

## 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 The U.S. Food and Drug Administration (FDA) released a revised draft guidance for industry on DDIs in 2012 (FDA 2012) that recommends new drug candidates should be evaluated for their potential to inhibit specific transporters.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 transporters 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-17 $\beta$ -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-17 $\beta$ -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  $\times 10^6$  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] ( $\mu$ M)	$K_m$ ( $\mu$ M)	Inhibitor	[Inhibitor] ( $\mu$ M)	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] ( $\mu$ M)	$K_m$ ( $\mu$ M)	Inhibitor	[Inhibitor] ( $\mu$ M)	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  $\mu$ M, 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  $\mu$ M, 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  $\mu$ M, respectively). Similarly, inhibition of pravastatin uptake by rifampin yielded comparable  $IC_{50}$  and  $K_i$  values (0.14 and 0.28  $\mu$ M, respectively). However, the  $IC_{50}$  and  $K_i$  values observed with E2G (0.79 and 1.1  $\mu$ M, respectively) were approximately 4 to 5-fold higher than the  $IC_{50}$  and  $K_i$  values observed with pravastatin (0.14 and 0.28  $\mu$ M, 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  $\mu$ M, 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  $\mu$ M, respectively (Figure 1E and 1F). OATP1B3 experiments conducted with E2G yielded equivalent  $IC_{50}$  and  $K_i$  values (0.19  $\mu$ M). These values were similar to the results observed with pravastatin ( $IC_{50}$  and  $K_i$  values of 0.16 and 0.14  $\mu$ M, 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  $\mu$ M, respectively) were confirmed to be within 2-fold of the experimentally determined  $K_i$  values of 4.7 and 50  $\mu$ M, respectively (Figure 2A and 2D). However, the  $K_i$  for probenecid was 25  $\mu$ M (Figure 2B) with OAT3 cells and was approximately 10-fold higher than the  $IC_{50}$  (2.8  $\mu$ M). In the case of OCT1, the  $IC_{50}$  for quinidine (15  $\mu$ M) was approximately 5-fold higher than the  $K_i$  of 3.2  $\mu$ M (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

