Model:** Full PBPK Model

**V<sub>SS</sub> (L/kg):** 5.37
### Unravelling Pleiotropic Effects of Rifampicin by Utilising Physiologically Based Pharmacokinetic Modelling: Assessing the Induction Magnitude of P-glycoprotein-CYP3A4 Dual Substrates

| Parameter | Value | Method/Reference |
|-----------|-------|------------------|
| $V_{\text{max}}$ (pmol/min/pmol isoform) | 174 | |
| $K_m$ (μM) | 99.5 | |
| Enzyme | CYP3A5 | |
| Pathway | D-617 | |
| $V_{\text{max}}$ (pmol/min/pmol isoform) | 117.7 | |
| $K_m$ (μM) | 73 | |
| $V_{\text{max}}$ (pmol/min/pmol isoform) | 174 | |
| $K_m$ (μM) | 99.5 | |
| CL$_{\text{int}}$ (HLM) (μL/min/mg protein) | 79.57 | (51) |
| CL$_R$ (L/h) | 2.52 | (52, 53) |

### Interaction

| Enzyme | CYP3A4 | |
| $K_{\text{app}}$ (μM) | 2.21 | (54) |
| $k_{\text{inact}}$ (1/h) | 2 | (54) |
| Enzyme | CYP3A5 | |
| $K_{\text{app}}$ (μM) | 3.99 | Estimated by extrapolating the CYP3A4 MBI parameters based on the correlation between CYP3A4 and CYP3A5 metabolic activities (55-57) |
| $k_{\text{inact}}$ (1/h) | 1.84 | |

### Transporter (gut)

| Transporter (gut) | ABCB1 (P-gp/MDR1) | |
| Ki (μM) | 0.16 | (58, 59) |

### Transporter (liver)

| Transporter (liver) | ABCB1 (P-gp/MDR1) | |
| Ki (μM) | 0.16 | Assumed the same as in gut |

### Norverapamil

| Parameter | Value | Method/Reference |
|-----------|-------|------------------|
| Molecular weight (g/mol) | 440.6 | Pubchem |
| log P | 4.66 | Predicted (ChemAxon) |
| Compound type | Monoprotic Base | |
| pKa | 10.29 | ChemAxon |
| B/P | 0.675 | (33) |
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| Parameter          | Value  | Source |
|--------------------|--------|--------|
| fu                 | 0.083  | (33, 37) |
| Main plasma binding protein | Human serum albumin | Assumed the same as parent |
| fu\text{gut}      | 1      | Assumed |
| Distribution Model | Minimal PBPK Model |
| V_{SS} (L/kg)     | 4.166812 | Predicted - Method 1 |
| Q (L/h)           | 18     | Optimised to recover the observed PK profile following single oral dose |
| V_{SAC} (L/kg)    | 2      | (43) |
| Enzyme Pathway    | CYP2C8 | A retrograde approach was used to calculate CL_{int} for CYP3A4/5 and CYP2C8, then converted to V_{max} using reported K_{m} (50) |
| V_{max} (pmol/min/pmol isoform) | 38.5 | |
| K_{m} (μM)        | 68     | |
| Enzyme Pathway    | CYP3A4 | |
| V_{max} (pmol/min/pmol isoform) | 46 | |
| K_{m} (μM)        | 90     | |
| Enzyme Pathway    | CYP3A5 | |
| V_{max} (pmol/min/pmol isoform) | 18.8 | |
| K_{m} (μM)        | 19.5   | |
| Enzyme Pathway    | CYP2C8 | |
| V_{max} (pmol/min/pmol isoform) | 113.2 | |
| K_{m} (μM)        | 59     | |
| CL_{R} (L/h)      | 1.91   | (51, 53) |
| Interaction Enzyme | CYP3A4 | |
| K_{app}(μM)       | 10.3   | (60) |
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| $k_{\text{inact}}$ (1/h) | 18 | (60) |
|-------------------------|----|-----|
| Enzyme                  | CYP3A5 |
| $K_{\text{app}}$ (μM)   | 4.53 | (60) |
| $k_{\text{inact}}$ (1/h) | 4.2 | (60) |
| Transporter (gut)       | ABCB1 (P-gp/MDR1) |

**Ki (μM)**: 0.04

Scaled based on the reported difference of IC50 between verapamil and norverapamil (61) and the Ki input value in the verapamil model

| Transporter (liver)     | ABCB1 (P-gp/MDR1) |
|------------------------|-------------------|
| **Ki (μM)**            | 0.04              |

Assumed the same as in gut

The PBPK models of dabigatran etexilate, dabigatran, and talinolol were adopted from Yamazaki et al. (2019) (15). The PBPK model of digoxin and naloxegol were adopted from Neuhoff et al. (2016) (62) and Zhou et al. (2016) (63), respectively.

