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In vitro **ADME & PK**

Cytochrome P450 Time Dependent Inhibition (Single Point)

Background Information

'TDI results from irreversible covalent binding or quasi-irreversible noncovalent tight binding of a chemically reactive intermediate to the enzyme that catalyzes its formation, resulting in loss of enzyme function.'

[1]Grimm SW *et al.*, (2009) *Drug Metab Dispos* **37(7)**; 1355-1370

- The inhibition of human cytochrome P450s is one of the most common mechanisms which can lead to drug-drug interactions.
- Time dependent inhibition defines an interaction where there is enhanced inhibition if the test compound is pre-incubated with the metabolising system prior to the addition of substrate.
- If an irreversible interaction occurs, the consequences of time dependent inhibition are considered to be more serious because the inactivated enzyme must be re-synthesised before activity is restored.
- Cyprotex's time dependent inhibition (single point) assay uses industry accepted probe substrates and human liver microsomes.

Related Services

- Cytochrome P450 Time Dependent Inhibition (IC_{50} Shift)
- Cytochrome P450 Time Dependent Inhibition (k_{inact}/K_I)

Protocol

CYP Isoforms Available
CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4

Substrates
See table 1

Number of Replicates
2

Pre-incubation Time
30 min

Test Article Concentration
25 µM

Positive Controls
See Table 1

Test Article Requirements
Dependent on number of isoforms assessed

Analysis Method
LC-MS/MS (with the exception of ethoxyresorufin for CYP1A)

Data Delivery
Mean percentage inhibition following pre-incubation

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Irreversible and quasi-irreversible inhibition are often viewed as more serious than reversible inhibition, since the inhibitory effect remains after elimination of the parent drug from the body[2].

Cytochrome P450 Time Dependent Inhibition (Single Point)

For the validation, a literature search was performed to identify a selection of compounds which were time dependent inhibitors of the main cytochrome P450 isoforms. These inhibitors were selected and were screened in triplicate on the plate on 3 separate occasions. The results show that there is a high level of consistency over a range of inhibition values.

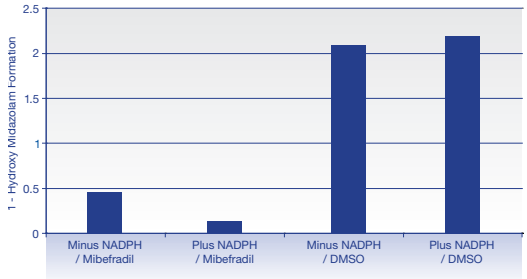
Table 1

Cytochrome P450 isoform specific substrates and positive control compouds used in the Cyprotex cytochrome P450 time depenedent inhibition (single point) assay.

Isoform	Substrate	Positive Control
CYP1A2	Ethoxyresorufin	Furafylline
CYP2B6	Bupropion	Ticlopidine
CYP2C8	Paclitaxel	Gemfibrozil glucuronide
CYP2C9	Diclofenac	Tienilic Acid
CYP2C19	S-Mephenytoin	Ticlopidine
CYP2D6	Dextromethorphan	Paroxetine
CYP3A4	Midazolam	Mifepristone
CYP3A4	Testosterone	Mifepristone

Figure 2

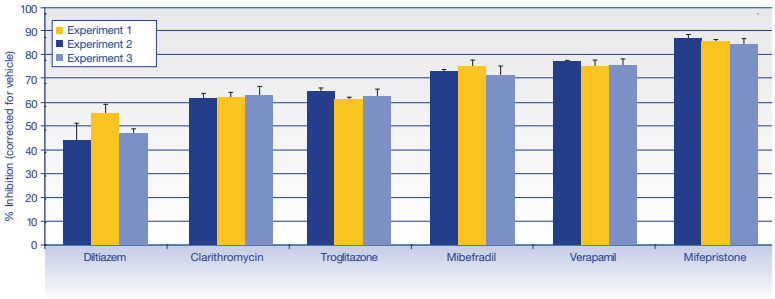
Discrimination between reversible and time dependent CYP3A4 inhibition by mibefradil using midazolam as substrate.



Mibefradil is both reversible and time dependent CYP3A4 inhibitor as it exhibits inhibitory potential in both the absence and presence of NADPH in the pre-incubation with the inhibition greater in the latter incubation. It is therefore possible to both detect and discriminate between the reversible cytochrome P450 inhibition and the time dependent cytochrome P450 inhibition associated with this test compound.

Figure 1

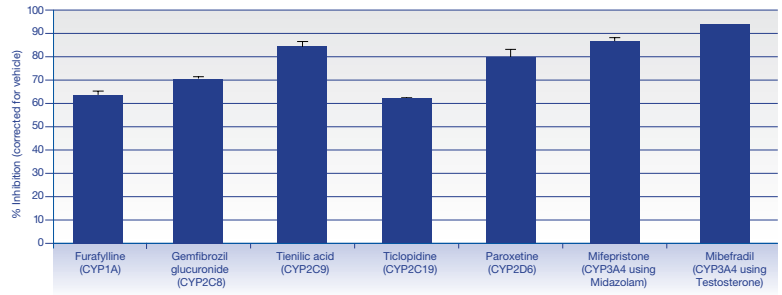
Mean % inhibition of the CYP3A4 probe substrate, midazolam, by 6 known time dependent inhibitors. Data were generated by the Cyprotex CYP3A4 time dependent inhibition (single point) assay.



The graph shows the mean % inhibition over 3 experiments with the error bars representing the standard deviation of the 3 replicates on the plate for 6 time dependent CYP3A4 inhibitors.

Figure 3

Data generated for known time dependent inhibitors in the Cyprotex cytochrome P450 time dependent inhibition (single point) assay (the mean of 3 replicates is displayed with the error bars representing the standard deviation).



All inhibitors shown in Figure 3 were screened at a concentration of 25 µM with the exception of gemfibrozil glucuronide which was screened at 10 µM.

References

[1] Grimm SW *et al.* (2009) *Drug Metab Dispos* **37(7)**; 1355-70
[2] Atkinson A *et al*, (2005) *Drug Metab Dispos* **33**; 1637-1647

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