

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

¹Ogilvie 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 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, 0.25x IC_{50} , 0.5x IC_{50} , 0.75x IC_{50} , 1x IC_{50} , 2.5x IC_{50} , 5x IC_{50}

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

Typical Substrate Concentrations
0.3 K_m , 1x K_m , 3x K_m , 6x K_m and 10x K_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.²



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	110	Non-competitive
Gibbs <i>et al.</i> (1999) ⁴	14.9	Non-competitive
Brown <i>et al.</i> (2007) ⁵	80	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

- Ogilvie BW *et al.*, (2008) *In Drug-Drug Interactions* Second Edition (Ed. Rodrigues AD) Informa Healthcare USA New York 231-358.
- FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (February 2012)
- Wrighton SA and Ring BJ. (1994) *Pharmaceutical Research* **11** (6): 921-924.
- Gibbs MA *et al.*, (1999) *Drug Metab Dispos* **27** (2): 180-187.
- Brown HS *et al.*, (2007) *Drug Metab Dispos* **35** (11): 2119-2126.