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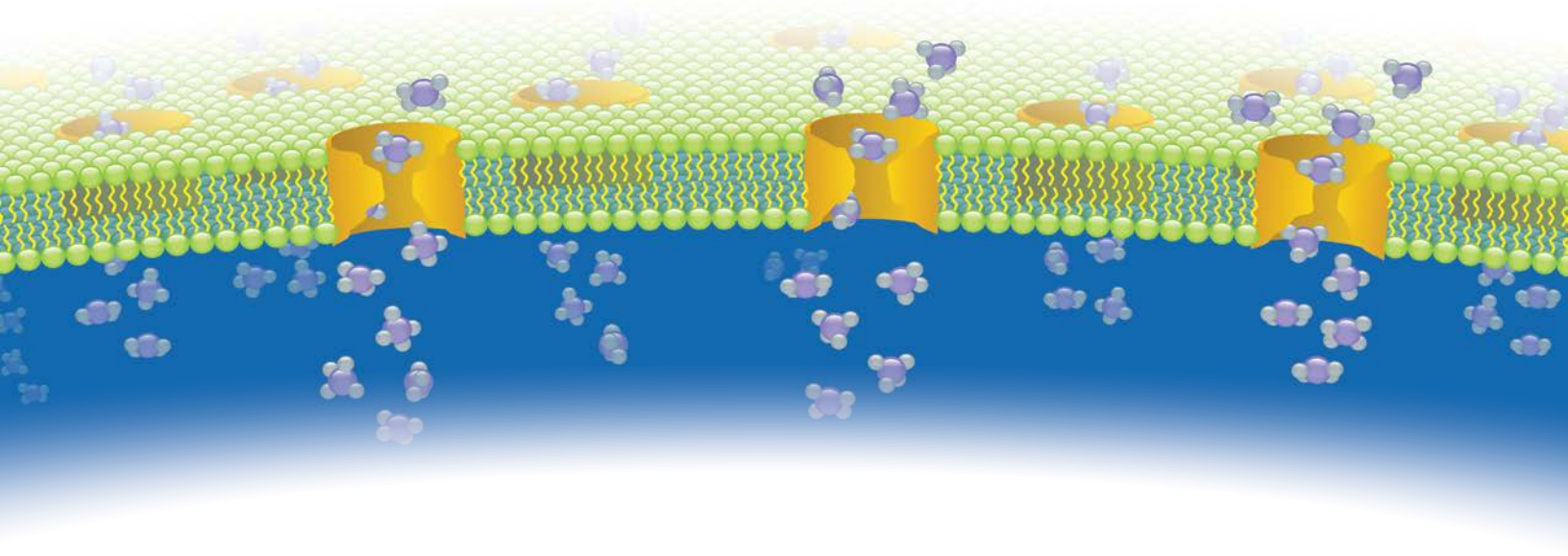
Drug Transporter Assays at XenoTech

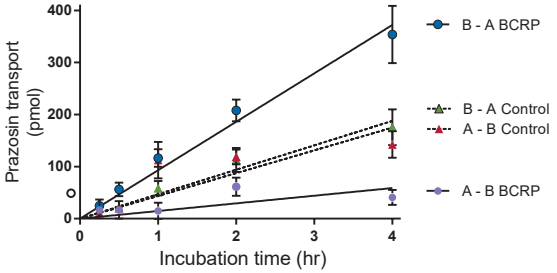


Transporters have become increasingly important in drug development due to the major role they play in absorption, distribution and excretion of endogenous and exogenous compounds, inasmuch as transporter-mediated drug-drug interactions (DDI) are associated with potential toxicological and pharmacological consequences. As evidenced in numerous publications, the effects of transporters on the pharmacokinetics of several drugs, and associated DDI, have been reported. Consequently, recent guidance documents released by the US FDA, European Medicines Agency (EMA) and Pharmaceuticals & Medical Devices Agency (PMDA) of Japan emphasize the importance of evaluating the potential of new drug candidates for transporter-mediated DDI with a determination of victim (substrate) and perpetrator (inhibition) potential.

