XENOTECH
A BioIVT Company

Comparison of K_i and IC_{50} Values for Prototypical Inhibitors of the Major Drug Uptake Transporters

Andrea R. Wolff, Caleb Isringhausen, Joel Widmer, Alison Coons, Greg Loewen, Brian W. Ogilvie and David B. Buckley
XenoTech, LLC, 16825 W 116th St, Lenexa, KS, USA

Introduction

Transporters are membrane-bound proteins that can influence the pharmacokinetic properties of drugs. Compounds that are inhibitors of these transporters may be involved in clinically significant drug interactions (Hillgren *et al.*, 2013, Giacomini *et al.*, 2010). The clinical implications of such interactions can be detrimental. The U.S. Food and Drug Administration (FDA) released a revised draft guidance for industry on DDIs in 2012 (FDA 2012) that recommends new drug candidates should be evaluated for their potential to inhibit specific transporters. To evaluate the inhibition of transporters *in vitro*, the 2012 FDA guidance document recommends the calculation of IC_{50} values whereas the recent EMA Guideline on the Investigation of Drug Interactions (EMA 2013) recommends the calculation of K_i values. The difficulty of experimental design coupled with intricate mathematical extrapolation can make the determination of intrinsic K_i values difficult and oftentimes reverts to the determination of extrinsic IC_{50} values. Also, it is important to consider clinically relevant substrates rather than prototypical probe substrates when evaluating potential drug-drug interactions (DDIs) of investigational drugs at the transporter level due to the fact that transporters have multiple substrate binding sites (Brouwer *et al.*, 2013). In this study, K_i and IC_{50} values were determined for the major hepatic and renal uptake transports OATP1B1, OATP1B3, OCT1, OAT1, OAT3 and OCT2 with prototypical substrates. In addition, K_i and IC_{50} values were determined with a clinically-relevant substrate of the hepatic uptake transporters OATP1B1 and OATP1B3. K_i values were calculated from the IC_{50} value using the Cheng-Prusoff equation (Cheng and Prusoff, 1973) and were determined with an experimental design incorporating multiple substrate and inhibitor concentrations.

Materials & Methods

Materials

Bovine serum albumin, butyric acid, estradiol-17β-glucuronide, estrone-3-sulfate, HEPES, pravastatin, quinidine, rifampin and sodium hydrogencarbonate were obtained from Sigma (St. Louis, MO, USA). [3H] p-Aminohippuric acid (PAHA), [³H]-estradiol-17β-glucuronide (E2G), [³H]-estrone-3-sulfate (E3S), [¹⁴C]-metformin (MET) and [¹⁴C]-tetraethylammonium bromide (TEA) was obtained from Perkin Elmer Life and Analytical Sciences (Waltham, MA, USA) or Arc, Inc. (St. Louis, MO, USA). HBSS was purchased from Invitrogen (Carlsbad, CA, USA). HEK293 cells over-expressing either OATP1B1, OATP1B3, OAT1, OAT3, OCT1 or OCT2 were obtained from Sekisui Medical Division, LTD (Tokai, Japan).

Methods

HEK293 cells over-expressing the transporter were cultured onto standard 24-well tissue culture plates at a density of 0.2 to 0.4 × 10^6 cells per well in cell culture media 1 to 3 days prior to the experiment. OATP1B1 and OATP1B3 cells were incubated with butyric acid for 24 hours prior to the experiments to inhibit suppression of the transporter. Incubations were carried out in HBSS buffer containing sodium hydrogencarbonate (4 mM) and HEPES (8.4 mM). IC_{50} determinations were conducted as described in Table 1. K_i determinations were conducted as described in Table 2. Uptake was terminated by washing the cells three times with ice-cold 1x PBS. In incubations with radiolabeled probe substrates, cells were lysed with 0.1 M NaOH and an aliquot was added to scintillation cocktail for analysis by scintillation counting. For unlabeled probe substrates, cells were extracted with 50:50 v/v methanol:water containing internal standard and analyzed by LC-MS/MS. IC_{50} values were determined with GraphPad Prism software (version 6.01). K_i values were determined with GraFit software (version 7.0.2). K_i values were also calculated from the IC_{50} values using the Cheng-Prusoff equation:

