IND-Enabling Bundle of *In Vitro* Assays to Assess Drug-Drug Interaction Risk

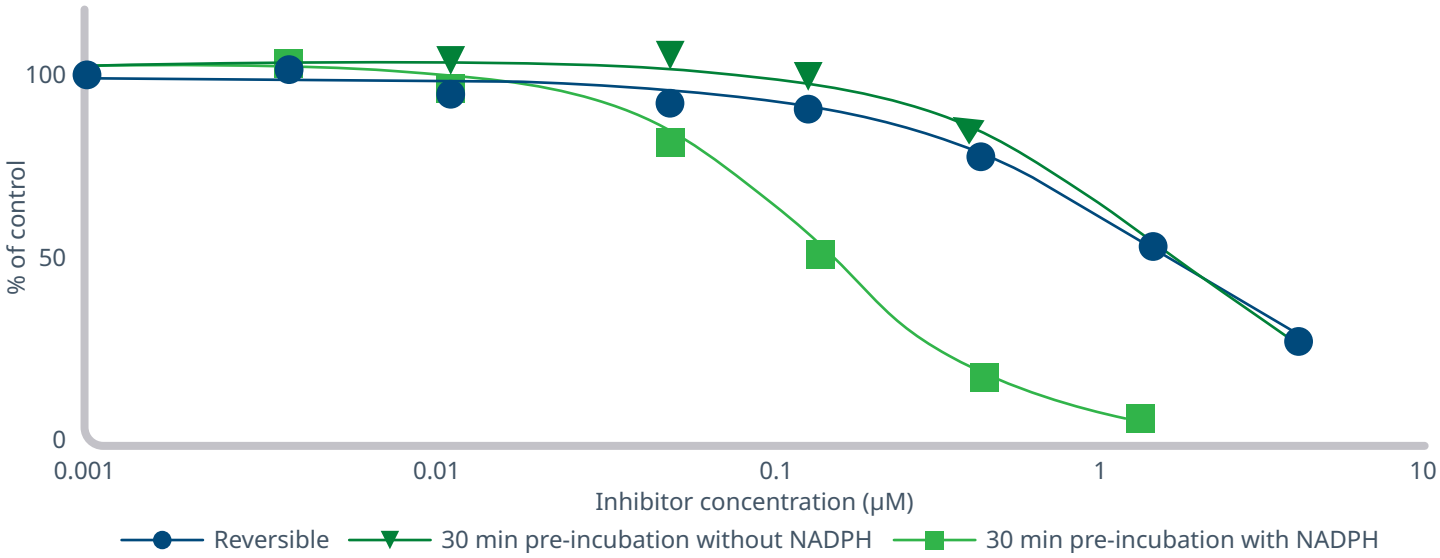
During development of New Chemical Entities (NCEs), an investigation into the likelihood of drug-drug Interactions (DDI) is vital to the progression of these drug candidates. Possible drug-drug Interactions can alter the pharmacokinetics of concomitantly administered medications, altering efficacy or toxicity. A definitive assessment of these risks should be performed early after selection of a lead candidate. Our scientists routinely evaluate these risks through validated, *in vitro* assays to determine the cytochrome P450 enzymes responsible for metabolism of NCEs and to assess the potential for NCEs to inhibit and/or induce these enzymes. These data will be included in the filing of the Investigational New Drug (IND) application to provide guideposts for clinical DDI assessments during the further development of drug candidates.

In Vitro IND-Enabling DDI Assays

Inhibition of Cytochrome P450 (CYP) enzymes

- Assess the potential of test article to inhibit the main cytochrome P450 isoforms: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (up to 3 probe substrates)
- Test article is incubated with pooled human liver microsomes (HLM) and NADPH in the presence of a cytochrome P450 isoform-specific probe substrate (~K_m)
- Optional assessment of Time-Dependent Inhibition (TDI) following pre-incubation of 30 minutes
- Sample analysis and quantitation by validated and optimized LC-MS/MS methods
- IC_{50} values are generated to assess reversible inhibition and signals for TDI (IC_{50} shift ≥ 1.5)
- If inhibition observed for any CYP enzyme, further kinetic analysis can be performed to determine the Ki value and type of reversible inhibition observed (competitive, etc.)
- Full QC reviewed, regulatory style report

