Do differences in cell lines and methods used for calculation of IC\textsubscript{50} values influence categorisation of drugs as P-glycoprotein substrates and inhibitors?

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**ABSTRACT**

1. *In vitro* bidirectional assays are employed to determine whether a drug is a substrate and/or inhibitor of P-glycoprotein (P-gp) transport. Differences between cell lines and calculation methods can lead to variations in the determination of efflux ratios (ER) and IC\textsubscript{50} values used to classify a drug as a P-gp substrate and inhibitor, respectively.

2. Information was collected from the literature on ER and IC\textsubscript{50} values with digoxin as the probe substrate using different cell lines and inhibition calculation methods. Predictive performance was evaluated by comparing [I\textsubscript{gut}]/IC\textsubscript{50} ratios versus reported *in vivo* results.

3. For known P-gp substrates, 50% of the drugs had their highest ER value in MDCK-MDR1 cells while 81% had their lowest ER value in Caco-2 cells. For 30 drugs with inhibition data, lower mean IC\textsubscript{50} values were often observed with the Caco-2 cells and calculations based on ER. Based on the cut-off criteria of [I\textsubscript{gut}]/IC\textsubscript{50} \geq 10, there were no significant differences in positive or negative predictive values based on either cell line or calculation method for the drugs.

4. Within this limited dataset, differences between cell lines or IC\textsubscript{50} calculation methods do not seem to impact the prediction of *in vivo* P-gp inhibitor classification.

**Introduction**

Drug–drug interactions (DDI) occur when one drug (‘perpetrator’ or ‘precipitant’) affects the pharmacokinetics of another drug (‘victim’ or ‘object’). The importance of comprehensively assessing metabolism-based DDIs is to ensure the safe and effective use of drugs that has been extensively documented (Obach et al. 2006; Galetin et al. 2008). More recently, the critical role played by drug transporters in affecting the absorption and disposition of drugs and endogenous substances, and toxicity has been clearly recognised (Estudante et al. 2013; Arya and Kiser 2016; Cheng et al. 2016; Lee et al. 2017). The International Transporter Consortium (ITC) has proposed a clear framework regarding the prospective evaluation of transporters in drug development (Giacomini et al. 2010; Brouwer et al. 2013). In addition, regulatory guidances underscore the importance of assessing transporter-mediated DDIs during drug development (European Medicinal Agency 2012; Pharmaceuticals and Medical Devices Agency 2018; Food and Drug Administration 2020; ICH Harmonised Guideline 2022).

One of the clinically relevant transporters is P-glycoprotein (P-gp, ABCB1), or MDR1, a member of the ATP-binding cassette (ABC) family of efflux transporters. This transporter is found on the apical surfaces of enterocytes, hepatocytes, proximal renal tubular cells, and brain endothelial cells (Giacomini et al. 2010). The P-gp transporter plays an important role in (i) the efflux of drugs from the intestinal lumen (Estudante et al. 2013); (ii) the elimination of endogenous compounds/metabolites, drugs and metabolites into the bile (Köck and Brouwer 2012); (iii) the clearance of compounds into the urine (Morrissay et al. 2013); and/or (iv) the access of toxins and drugs into the central nervous system (Mahringer and Fricker 2016).

To assess whether a drug has the potential to inhibit P-gp transporters in the intestinal lumen, regulatory guidances recommend determining whether [I\textsubscript{gut}]/IC\textsubscript{50} ratio is greater than or equal to 10 (European Medicinal Agency 2012; Pharmaceuticals and Medical Devices Agency 2018; Food and Drug Administration 2020). [I\textsubscript{gut}] refers to the theoretical maximum gastrointestinal drug concentration calculated at the highest dose per administration (HDPA) divided by a volume of 250 mL and IC\textsubscript{50} refers to the concentration causing half-maximal inhibition of P-gp transporters *in vitro*. Of note, although there have been several efforts to assess the performance of other *in vitro* prediction methods, based on the available data, [I\textsubscript{gut}]/IC\textsubscript{50} appears to reasonably predict the potential for orally administered drugs as P-gp inhibitors (Agarwal et al. 2013; Zhou et al. 2019). Digoxin is commonly used as a probe *in vitro* and *in vivo* substrate to evaluate whether a drug is a P-gp inhibitor (Taub et al. 2005; Rautio et al. 2006; Ma et al. 2010; Nader and Foster 2014).
In vitro transporter assays are used during drug development to assess whether a drug is a substrate and/or inhibitor of P-gp transporters. These include cell-based bidirectional assays that are the most direct and functionally relevant models available to evaluate whether a new drug is a substrate or inhibitor of efflux transporters (Brouwer et al. 2013; Volpe 2016). The basic setup of a cell-based assay employs a dual-chamber apparatus with a cell monolayer grown on a semi-porous membrane separating the apical (AP) and basolateral (BL) chambers. Based upon the apparent permeability ($P_{app}$) in the secretive ($P_{app,BL-AP}$) and absorptive ($P_{app,AP-BL}$) directions, an efflux ratio (ER) is calculated. A drug is generally considered to be a substrate of an efflux transporter if its ER is greater than or equal to 2 (Giacomini et al. 2010; Food and Drug Administration 2020). IC$_{50}$ values for the drugs are calculated from the inhibition of digoxin efflux in the citations based on the change of ER, net secretory flux (NSF), or $P_{app,BL-AP}$ over a range of drug concentrations (Balimane et al. 2008).

