

CYTOCHROME P450 INHIBITION STUDIES USING FDA RECOMMENDED PROBE SUBSTRATES IN HUMAN LIVER MICROSOMES

Background

Many drug-drug interactions are metabolism based and mediated primarily via the Cytochrome P450 (CYP) family of enzymes. Ten CYP isoforms are expressed in a typical human liver (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1, and CYP3A4). Of these, six principle enzymes (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) appear to be the most commonly responsible for the metabolism of most drugs and the associated drug-drug interactions. The inhibition of these enzymes may have important clinical consequences such that inhibition of a CYP isoenzyme(s) by a xenobiotic may decrease the metabolic clearance of a co-administered drug resulting in elevated blood concentrations of the drug. These elevated concentrations of the drug may result in adverse drug effects or toxicity. As detailed in the FDA's Draft Guidance document for Drug-Drug Interactions (2006)¹, the FDA has placed emphasis on evaluating the inhibition potential of a new chemical entity (NCE) at an earlier stage in drug development in order to avoid developing compounds with the potential to yield adverse drug interactions. Early assessment of an NCE's ability to inhibit the activity of a particular CYP subtype can be achieved using human liver microsomes.

Assay Outline

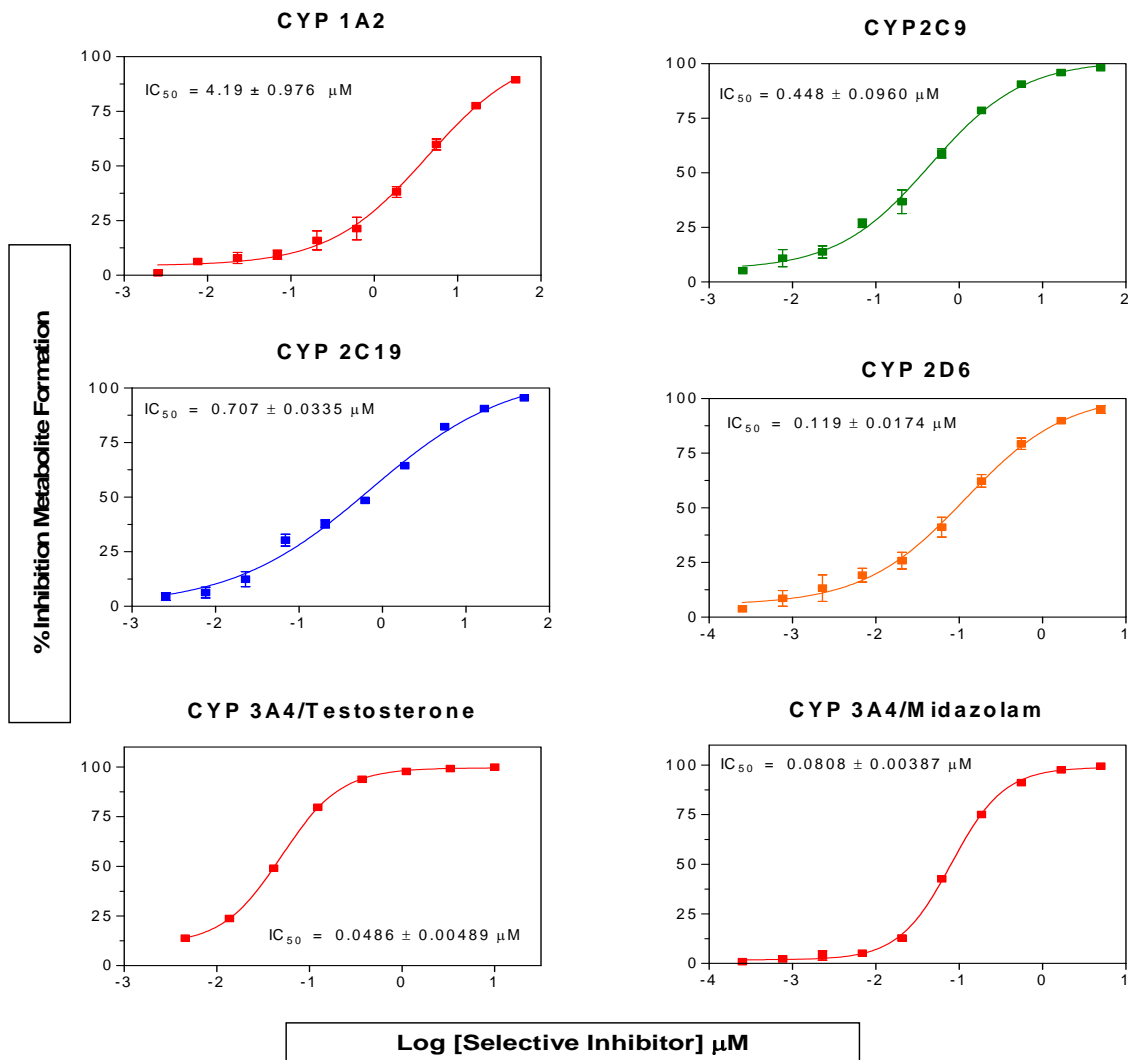
In accordance with the FDA Draft Guidance for Drug-Drug Interactions Definitive CYP Inhibition Studies are carried out as follows:

