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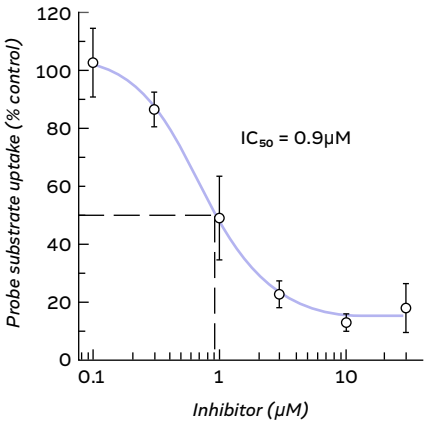
AN EVOTEC COMPANY

Drug Transporter Inhibition

Understanding whether your investigational drug has the potential to perpetrate (precipitate) transporter-mediated drug-drug interactions with co-administered victim (object) drugs that are substrates of transporters starts with evaluation of your drug as an inhibitor of drug transporters in vitro. Cyprotex has well-validated transporter inhibition assay methodology and test systems that conform to the recommendations highlighted by regulatory authorities. We ensure that the correct, most accurate IC_{50} (=K_i) is obtained for robust DDI risk assessment.

ABC transporters (BSEP, MRPs, P-gp, BCRP)	**Vesicle transport assay**	▸ Uptake rate (+ATP) minus uptake rate (+AMP) ▸ Corrected transporter-mediated uptake rate (pmol/mg) in absence (vehicle control) and presence of inhibitor
SLC transporters (OATPs, OATs, OCTs, MATEs, OCTN2, PEPTs, NTCP)	**Cell uptake assay**	▸ Uptake rate in transporter expressing cells minus uptake rate in control cells or passive uptake in transporter expressing cells ▸ Corrected transporter-mediated uptake rate (pmol/mg) in absence (vehicle control) and presence of inhibitor
ABC transporters (P-gp, BCRP)	**Unidirectional transport assay (Caco-2)**	▸ Corrected transporter-mediated B>A P_{app} (B>A P_{app} - passive P_{app}) in absence (vehicle control) and presence of inhibitor

Converted to % control transport activity



Intestine
P-gp*, BCRP*, OATP2B1, OCT1, MRP2, MRP3

Kidney
OAT1*, OAT3*, OCT2*, MATE1*, MATE2-K*, P-gp*, BCRP*, OAT2, OAT4, PEPT1, PEPT2, OCTN2, MRP2, MRP4

Liver
OATP1B1*, OATP1B3*, OCT1*, P-gp*, BCRP*, BSEP, OATP2B1, OAT2, NTCP, MRP2, MRP3, MRP4, preclinical Oatp1b

Blood Brain Barrier
P-gp*, BCRP*, OATP1A2, OATP2B1, MRP4

** regulatory required transporters*

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Your Partner in Understanding Transporter Drug-Drug Interactions

- **Extensive experience:** Our team of experts have decades of combined published experience in transporter-mediated DDIs and contextualisation of *in vitro* data to clinical risk.
- **From Discovery to Development:** We offer a comprehensive range of transporter inhibition assay formats applicable to either early discovery (screening; % inhibition) or to regulatory profiling stages (IC_{50} determination) during preclinical development, clinical development and on to new drug application.
- **Regulatory compliance:** We adhere to global regulatory guidance/guideline recommendations, including:
  - Full panel of transporters (P-gp, BCRP, OATP1B1/3, OAT1/3, OCT1/2, MATE1/2K): regardless of investigational drug's BCS class, transporter substrate status, or principal elimination route(s).
  - Use of clinically relevant, or published good surrogates of clinically relevant *in vivo* substrate, as *in vitro* probe for P-gp/BCRP/OATP1B/OCT2/MATE1, or conservative probes for OATs/OCT1/MATE2-K, all run at ~10× lower than their K_m so IC_{50} equates to K_i.
  - Use of radiolabelled probe substrates such there is no interference of the investigational drug on analytical response so no risk of artefactual false positives or negatives in identifying inhibitors

Assessing Perpetrator (Transporter Inhibitor) Potential along the Drug Discovery/ Development Value Chain

Inhibition Screening Assays

(if critical co-meds have to be co-dosed)

INHIBITION SCREENING

– Assay formats (flexible)

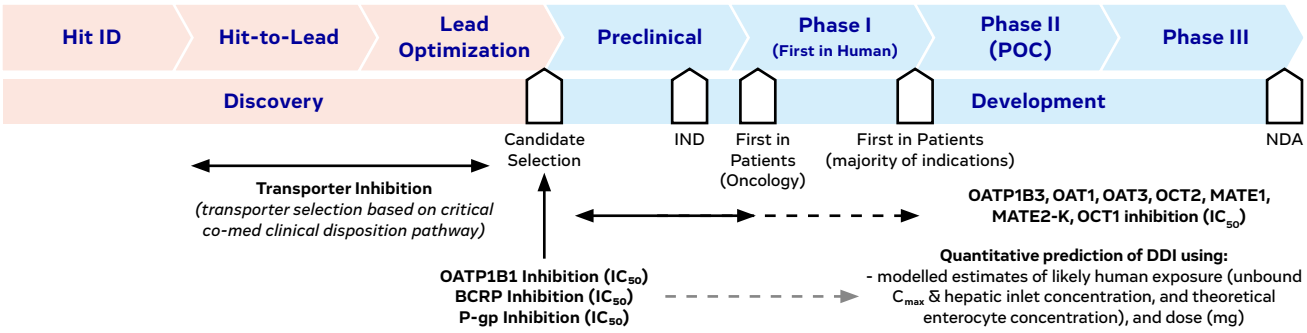
- IC_{50} – 7 concentrations plus 0 *µM* (singlicate wells) for P-gp/BCRP/regulatory guidance SLCs
- IC_{50} – 6 concentrations, plus 0 *µM* (duplicate wells) for BSEP/MRPs
- 1 or 2 concentraions (duplicate wells) for regulatory guidance SLCs
- 1 or 2 concentraions (triplicate wells) for non-regulatory guidance SLCs

Inhibition Profiling Assays

INHIBITION PROFILING

– Assay formats

- IC_{50} – 7 concentrations plus 0 *µM* (triplicate wells)
- Includes pre-incubation step with investigational drug as standard for all ABC/SLC transporters to give correct IC_{50} for robust risk assessment
- Prediction of transporter DDI risk – reports based on regulatory basic static equations
- Quantitative prediction of transporter DDI – AUCR prediction of statin DDIs as victim drugs using mechanistic static equations. Hypothetical AUCR predictions for potential victim co-meds whose transporter contribution to disposition is not defined.



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