

# Cloe Select UGT1A1 Inhibition

experts in **ADME**

## 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.'

<sup>1</sup>Rommel R, Nagar S and Argikar U. (2008) 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
- UGT1A1 is involved in glucuronidation of the endogenous product, bilirubin, as well as estrogens and flavanoids
- Inhibition of UGT1A1 enzyme has the potential to produce increased levels of bilirubin in the circulation which can lead to toxicological effects
- UGT1A1 genotype is a useful safety biomarker for lower therapeutic drugs that are primarily cleared by UGT1A1<sup>1</sup>
- In the Cloe Select UGT1A1 Inhibition assay, a decrease in the formation of the metabolite compared to the vehicle control is used to calculate an  $IC_{50}$  value (test compound which produces 50% inhibition)



### Protocol

**Substrate**  
Estradiol

**Metabolite**  
Estradiol 3-glucuronide

**Test Compound Concentration**  
0, 0.4, 1, 4, 10, 40 and 100 $\mu$ M  
(different concentrations available)

**Enzyme source**  
Human UGT1A1 Supersomes™

**Cofactors**  
UDPGA

**Compound Requirements**  
50 $\mu$ L of 20mM solution

**Positive Control**  
Silybin

**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<sup>2</sup>

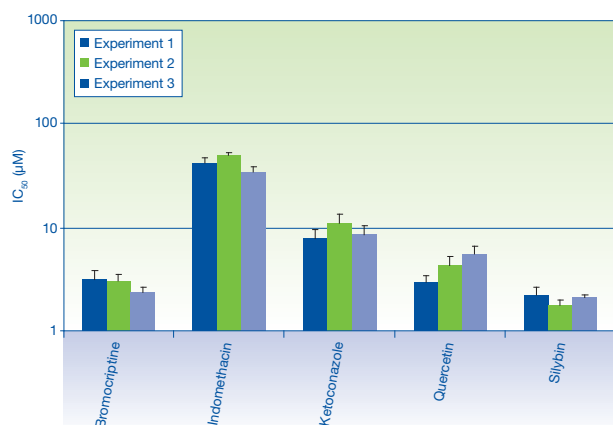


### Cloe Select UGT1A1 Inhibition

Time and protein linearity studies and enzyme kinetics studies were performed initially to set the final conditions for the IC<sub>50</sub> assessments. Known UGT1A1 inhibitors were assessed in the Cloe Select UGT1A1 Inhibition assay over 3 separate occasions.

**Figure 1**

Cloe Select UGT1A1 Inhibition validation data



The effect of 5 known inhibitors (bromocriptine, indomethacin, ketoconazole, quercetin and silybin) on the 3-glucuronidation of estradiol was investigated on 3 separate occasions. The error bars represent the standard error of the IC<sub>50</sub> determination. The inhibitors show good consistency between different assay runs.

**Table 1**

Comparison of Cloe Select UGT1A1 Inhibition data for known inhibitors with literature values.

Inhibitor	Mean Cloe Select IC <sub>50</sub> (n=3) (µM)	Literature IC <sub>50</sub> * (µM)
Quercetin	4.22	6.6 <sup>(5)</sup>
Bromocriptine	2.83	12.2 <sup>(5)</sup>
Indomethacin	42.1	51.5 <sup>(4)</sup>
Silybin	2.04	1.4 <sup>(5)</sup>

\* Due to the limited amount of data available, it has been necessary to quote literature values extracted from a number of different sources where the protocols vary considerably.

### References

- Rommel *et al.*, (2008) Conjugative Metabolism of Drugs, in *Drug Metabolism in Drug Design and Development*, (Zhang D *et al.*, eds); pp 37-88, John Wiley & Sons, Inc.
- Williams *et al.*, (2004) *Drug Metab Dispos* **32** (11); 1201-1208.
- Trubetskoy *et al.*, (2007) *Assay and Drug Development Technologies* **5**(3); 343-354.
- Mano *et al.*, (2005) *Biopharm Drug Dispos* **26**; 35-39.
- Sridar *et al.*, (2004) *Drug Metab Dispos* **32** (6); 587-594.