### Abbreviations:

LogP: Log of the octanol:water partition coefficient;

$f_{u,p}$: fraction unbound in plasma;

B:P: blood-to-plasma partition ratio;

ADAM: Advanced Dissolution, Absorption and Metabolism

$f_{u,gut}$: unbound fraction in enterocyte;

$P_{\text{eff,man}}$: effective permeability in human jejunum;

PSA: polar surface area;

HBD: hydrogen bound donor;

$K_{m,u}$: Michaelis-Menten constant accounting for the binding in vitro system;

$J_{\text{max}}$: in vitro maximum rate of transporter-mediated efflux or uptake correcting for the insert growth area of the Transwell;

$\text{CL}_{\text{int,T}}$: in vitro intestinal transporter-mediated intrinsic clearance;

RAF/REF: relative activity or expression factor;

$V_{\text{sac}}$: volume of the single-adjusting compartment;

$Q$: flow rate in single-adjusting compartment;
Kp scalar: scalar applied to all predicted tissue to plasma partition coefficients;

$V_{\text{max}}$: maximum rate of metabolite formation;

$\text{CL}_{\text{int}}$: \textit{in vitro} intrinsic clearance;

HLM: human liver microsomes;

$\text{CL}_R$: renal clearance;

$K_i$: competitive inhibition constant;

$K_{\text{app}}$: concentration of mechanism-based inhibitor associated with half maximal inactivation rate;

$k_{\text{inact}}$: inactivation rate of the enzyme;

Ind$_{\text{max}}$: maximal fold induction over vehicle;

IndC$_{50}$: concentration that supports half maximal induction
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**Table S2. Summary of clinical DDI studies with rifampicin**

| Victim       | Victim drug dose regimen                          | Rifampicin dose regimen          | Reference |
|--------------|---------------------------------------------------|----------------------------------|-----------|
| Abemaciclib  | 200 mg oral single dose on day 5                   | 600 mg oral QD for 14 days       | (1)       |
| Acalabrutinib| 100 mg oral single dose on day 9                   | 600 mg oral QD for 9 days        | (2)       |
| Bosutinib    | 500 mg oral single dose on day 7                   | 600 mg oral QD for 10 days       | (3)       |
| Crizotinib   | 250 mg oral single dose on day 9                   | 600 mg oral QD for 14 days       | (4)       |
| Naldemedine  | 0.2 mg oral single dose on day 15                  | 600 mg oral QD for 17 days       | (5)       |
| Naloxegol    | 25 mg oral single dose on day 10                   | 600 mg oral QD for 10 days       | (6)       |
| Olaparib     | 300 mg oral single dose given 216 hours after initial rifampicin dose | 600 mg oral QD for 13 days       | (7)       |
| Quinidine    | 6 mg/kg (quinidine sulfate) oral single dose on day 8 | 500 mg oral QD for 7 days        | (8)       |
| Verapamil    | 120 mg oral single dose on day 16                  | 600 mg oral QD for 15 days       | (9)       |
| Digoxin      | 1 mg single oral dose on day 11                    | 600 mg oral QD for 16 days       | (10)      |
| Dabigatran   | 150 mg single oral dose on day 8                   | 600 mg oral QD for 7 days        | (11)      |
| Etexilate    |                                                    |                                  |           |
| Talinolol    | 100 mg oral QD from day 2 to day 7 at 7AM          | 600 mg oral QD from day 1 to day 9 at 6PM | (12)      |
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### Table S3. Base model of the prototypical compound file in sensitivity analysis

| Parameter                                    | Value                                                                 |
|----------------------------------------------|----------------------------------------------------------------------|
| Molecular weight (g/mol)                     | 500                                                                 |
| log P                                        | 4                                                                   |
| Compound type                                | Diprotic Base                                                         |
| pKa                                          | 8, 4                                                                |
| B/P                                          | 0.55                                                                |
| fu                                           | 0.05                                                                |
| Absorption Model                             | ADAM Model                                                           |
| fu_{gut}                                     | 1                                                                   |
| P_{trans,0} (10^{-6} cm/s)                   | Ranged from 10 to 100000                                             |
| P_{eff,man} (10^{-4} cm/s)                   | Predicted by MechPeff model based on P_{trans,0}                      |
| Formulation                                  | Solution                                                             |
| Distribution Model                           | Minimal PBPK                                                          |
| VSS (L/kg)                                   | Predicted by Method 2 (Rodgers and Rowland method)                    |
| Enzyme kinetics                              |                                                                      |
| CYP3A4 CL_{int} (μL/min/mg protein)          | Equal to 0 for P-gp pure substrate                                    |
| Additional CL_{int} (HLM) (μL/min/mg protein)| Varied to obtain different E_{H} (0.1, 0.3, 0.7) and fm_{CYP3A4} (0.6 ~ 1) |
| CL_{R} (L/h)                                 | 0                                                                   |
| Transporter (gut)                            | ABCB1 (P-gp/MDR1)                                                     |
| CL_{int,T} (μL/min)                          | Ranged from 0 to 600                                                 |
Table S4. Dose normalised AUC of talinolol, bosutinib, quinidine and verapamil