$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}}$$

Table 1. Summary of incubation conditions for IC_{50} determinations

Transporter	Substrate	[Substrate] (μM)	K_m (μM)	Inhibitor	[Inhibitor] (μM)	Incubation time (min)
OATP1B1	[³H]-E2G	0.05	6.0	Rifampin	0.01 - 10	2
OATP1B1	Pravastatin	10	97	Rifampin	0.03 - 30	2
OATP1B3	[³H]-E2G	0.05	13	Rifampin	0.01 - 10	2
OATP1B3	Pravastatin	10	110	Rifampin	0.03 - 30	2
OAT1	[¹⁴C]-PAHA	1	48	Probenecid	0.01 - 100	1
OAT3	[³H]-E3S	0.05	20	Probenecid	0.01 - 100	2
OCT1	[¹⁴C]-TEA	5	510	Quinidine	1 - 100	15
OCT2	[¹⁴C]-MET	10	1800	Quinidine	1 - 300	2

Table 2. Summary of incubation conditions for K_i determinations

Transporter	Substrate	[Substrate] (μM)	K_m (μM)	Inhibitor	[Inhibitor] (μM)	Incubation time (min)
OATP1B1	[³H]-E2G	1 - 36	6.0	Rifampin	0.133 - 4.8	2
OATP1B1	Pravastatin	1 - 300	97	Rifampin	0.1 - 10	2
OATP1B3	[³H]-E2G	2.2 - 78	13	Rifampin	0.033 - 1.2	2
OATP1B3	Pravastatin	1 - 300	110	Rifampin	0.1 - 10	2
OAT1	[¹⁴C]-PAHA	6.67 - 240	48	Probenecid	1.67 - 60	1
OAT3	[³H]-E3S	4 - 144	20	Probenecid	0.833 - 48	2
OCT1	[¹⁴C]-TEA	83 - 3000	510	Quinidine	2.5 - 90	15
OCT2	[¹⁴C]-MET	330 - 12000	1800	Quinidine	4.17 - 150	2

Results

The prototypical probe substrate E2G and clinically relevant probe substrate pravastatin were evaluated with OATP1B1 and OATP1B3 to compare the inhibitory constants. Rifampin inhibited E2G and pravastatin uptake into OATP1B1-expressing cells with IC_{50} values of 0.79 and 0.14 μM, respectively (Figure 1A and Table 3). Subsequent K_i determinations with rifampin resulted in inhibition of OATP1B1-mediated uptake of E2G and pravastatin with K_i values of 1.1 and 0.28 μM, respectively (Figure 1B and 1C). Inhibition of OATP1B1 uptake of E2G yielded comparable (within 2-fold) IC_{50} and K_i values (0.79 and 1.1 μM, respectively). Similarly, inhibition of pravastatin uptake by rifampin yielded comparable IC_{50} and K_i values (0.14 and 0.28 μM, respectively). However, the IC_{50} and K_i values observed with E2G (0.79 and 1.1 μM, respectively) were approximately 4 to 5-fold higher than the IC_{50} and K_i values observed with pravastatin (0.14 and 0.28 μM, respectively) displaying substrate-dependent inhibition.

Rifampin inhibited E2G and pravastatin uptake into OATP1B3-expressing cells with IC_{50} values of 0.19 and 0.16 μM, respectively (Figure 1D and Table 3). Rifampin inhibited the uptake of E2G and pravastatin by OATP1B3 with K_i values of 0.19 and 0.14 μM, respectively (Figure 1E and 1F). OATP1B3 experiments conducted with E2G yielded equivalent IC_{50} and K_i values (0.19 μM). These values were similar to the results observed with pravastatin (IC_{50} and K_i values of 0.16 and 0.14 μM, respectively) thus demonstrating no apparent substrate-dependent inhibition.