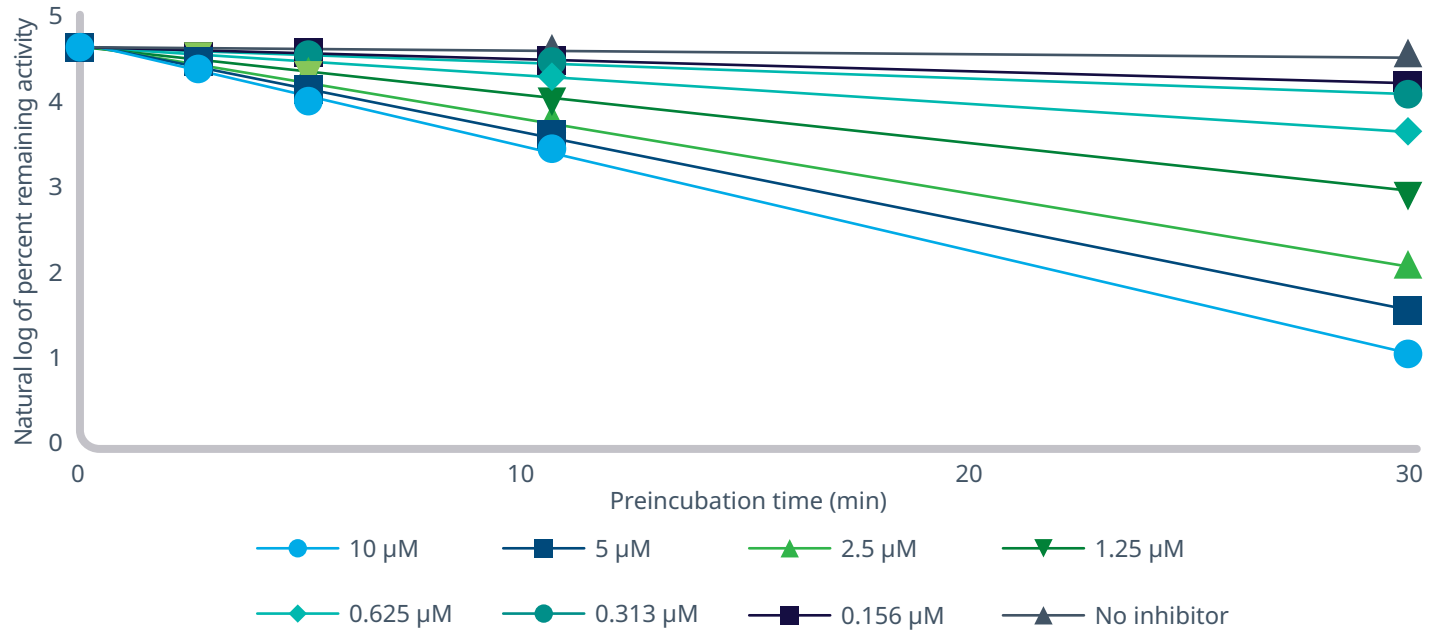
Determination of IC_{50} values



Time-dependent inhibition of CYP enzymes

- Determine the kinetics of time-dependent inhibition of the main CYP isoforms: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4
- Test article is incubated with pooled Human Liver Microsomes (HLM) and NADPH for various time points for inactivation phase before dilution into the activity assay mixture containing a cytochrome P450 isoform-specific probe substrate
- Sample analysis and quantitation by validated and optimized LC-MS/MS methods
- The percent remaining enzyme activity at each inactivation timepoint will be determined for each inhibitor concentration (k_{obs} rate determination) and used to calculate the kinetic parameters (K_I and k_{inact}) using nonlinear regression
- Full QC reviewed, regulatory style report

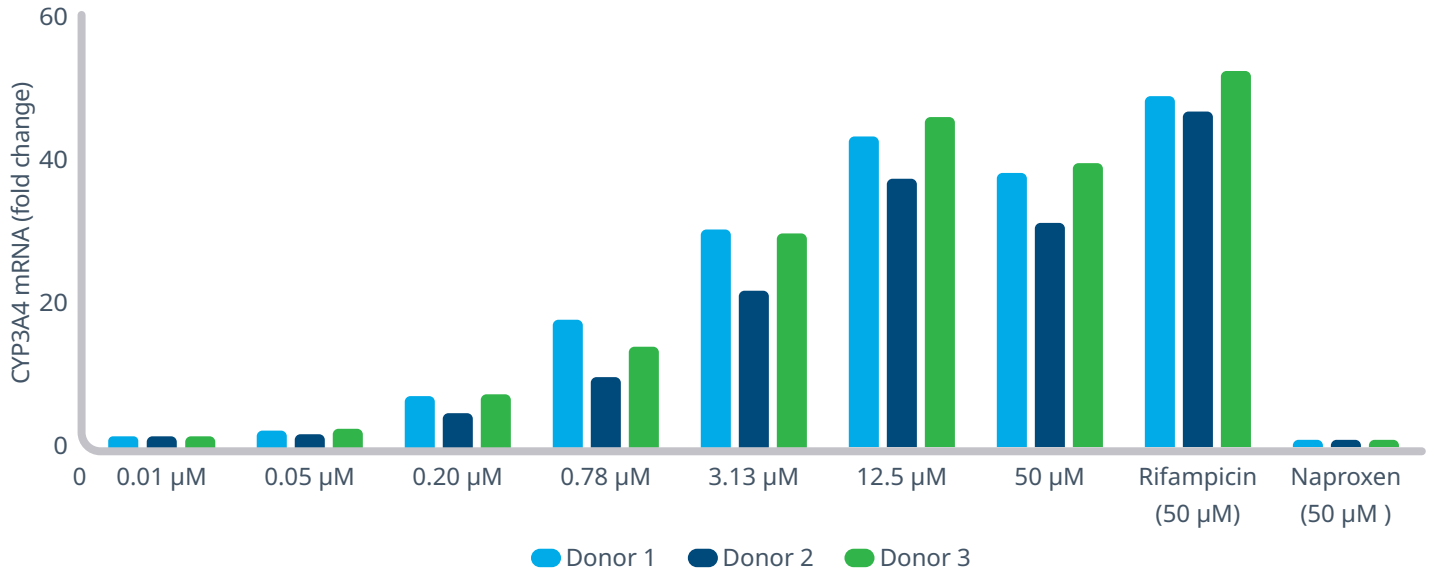
Observed loss of enzyme activity

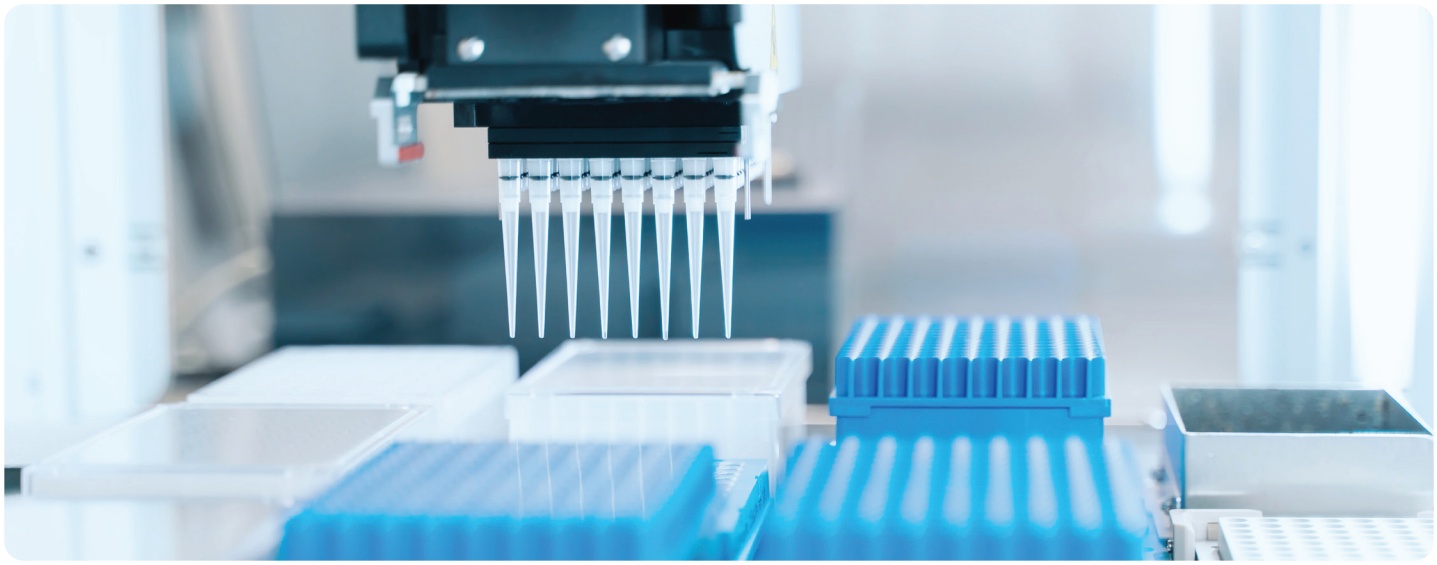


Induction of CYP enzymes

- Assess the potential of test article to induce CYP1A2, CYP2B6, and CYP3A4 through mRNA analysis and/or enzymatic activity
- Pretests to inform top concentration of test article include assessment of solubility in incubation media and cytotoxicity in a single lot of hepatocytes
- Test article is incubated with plated cryopreserved human hepatocytes pre-characterized to be responsive to CYP1A2, CYP2B6, and CYP3A4 induction before analysis of mRNA levels and/or addition of probe substrate(s) to assess enzymatic activity
- Optional determination of the induction of CYP2C8, CYP2C9, and CYP2C19 is available
- Fold-induction over vehicle control is calculated. EC_{50} and E_{max} values to be calculated if induction is concentration-dependent and >2-fold at max
- Full QC reviewed, regulatory style report

mRNA expression levels

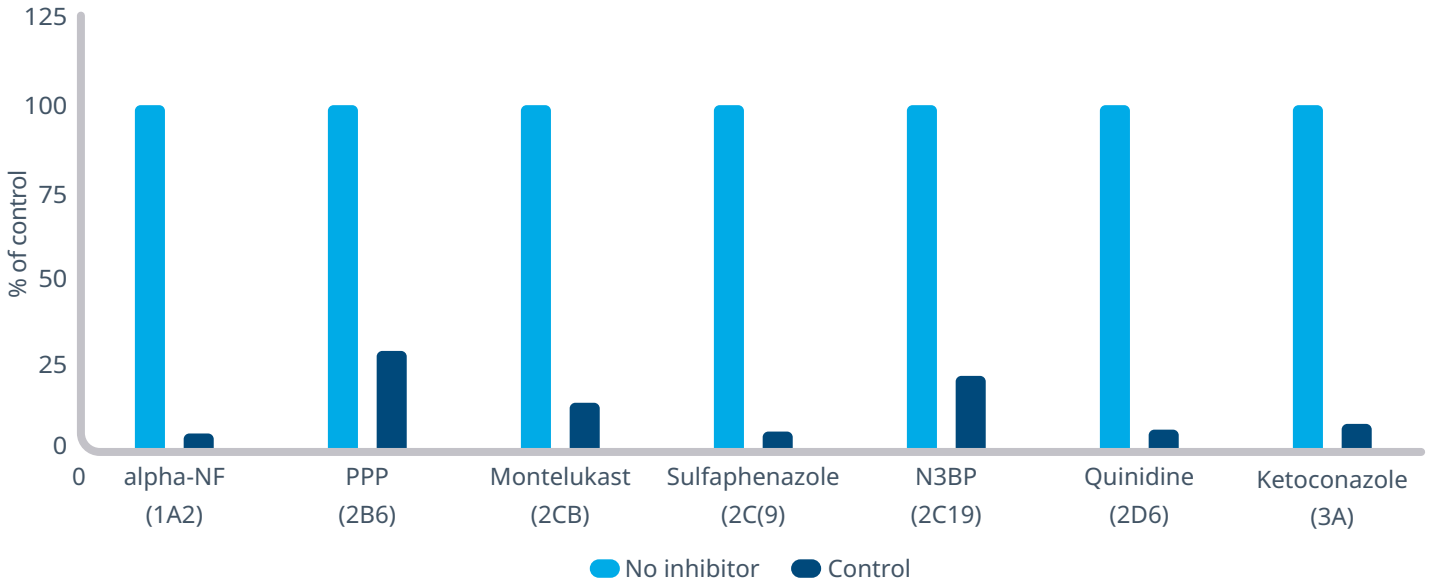




CYP phenotyping

- Determine the CYP enzymes involved in the metabolism of a test article using two complementary assay designs (Recombinant CYP phenotyping and CYP Inhibition using CYP specific chemical inhibitors)
- Evaluating the percent contribution of CYP enzymes in the metabolism of the test article using recombinantly expressed CYP enzymes
- Individual CYP Relative Activity Factors (RAF) will be used to estimate fraction metabolized by each CYP enzyme
- Fraction metabolized by each CYP enzyme will be calculated ($f_{m,CYP}$)
- Evaluating the percent contribution of each of CYP enzymes in the metabolism of the test article by sequentially inhibiting CYP activity
- Test article is incubated with HLM and NADPH in the absence and presence of selective CYP-specific inhibitors
- Full QC reviewed, regulatory style report

Reaction phenotyping with chemical inhibitors



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