cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

BSEP, MRP2, MRP3 and MRP4 Inhibition

Background Information

'Proactive evaluation and understanding of BSEP inhibition is recommended in drug discovery and development to aid internal decision making on DILI risk..'

[3]Kenna JG *et al*., (2018) *Clin Pharmacol Ther* **104(5)**: 916-932.

- BSEP (bile salt export pump; ABCB11) is an ATP binding cassette (ABC) efflux transporter located on the canalicular membrane of hepatocytes, and is the major transporter for the secretion of bile acids from hepatocytes into bile in humans[1].
- Because of the link between BSEP inhibition and initiation of cholestatic DILI, the European Medicines Agency Guideline on the Investigation of Drug Interactions (2012) recommends *in vitro* screening of BSEP inhibition[2].
- MRP2 (multidrug resistance associated protein 2; ABCC2), MRP3 (ABCC3) and MRP4 (ABCC4) are ATP binding cassette (ABC) efflux transporters located on the canalicular membrane (MRP2) or sinusoidal membrane (MRP3, MRP4) of hepatocytes[3,4].
- MRP3 and MRP4 efflux transporters are upregulated under cholestatic conditions suggesting they provide a protective role aginst bile acid-mediated hepatotoxicity by alleviating increases in intracellular bile acid concentrations, which may occur as a result of impaired biliary excretion due to inhibition of BSEP[3,4,5]. Understanding whether a compound is able to inhibit MRP transporters may therefore provide useful additional information towards helping evaluate the risk of DILI.
- Cyprotex offer BSEP, MRP2, MRP3 and MRP4 inhibition assays which investigate inhibition of the uptake of prototypical probe substrates (taurocholic acid for BSEP and estradiol 17β-D-glucuronide for MRPs) into inside-out membrane vesicles overexpressing the human ABC-transporter of interest.

Related Services

P-gp
BCRP
Human SLC Transporters

Protocol

Test System
Sf9 insect cell-derived or mammalian (HEK293) cell-derived inside-out membrane vesicles overexpressing a single transporter (BSEP, MRP2, MRP3 or MRP4) incubated in the presence of ATP and AMP (absence of ATP).

Probe Substrate
[^{3}H]-Taurocholic acid
[^{3}H]-Estradiol 17β-glucuronide

Test Article Concentrations
6 concentrations plus 0 µM (triplicate wells) (final test article concentrations dependent on customer requirements)

Time Points
Dependent on transporter

Analysis Method
Radiochemical detection using scintillation counting

Data Delivery
IC_{50}
Written report available on request

Figure 1

BSEP-mediated taurocholic acid (A) and MRP-mediated estradiol 17ß-D-glucuronide (B-D) transport in the presence of a range of concentrations of inhibitor expressed as a percentage of vehicle control (mean ± standard deviation; n=3-9 wells, triplicate incubations performed on 3 separate occasions).