It is well known that the results of in vitro cell-based transporter assays are influenced by multiple factors such as physicochemical characteristics of test compounds (e.g. solubility, stability), test systems (e.g. cells), experimental design (e.g. drug concentration, buffer pH) (Bhoopathy et al. 2014; Volpe 2016), and calculation methods (ER, $P_{app,BL-AP}$ or NSF). Differences in these factors can lead to variability in the determination of IC$_{50}$ values (Balimane et al. 2008; Sugimoto et al. 2011; Bentz et al. 2013; Kishimoto et al. 2014; Volpe et al. 2014). The research presented here focuses on two of these factors that may influence the results of in vitro cell-based transporter assays and by extension, the reliability of predicting the in vivo potential of a drug to inhibit P-gp: cell lines and calculation methods.

The cell line factor was selected as the expression of efflux transporters can vary between cells (Shirasaka et al. 2008; Bentz et al. 2013) and this can lead to different efflux ratios of a substrate drug (Tang et al. 2002; Siissalo et al. 2007; Patil et al. 2011) and IC$_{50}$ values of an inhibitor drug (Tang et al. 2002). The human epithelial colorectal adenocarcinoma (Caco-2) and Madin-Darby canine kidney (MDCK) cells are two cell lines that are most commonly used to assess whether a new drug is a substrate and/or inhibitor of the P-gp efflux transporter in bidirectional cell assays (Volpe 2011, 2016). Given the canine origin of MDCK cells and low expression of transporter proteins, they are often transfected with human transporter proteins such as P-gp (MDR1-MDCK cells) for use in transport assays (Tang et al. 2002; Volpe 2011). Additionally, Lilly Laboratories cell-porcine kidney 1 (LLC-PK1) cells are transfected with MDR1 for use in P-gp assays (Sugimoto et al. 2011).

The calculation method factor was selected as there are multiple methods reported in the literature for calculating IC$_{50}$ values for a drug in the bidirectional assay (Balimane et al. 2008). However, there is limited information in the literature on whether the type of method selected has an influence on the IC$_{50}$ value to the extent that it can differ in classifying a drug as a P-gp inhibitor or a non-inhibitor. Three methods selected for this research were the ER, $P_{app,BL-AP}$, or NSF methods (Balimane et al. 2008).

Overall, the purpose of our work was to assess whether differences between cell lines and calculation methods can lead to variations in the computation of ER and IC$_{50}$ values used to classify a drug as a P-gp substrate and inhibitor, respectively. Further, our goal was to assess whether a specific cell line and calculation method are more accurate in predicting if a drug is a substrate and/or inhibitor of P-gp in vivo.

### Materials and methods

We conducted a literature survey to collect in vitro information regarding the use of Caco-2, LLC-PK1-MDR1, and MDCK-MDR1 cells to predict whether a new drug is a P-gp substrate or inhibitor and in vivo information from DDI trials conducted to evaluate the effect of drugs on digoxin pharmacokinetics. Sources of the in vitro and in vivo interaction studies included the University of Washington Drug Interaction Solutions (https://www.druginteractionsolutions.org/), PubMed (https://www.ncbi.nlm.nih.gov), EMBASE (https://www.embase.com), Google Scholar (https://scholar.google.com/), Web of Science (https://apps.webofknowledge.com), and Drugs@FDA (https://www.accessdata.fda.gov/scripts/cder/daf/).

