cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

Cytochrome P450 K_i

Background Information

'K_i values are intrinsic constants, whereas IC_{50} values are extrinsic constants. Theoretically, IC_{50} values, in contrast to K_i values, are dependent on the type of substrate, the concentration of substrate, and incubation conditions (protein concentration or incubation times, etc).'

[1]Ogilvie BW, Usuki E, Yerino P and Parkinson A (2008). *In* Drug-Drug Interactions Second Edition (Ed. Rodrigues AD) Informa Healthcare USA New York 231-358

- 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_i) of a compound is the current recommended approach by the FDA[2] for studying the clinical relevance of reversible cytochrome P450 inhibitors.
- Cyprotex's Cytochrome P450 K_i assay delivers a written report detailing graphical representation of the data and calculation of the K_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_{50}, 0.5xIC_{50}, 0.75xIC_{50}, 1xIC_{50}, 2.5xIC_{50}, 5xIC_{50}

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

Typical Substrate Concentrations
0.3K_m, 1xK_m, 3xK_m, 6xK_m and 10xK_m

Number of Replicate
n=2

Test Article Requirements
Dependent on IC_{50}

Analysis Method
LC-MS/MS

Data Delivery
K_i
Standard error of K_i
Identification of type of inhibition
Written report

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

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 1

Rate equations for different inhibition model types.

Inhibition type	Rate equation
Competitive	$v = \frac{V_{max}[S]}{[S] + K_m\left(1+\frac{[I]}{K_i}\right)}$
Non-Competitive	$v = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_i}\right) + [S]\left(1+\frac{[I]}{K_i}\right)}$
Uncompetitive	$v = \frac{V_{max}[S]}{K_m + [S]\left(1+\frac{[I]}{K_i}\right)}$
Mixed	$v = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_i}\right) + [S]\left(1+\frac{[I]}{\alpha K_i}\right)}$

Where v is the rate, V_{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 α is the interaction parameter which determines the degree to which the binding of inhibitor changes the affinity of the enzyme for the substrate.

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.

Literature source	K_i (nM)	Type of inhibition
Wrighton and Ring (1994)[3] Gibbs *et al*. (1999)[4] Brown *et al*. (2007)[5]	110 14.9 80	Non-competitive Non-competitive Not available
Mean of literature data	**68.3**	
Cyprotex's CYP3A4 K_i	53.1	Non-competitive

In Cyproex'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

1 Ogilvie BW *et al*, (2008) *In* Drug-Drug Interactions Second Edition (Ed. Rodrigues AD) Informa Healthcare USA New York 231-358.

2 FDA Guidance for Industry – In Vitro Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (January 2020)

3 Wrighton SA and Ring BJ. (1994) *Pharmaceutical Research* **11 (6)**; 921-924.

4 Gibbs MA *et al*, (1999) *Drug Metab Dispos* **27 (2)**; 180-187.

5 Brown HS *et al*, (2007) *Drug Metab Dispos* **35 (11)**; 2119-2126.

Read online: