

In vitro ADME & PK

Human SLC Uptake Transporter Inhibition (OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2-K, OATP1A2, OATP2B1, OAT2, OAT4, OCTN2, PEPT1, PEPT2 or NTCP) for Screening or Regulatory Reporting

Background Information



'Membrane transporters can have clinically relevant effects on the pharmacokinetics and pharmacodynamics of a drug in various organs and tissues by controlling its absorption, distribution and elimination.'

²Draft FDA Guidance for Industry – In vitro metabolism- and transporter-mediated drug-drug interaction studies, October 2017.

- The SLC (solute carrier) family transport a wide range of different solutes across biological membranes using diverse energy coupling mechanisms¹.
- Members of the SLC transporters include the OATP, OAT, OCT, MATE, OCTN, and the PEPT transporters. These transporters are based predominantly in the intestine, the blood brain barrier, the kidneys and the liver where they influence the absorption, distribution, metabolism and excretion of drugs within the body.
- The draft FDA guidance² and the EMA guidance³ recommend investigating for potential OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K inhibition due to the role of these transporters in clinical drug-drug interactions and the impact of genetic polymorphism of some of these transporters on therapy outcome and toxicity.
- The EMA³ and the International Transporter Consortium (ITC)⁴ also suggests that potential interactions with OCT1 should be considered.
- Cyprotex's SLC transporter inhibition assay determines if your compound is an inhibitor of the key transporters recommended in the regulatory guidelines. Additionally since 2016 and ahead of guidance recommendations, for IC₅₀ determination, the assay has incorporated a 15 min preincubation step with test compound for OATP1B1, OATP1B3 and all other SLC transporters. Inclusion of this 15 min inhibitor preincubation step produces an identical IC₅₀ value for the OATP inhibitor cyclosporin A when compared to that determined with inclusion of a 30 min preincubation step recommended in the 2017 draft FDA guidance², indicating that the preincubation methods are equivalent for DDI risk assessment. Furthermore, the use of a shorter 15 min preincubation time might lessen any impact of potential cytotoxic effects during cell incubations.

Related Services

P-gp
BCRP
BSEP
MRPs

Protocol

Test System

Mammalian HEK293 cells transiently overexpressing a single transporter (OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2-K, OATP1A2, OATP2B1, OAT2, OAT4, OCTN2, PEPT1, PEPT2 or NTCP – other transporters available on request)

Control vector-transfected HEK293 cells

Probe Substrate

[³H]-Estradiol 17β-glucuronide (OATP1B1, OATP1B3)

[³H]-PAH (OAT1)

[³H]-Estrone 3-sulfate (OAT3, OAT4, OATP1A2, OATP2B1)

[¹⁴C]-Metformin (OCT2, MATE1) (upon request for MATE2-K)

[¹⁴C]-TEA (OCT1, MATE2-K) (upon request for MATE1)

[³H]-cGMP (OAT2)

[³H]-L-Carnitine (OCTN2)

[³H]-Glycyl sarcosine (PEPT1, PEPT2)

[³H]-Taurocholic acid (NTCP)

Test Article Concentrations

Single concentration (for batches of 6 compounds) OR

6 concentrations plus 0 μM (final test compound concentrations dependent on customer requirements)

Time Point

Dependent on transporter

Analysis Method

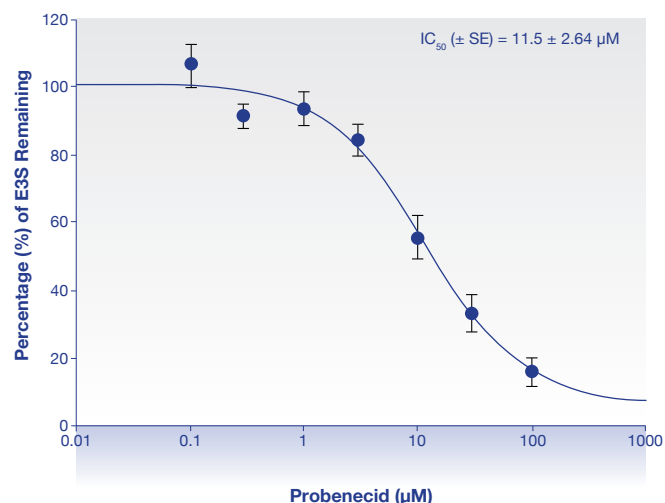
Radiochemical detection using scintillation counting