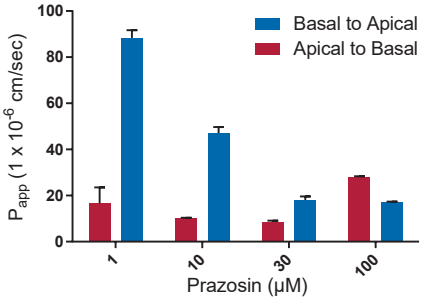
XenoTech performs *in vitro* transporter studies to determine if compounds are substrates or inhibitors of clinically-relevant transporters with validated, and industry accepted, test systems. In addition to a dedicated drug transport team, XenoTech employs an experienced analytical department with capabilities to conduct studies with labeled or unlabeled drug candidates, a Quality Assurance department to oversee GLP studies and a knowledge management group to facilitate and expedite production of high quality data reports.

P-gp (MDR1/ABCB1), BCRP (ABCG2), MRP2 (ABCC2) and BSEP (sPgp/ABCB11) are members of the ATP-binding cassette superfamily of transporters and are expressed on the apical membrane of cells in a number of tissues. P-gp and BCRP are expressed in the luminal membrane of enterocytes, endothelial cells in the brain, brush border membrane of renal proximal tubules and canalicular membrane of hepatocytes where they limit the intestinal absorption, blood-brain barrier penetration and facilitate excretion into the bile and urine. MRP2 and BSEP are mainly expressed in the canalicular membrane of hepatocytes where they facilitate excretion into the bile.

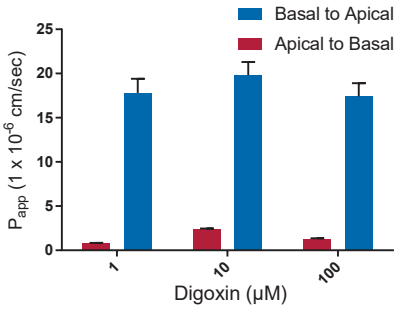
To determine if a compound is a substrate of P-gp or BCRP, the bidirectional permeability of the compound across MDCKII-MDR1 or MDCKII-BCRP and MDCKII control cells is measured. Prazosin efflux across BCRP-expressing MDCKII cells is linear over time and saturable. The prototypical P-gp substrate, digoxin, is not saturatable in Caco-2 or MDCKII cells (Caco-2 data shown below). BSEP and MRP2 substrate experiments are evaluated in membrane vesicles by measuring accumulation of the test compound in the vesicle.



Prazosin Time Course Evaluation in BCRP (MDCKII cells)

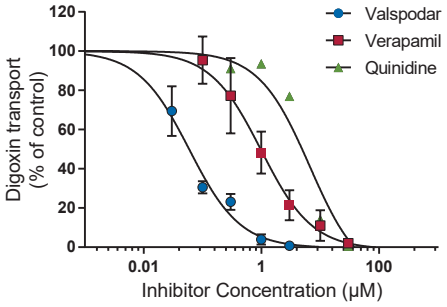


Bidirectional Permeability of Prazosin in BCRP (MDCKII cells)

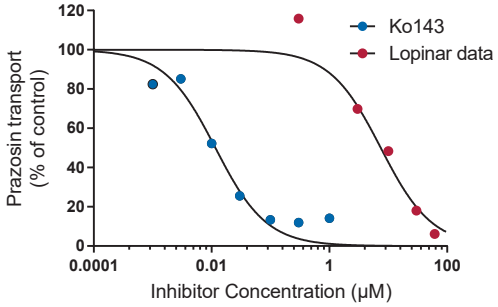


Bidirectional Permeability of Digoxin in P-gp (Caco-2 cells)

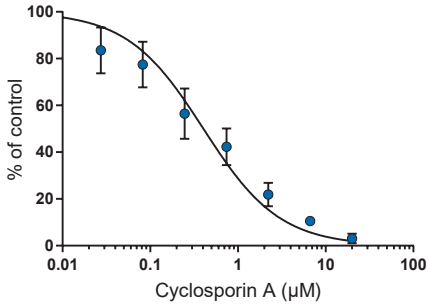
P-gp inhibition is evaluated by measuring the bidirectional permeability of digoxin across Caco-2 cells in the presence of the test compound. BCRP inhibition is evaluated by measuring the bidirectional permeability of prazosin across MDCKII-BCRP cells in the presence of the test compound. BSEP and MRP2 inhibition experiments are evaluated in membrane vesicles by measuring the effect of the test compound on the accumulation of a probe substrate. IC_{50} curves for inhibition of digoxin bidirectional permeability in Caco-2 cells and prazosin in MDCKII cells and Taurocholate in BSEP vesicles in the presence of the test compound are shown below.