The experimentally determined IC_{50} and K_i values were also determined for OAT1, OAT3, OCT1 and OCT2 with only prototypical probe substrates and are summarized in Table 3. The K_i results are illustrated in Figure 2. The IC_{50} values for OAT1 and OCT2 (8.3 and 25 μM, respectively) were confirmed to be within 2-fold of the experimentally determined K_i values of 4.7 and 50 μM, respectively (Figure 2A and 2D). However, the K_i for probenecid was 25 μM (Figure 2B) with OAT3 cells and was approximately 10-fold higher than the IC_{50} (2.8 μM). In the case of OCT1, the IC_{50} for quinidine (15 μM) was approximately 5-fold higher than the K_i of 3.2 μM (Figure 2C).

The mechanism of inhibition for OATP1B1, OATP1B3, OAT1, OAT3 and OCT1-mediated uptake was determined to be competitive according to the best fit of the experimental data, whereas the mechanism of inhibition was noncompetitive for OCT2-mediated uptake (Table 3).

Figure 1. IC_{50} and K_i determinations for OATP1B1 and OATP1B3

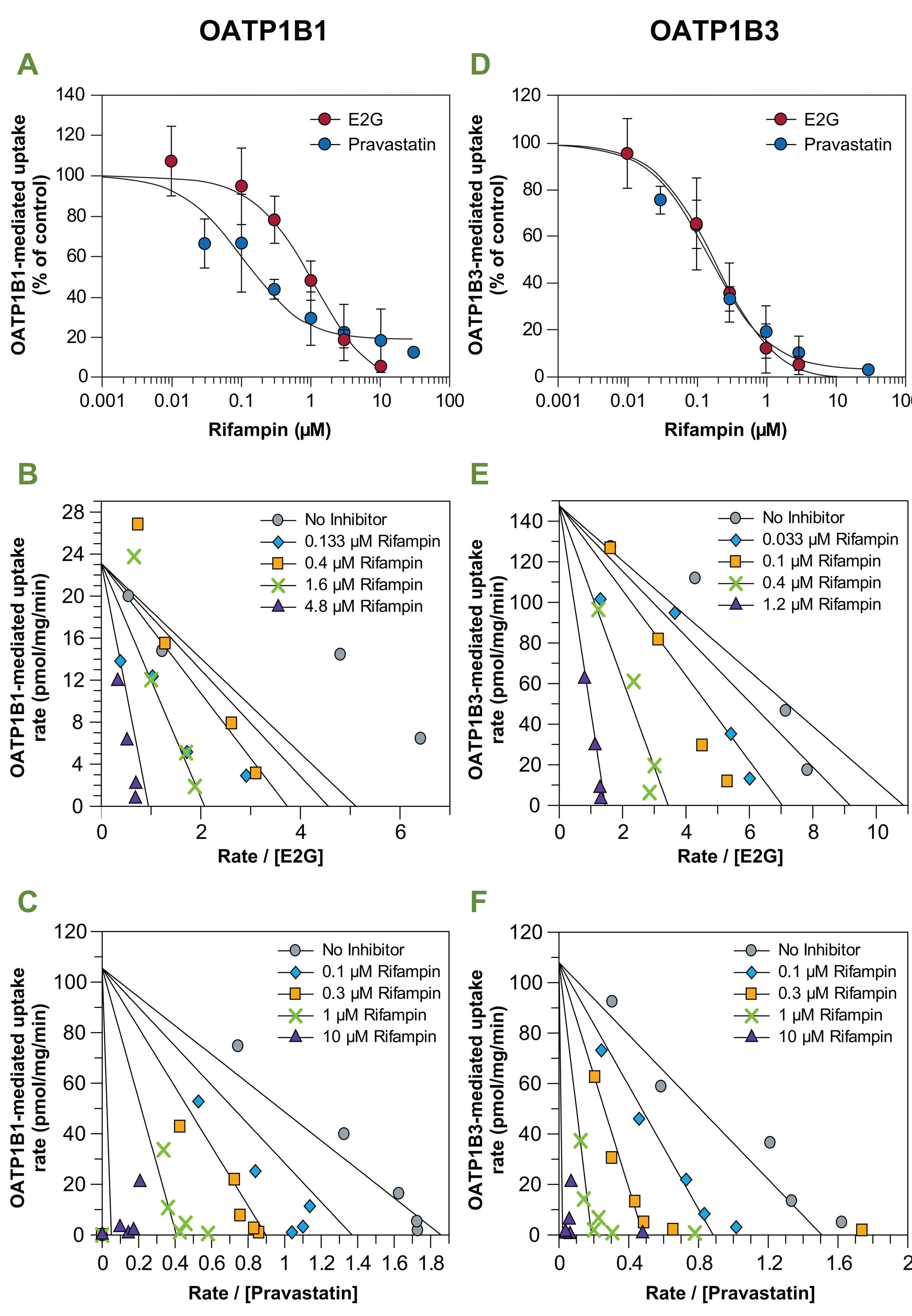
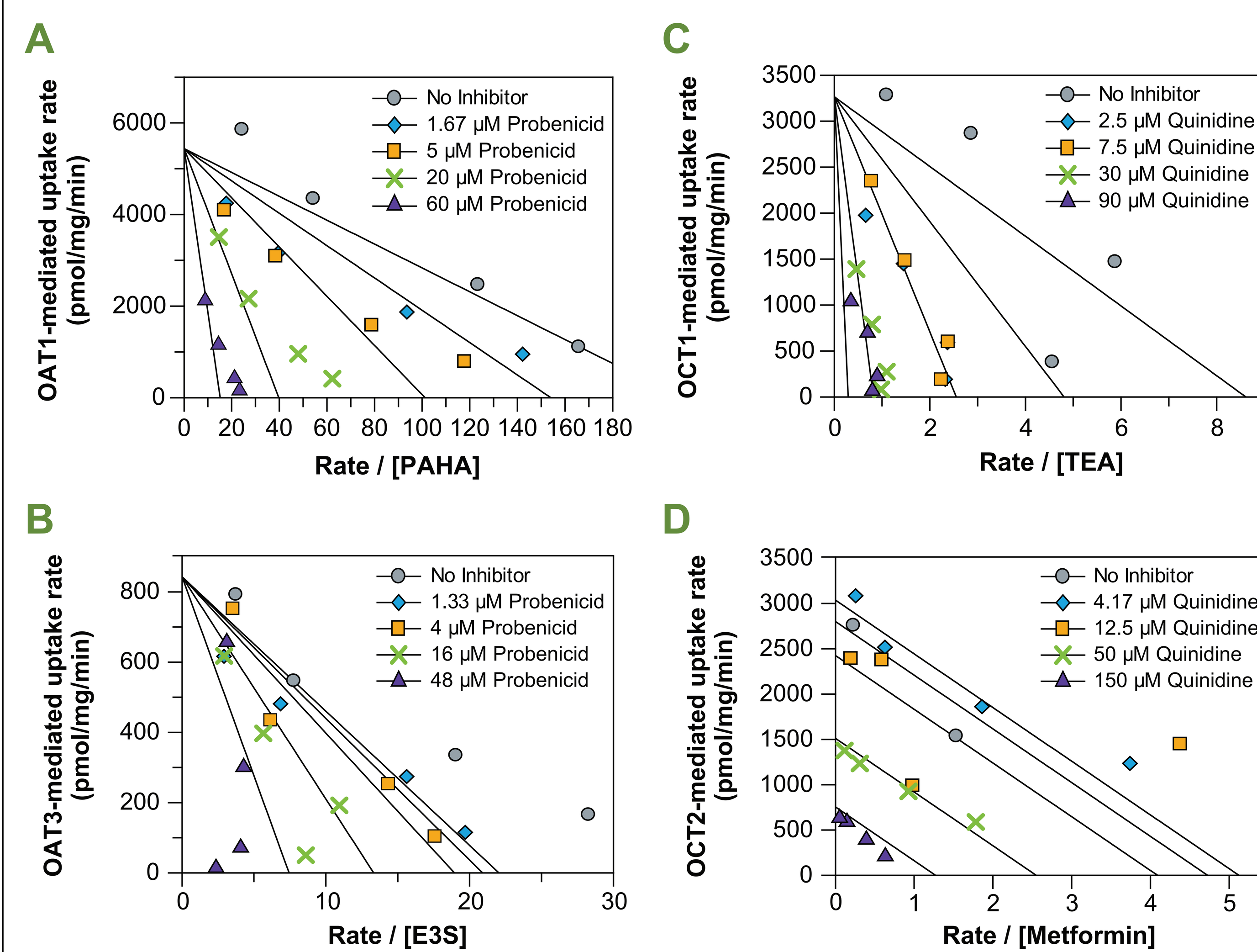


