

**In vitro ADME & PK**

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

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**Background Information**

- 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\textsubscript{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.

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**Protocol**

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

**Enzyme Source**
- Human UGT Supersomes\textsuperscript{TM}

**Cofactors**
- UDPGA

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

**Analysis Method**
- LC-MS/MS

**Data Delivery**
- IC\textsubscript{50}
- Standard error of IC\textsubscript{50}

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\'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.'

Glucuronidation is a listed clearance mechanism for 1 in 10 of the top 200 prescribed drugs\textsuperscript{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\textsubscript{50} data (n=3) for known UGT inhibitors in Cyprotex’s UGT inhibition assay.

<table>
<thead>
<tr>
<th>UGT Isoform</th>
<th>Substrate</th>
<th>Inhibitor</th>
<th>Mean IC\textsubscript{50} ± standard deviation (n=3) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>Estradiol</td>
<td>Silybin</td>
<td>4.7 ± 3.5</td>
</tr>
<tr>
<td>UGT1A3</td>
<td>Sulindac sulfone</td>
<td>Ritonavir Quinidine</td>
<td>0.5 ± 0.1, 35 ± 9.3</td>
</tr>
<tr>
<td>UGT1A4</td>
<td>Trifluoperazine</td>
<td>Diclofenac</td>
<td>61 ± 7.8</td>
</tr>
<tr>
<td>UGT1A6</td>
<td>Naphthol</td>
<td>Diclofenac</td>
<td>221 ± 32</td>
</tr>
<tr>
<td>UGT1A9</td>
<td>Propofol</td>
<td>Diclofenac Mycophenolic acid</td>
<td>29 ± 3.8, 66 ± 22</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>Naloxone</td>
<td>Diclofenac Quinidine</td>
<td>24 ± 11, 139 ± 33</td>
</tr>
</tbody>
</table>

References
\textsuperscript{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.