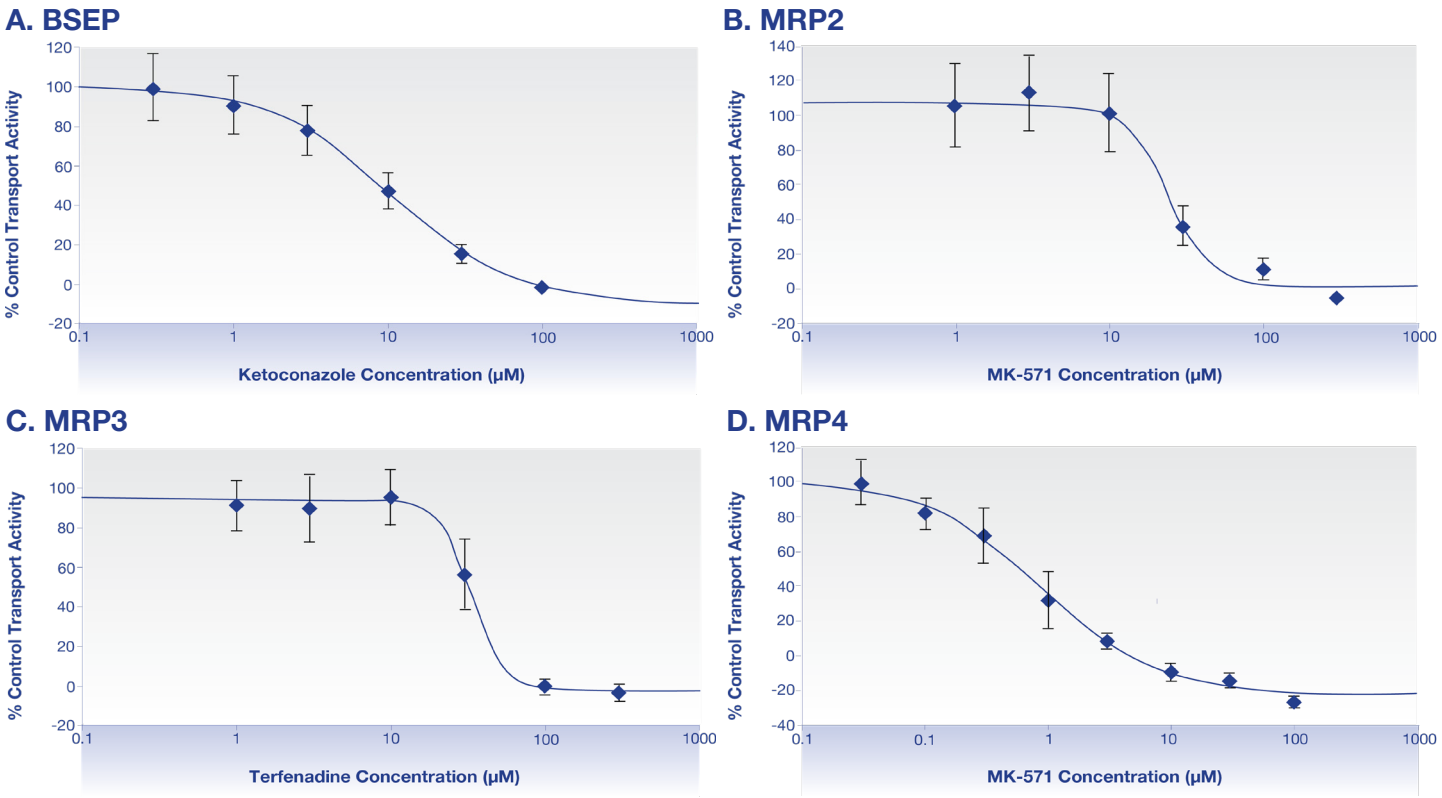

Table 1

Inhibition of human BSEP- and MRP-mediated transport of the prototypical substrates, taurocholic acid and estradiol 17ß-glucuronide, respectively.

Transporter	Substrate	Inhibitor	IC_{50} ± Standard Deviation (µM)
BSEP	Taurocholic acid	Ketoconzole	8.78 ± 1.25
MRP2	Estradiol 17β-glucuronide	MK-571	22.6 ± 6.38
MRP3	Estradiol 17β-glucuronide	Terfenadine	33.5 ± 6.77
		MK-571	56.8 ± 7.23
		Fidaxomicin	1.06 ± 0.117
MRP4	Estradiol 17β-glucuronide	MK-571	0.555 ± 0.238
		Indomethacin	3.79 ± 0.342
		Ibuprofen	42.0 ± 23.7

The incubation conditions for each of the species have been fully characterised for the chosen substrates based on time linearity and uptake kinetics (V_{max} and K_m).

The chosen substrate concentration is much lower than the determined K_m, and as such IC_{50} equates to K_i (assuming competitive inhibition).

References

1 Wang L *et al*., (2002) The role of bile salt export pump mutations in progressive familial intrahepatic cholestasis type II. *J Clin Invest* **110(7)**; 965-972.

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

3 Kenna JG *et al*., (2018) Can bile salt export pump inhibition testing in drug discovery and development reduce liver injury risk? An International Transporter Consortium perspective. *Clin Pharmacol Ther* **104(5)**: 916-932.

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

5 Morgan RE *et al*., (2013) A multifactorial approach to hepatobiliary transporter assessment enables improved therapeutic compound development. *Tox Sci* **136**: 216-241