For in vitro studies, information on the cell line used, culture and transport conditions, and resultant ER or IC$_{50}$ values were compiled and tabulated. ER and NSF were defined for the in vitro inhibitors according to the equations in Table 1. IC$_{50}$ values were calculated in the citations for a drug in the bidirectional assay based on the ER, $P_{app,BL-AP}$, or net secretory flux (NSF) methods (Balimane et al. 2008; Bentz et al. 2013).

For DDI trials, information included the dosing regimen of digoxin and the inhibitor drug as well as digoxin AUC (area under the concentration-time curve) values in the absence and presence of the inhibitor drug. The DDI studies included those using the inhibitor drug at its HDPA and recommended dosing schedule. While compiling the dataset for this research, studies that evaluated the multiple doses of inhibitor drugs were selected over single-dose interaction studies as the inhibitor drug concentrations as the former are expected to be relatively better aligned with the drug’s therapeutic use than the latter. Each drug’s intestinal concentration ($I_{gut}$) was determined based on the respective drug’s HDPA in 250 mL volume and these $I_{gut}$ values were compared with the pre-defined cut-off criteria. Specifically, a drug was classified as in vitro inhibitor if its $I_{gut}/IC_{50}$ ratio was greater than or equal to 10 (European Medicinal Agency 2012; Pharmaceuticals and Medical Devices Agency 2018; Food and Drug Administration 2020; ICH Harmonised Guideline 2022). A drug was included in the dataset if (1) clinical drug interaction data with digoxin AUC values were available and (2) in vitro IC$_{50}$ values in at least two of the cell lines and at least two calculation methods were available. A drug was classified to be an in vivo inhibitor if the ratio of digoxin AUC (AUCR) in the presence and absence of a drug
was greater than or equal to 1.25-fold and an in vivo non-inhibitor when the AUCR was less than 1.25-fold (Agarwal et al. 2013; Zhou et al. 2019).

Predictive performance was evaluated by comparing in vitro and in vivo classifications. Specifically, to assess performance, true-positive (TP, in vitro and in vivo inhibitor), true-negative (TN, in vitro and in vivo non-inhibitor), false-positive (FP, in vitro inhibitor and in vivo non-inhibitor), and false-negative (FN, in vitro non-inhibitor and in vivo inhibitor) results were calculated. From these values, accuracy, specificity, sensitivity, negative predictive value (NPV), and positive predictive value (PPV) were calculated (Trevelyan 2017) (Table 1). The geometric mean was utilised to generate an $\frac{[I_{gut}]}{IC_{50}}$ ratio for each drug for the performance calculations with similar units ($\mu$M) for the $[I_{gut}]$ and $IC_{50}$ values.

### Results

Thirty-two substrates with ER values in at least two cell lines were collected for a total of 1097 data points. The majority of the data originated from Caco-2 cells (64%), followed by MDCK-MDR1 (25%) and LLC-PK1-MDR1 (11%) cells. No one cell line produced the highest or lowest ER values across all the drugs. Over half of the substrates had their maximum ER value in MDCK-MDR1 cells while 38% had their highest ER value in Caco-2 cells. All the drugs had their minimum individual ER value in Caco-2 cells except colchicine, doxorubicin, erythromycin, etoposide, ritonavir, and vincristine. Where individual ER value in Caco-2 cells except colchicine, doxorubicin, erythromycin, etoposide, ritonavir, and vincristine. Where IC50 values with at least two cell lines and two calculation methods. The number of data points for a drug ranged from 79 (for verapamil) to 3 (for atorvastatin, linagliptin, and pantoprazole). Twelve of the drugs were inhibitory in vivo with 331 IC50 data points (48%) and the remaining 18 drugs were not inhibitory in vivo with 360 data points (52%). Caco-2 was the most common cell line with 411 (59%) of the IC50 values followed by LLC-PK1-MDR1 and MDCK-MDR1 with 148 (21%) and 132 (19%), respectively. The use of different calculation methods was more evenly distributed among NSF (274, 40%), P_app,BL-AP (224, 32%), and ER (193, 28%). 