Table 3. Summary of experimentally-determined and estimated inhibitory constants for the uptake transporters

Transporter	Substrate	Inhibitor	IC_{50} (μM)	Experimental K_i (μM)[a]	Estimated K_i (μM)[b]	Mechanism (best fit)
OATP1B1	[³H]-E2G	Rifampin	0.79	1.1	0.78	Competitive
OATP1B1	Pravastatin	Rifampin	0.14	0.28	0.13	Competitive
OATP1B3	[³H]-E2G	Rifampin	0.19	0.19	0.19	Competitive
OATP1B3	Pravastatin	Rifampin	0.16	0.14	0.15	Competitive
OAT1	[¹⁴C]-PAHA	Probenecid	8.3	4.7	8.1	Competitive
OAT3	[³H]-E3S	Probenecid	2.8	25	2.8	Competitive
OCT1	[¹⁴C]-TEA	Quinidine	15	3.2	15	Competitive
OCT2	[¹⁴C]-MET	Quinidine	25	50	25	Noncompetitive

[a] Experimentally determined [b] Derived from Cheng-Prusoff equation

Figure 2. K_i determinations for OAT1, OAT3, OCT1 and OCT2



Conclusions

- Substrate-dependent inhibition by rifampin was observed in OATP1B1 cells but not in OATP1B3 cells inasmuch as rifampin inhibited OATP1B1-mediated pravastatin uptake with approximately four to five-fold more potency than observed with E2G. In some cases, IC_{50} and K_i experiments should be determined with a clinically relevant substrate because substrate-dependent inhibition has been demonstrated for OATP1B1.
- Experimentally determined K_i values were similar to IC_{50} values (within two-fold) for inhibition of OATP1B1, OATP1B3, OAT1 and OCT2, but not for inhibition of OAT3 and OCT1. In most cases, experimentally determined IC_{50} values may be as accurate as K_i values for DDI predictions when IC_{50} experiments are conducted with a probe substrate concentration well below the K_m.

References

1 Brouwer KLR, Keppler D, Hoffmaster KA, Bow DAJ, Cheng Y, Lai Y, Palm JE, Stieger B, Evers R (2013) *In Vitro* Methods to Support Transporter Evaluation in Drug Discovery and Development. *Clin Pharmacol Ther* **94**:95-112.

2 Cheng Y, Prusoff WH (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem Pharmacol* **22**:3099–3108.

3 [EMA] European Medicines Agency (2013) Guideline on the Investigation of Drug Interactions. European Medicines Agency, London. 60 p. EMA Guideline No.: CPMP/EWP/560/95/Rev. 1 Corr.

4 [FDA] Food and Drug Administration (2012) Draft Guidance for Industry: Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations, U.S. Department of Health and Human Services, Rockville, MD. 79 p.

5 International Transporter Consortium: Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM, Hoffmaster KA, Ishikawa T, Keppler D, Kim RB, Lee CA, Niemi M, Polli JW, Sugiyama Y, Swaan PW, Ware JA, Wright SH, Yee SW, Zamek-Gliszczynski MJ, Zhang L (2010) Membrane transporters in drug development. *Nat Rev Drug Discov* **9**:215-236.

6 Hillgren KM, Keppler D, Zur AA, Giacomini KM, Stieger B, Cass CE, Zhang L (2013) Emerging Transporters of Clinical Importance: An Update From the International Transporter Consortium. *Clin Pharmacol Ther* **94**:52-63.

7 Tibbets G, Isringhausen C, Widmer J, Coons A, Lyon KC, Wolff A, Hays A, Loewen G and Buckley DB (2013) *ISSX Poster Presentation*.