Data Delivery

Percentage inhibition (single concentration) OR IC₅₀
Written report available on request

Figure 1

Representative graph showing inhibition of OAT3-mediated transport of estrone 3-sulfate by the OAT3 inhibitor, probenecid. Data shown represents the mean of 3 replicates \pm standard deviation.

**Table 1**

Inhibition of OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2-K, OATP1A2, OATP2B1, OAT2, OAT4, OCTN2, PEPT1, PEPT2 and NTCP-mediated transport of prototypical substrates.

Transporter	Substrate	Inhibitor	IC ₅₀ \pm Standard Error (μ M)
OATP1B1	Estradiol 17 β -glucuronide	Rifamycin SV	0.67 \pm 0.18
		Cyclosporin A	1.53 \pm 0.22
OATP1B3	Estradiol 17 β -glucuronide	Rifampicin	0.79 \pm 0.11
		Cyclosporin A	0.96 \pm 0.24
OAT1	PAH	Probenecid	16.6 \pm 11.7
		Diclofenac	1.00 \pm 0.36
OAT3	Estrone 3-sulfate	Probenecid	11.5 \pm 2.64
		Diclofenac	18.7 \pm 3.91
OCT1	TEA	Verapamil	7.59 \pm 2.42
		Quinidine	30.5 \pm 4.20
OCT2	Metformin	Verapamil	26.3 \pm 2.42
		Quinidine	35.6 \pm 2.03
MATE1	Metformin	Cimetidine	1.22 \pm 0.09
		Trimethoprim	2.64 \pm 0.27
MATE2-K	Metformin	Cimetidine	3.34 \pm 1.02
		Trimethoprim	0.35 \pm 0.06
MATE1	TEA	Cimetidine	0.92 \pm 0.10
		Verapamil	17.9 \pm 3.88
MATE2-K	TEA	Cimetidine	7.02 \pm 5.27
		Verapamil	21.6 \pm 1.79

Transporter	Substrate	Inhibitor	IC ₅₀ \pm Standard Error (μ M)
OATP1A2	Estrone 3-sulfate	Rifamycin SV	3.44 \pm 0.78
		Ritonavir	1.41 \pm 0.77
OATP2B1	Estrone 3-sulfate	Rifamycin SV	2.72 \pm 0.33
		Ritonavir	4.88 \pm 0.85
		Atorvastatin	0.23 \pm 0.08
OAT2	cGMP	Indomethacin	8.31 \pm 0.62
		Cimetidine	239 \pm 35.80
OAT4	Estrone 3-sulfate	Losartan	46.4 \pm 3.12
		Furosemide	159 \pm 25.70
OCTN2	L-Carnitine	Meldonium	32.3 \pm 6.15
		Verapamil	111 \pm 42.0
PEPT1	Glycyl sarcosine	Losartan	399 \pm 62.55
		Cefadroxil	629 \pm 157.50
PEPT2	Glycyl sarcosine	Losartan	34.9 \pm 5.92
		Cefadroxil	22.6 \pm 7.43
NTCP	Taurocholic acid	Pioglitazone	10.2 \pm 0.98
		Cyclosporin A	7.21 \pm 1.75

References

- Schlessinger A *et al.*, (2013) Molecular modeling and ligand docking for solute carrier (SLC) transporters. *Curr Top Med Chem* **13**(7): 843-856.
- Draft FDA Guidance for Industry – In vitro metabolism- and transporter-mediated drug-drug interaction studies, October 2017.
- The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012).
- Zamek-Gliszczyński MJ *et al.*, (2018) Transporters in drug development: 2018 ITC recommendations for transporters of emerging clinical importance. *Clin Pharmacol Ther* **104**(5): 890-899.