#### Table 1. Definitions and equations.

| Measure | Definition | Formula |
|---------|------------|---------|
| ER      | Ratio of secretory to absorptive permeability | $\frac{P_{app,AP-BL}}{P_{app,AP-BL}}$ |
| NSF     | Secretive minus absorptive permeability | $\frac{P_{app,Clark}-P_{app,AP-BL}}{P_{app,AP-BL}}$ |
| Inhibitor | In vivo inhibitor if digoxin AUCR $\geq$ 1.25-fold in the presence of the drug | AUCR $\geq$ 1.25 |
| Non-inhibitor | In vivo non-inhibitor if digoxin AUCR < 1.25-fold in the presence of the drug | AUC < 1.25 |
| Accuracy | Proportion of true results in all cases | $\frac{TP}{TP+FP+FN}$ |
| Sensitivity | Proportion of positive clinical DDI that had a positive in vitro result | $\frac{TP}{TP+FP}$ |
| Specificity | Proportion of negative clinical DDI that had a negative in vitro result | $\frac{TN}{TN+FP}$ |
| PPV     | Proportion of positive in vitro result that had a positive clinical DDI | $\frac{TN}{TN+FP}$ |
| NPV     | Proportion of negative in vitro result that had a negative clinical DDI | $\frac{FP}{FP+FN}$ |

#### Table 2. Substrate drug efflux ratios (geometric means).

| Drug         | Overall | Caco-2 | LLC-PK1-MDR1 | MDCK-MDR1 |
|--------------|---------|--------|--------------|-----------|
| Amprenavir   | 13.83   | 3.25   | 11.70        | 40.12     |
| Atazanavir   | 7.35    | 6.92   | 14.1         |           |
| Atorvastatin | 6.31    | 3.83   | 6.14         | 9.53      |
| Cetirizine   | 6.60    | 2.43   | 13.49        |           |
| Cimetidine   | 2.97    | 2.65   | 4.30         | 3.29      |
| Colchicine   | 11.81   | 16.82  | 4.07         | 9.07      |
| Cyclosporine | 5.76    | 5.61   | 6.18         | 5.80      |
| Dabigatran   | 5.75    | 5.87   | 15           | 1.8       |
| Dexamethasone| 3.19    | 1.81   | 3.01         | 6.99      |
| Digoxin      | 11.60   | 9.67   | 11.81        | 19.41     |
| Donperidone  | 13.83   | 11.48  | 18.7         | 31.2      |
| Doxorubicin  | 5.91    | 6.96   | 3.12         | 1.72      |
| Erythromycin | 11.62   | 19.43  | 5.17         | 7.01      |
| Etoposide    | 9.26    | 10.99  | 4.1          | 7.56      |
| Fexofenadine | 8.31    | 9.45   | 4.36         | 5.27      |
| Imatinib     | 9.27    | 2.95   | 5.75         | 18.58     |
| Indinavir    | 11.81   | 9.21   | 14.30        | 27.26     |
| Imnetocan    | 10.96   | 6.54   | 29.10        | 18.54     |
| Loperamide   | 6.75    | 2.93   | 5.81         | 34.02     |
| Paclitaxel   | 13.86   | 11.50  | 14.05        | 26.48     |
| Prazosin     | 4.54    | 3.79   | 5.87         | 5.11      |
| Quinidine    | 5.43    | 3.37   | 2.27         | 16.10     |
| Rhodamine 123| 7.96    | 7.99   | 4.37         | 10.30     |
| Risperidone  | 2.24    | 2.16   | 3.29         | 2.01      |
| Ritonavir    | 18.69   | 12.23  | 21.65        | 32.70     |
| Rivanoxaban  | 8.18    | 5.91   | 5.46         | 13.76     |
| Saquinavir   | 21.25   | 16.57  | 18.01        | 38.86     |
| Tacrolimus   | 3.34    | 2.64   | 4.14         | 4.6       |
| Talinol      | 9.52    | 10.06  | 6.32         | 5         |
| Verapamil    | 2.38    | 2.01   | 4.16         | 2.48      |
| Vinblastine  | 17.85   | 14.57  | 9.74         | 45.14     |
| Vincristine  | 6.99    | 9.78   | 2.37         | 6.35      |

Note. ‘Overall’ refers to all efflux data regardless of cell line.
Over two-thirds of the drugs had their lowest geometric mean IC50 values in Caco-2 cells and with the ER calculation method for 80% of all the drugs. With respect to the \textit{in vivo} classification data for the 30 drugs, 12 (40\%) drugs were inhibitory \textit{in vivo} (\textit{i.e.} AUCR \textless 1.25) and while the other 18 (60\%) drugs did not affect digoxin exposure (\textit{i.e.} AUCR \textgreater 1.25). Of note, several of these drugs are also \textit{in vitro} P-gp substrates including atorvastatin, lapatinib, linagliptin, omeprazole, pantoprazole, quinidine, quinine, ranolazine, ritonavir, and verapamil. Supplemental file 3 summarises the clinical drug–drug interaction data with digoxin for these drugs.

