Cytochrome P450 Time Dependent Inhibition (IC50 shift)

Background: Time dependent inhibition (TDI) gains an increasingly greater attention as a predictor of the drug-drug interaction potential of clinical candidates. IC50 shift assay is a current standard approach for preliminary assessment of TDI. In addition to competitive inhibition of CYP450, some compounds display time dependent inhibition. CYP450 mediated transformation of these compounds results in metabolites which act as reversible inhibitors or modify chemically enzyme (e.g. via covalent bond formation). Their inhibitory potency increases with incubation time. TDI is manifested by difference in IC_{50} values measured under two different conditions: pre-incubation of a test article with an enzyme and a cofactor NADPH, which produces metabolites, and pre-incubation without NADPH. TDI can be divided into a number of mechanistic categories including irreversible (covalent) modification of the enzyme (mechanism based inactivation), quasi-irreversible (metabolite intermediate complex), reversible (metabolite more potent inhibitor than parent), etc. TDI inhibition is of particular importance because it may result in a long lasting inhibition since usually the enzyme re-synthesis is required to recover the CYP450 activity.

Service Details: Time dependent inhibition of 5 major cytochromes CYP1A2, 2C9, 2C19, 2D6, and 3A4 is studied using IC50 shift approach. In this assay, the IC50 value (concentration which produces 50% inhibition) of a test compound is determined under two different experimental conditions: 1) 30 min pre-incubation of test article with enzyme without NADPH, 2) 30 min pre-incubation of test article with enzyme and NADPH. Following the pre-incubation step, the specific substrates are added to the incubation mixture. The experiment is performed using non-dilution method (Kozakai et al, Drug Metab. Pharmacokinet. 2014, 198-207). If the compound is a time dependent inhibitor, an increase in potency will occur between the measurements done at 30 min pre-incubation “minus NADPH” (reversible inhibition component) vs. 30 min pre-incubation “plus NADPH” (time dependent inhibition component). The ratio of these two values yields the IC50 shift:

IC50 shift = IC50 “minus NADPH” / IC50 “plus NADPH”

The ratio higher than the cut-off value (from 1.5 to 2-fold) indicates a time dependent inhibition. Accordingly to FDA recommendations, the time dependent inhibition experiments are performed using LC-MS/MS based assay, in which biotransformations of the CYP450 specific substrates are used as markers to quantify the enzymatic activity. The extensivelycharacterized pooled human liver microsomes (50 or 200 donors) are used in the assay to provide consistent and reproducible results. Quantification of a decrease in the formation of a metabolite in the presence of an inhibitor is used to determine the IC50.

CYP450	Substrate	Metabolite	Reference time dependent inhibitor
CYP1A2	Phenacetin	Acetaminophen	Furafylline
CYP2C9	Diclofenac	4′-Hydroxydiclofenac	Tienilic acid
CYP2C19	S-Mephenytoin	S-4-Hydroxymephenytoin	Fluoxetine
CYP2D6	Dextromethorphan	Dextrorphan	Paroxetine
CYP3A4	Testosterone	6β-Hydroxytestosterone	Verapamil

Deliverable: Dose-response inhibition curves (8 points, 3-fold serial dilution) of the test compound and the reference inhibitor starting from 100μM concentration. All test points are performed in duplicates. The IC50 values are calculated using Microsoft Excel and GraphPad Prism software. Data include tables with inhibition % for each compound concentration and SD values, two dose response inhibition curves (plus/minus NADPH), and IC50 shift value.

Sample Submission: A minimal accurately weighable quantity of dry compound (~2-3 mg or 5 μmol) or 100 μL of 40 mM stock DMSO solution is required for this assay. For multiple assays, lesser amount of compound per assay may be sufficient, which should be discussed for each particular project.