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CONCEPT LIFE SCIENCES

Concept Life Sciences:
CYP 450 Inhibition

Introduction

Inhibitory potency (IC50) provides invaluable information regarding drug interaction potential. The IC50 of test compound against the five most important drug metabolizing enzymes is determined using specific drug probe substrates. Analysis is by LC-MSMS.

Deliverable: IC50 (µM) of test compound against CYP1A2, CYP2C9, CYP2C19, CYP2D6 & CYP3A4.

Customer provides

Compound identifier and molecular formula.
Test: 70µLof 10mM in DMSO or 0.5mg solid.

Enzymes

Pooled recombinant E.coli membranes expressing human CYPs.

Test compound

Concentration range 0, 0.15, 0.5, 1.5, 5, 15 & 50µM.

Format

96-well plate, 200µL incubation volume.

Protocol

CYPs are pooled in a ratio such that biotransformation of each probe substrate is specific for the particular CYP450. Substrates (at their Km) are also pooled within the same solution to create an enzyme-substrate stock, which is aliquoted into each well of the microplate. Test compound is added to appropriate wells. The final solvent concentration is 1.0% DMSO. After equilibration to 37ºC, addition of NADPH and mixing initiates substrate biotransformation. Each concentration of test compound is assayed against five CYPs in the same well simultaneously. The incubation is stopped at t = 10min by removal of the plate from the shaking incubator, followed by the addition of 200µL of ice cold methanol containing internal standard, and mixing.

The quenched samples are centrifuged for 30 minutes 4000rpm at 4ºC to precipitate the protein.

Quantitation

The supernatants analyzed by LC-MS/MS using Concept Life Sciences generic analytical methods to measure metabolite formation.

CYP	Substrate at km	Metabolite produced	Standard inhibitor
1A2	Phenacetin	Acetaminophen	α-Naphthoflavone
2C9	Piclofenac	4'OH Diclofenac	Sulfaphenazole
2C19	S-mephenytoin	4'OH Mephenytoin	Tranylcypromine
2D6	Bufuralol	1'OH Bufuralol	Quinidine
3A4	Midazolam	1'OH Midazolam	Ketoconazole

Data analysis and results

The enzyme activity within each test compound incubation is compared with Control (contains solvent but no test compound). For each concentration of test:

$$\%\ \text{Control activity} = \frac{\text{Test peak area}}{\text{Internal standard peak area in test}} \times \frac{\text{Internal standard peak area in control}}{\text{Control peak area}} \times 100\%$$

% control activities obtained from the above calculation are plotted against test concentration.

IC50 = test compound concentration producing 50% control activity (=50% inhibition).

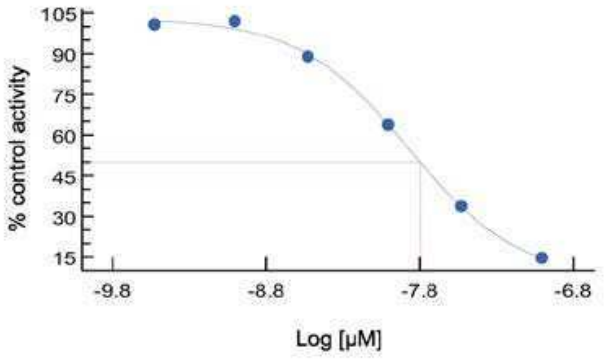
+VE Controls

Control compound, one for each CYP, is used in every assay run (see bar chart below for identities).

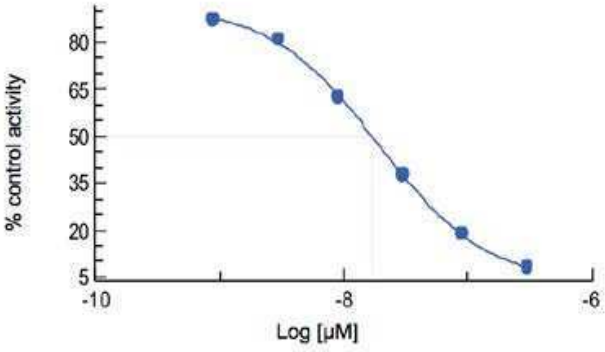
Results

6-point inhibition curves are used to calculate IC50's for all five CYPs.

3A4 Ketoconazole Inhibition



2D6 Quinidine Inhibition



Concept IC_{50}