In general, no prominent differences were noted when the $\|g_{\text{rel}}\|_{\text{IC}}$ cut-off criterion was compared based on a cell line or calculation method. The three cell lines had similar/comparable (\textit{difference \leq 15\%}) accuracy and sensitivity while the specificity was lower in the Caco-2 cells (Table 4). This may be due to the differences in P-gp expression between the cell lines. MDCK-MDR1 cells had slightly higher NPV and PPV results. MDCK-MDR1 cells had numerically higher and comparable NPV and PPV results to results from Caco-2 cells, respectively. However, MDCK-MDR1 cells had comparable NPV and PPV results with LLC-PK1-MDR1 results. Looking at the IC50 calculation methods, P app,BL-AP had a higher NPV than the ER and NSF (Table 5). There was a \textit{\leq 16\%} difference in the three methods for PPV, accuracy, specificity, or sensitivity.

### Discussion

It is well known that variability in experimental conditions and methodology can influence outcomes in efflux and inhibition experiments for the evaluation of a drug as a substrate or inhibitor of an efflux transporter (Volpe 2011, 2016). These include cell lines (Tang et al. 2002; Taub et al. 2005), P-gp expression levels (Hayeshi et al. 2008; Shirasaka et al. 2008), substrate/inhibitor concentrations (Keogh and Kunta 2006; Elsby et al. 2008; Crowe and Wright 2012; Hodin et al. 2018), culture conditions (Balimane et al. 2004; Siissalo et al. 2011).
tion methods (Balimane et al. 2008; Cook et al. 2010; Perloff et al. 2011; Sugimoto et al. 2011; Bentz et al. 2013; Kishimoto et al. 2014; Poirier et al. 2014; Volpe et al. 2014). For assays determining whether a drug is a P-gp substrate, investigators have found that efflux ratios can vary based on cell passage number, initial cell seeding, plate format, monolayer age, substrate concentration, and transport buffer pH (Neuhoff et al. 2003; Balimane et al. 2004; Crowe and Wong 2004; Korjamo et al. 2005; Sissalo et al. 2007; Elsby et al. 2008; Shirasaka et al. 2008; Kamiyama et al. 2009; Miliotis et al. 2011; Patil et al. 2011; Crowe and Wright 2012).

For the 30 P-gp substrates evaluated, geometric mean ERs ranged from 45.14 (vinblastine) to 1.72 (doxorubicin). Overall, there was no single cell line that yielded the highest or lowest ER values. An examination was also conducted on a small subset of these substrate drugs for those with low (~2–3) and higher (>10) geometric mean efflux ratios. There were no significant differences in logP values between the groups or physiological charge (data not shown). Similarly, there were no significant trends between the groups based on their permeability or solubility class according to the biopharmaceutics classification system (BCS; data not shown).

For assays determining whether a drug is a P-gp inhibitor, IC50 values can vary across cell lines, substrates, and calculation methods (Balimane et al. 2008; Cook et al. 2010; Perloff et al. 2011; Sugimoto et al. 2011; Bentz et al. 2013; Kishimoto et al. 2014; Poirier et al. 2014; Volpe et al. 2014). However, a common observation was that IC50 values based on ER were lower than those calculated from NSF or \( P_{\text{app,BL-AP}} \) (Balimane et al. 2008; Cook et al. 2010; Perloff et al. 2011; Sugimoto et al. 2011; Bentz et al. 2013; Kishimoto et al. 2014; Poirier et al. 2014; Volpe et al. 2014). A study with 23 labs, using four systems (Caco-2, LLC-PK1-MDR1, MDCK-MDR1, vesicles) with 15 test drugs, found substantial variability in IC50 values for the inhibition of digoxin efflux (Bentz et al. 2013). In the study, the lowest variability was seen for sertraline (20-fold) and isradipine (24-fold) with telmisartan (407-fold), and verapamil (796-fold) having the highest variability. The study concluded that a large amount of the variability was due to lab-to-lab procedural differences (e.g., cell source, passage number, culture/transport conditions) rather than P-gp expression in the cell systems (Bentz et al. 2013). Variability within a laboratory may be limited by uniform practices in terms of these multiple factors including cell source and calculation methods when conducting in vitro experiments to determine whether a new drug is a substrate or inhibitor of a transporter (Brouwer et al. 2013; Volpe 2016).