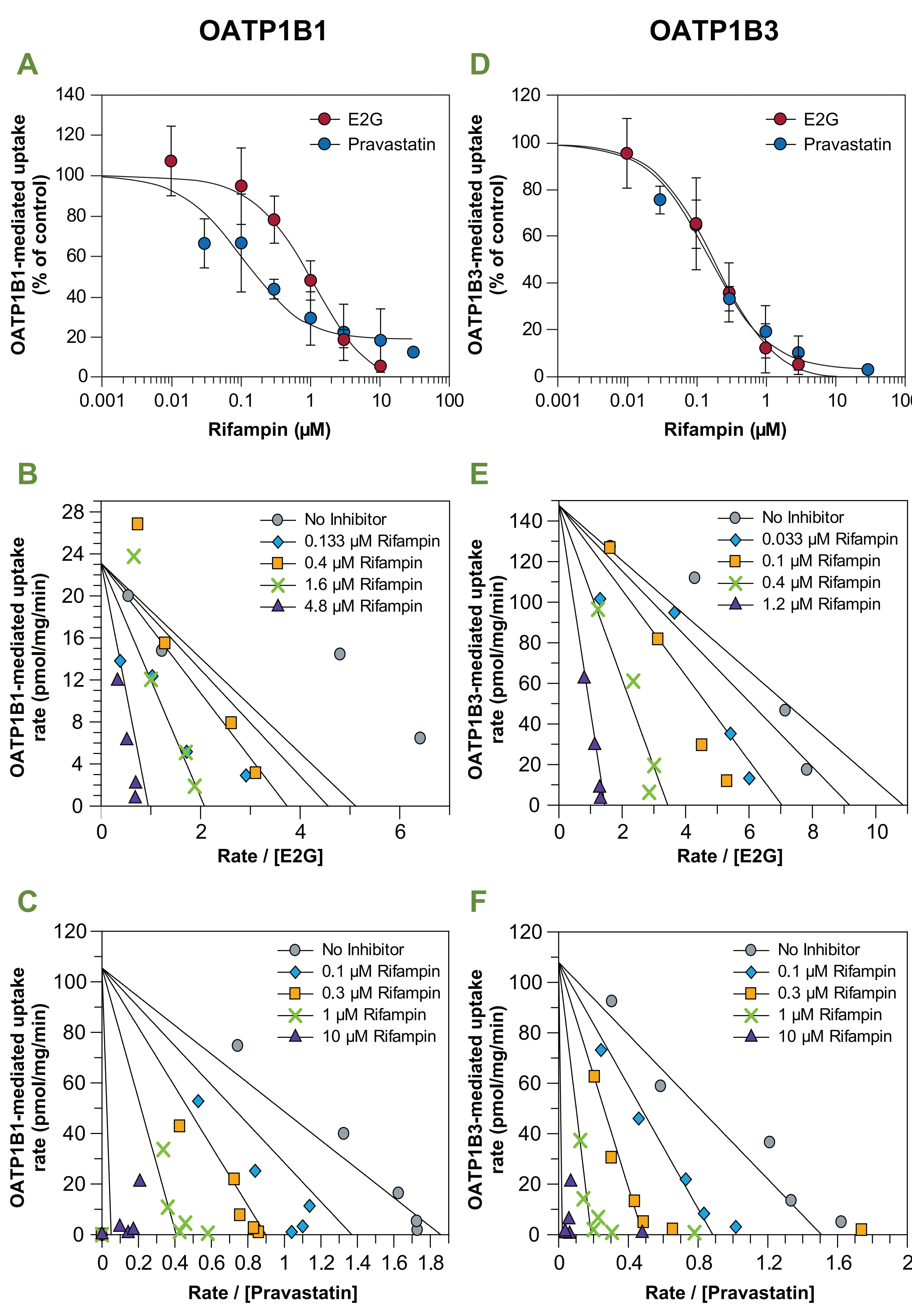


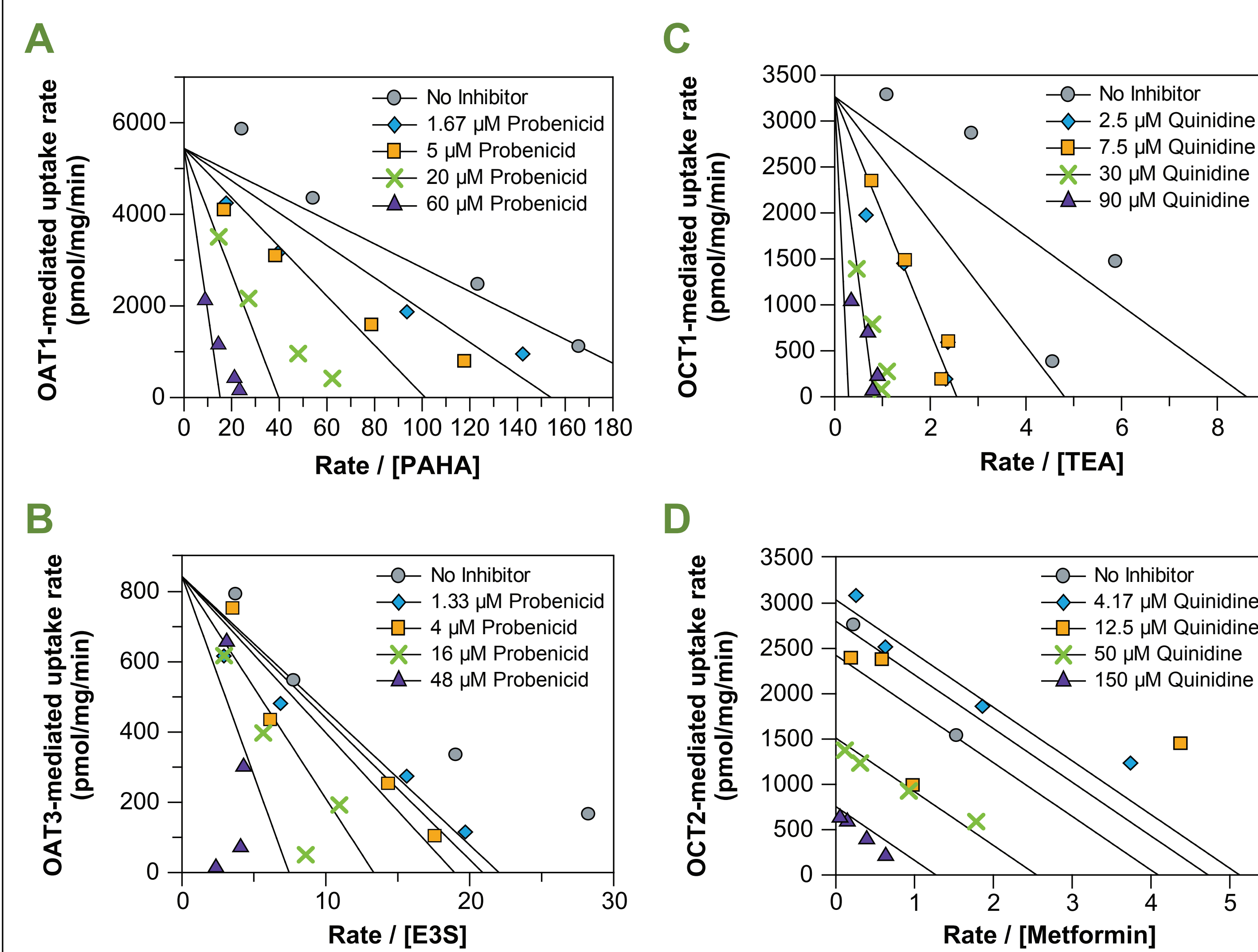
Table 3. Summary of experimentally-determined and estimated inhibitory constants for the uptake transporters

Transporter	Substrate	Inhibitor	$IC_{50}$ ( $\mu$ M)	Experimental $K_i$ ( $\mu$ M) <sup>a</sup>	Estimated $K_i$ ( $\mu$ M) <sup>b</sup>	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

<sup>a</sup> Experimentally determined

<sup>b</sup> 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

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