Inhibition of P-gp-mediated Digoxin Transport (Caco-2 cells)



Inhibition of BCRP-mediated Prazosin Transport (MDCKII cells)



Inhibition of BSEP-mediated Taurocholate Transport (Vesicles)

*Error bars represent means ± SD from 2-3 independent experiments

Transporter	Cell Line	Substrate	Incubation Time (min)	Inhibitor	IC_{50} (µM)
P-gp	Caco-2	10 µM Digoxin	120	Verapamil / Valspodar	0.81 ± 0.33 / 0.06 ± 0.02
P-gp	MDCKII-MDR1	10 µM Digoxin	120	Verapamil / Valspodar	21.7 ± 18.6 / 2.3
BCRP	MDCKII-BCRP	1 µM Prazosin	120	Ko143 / Lopinavir	0.012 ± 0.003 / 4.6
BSEP	Vesicles	0.4 µM Taurocholate	5	Cyclosporin A	0.4 ± 0.03

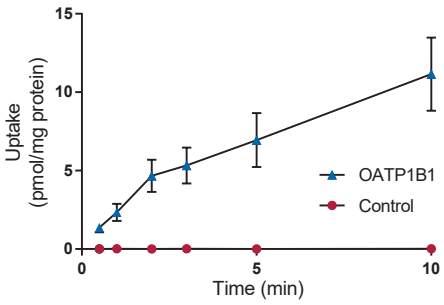
Hepatic & Renal Uptake Transporters (OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2)

OATP1B1 (OATP-C/OATP2/SLCO1B1), OATP1B3 (OATP-8/SLCO1B3) and OCT1 (SLC22A8) are expressed on the sinusoidal membrane of hepatocytes and facilitate the uptake of endogenous and xenobiotic compounds into hepatocytes for further metabolism or excretion into the bile. OAT1 (SLC22A6), OAT3 (SLC22A8) and OCT2 (SLC22A2) are expressed on the basolateral membrane of renal proximal tubules and facilitate the uptake of compounds into the proximal tubule for further excretion in the urine.

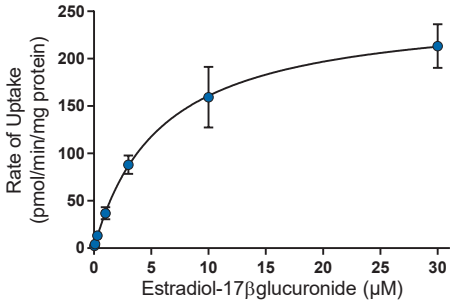
To determine if a compound is a substrate of an uptake transporter, the accumulation of the compound in cells over-expressing a single uptake transporter is measured.

Inhibition of uptake transporters is evaluated by measuring the accumulation of a probe substrate in the cells in the presence of the test compound. Examples of substrate accumulation and concentration-dependent inhibition are presented below.

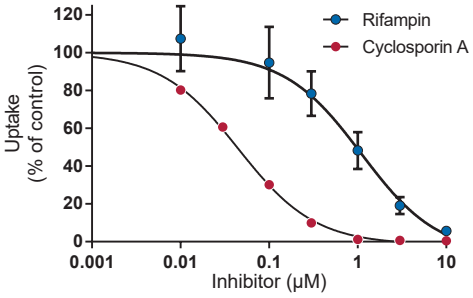
OATP1B1 (HEK-293)



