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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 draft 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 draft 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
20 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] Draft FDA Guidance for Industry – In Vitro Metabolism and Transporter-mediated Drug-Drug Interaction Studies, 2017
[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