| Drug        | Dose | AUC/Dose (h*ml⁻¹*1*10⁶) | Observed | Predicted | Predicted/Observed |
|-------------|------|-------------------------|----------|-----------|--------------------|
|             |      |                         |          |           |                    |
| Talinolol   | 50 mg| 18.8                    | 22.8     | 1.21      |                    |
|             | 100 mg| 22.6                    | 23.2     | 1.03      |                    |
|             | 400 mg| 27.1                    | 31.7     | 1.17      |                    |
| Bosutinib   | 100 mg| 3.23                    | 3.74     | 1.16      |                    |
|             | 500 mg| 4.66                    | 4.67     | 1.00      |                    |
| Quinidine   | 1 mg | 10.5                    | 17.6     | 1.68      |                    |
|             | 10 mg| 14.8                    | 19.6     | 1.32      |                    |
|             | 100 mg| 20.8                    | 24.5     | 1.18      |                    |
| Verapamil   | 3 mg | 1.65                    | 2.72     | 1.65      |                    |
|             | 16 mg| 2.55                    | 3.33     | 1.31      |                    |
|             | 80 mg| 3.20                    | 3.94     | 1.23      |                    |

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Figure S1. Simulated and observed plasma concentration-time profiles of each drug at clinically relevant dose. Simulated (black lines) and observed (data points) mean plasma concentration-time profile of each drug after a single oral dose. Observed data were extracted from literature: 200 mg Abemaciclib (1), 100 mg Acalabrutinib (2), 500 mg Bosutinib (3), 250 mg Crizotinib (4), 0.2 mg Naldemedine (5), 25 mg Naloxegol (6), 300 mg Olaparib (7), 332 mg Quinidine free base (8), 120 mg Verapamil (9), 1 mg Digoxin (10), Dabigatran (150 mg of Dabigatran Etexilate)(11), 100 mg Talinolol (12). Dashed lines represent the 5th and 95th percentile of the total virtual population.
**Supplementary Material**

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**Figure S2.** Predicted and observed AUC and $C_{\text{max}}$ of each drug at clinically relevant dose. Predicted and observed mean values of AUC and $C_{\text{max}}$ of each drug after a single oral dose. The observed data were reported in the literature: 200 mg Abemaciclib (1), 100 mg Acalabrutinib (2), 500 mg Bosutinib (3), 250 mg Crizotinib (4), 0.2 mg Naldemedine (5), 25 mg Naloxegol (6), 300 mg Olaparib (7), 332 mg Quinidine free base (8), 120 mg Verapamil (9), 1 mg Digoxin (10), Dabigatran (150 mg of Dabigatran Etexilate)(11), 100 mg Talinolol (12).
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Figure S3. Simulated luminal concentration for each intestinal segment of each drug. The profiles were simulated in a population representative (20 years old, male, healthy volunteer) after a single oral dose (shown in Table 1) of each drug.
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Figure S4. Simulated free enterocyte concentration for each intestinal segment of each drug. The profiles were simulated in a population representative (20 years old, male, healthy volunteer) after a single oral dose (shown in Table 1) of each drug.
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Figure S5. Simulated absorption rate and efflux rate for each intestinal segment of each drug. The profiles were simulated in a population representative (20 years old, male, healthy volunteer) after a single oral dose (shown in Table 1) of each drug. The solid line represents the absorption rate and the dashed lines represent P-gp-mediated efflux rate.
Figure S6. Simulated efflux clearance for each intestinal segment of each drug. The profiles were simulated in a population representative (20 years old, male, healthy volunteer) after a single oral dose (shown in Table 1) of each drug.
Figure S7. Simulated (lines) and clinically observed (data points) plasma concentration-time profiles of bosutinib with (red) and without (blue) coadministration of rifampicin. A single oral dose of bosutinib was administered to healthy subjects at the dose of 500 mg with and without repeated coadministration of 600 mg rifampicin once daily. Bosutinib plasma concentrations were predicted with a default rifampicin file (CYP3A4 Ind_max = 16, IndC50 = 0.32 μM and fu,gut = 1) (a, b and c) or a modified rifampicin file (CYP3A4 Ind_max = 30.6, IndC50 = 0.32 μM and fu,gut = 0.116) (d, e and f). Rifampicin-mediated intestinal P-gp fold-induction was assumed as 1 (a and d), 3.5 (b and e) or 10 (c and f). The error bars represent the standard deviation of the observed data. The dashed lines represent the predicted 5th and 95th percentiles of the virtual population.