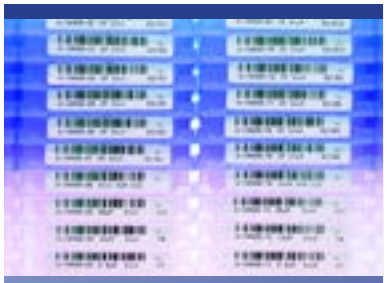
cyprotex AN EVOTEC COMPANY

In vitro **ADME**

BCRP Inhibition

Background Information



'*In vitro* inhibition studies are recommended to investigate whether the investigational drug inhibits any of the transporters known to be involved in clinically relevant *in vivo* drug interactions'

[5]The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012)

- BCRP (Breast Cancer Resistance Protein/ABCG2) is expressed in the gastrointestinal tract, liver, kidney, brain endothelium, mammary tissue, testis and placenta[1].
- Inhibition of intestinal BCRP has shown to be responsible for several clinical drug-drug interactions involving specific statin common co-medications such as rosuvastatin and atorvastatin, resulting in their increased absorption and subsequent exposure (up to 2 fold increase in AUC)[2,3]
- The International Transporter Consortium[1], the FDA guidance[4] and the EMA guideline[5] recommend investigating BCRP due to BCRP's clinical importance in the absorption and disposition of drugs.
- Cyprotex use Caco-2 cells to identify BCRP inhibitors using a range of test inhibitor concentrations in the presence of the probe substrate estrone 3-sulfate, a good surrogate for the clinically relevant BCRP substrate rosuvastatin. This method conforms with the recommended methods within the International Transporter Consortium white paper[1], the FDA drug interactions guidance[4] and the EMA drug interactions guideline[5].

Protocol

Substrate
1 µM [^{3}H]-estrone 3-sulfate (surrogate *in vitro* probe for clinically relevant BCRP substrate rosuvastatin[7])

Test Article Concentrations
Seven point IC_{50} (triplicate wells)

Direction
Unidirectional (basolateral to apical)

Inhibitor Preincubation Time
30 min

Incubation Time
90 min

Growth Period
18-22 days

Analysis Method
Liquid scintillation counting

Integrity Marker
Lucifer Yellow

Data Delivery
IC_{50} (derived from corrected B-A P_{app})

‘BCRP has been increasingly recognized for its important role in the absorption, elimination and tissue distribution of drugs and xenobiotics[6].’

Table 1

Inhibition of BCRP-mediated estrone 3-sulfate transport by literature inhibitors.

Inhibitor	Mean IC_{50} ± Standard Deviation (n=3)
Novobiocin (positive control)	2.06 ± 0.884
Fumitremorgin C	0.250 ± 0.0540
Pantoprazole	11.0 ± 0.737
Elacridar	0.581 ± 0.165

The Caco-2 cell test system using the BCRP substrate estrone 3-sulfate is able to correctly identify known literature BCRP inhibitors with a range of different potencies.

The incubation conditions have been fully characterised for our chosen BCRP substrate, estrone 3-sulfate, based on time linearity and chosen substrate concentration being approximately ten-times lower than the reported K_m previously determined in membrane vesicles[7], and as such IC_{50} equates to K_i (assuming competitive inhibition).

References

[1] The International Transporter Consortium (2010) *Nat Rev Drug Disc* **9**; 215–236
[2] Elsby R *et al.*, (2012) *Clin Pharmacol Ther* **92(5)**; 584-598
[3] Elsby R *et al.*, (2016) *Drug Metab Dispos* **44**; 398-408
[4] FDA Guidance for Industry – In Vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (January 2020)
[5] The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012)
[6] Zhanglin N *et al.*, (2010) *Curr Drug Metab* **11(7)**; 603-617
[7] Elsby R *et al.*, (2011) *Xenobiotica* **41(9)**; 764-783