- Assays are performed in 96-well microtiter plates with pooled human liver microsomes.
- Various concentrations of test compound are incubated together with probe substrate in the presence of NADPH, and the inhibition of metabolite formation by each CYP subtype is determined.

CYP Subtype	Probe Substrate	Metabolite	Known Inhibitor	Observed IC ₅₀ (μM) ± S.D
CYP 1A2	Phenacetin	Acetaminophen	Furafylline	4.19 ± 0.976
CYP 2C9	Diclofenac	4-hydroxy-Diclofenac	Sulfaphenazole	0.448 ± 0.0960
CYP 2C19	S-Mephenytoin	4-hydroxy-Mephenytoin	Ticlopidine	0.707 ± 0.0335
CYP 2D6	Dextromethorphan	Dextrorphan	Quinidine	0.119 ± 0.0174
CYP 3A4	(i) Testosterone	6-β-OH-Testosterone	Ketoconazole	0.0486 ± 0.00489
	(ii) Midazolam	1-hydroxy-Midazolam	Ketoconazole	0.0808 ± 0.00387

- A known inhibitor for each CYP subtype is run in parallel as a positive control.
- All incubations are performed in triplicate.
- Metabolite formation for each CYP subtype in the presence and absence of test compound is measured by validated LC/MS methods.
- Where possible, the IC₅₀ value (concentration which reduces the metabolism of the probe substrate by 50%) will be determined for the test compound for each CYP subtype.

As an example, to assess the inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 activity, the known selective inhibitors were incubated together with their respective probe substrates, in pooled human liver microsomes in the presence of NADPH. The IC₅₀ of each reference inhibitor for the CYP 450 enzyme subtype was determined as a measure of the inhibition of probe substrate metabolite formation. The data is depicted graphically in the figure below. The IC₅₀ of each reference compound is presented in the graphs below and in the table above. These values are consistent with data reported in the literature².



NoAb's CYP450 inhibition assay allows the evaluation of the inhibitory potential of NCEs, helping to identify and avoid developing candidates with potential adverse drug interaction properties. NoAb also offers a complementary activity based CYP450 induction assay, to evaluate the induction potential of a NCE. In addition, NoAb's proprietary DTEXTM Gene Expression Analysis allows investigators to simultaneously survey drug related induction and suppression of ADME related genes (such as CYP450s, drug transporters and transcription factors) at the gene expression level, which is useful where activity based assays are unreliable or unavailable. All of these services are examples of NoAb's commitment to providing the best drug discovery tools for our clients, helping to shape drug discovery.

References:

1. USFDA (2006) Draft Guidance for Industry: Drug Interaction Studies-Study Design, Data Analysis, and Implications for Dosing and Labeling, U.S. Food and Drug Administration Publication
2. Walsky and Obach, Drug Metabolism and Disposition, 2004; 32: 647-660

www.noabbiodiscoveries.com

For more information contact:

Sal Lemus, B.Sc.

NoAb BioDiscoveries Inc.

905.814.5238, Ext. 235

slemus@noabbiodiscoveries.com