**In vitro ADME & PK**

**Cytochrome P450 K\textsubscript{i}**

**Background Information**

- Assessment of the potential of a compound to inhibit a specific cytochrome P450 enzyme is important as co-administration of compounds may result in one or both inhibiting the other’s metabolism. This may affect plasma levels in vivo and potentially lead to adverse drug reactions or toxicity.

- Determination of the inhibition constant (K\textsubscript{i}) of a compound is the current recommended approach by the FDA for studying the clinical relevance of reversible cytochrome P450 inhibitors.

- Cyprotex’s Cytochrome P450 K\textsubscript{i} assay delivers a written report detailing graphical representation of the data and calculation of the K\textsubscript{i} value. The type of inhibition is determined by fitting statistics for the enzyme inhibition models (i.e., competitive, non-competitive, uncompetitive and mixed).

**Protocol**

**Typical Test Article Concentrations**

- 0, 0.25xIC\textsubscript{50}, 0.5xIC\textsubscript{50}, 0.75xIC\textsubscript{50}, 1xIC\textsubscript{50}, 2.5xIC\textsubscript{50}, 5xIC\textsubscript{50}

**CYP Isoforms**

- CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4
  (other isoforms are available)

**Typical Substrate Concentrations**

- 0.3K\textsubscript{m}, 1xK\textsubscript{m}, 3xK\textsubscript{m}, 6xK\textsubscript{m} and 10xK\textsubscript{m}

**Number of Replicate**

n=2

**Test Article Requirements**

Dependent on IC\textsubscript{50}

**Analysis Method**

LC-MS/MS

**Data Delivery**

- Identification of type of inhibition
- Written Report

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"K\textsubscript{i} values are intrinsic constants, whereas IC\textsubscript{50} values are extrinsic constants. Theoretically, IC\textsubscript{50} values, in contrast to K\textsubscript{i} values, are dependent on the type of substrate, the concentration of substrate, and incubation conditions (protein concentration or incubation times, etc)."

For reversible inhibition, a simple classification, based on $[I]/K_i$ ratio, is commonly used to predict clinical drug-drug interactions.  

Table 1
Rate equations for different inhibition model types.

<table>
<thead>
<tr>
<th>Inhibition type</th>
<th>Rate equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive</td>
<td>$v = \frac{V_{\text{max}}[S]}{[S]+K_m\left(1+\frac{[I]}{K_i}\right)}$</td>
</tr>
<tr>
<td>Non-Competitive</td>
<td>$v = \frac{V_{\text{max}}[S]}{K_m\left(1+\frac{[I]}{K_i}\right)+[S]\left(1+\frac{[I]}{K_i}\right)}$</td>
</tr>
<tr>
<td>Uncompetitive</td>
<td>$v = \frac{V_{\text{max}}[S]}{K_m/[S]+[S]\left(1+\frac{[I]}{K_i}\right)}$</td>
</tr>
<tr>
<td>Mixed</td>
<td>$v = \frac{V_{\text{max}}[S]}{K_m\left(1+\frac{[I]}{K_i}\right)+[S]\left(1+\frac{[I]}{K_i}\alpha\right)}$</td>
</tr>
</tbody>
</table>

Where $v$ is the rate, $V_{\text{max}}$ is the maximal rate, $K_m$ is the affinity constant, $[S]$ is the substrate concentration, $[I]$ is the inhibitor concentration, $K_i$ is the inhibition constant and $\alpha$ is the interaction parameter which determines the degree to which the binding of inhibitor changes the affinity of the enzyme for the substrate.

Cytochrome P450 Ki

Compounds are evaluated at up to 6 inhibitor concentrations and 5 substrate concentrations in duplicate. Regression analysis is used to identify the type of inhibition (competitive, non-competitive, uncompetitive or mixed).

To select the most appropriate inhibition model, the goodness of fit criteria comprises of visual inspection of the data, correlation of determination ($R^2$) and corrected Akaike’s Information Criterion (AICc). For visual inspection, data are presented as a direct plot of response against substrate concentration, in addition to Eadie-Hofstee and Lineweaver-Burk plots.

Table 2
Comparison of the $K_i$ of ketoconazole determined in Cyprotex’s CYP3A4 $K_i$ assay with values sourced from the literature. The $K_i$ was performed using human liver microsomes with midazolam as the CYP3A4 probe substrate.

<table>
<thead>
<tr>
<th>Literature source</th>
<th>$K_i$ (nM)</th>
<th>Type of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrighton and Ring (1994)</td>
<td>110</td>
<td>Non-competitive</td>
</tr>
<tr>
<td>Gibbs et al. (1999)</td>
<td>14.9</td>
<td>Non-competitive</td>
</tr>
<tr>
<td>Brown et al. (2007)</td>
<td>80</td>
<td>Not available</td>
</tr>
<tr>
<td>Mean of literature data</td>
<td>68.3</td>
<td></td>
</tr>
<tr>
<td>Cyprotex’s CYP3A4 $K_i$</td>
<td>53.1</td>
<td>Non-competitive</td>
</tr>
</tbody>
</table>

In Cyprotex’s CYP3A4 $K_i$ assay, the type of inhibition of CYP3A4-mediated midazolam-1’-hydroxylation by ketoconazole in human liver microsomes was identified as being non-competitive in nature. The $K_i$ of ketoconazole was determined to be 53.1 nM. Table 2 illustrates that data generated in Cyprotex’s CYP3A4 $K_i$ assay for ketoconazole are comparable with data reported in the literature.

References

2 FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (February 2012)