cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

Cytochrome P450 Time Dependent Inhibition (k_{inact}/K_I)

Background Information

'Metabolic drug-drug interactions resulting from TDI can display a delayed onset due to the time dependence in inhibition and can persist even after the inhibitor has been eliminated because enzymatic activity is only restored by de novo protein synthesis.'

[2]Grimm SW, Einolf HJ, Hall SD, He K, Lim H-K, John Ling, K-H, Lu C, Nomeir AA, Seibert E, Skordos KW, Tonn GR, Horn RV, Wang RW, Wong YN, Yang TJ and Obach RS. (2009) *Drug Metab Dispos* **37**; 1355-1370

- Time dependent inhibition of cytochrome P450, often caused by an irreversible or quasi-irreversible interaction, can lead to clinically relevant drug-drug interactions or non-linear pharmacokinetics of a drug. In addition, these interactions are typically a consequence of reactive metabolite formation which is also associated with toxicity via covalent binding to cellular macromolecules[1].
- The FDA guidance for drug interactions (2020)[3] and the EMA guideline on the investigation of drug interactions (adopted 2012)[8] recommend evaluating time dependent inhibition for investigational drugs.
- Characterisation of the k_{inact} (maximal inactivation) and K_I (concentration at 50% k_{inact}) parameters is frequently performed during drug development to evaluate risk of time dependent inhibition and decide if a clinical interaction study is required.
- Cyprotex's k_{inact}/K_I assay evaluates the inactivation kinetics of time dependent inhibition at 5 inhibitor concentrations and 7 pre-incubation times.

Related Services

- Cytochrome P450 Time Dependent Inhibition (Single Point)
- Cytochrome P450 Time Dependent Inhibition (IC_{50} Shift)

Protocol

Substrates and CYP Isoforms
Phenacetin (CYP1A2), bupropion (CYP2B6), paclitaxel (CYP2C8), diclofenac (CYP2C9), S-mephenytoin (CYP2C19), dextromethorphan (CYP2D6), midazolam (CYP3A4)
(others available on request)

Test System
Human liver microsomes

Pre-incubation Times
7 Pre-incubation times (including 0 min)

Test Article Concentrations
5 Concentrations plus vehicle control

Number of Replicates
2

Analysis Method
LC-MS/MS

Data Delivery
k_{inact}
K_I

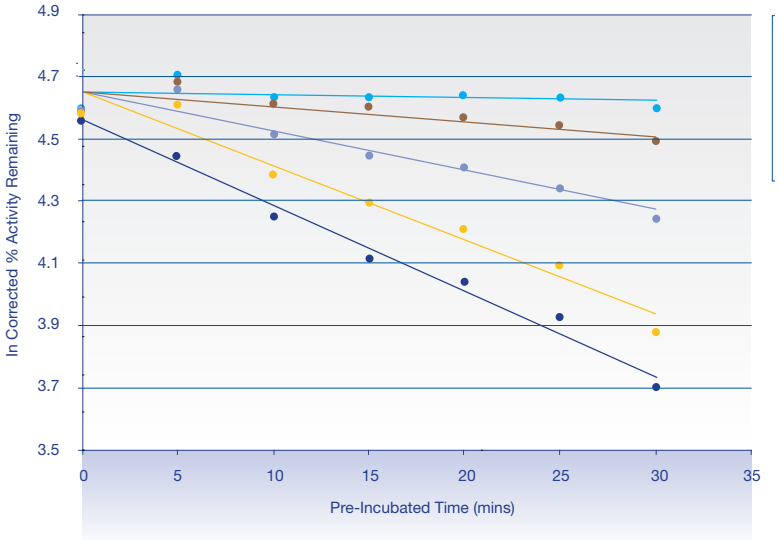

Figure 1

Inactivation plot (natural logarithm of the corrected % remaining activity versus pre-incubation time) for the CYP3A4 time dependent inhibitor, diltiazem, using midazolam as the probe substrate for CYP3A4.

Data illustrated are the mean of duplicate incubations.

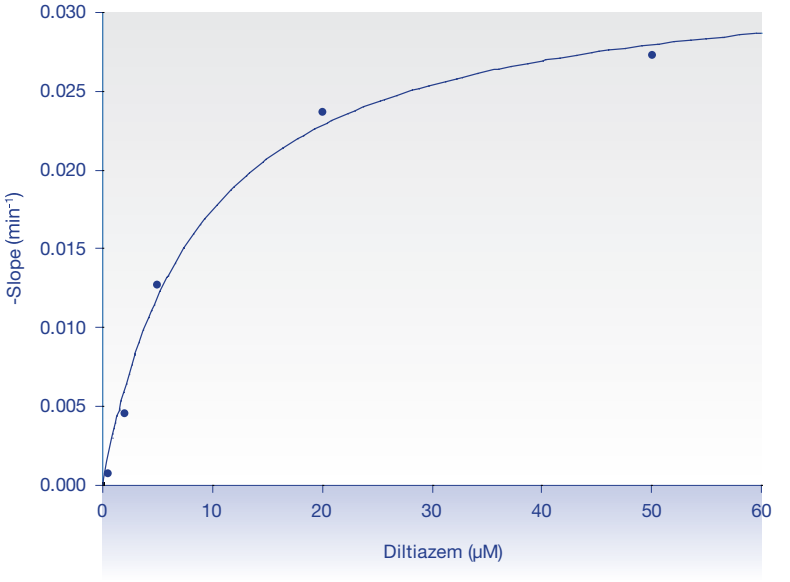

Figure 2

Non-linear regression analysis of the negative slopes versus inhibitor concentration (in the pre-incubation) performed to obtain k_{inact} and K_I values using the data illustrated in Figure 1.

Table 1

Experimental conditions selected for 4 known CYP3A4 time dependent inhibitors and comparison of the inactivation parameters, K_I, k_{inact} and k_{inact}/K_I ratio with published literature values. Parameters (Cyprotex values) are derived from the mean of duplicate incubations. The table illustrates that data generated at Cyprotex compares well with literature data.

Compound	Experimentally Determined Values at Cyprotex						Literature Values		
	Dilution factor	Concentration range (μM)	Pre-incubation Times (min)	K_I (μM)	k_{inact} (min^{-1})	k_{inact}/K_I (ml/min/μmol)	K_I (μM)	k_{inact} (min^{-1})	k_{inact}/K_I (ml/min/μmol)
Diltiazem	1:10	0.5-50	0, 5, 10, 15, 20, 25, 30	8.8	0.0329	3.74	4.5	0.012	2.67[4]
Mibefradil	1:20	0.2-20	0, 1, 2.5, 5, 10, 15, 20	4.8	0.312	65.0	2.6	0.4	174[5]
Mifepristone	1:10	0.2-20	0, 5, 10, 15, 20, 25, 30	1.6	0.0700	43.8	1.3	0.061	47[6]
Verapamil	1:10	0.3-30	0, 5, 10, 15, 20, 25, 30	3.9	0.0619	15.9	1.8	0.043	11.5[7]

References

1 Kalgutkar AS *et al*, (2007) *Curr Drug Metab* **8**; 407-447.

2 Grimm SW *et al*, (2009) *Drug Metab Dispos* **37**; 1355-1370.

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

4 Obach RS *et al.* (2007) *Drug Metab Dispos* **35 (2)**; 246-255.

5 Prueksaritanont T *et al*, (1999) *Br J Clin Pharmacol* **47**; 291-298.

6 Berry LM *et al*, (2008) *Drug Metab Lett* **2**; 51-59.

7 Perloff ES *et al*, (2009) *Xenobiotica* **39 (2)**; 99-112.

Read online: