

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). The inhibition of these enzymes may have important clinical consequences as 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 leading to adverse drug effects or toxicity. 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In accordance with the FDA Draft Guidance for Drug-Drug Interactions Definitive CYP Inhibition Studies are carried out as follows: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.
- 12 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.

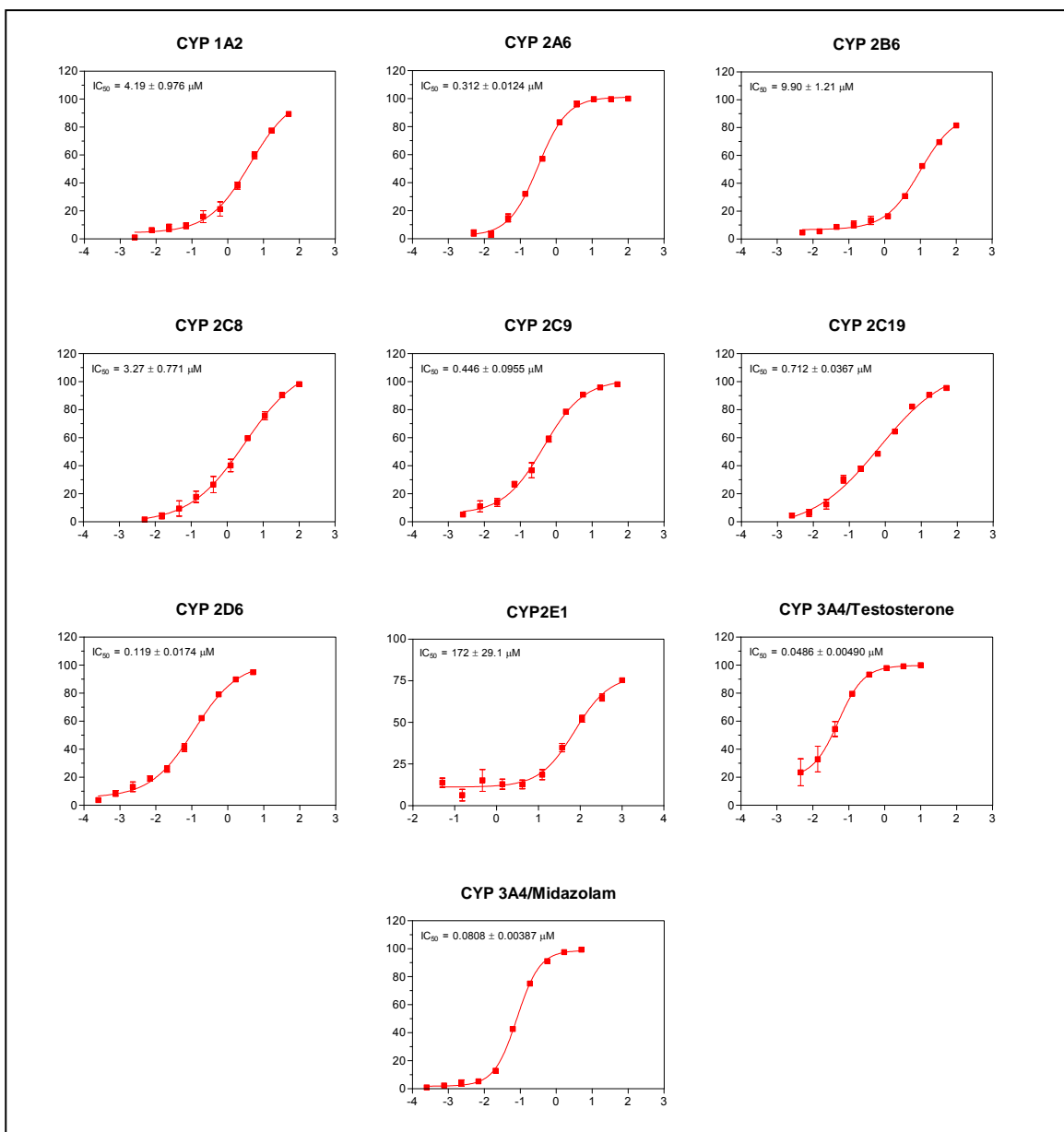
CYP Subtype	Probe Substrate	Metabolite	Known Inhibitor	Observed IC ₅₀ (μM) ± S.D
CYP 1A2	Phenacetin	Acetaminophen	Furafylline	4.19 ± 0.976
CYP2A6	Coumarin	7' Hydroxycoumarin	Tranlycypromine	0.312 ± 0.012
CYP2B6	Bupropion	Hydroxybupropion	Thio-TEPA	9.90 ± 1.21
CYP2C8	Paclitaxel	6α Hydroxypaclitaxel	Quercetin	3.27 ± 0.771
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
CYP2E1	Chlorzoxazone	6 Hydroxychlorzoxazone	Clomethiazole	172 ± 29.1
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, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, 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 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.

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



Log (Selective Inhibitor) μM

NoAb also offers a complementary activity and mRNA based CYP450 induction assay, to evaluate the CYP1A2, CYP2B6, and CYP3A4 induction potential of NCEs. 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.

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