Time Course Evaluation

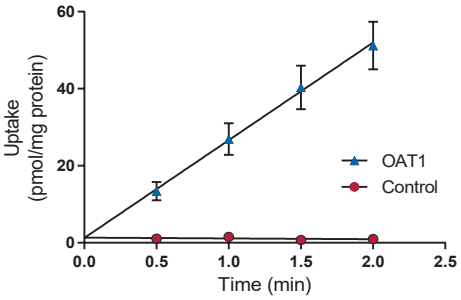


K_m/V_{max}

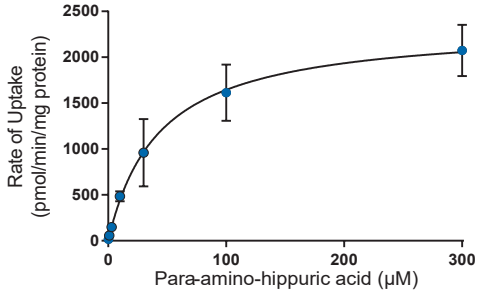


IC_{50}

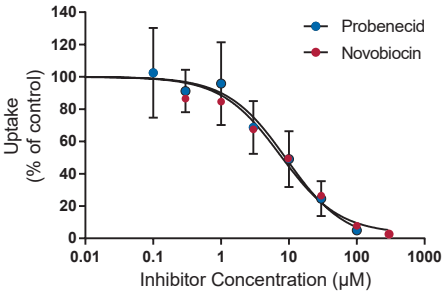
OAT1 (HEK-293)



Time Course Evaluation



K_m/V_{max}



IC_{50}

*Error bars represent means ± SD from 2-3 independent experiments

Transporter	Cell Line	Substrate	K_m (μM)	Incubation Time (min)	Inhibitor	IC_{50} (μM)
OATP1B1	HEK-293	50 nM Estradiol-17βglucuronide	6.0 ± 1.8	2	Rifampin / Cyclosporin A	0.80 ± 0.13 / 0.04 ± 0.002
OATP1B3	HEK-293	50 nM Estradiol-17βglucuronide	13.2 ± 2.5	2	Rifampin / Cyclosporin A	0.19 ± 0.09 / 0.09 ± 0.002
OAT1	HEK-293	1 μM p-Aminohippuric acid	42.9 ± 8.9	1	Probenecid / Novobiocin	8.7 ± 3.2 / 8.2
OAT3	HEK-293	50 nM Estrone-3-Sulfate	24.4 ± 11.9	2	Probenecid / Ibuprofen	2.8 ± 1.1 / 4.5
OCT1	HEK-293	5 μM Tetraethylammonium	0.5 ± 1.8	15	Quinidine / Verapamil	15 ± 3 / 0.5
OCT2	HEK-293	10 μM Metformin	1840 ± 640	2	Quinidine / Cimetidine	25 ± 1 / 280

Selection of test article concentrations for *in vitro* transporter inhibition assays

The 2012 FDA Guidance for Industry (Drug Interaction Studies- Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations) and the 2013 EMA Guideline on the Investigation of Drug Interactions describe criteria for investigating possible DDIs based on *in vitro* inhibition data of compounds. The criteria relate pharmacokinetic data and the IC_{50} or K_i of the compound. Based on those criteria, the maximum concentration of the compound that needs to be tested in an *in vitro* inhibition experiment can be inferred. The highest concentration that can reasonably be tested in each experiment should also be considered as it may be limited by *in vitro* solubility or cell toxicity or estimated maximum *in vivo* solubility at gastrointestinal or physiological pH. The criteria outlined below are described in the EMA and FDA guidance documents.

Transporter expression	EMA criteria	Relevant concentration	FDA criteria	Relevant concentration
P-gp, BCRP intestinal	K_i < 0.1x dose/250 mL	0.1x dose/250 mL	$[I]_2/IC_{50}$ is > 10	0.1x dose/250 mL
P-gp, BCRP systemic	K_i ≤ 50x unbound C_{max}	50x unbound C_{max}	$[I]_{1\ total}/IC_{50}$ is ≥ 0.1 (equivalent to IC_{50} ≤ 10x total C_{max})	10x total C_{max}
OATP1B1 and OATP1B3 (hepatic uptake)	K_i ≤ 25x unbound portal vein concentration or 50x the unbound C_{max} for iv drugs	25x unbound portal vein concentration or 50x unbound C_{max} of iv dugs	$[I]_{1\ total}/IC_{50}$ is ≥ 0.1 (equivalent to IC_{50} ≤ 10x total C_{max})	10x total C_{max}
OAT1, OAT3 and OCT2 (renal uptake)	K_i ≤ 50x unbound C_{max}	50x unbound C_{max}	$[I]_{1\ unbound}/IC_{50}$ is ≥ 0.1 (equivalent to IC_{50} ≤ 10x unbound C_{max})	10x unbound C_{max}

For reference only:
$[I]_1$ Concentration equal to the unbound or the total C_{max}
$[I]_2$ Concentration equal to the maximum dose in 250 mL, apparent intestinal concentration

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