Given the aforementioned reports of various factors shown to have an impact on ER and IC50 values, we evaluated whether differences between cell lines and calculation methods can impact a drug’s classification as a P-gp substrate or inhibitor. Our research shows that there were no differences in predictive performances in this small dataset based on a cell line or IC50 calculation method used. It is also noteworthy that a number of drugs routinely had false or true predictions based on the \( \frac{|\text{Igut}|}{\text{IC50}} \geq 10 \) cut-off criterion, that is, bepridil, conivaptan, dipyriramole, ketoconazole, and troglitazone nearly always had FPs or FNs. Additionally, mibebradil, nicardipine, propafenone, sertraline, spiranolaclactone, and telmisartan mostly had FPs or FNs with this criterion. It is also notable that bepridil, propafenone, and telmisartan had in vivo AUCRs that were close to the 1.25 cut-off. Conversely, amiodarone, atorvastatin, carvedilol, clarithromycin, diltiazem, isradipine, itraconazole, lapatinib, linagliptin, pantoprazole, quinidine, ranolazine, ritonavir, spironolactone, telmisartan, and verapamil nearly always had TP or TN results.

Overall, the NPV (86%) was greater than PPV (48%) with the 30 drugs in the dataset. IC50 values from the Caco-2 cells tended to result in an NPV and PPV numerically lower than the LLC-PK1-MDR1 and MDCK-MDR1 cells. Both the LLC-PK1-MDR1 and MDCK-MDR1 cells had the same NPV (100%) and sensitivity (100%) results. Using \( P_{\text{app,BL-AP}} \) for calculations of IC50 values resulted in somewhat better NPV and PPV than the ER or NSF methods. Even though there were differences in predictive performances in this small dataset, there were no significant differences in the PPV and NPV values regardless of the use of a particular cell line or IC50 calculation method.

We do acknowledge several important limitations with our research presented here. First, this study was limited by the low number of evaluated drugs (30) of which 60% were in vivo non-inhibitors of P-gp in digoxin DDI studies. However, it should be noted that for the in vitro IC50 values there was a similar number of data points for the in vivo inhibitors (48%) and non-inhibitors (52%). Secondly, the dataset collated was from various laboratories which could potentially introduce variability from the experimental procedures such as incubation time, inhibitor concentration, and digoxin concentration. However, this closely mimics the situation frequently encountered during drug development where in vitro characterisation of drugs is conducted in different laboratories under different experimental conditions. A third limitation is that the findings failed to identify a combination of cell line and calculation method as superior in reducing the FN rate. This could be due to the fact that the number of FN and TN results was relatively low compared to FP and TP results. The last limitation is that although digoxin is widely used as a probe substrate for P-gp clinical interaction studies and the majority of available in vitro and in vivo data is based on using digoxin as a substrate (Taub et al. 2005; Rautio et al. 2006), it has a few limitations. This includes high interlaboratory results with digoxin and uptake mechanisms of digoxin in the liver and kidney (Bentz et al. 2013; Lee et al. 2014; Nader and Foster 2014). Hence, if a different P-gp substrate was to be used in in vitro and in vivo studies, whether differences in cell lines or calculation methods will influence the categorisation of drugs as P-gp substrates or inhibitors, needs to be investigated.

Overall, we conducted an analysis of literature data of P-gp substrates and inhibitors to examine differences in ER or IC50 values due to cell lines and calculation methods and to compare the resultant \( |\text{Igut}|/\text{IC50} \) ratios to predict the potential for a drug to inhibit P-gp transporters. For 30 drugs, lower mean IC50 values were often observed with the Caco-2
cells and calculations based on efflux ratios. Both positive and negative predictive values were comparable regardless of the use of a particular cell line or calculation method for a drug’s IC₅₀ value. This has implications in drug development and regulatory review in that different cell lines or IC₅₀ calculation methods may be used in predicting P-gp-mediated drug–drug interactions.

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Data availability statement

The authors confirm that all the literature references for the data supported in this article are available in the supplementary materials.

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