cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

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

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 substrate identification 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 International Transporter Consortium (ITC)[4] also suggests that potential interactions with OCT1 should be considered.
- It is only necessary to evaluate potential OAT1, OAT3, OCT2, MATE1 and MATE2-K substrates when renal active secretion of the investigational drug is significant (e.g., active secretion by the kidney is more than or equal to 25% of total clearance) and it is only necessary to evaluate potential OATP1B1, OATP1B3 and OCT1 substrates when hepatic or biliary secretion is more than or equal to 25% of total clearance.[2,3,4]
- Cyprotex's SLC transporter substrate identification assay determines if your compound is a substrate of the key transporters recommended in the regulatory guidelines.

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

Test Article Concentrations
Screening study - single concentration (typically, 1 µM), single time point for 7 compounds

Screening study - two concentrations (typically, 1 and 10 µM), single time point for 3 compounds

Screening study - two concentrations (typically, 1 and 10 µM), two time points for single compound

Regulatory study - typically 1, 10, 50 and 100 µM (depending on customer requirements) plus inhibition at two substrate concentrations (two time points)

Time Points
Typically, 2 min or 2 and 20 min (depending on customer requirements)

Analysis Method
MicroBeta® scintillation counter (radiolabelled substrates)

LC-MS/MS analysis (non-radiolabelled substrates)

Data Delivery
Cellular uptake and fold accumulation

Written report available on request

Figure 1

Uptake of ^{3}H-estradiol 17ß-glucuronide (1 µM) in OATP1B1-transfected HEK293 cells and control HEK293 cells over 20 min in the presence and absence of the inhibitor rifamycin (100 µM).

To confirm transporter involvement in the uptake of estradiol 17**ß**-glucuronide in the OATP1B1 transfected cells, the inhibitor rifamycin was included in the incubations. This reduced the uptake to similar levels (< 2 fold) as observed in the control cells.

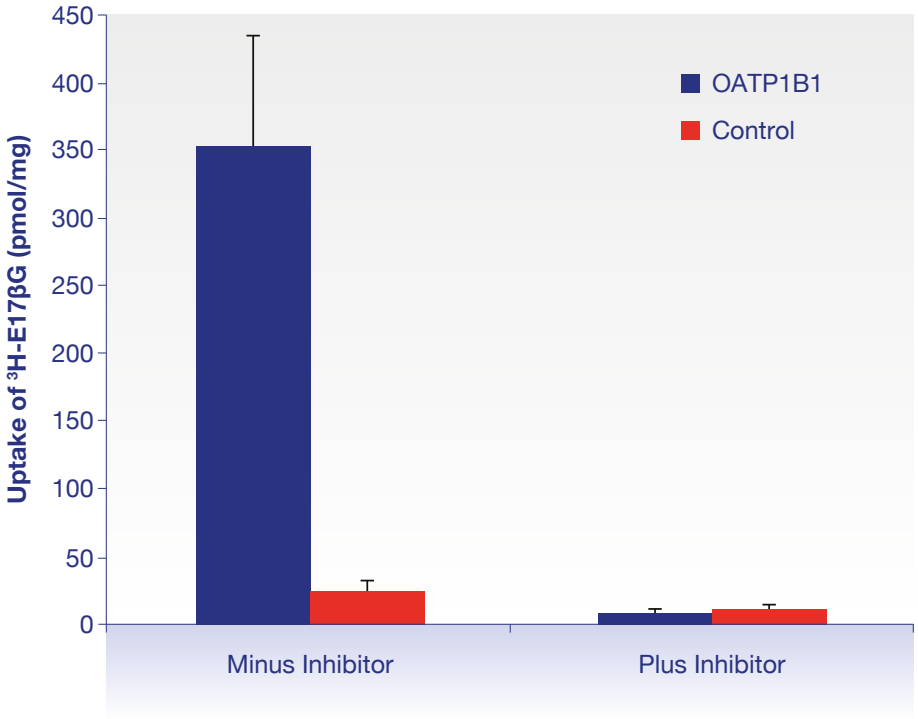
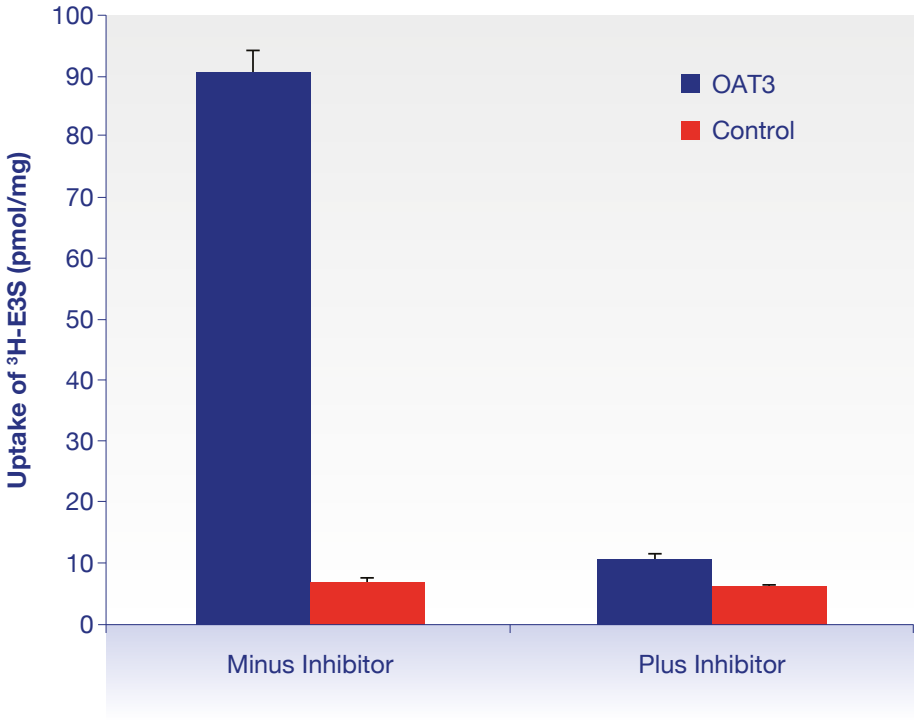


Figure 2

Uptake of ^{3}H-estrone 3-sulfate (1 µM) in OAT3-transfected HEK293 cells and control HEK293 cells over 20 min in the presence and absence of the inhibitor probenecid (100 µM).

To confirm transporter involvement in the uptake of estrone 3-sulfate in the OAT3-transfected cells, the inhibitor probenecid was included in the incubations. This reduced the uptake to similar levels (< 2 fold) as observed in the control cells.



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: