

Cloe Select Cytochrome P450 K_i

Background Information



'K_i values are intrinsic constants, whereas IC₅₀ values are extrinsic constants. Theoretically, IC₅₀ 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).'

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 for studying the clinical relevance of reversible cytochrome P450 inhibitors.
- The Cloe Select 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 Compound Concentrations
0, 0.25xIC₅₀, 0.5xIC₅₀, 0.75xIC₅₀, 1xIC₅₀, 2.5xIC₅₀, 5xIC₅₀

CYP Isoforms
CYP1A, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4

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

Number of Replicates
n=2

Compound Requirements
Dependent on IC₅₀

Analysis Method
LC-MS/MS (Fluorescent endpoint for CYP1A)

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 (where $[I]$ is the inhibitor concentration at steady state C_{max} of the highest clinical dose), is commonly used to predict clinical drug-drug interactions (Bjornsson *et al.*, 2003)²



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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 the Cloë Select 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	
Cloë Select CYP3A4 K_i	53.1	Non-competitive

In the Cloë Select 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 the Cloë Select 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
- Bjornsson TD *et al.* (2003) *Drug Metab Dispos* **31**; 815-832.
- 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.