In vitro ADME & PK

Cytochrome P450 Time Dependent Inhibition ($IC_{50}$ Shift)

**Background Information**

- Inhibition of cytochrome P450 enzymes is one of the most common mechanisms resulting in clinically relevant drug-drug interactions. This inhibitory effect can either be a reversible or irreversible (time dependent) interaction.

- Time dependent inhibition (TDI) of cytochrome P450 is of particular concern as typically de novo synthesis of the enzyme is required in order to restore activity. The consequences of TDI can be termination of drug development, drug withdrawal or serious restrictions of use.

- Cyprotex’s $IC_{50}$ shift assay determines the $IC_{50}$ (inhibitor concentration which results in 50% inhibition of activity) following a pre-incubation in the absence and presence of NADPH. This assay enables discrimination between compounds which cause reversible, irreversible, or both reversible and irreversible inhibition.

**Protocol**

- CYP Isoforms Available
  - CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4

- Substrates
  - See table 1

- Test System
  - Human liver microsomes

- Pre-Incubation Time
  - 30 min (+/- NADPH) and 0 min

- Test Article Concentration
  - 0.1, 0.25, 1, 2.5, 10, 25 µM (different concentrations available)

- Positive Controls
  - See Table 1

- Test Article Requirements
  - 150 µL of 10 mM solution

- Analysis Method
  - LC-MS/MS

- Data Delivery
  - $IC_{50}$
  - Standard error of $IC_{50}$
  - Shifted $IC_{50}$

**Related Services**

- Cytochrome P450 Time Dependent Inhibition (Single Point)
- Cytochrome P450 Time Dependent Inhibition ($k_{inact}/K_I$)

To find out more contact enquiries@cyprotex.com
Failure of several late stage clinical candidates has been attributed to TDI, and this mechanism is also suspected to play a role in liver toxicities often observed in preclinical species.

Cytochrome P450 Time Dependent Inhibition (IC$_{50}$ Shift)

A number of known time dependent inhibitors were screened in the IC$_{50}$ shift assay in triplicate on the plate on three separate occasions. Inhibitors which were known to be solely reversible inhibitors were screened as negative controls alongside the time dependent inhibitors. The results show a high level of consistency over a range of inhibition values.

Figure 1
IC$_{50}$ shift data for phenacetin O-deethylation inhibition by furafylline, a time dependent inhibitor.

Furafylline was pre-incubated with human liver microsomes in the presence and absence of NADPH prior to the addition of the CYP1A2 substrate, phenacetin. The 17 fold shift in IC$_{50}$ value (IC$_{50}$ (30 Minus) = 10.1 µM; reversible inhibition component, IC$_{50}$ (30 Plus) = 0.586 µM; time-dependent effect) when NADPH is included in the pre-incubation indicates that furafylline is a time dependent inhibitor.

It is recommended that compounds which exhibit an IC$_{50}$ shift of ≥ 1.5 with a 30 min pre-incubation are classified as time dependent inhibitors. Tienilic acid, a known reversible and time dependent inhibitor, gives a mean fold shift of 6.8, whereas sulphaphenazole, which is solely a reversible inhibitor, gives a mean fold shift of 0.84.

Table 1
Cytochrome P450 isomor specific substrates and positive control compounds used in the Cyprotex IC$_{50}$ Shift assay.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Substrate</th>
<th>Positive Control</th>
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<tbody>
<tr>
<td>CYP1A2</td>
<td>Phenacetin</td>
<td>Furafylline</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Bupropion</td>
<td>Thiotepa</td>
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<tr>
<td>CYP2C8</td>
<td>Paclitaxel</td>
<td>Gemfibrozil glucuronide</td>
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<td>CYP2C9</td>
<td>Diclofenac</td>
<td>Tienilic acid</td>
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<td>CYP2C19</td>
<td>S-Mephenytoin</td>
<td>Fluoxetine</td>
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<td>CYP2D6</td>
<td>Dextromethorphan</td>
<td>Paroxetine</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Midazolam</td>
<td>Mifepristone</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Testosterone</td>
<td>Mifepristone</td>
</tr>
</tbody>
</table>

Graph illustrates the fold shift in IC$_{50}$ for tienilic acid (a reversible and time dependent inhibitor) and sulphaphenazole (a reversible inhibitor). Data are mean ± standard deviation of triplicate incubations for three separate experiments.

Graph illustrating mean fold shift in IC$_{50}$ of midazolam 1-hydroxylation by four known CYP3A4 time dependent inhibitors.

The data illustrate the mean fold shift in IC$_{50}$ over three experiments with the error bars representing the triplicate incubations on each run of the assay.

References