cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

UGT Inhibition (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7)

Background Information

'Inhibitory interactions can occur when glucuronidation is a predominant metabolic elimination pathway, when the glucuronidation is catalysed by a single enzyme and when the therapeutic concentrations of the inhibitor are close to the K_i of the target UGT.'

[1]Remmel R *et al*., (2007) Conjugative Metabolism of Drugs, in *Drug Metabolism in Drug Design and Development*, (Zhang D *et al*., eds); pp 37-88, John Wiley & Sons, Inc.

- Uridine glucuronyl transferases (UGT) are a family of enzymes which play a major role in the Phase II metabolism of drugs.
- One in ten of the top two hundred prescribed drugs have glucuronidation as a clearance mechanism illustrating the importance of UGTs in drug metabolism.
- Functionally relevant polymorphisms have been demonstrated for the UGT genes. For example, the polymorphism in UGT1A1 can lead to toxicity associated with Gilbert's syndrome or the more severe Criglar-Najar syndrome where levels of bilirubin are elevated.
- The regulatory authorities are now recommending that UGT inhibition is evaluated as part of *in vitro* drug-drug interaction (DDI) packages to determine if clinical DDI studies are required.
- In Cyprotex's UGT inhibition assay, a decrease in the formation of the UGT-specific metabolite compared to the vehicle control is used to calculate an IC_{50} value (test compound which produces 50% inhibition). Follow-up Ki determination is also available if required.
- Cyprotex can offer either early stage UGT inhibition screening or regulatory UGT inhibition assessments as part of a DDI package for IND or NDA submissions.

Protocol

Substrates
Estradiol (UGT1A1), sulindac sulfone (UGT1A3), trifluoperazine (UGT1A4), naphthol (UGT1A6), propofol (UGT1A9), naloxone (UGT2B7)

Enzyme Source
Human UGT Supersomes™

Cofactors
UDPGA

Positive Controls
Silybin or atazanavir (UGT1A1), quinidine (UGT1A3), diclofenac (UGT1A4, UGT1A6, UGT1A9 and UGT2B7)

Analysis Method
LC-MS/MS

Data Delivery
IC_{50}
Standard error of IC_{50}

Glucuronidation is a listed clearance mechanism for 1 in 10 of the top 200 prescribed drugs[2]

Figure 1

Graphs showing the inhibition of UGT isoforms by the positive control inhibitors in Cyprotex's UGT inhibition assay. Data show the mean ± standard deviation of 3 replicates.

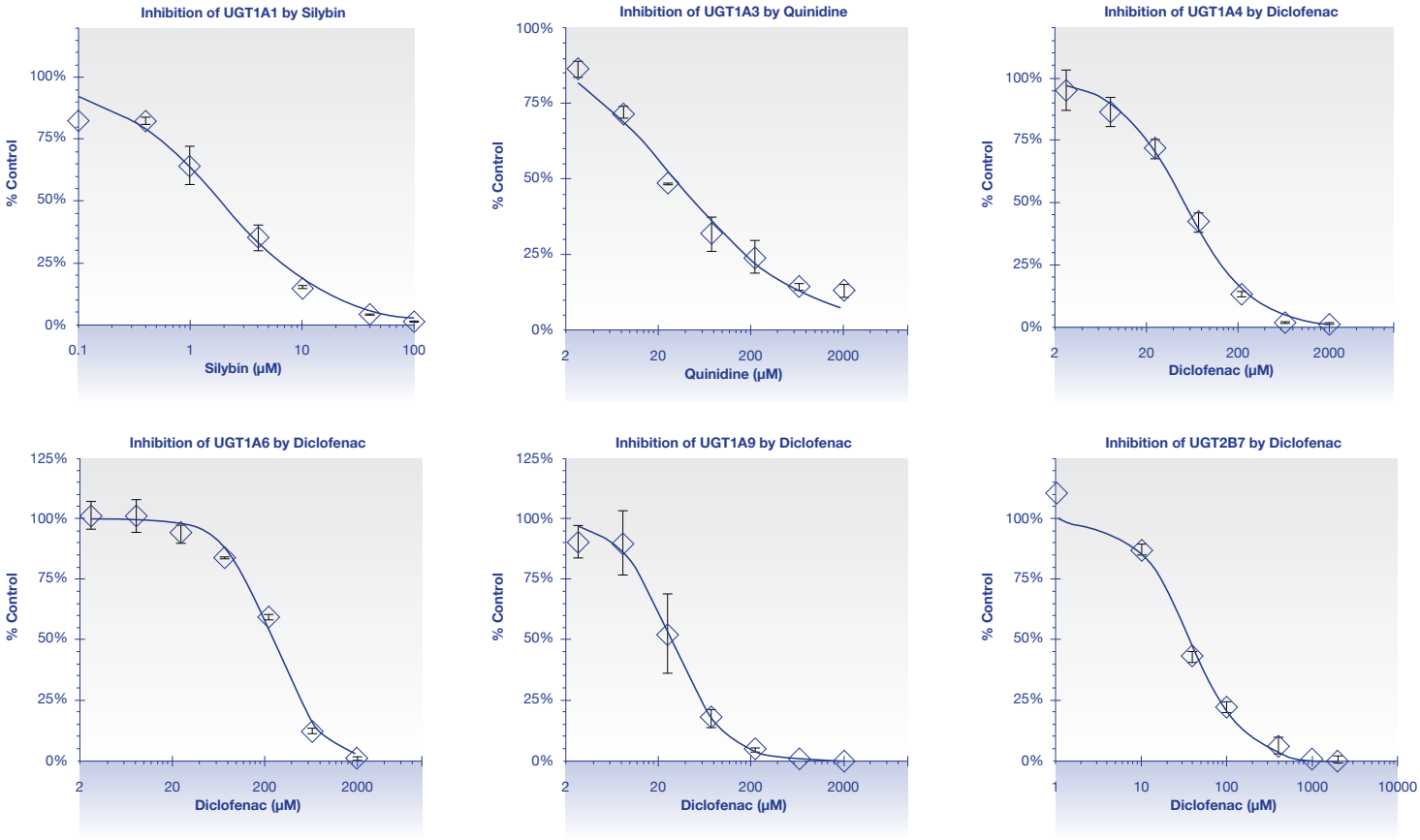

Table 1

Summary of IC_{50} data (n=3) for known UGT inhibitors in Cyprotex's UGT inhibition assay.

UGT Isoform	Substrate	Inhibitor	Mean IC_{50} ± standard deviation (n=3) (µM)
UGT1A1	Estradiol	Silybin	4.7 ± 3.5
UGT1A3	Sulindac sulfone	Ritonavir Quinidine	0.5 ± 0.1 35 ± 9.3
UGT1A4	Trifluoperazine	Diclofenac	61 ± 7.8
UGT1A6	Naphthol	Diclofenac	221 ± 32
UGT1A9	Propofol	Diclofenac Mycophenolic acid	29 ± 3.8 66 ± 22
UGT2B7	Naloxone	Diclofenac Quinidine	24 ± 11 139 ± 33

References

1 Remmel R *et al.*, (2007) Conjugative Metabolism of Drugs in *Drug Metabolism in Drug Design and Development*, (Zhang D *et al.*, eds); pp 37-88, John Wiley & Sons, Inc.

2 Williams JA *et al.*, (2004) Drug-drug interactions for UDP-glucuronosyltransferase substrates: A pharmacokinetic explanation for typically observed low exposure (AUCI/AUC) ratios *Drug Metab Dispos* **32(11)**; 1201-1208.