cyprotex AN EVOTEC COMPANY

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 controllong its absorption, distribution and elimination.'

[2]FDA Guidance for Industry – In Vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (January 2020)

- The SLC (solute carrier) family transport a wide range of different solutes across biological membranes using diverse energy coupling mechanisms[1].
- 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 FDA guidance[2] and the EMA guidance[3] 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 EMA[3] 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 IC_{50} determination, the assay has incorporated a preincubation step with test compound for OATP1B1, OATP1B3 and all other SLC transporters.

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
[^{3}H]-Estradiol 17ß-glucuronide (OATP1B1, OATP1B3)
[^{3}H]-PAH (OAT1)
[^{3}H]-Estrone 3-sulfate (OAT3, OAT4, OATP1A2, OATP2B1)
[^{14}C]-Metformin (OCT2, MATE1) (upon request for MATE2-K)
[^{14}C]-TEA (OCT1, MATE2-K) (upon request for MATE1)
[^{3}H]-cGMP (OAT2)
[^{3}H]-L-Carnitine (OCTN2)
[^{3}H]-Glycyl sarcosine (PEPT1, PEPT2)
[^{3}H]-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 IC_{50}
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 ± standard deviation.

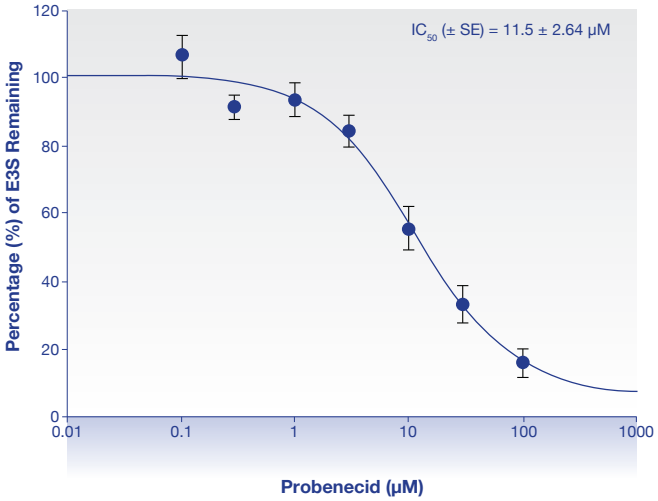

Table 1

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} ± Standard Error (µM)
OATP1B1	Estradiol 17β-glucuronide	Rifamycin SV	0.67 ± 0.18
		Cyclosporin A	1.53 ± 0.22
OATP1B3	Estradiol 17β-glucuronide	Rifampicin	0.79 ± 0.11
		Cyclosporin A	0.96 ± 0.24
OAT1	PAH	Probenecid	16.6 ± 11.7
		Diclofenac	1.00 ± 0.36
OAT3	Estrone 3-sulfate	Probenecid	11.5 ± 2.64
		Diclofenac	18.7 ± 3.91
OCT1	TEA	Verapamil	7.59 ± 2.42
		Quinidine	30.5 ± 4.20
OCT2	Metformin	Verapamil	26.3 ± 2.42
		Quinidine	35.6 ± 2.03
MATE1	Metformin	Cimetidine	1.22 ± 0.09
		Trimethoprim	2.64 ± 0.27
MATE2-K	Metformin	Cimetidine	3.34 ± 1.02
		Trimethoprim	0.35 ± 0.06
MATE1	TEA	Cimetidine	0.92 ± 0.10
		Verapamil	17.9 ± 3.88
MATE2-K	TEA	Cimetidine	7.02 ± 5.27
		Verapamil	21.6 ± 1.79
OATP1A2	Estrone 3-sulfate	Rifamycin SV	3.44 ± 0.78
		Ritonavir	1.41 ± 0.77
OATP2B1	Estrone 3-sulfate	Rifamycin SV	2.72 ± 0.33
		Ritonavir	4.88 ± 0.85
		Atorvastatin	0.23 ± 0.08
OAT2	cGMP	Indomethacin	8.31 ± 0.62
		Cimetidine	239 ± 35.80
OAT4	Estrone 3-sulfate	Losartan	46.4 ± 3.12
		Furosemide	159 ± 25.70
OCTN2	L-Carnitine	Meldonium	32.3 ± 6.15
		Verapamil	111 ± 42.0
PEPT1	Glycyl sarcosine	Losartan	399 ± 62.55
		Cefadroxil	629 ± 157.50
PEPT2	Glycyl sarcosine	Losartan	34.9 ± 5.92
		Cefadroxil	22.6 ± 7.43
NTCP	Taurocholic acid	Pioglitazone	10.2 ± 0.98
		Cyclosporin A	7.21 ± 1.75

References

1 Schlessinger A *et al*., (2013) Molecular modeling and ligand docking for solute carrier (SLC) transporters. *Curr Top Med Chem* **13(7)**; 843-856.
2 FDA Guidance for Industry – In Vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (January 2020).
3 The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012).
4 Zamek-Gliszczynski MJ *et al*., (2018) Transporters in drug development: 2018 ITC recommendations for transporters of emerging clinical importance. *Clin Pharmacol Ther* **104(5)**; 890-899.

Read online: