

Cost-effective *In Vitro* Methods for Evaluating the DDI Potential in Drug Discovery

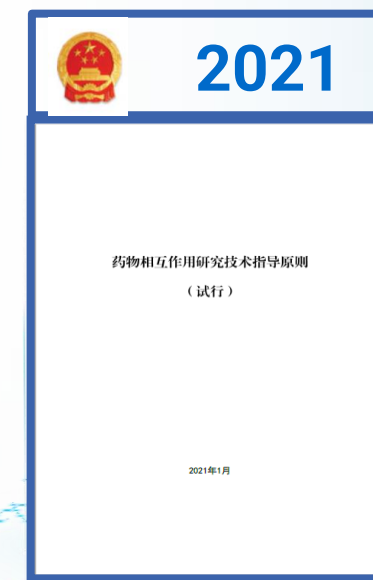
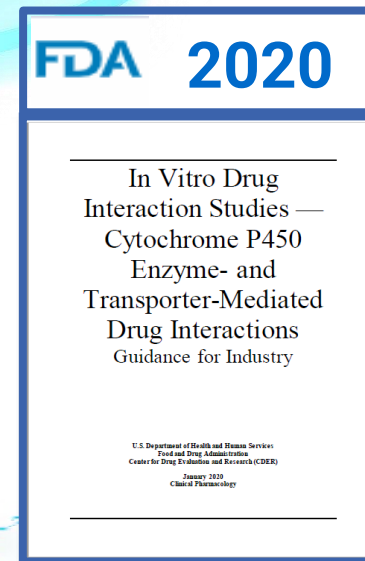
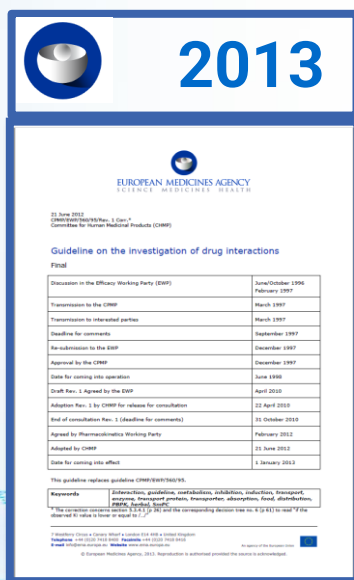
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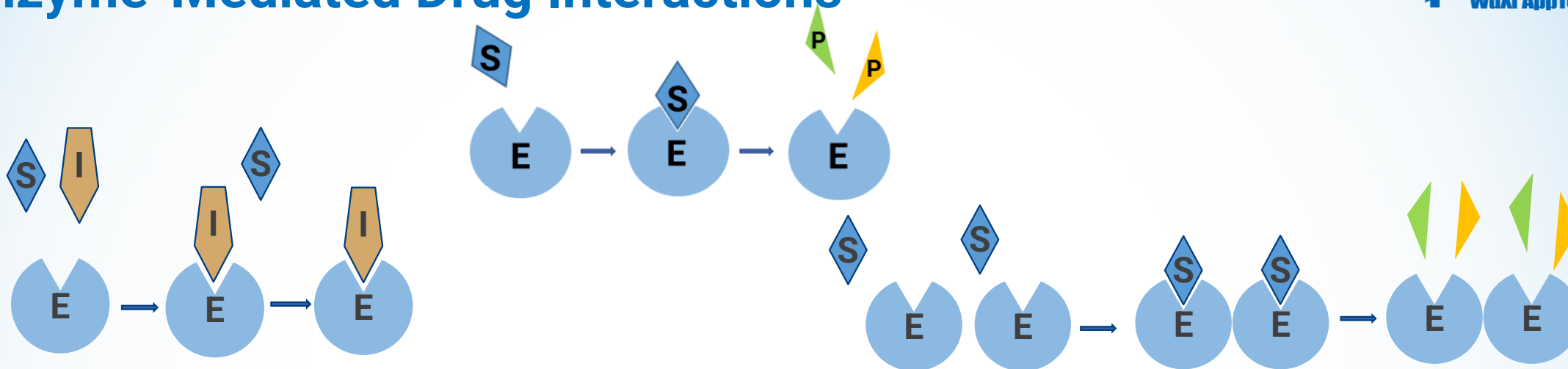
Email: jiang_lifang@wuxiapptec.com



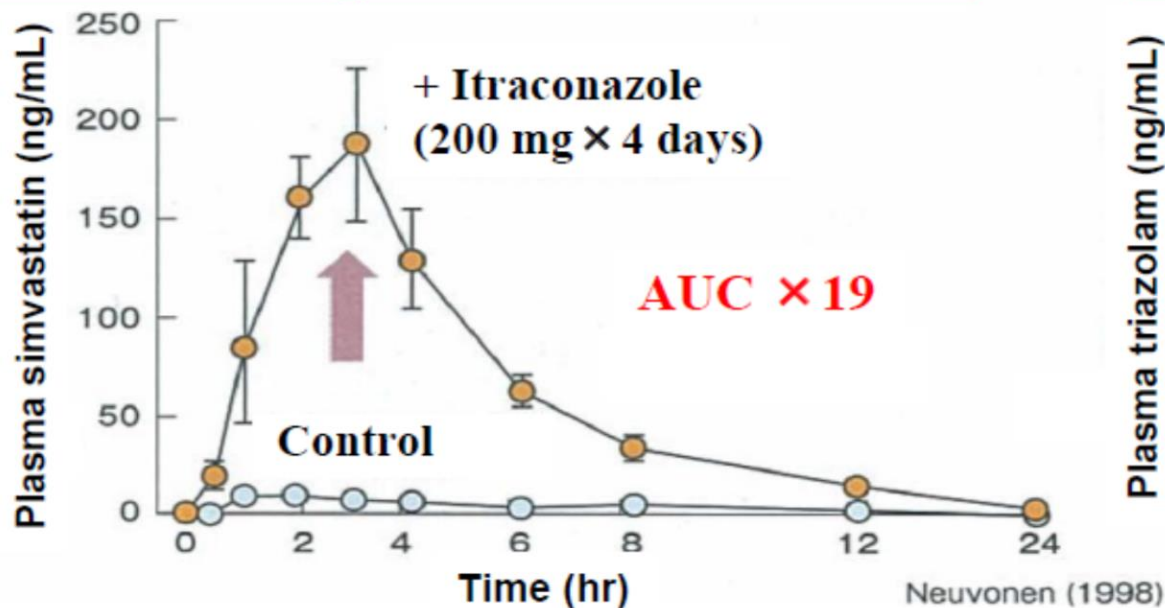
A Harmonized Approach to Assessing DDI: ICH M12



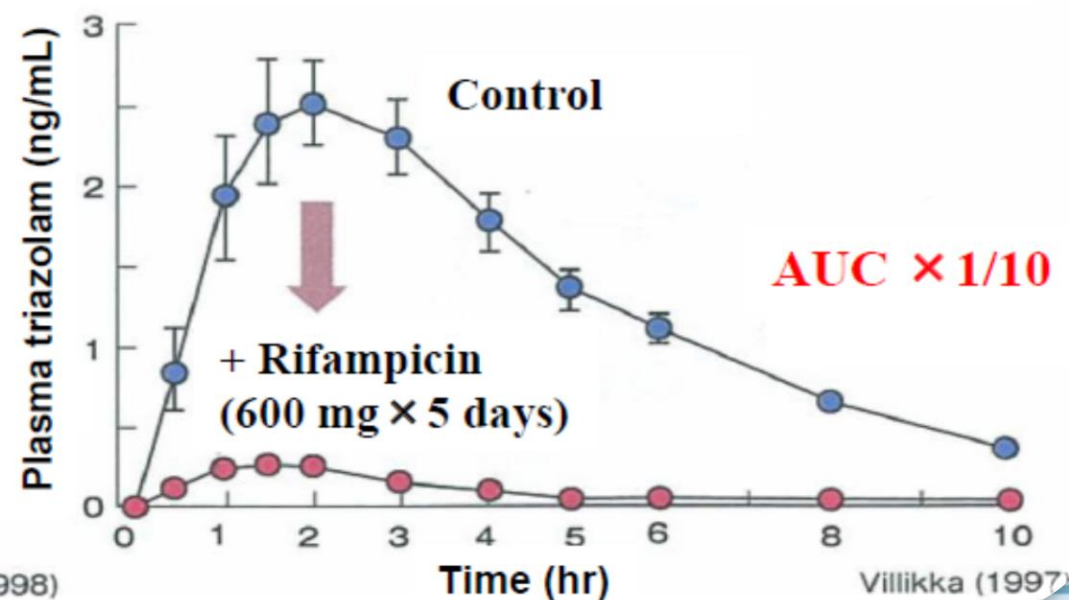
Enzyme-Mediated Drug Interactions



DDI involving CYP3A4 inhibition



DDI involving CYP3A4 induction



Is the Dilution Method a More Sensitive Approach for Evaluating Time-dependent Inhibition?

For example, TDI can be detected by assessing a difference in IC_{50} curves generated with and without a pre-incubation with nicotinamide adenine dinucleotide phosphate (NADPH) (i.e., IC_{50} shift), decreases in enzyme activity (measurement of the pseudo first-order rate constant, k_{obs}) or percent activity loss with the inactivator over time (also called **standard dilution methods**).

-----ICH M12 Draft version Endorsed on 24 May 2022 Currently under public Consultation

Perspective

The Conduct of in Vitro Studies to Address Time-Dependent Inhibition of Drug-Metabolizing Enzymes: A Perspective of the Pharmaceutical Research and Manufacturers of America

Scott W. Grimm, Heidi J. Einolf, Steven D. Hall, Kan He, Heng-Keang Lim, Kah-Hiing John Ling, Chuang Lu, Amin A. Nomeir, Eleanore Seibert, Konstantine W. Skordos, George R. Tonn, Robert Van Horn, Regina W. Wang, Y. Nancy Wong, Tian J. Yang, and R. Scott Obach

Dilution methods 50%
vs
Non-dilution methods 50%



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Overview of comments received ICH Harmonized Guideline Drug Interactions M12
(EMA/CHMP/ICH/652460/2022)

Please note that comments will be sent to the ICH M12 EWG for consideration in the context of Step 3 of the ICH process.

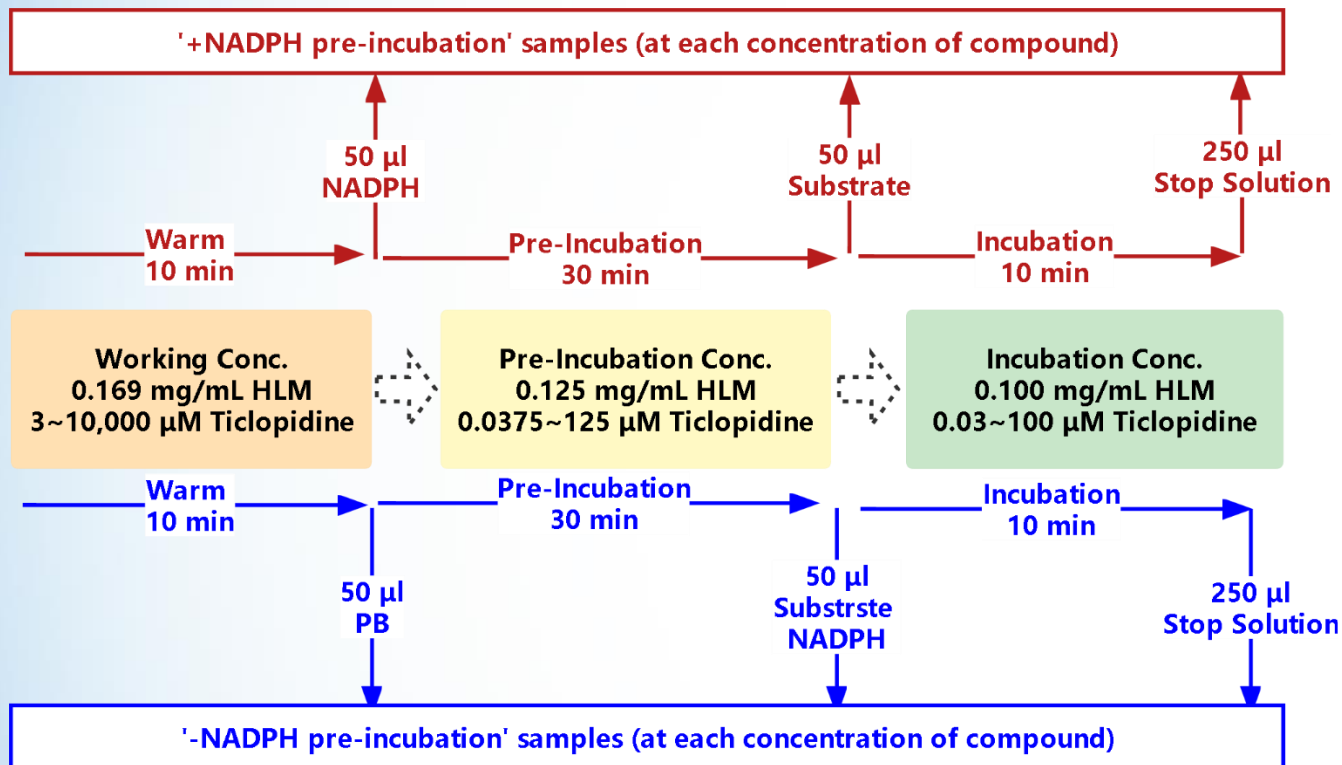
1. General comments – overview

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
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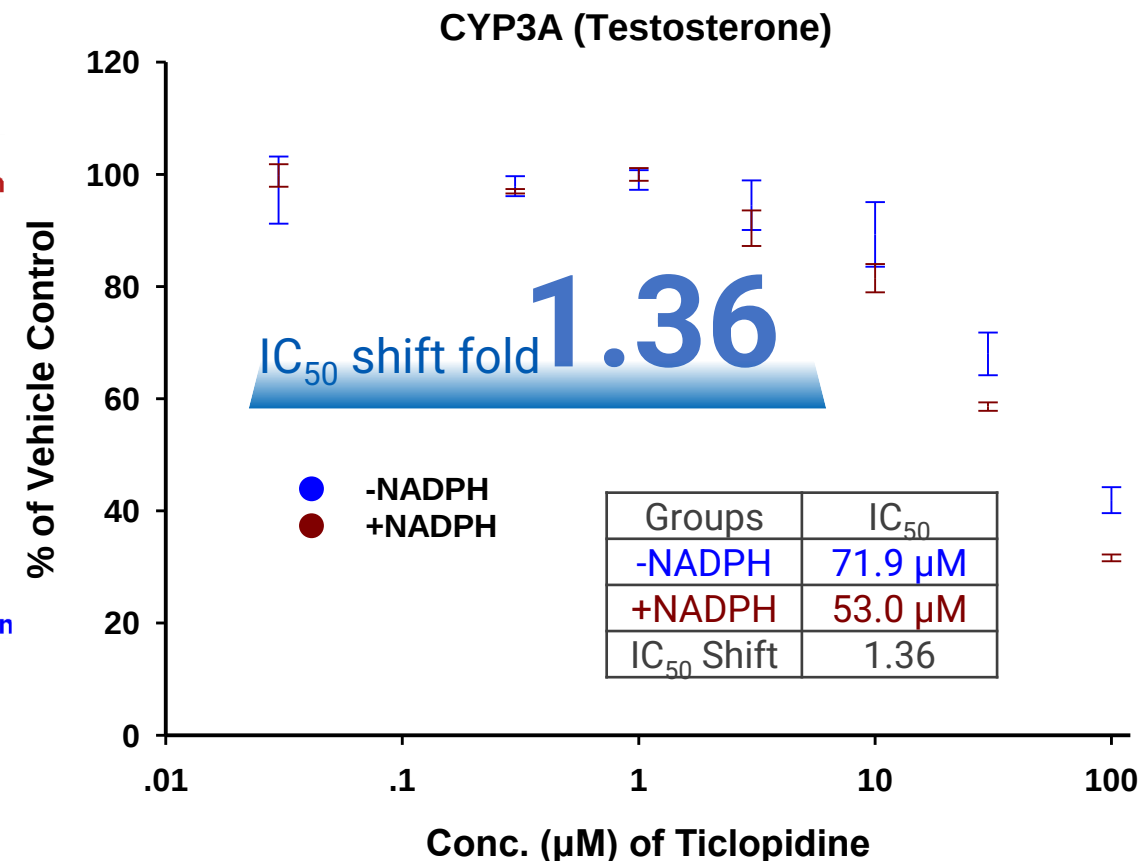
4 Comments

Evaluating CYP3A (Testosterone) Time-Dependent Inhibition (IC_{50} Shift Assay): Non-dilution Method

Experimental diagram



Results

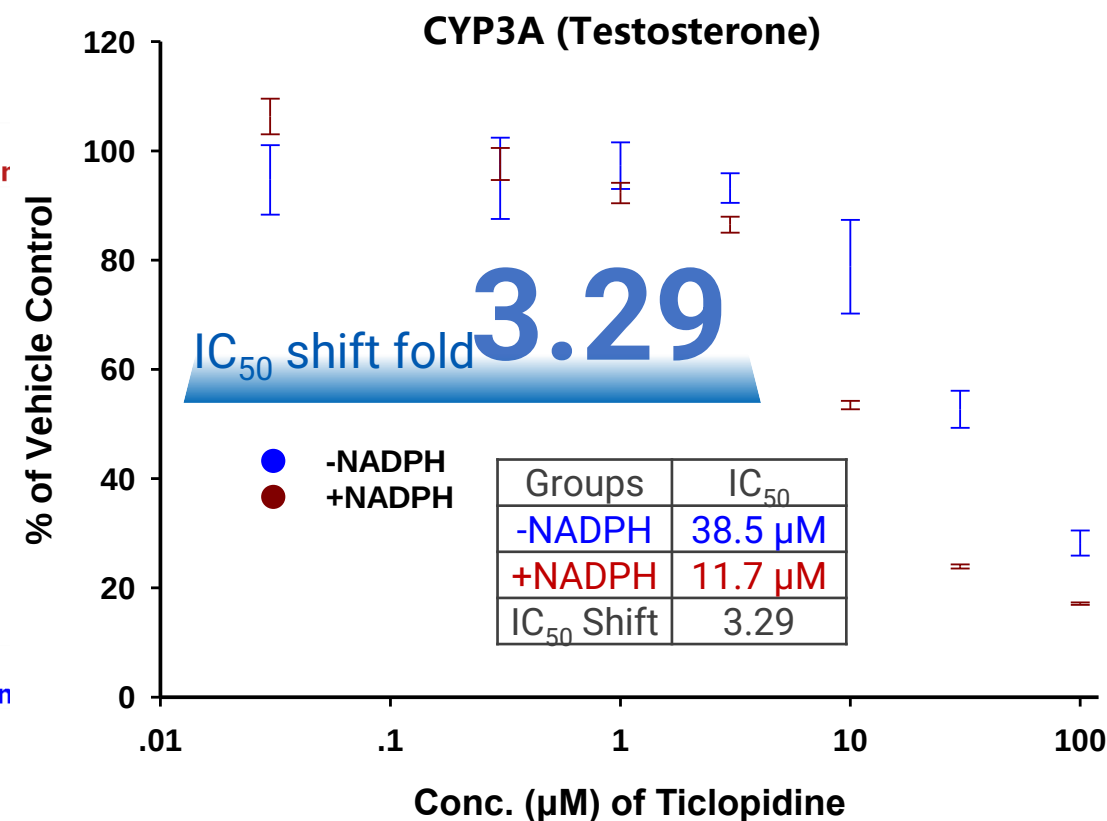
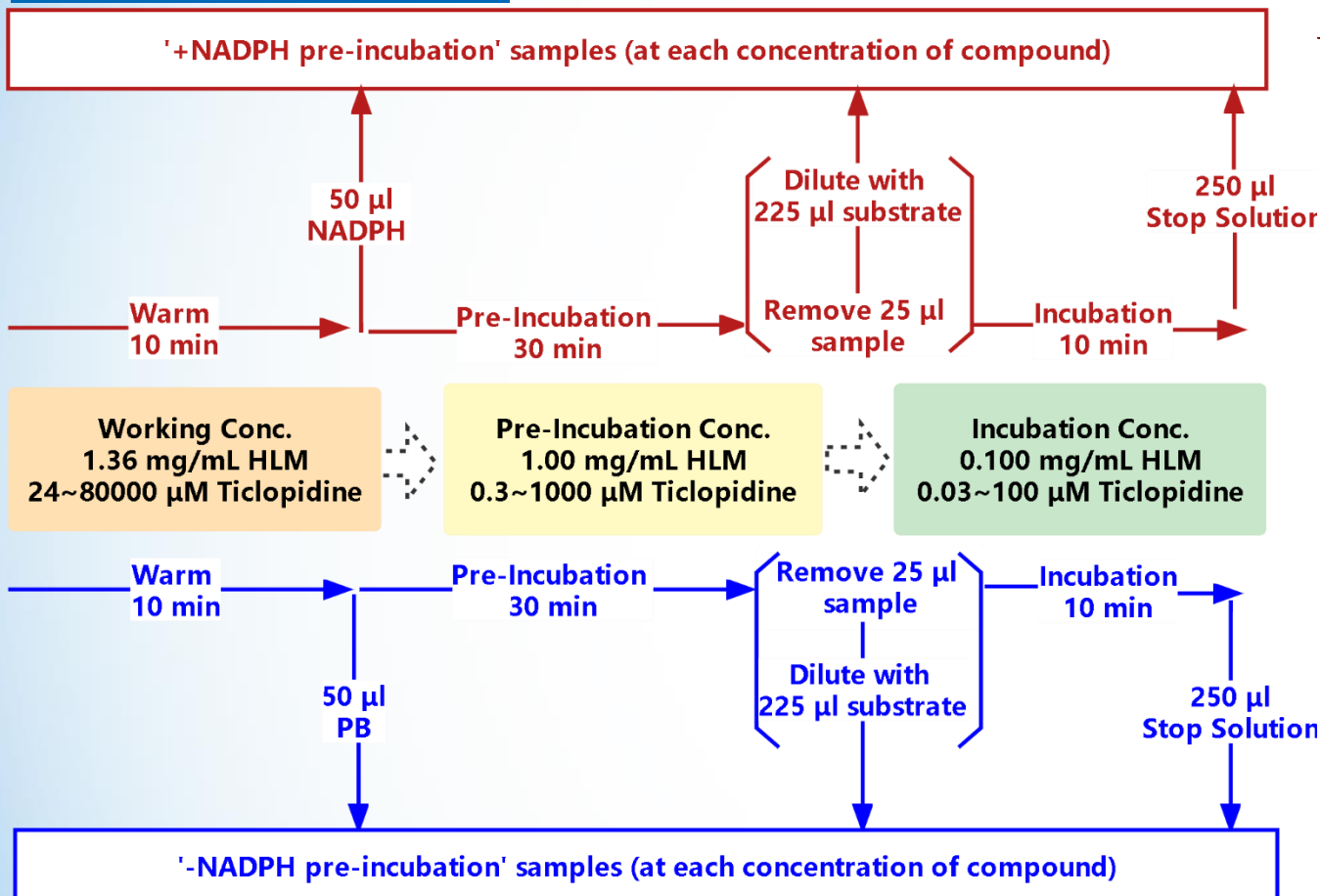


- Conc.: concentration
- HLM: human liver microsomes
- PB: potassium phosphate buffer
- Ticlopidine: a known time-dependent inhibitor for CYP2B6 & CYP2C19, as a negative control for CYP3A (IC_{50} shift cut off value of 1.5)

Evaluating CYP3A (Testosterone) Time-Dependent Inhibition (IC_{50} Shift Assay): Dilution Method

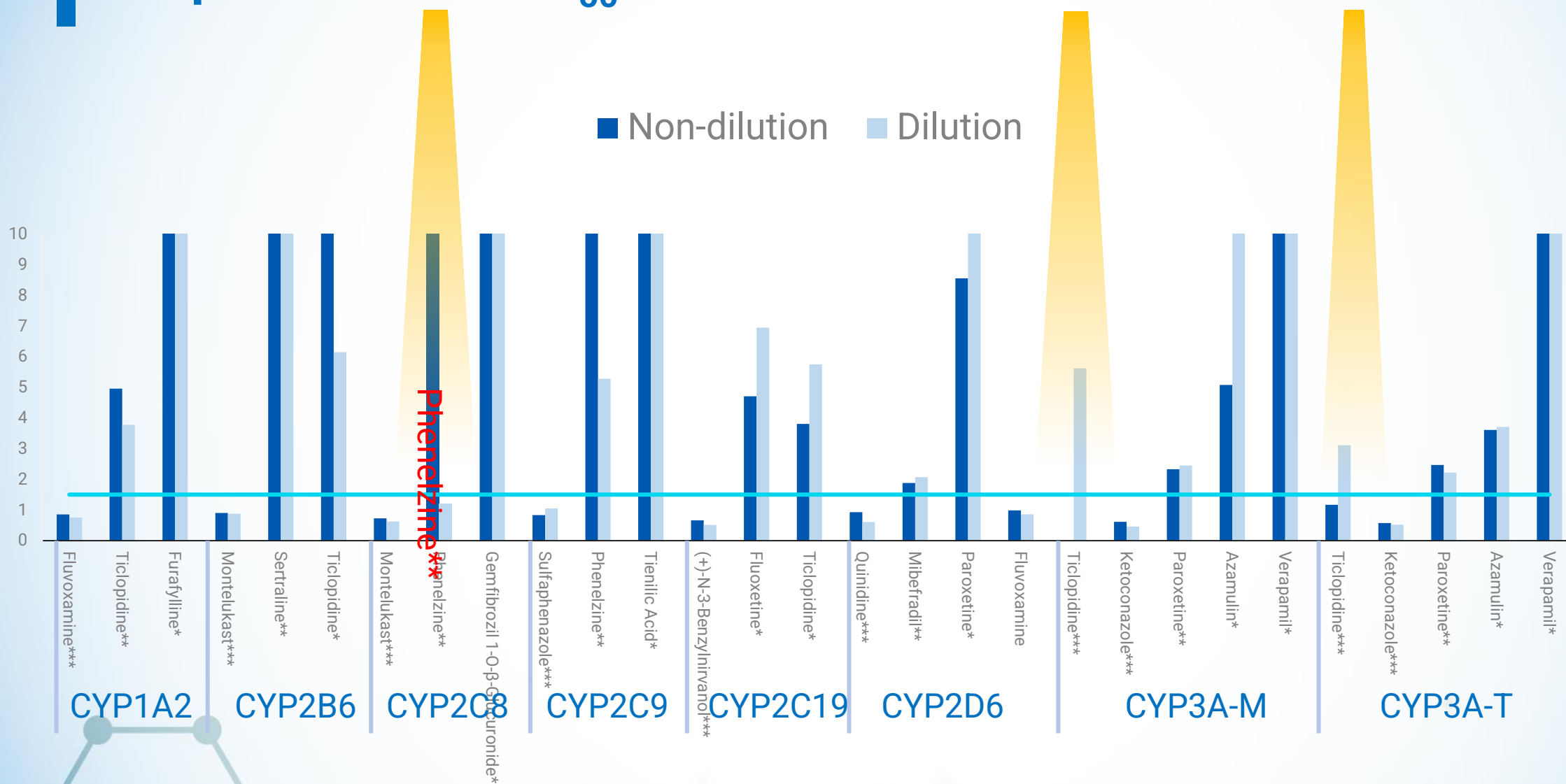
Experimental diagram

Results



- Ticlopidine: a known time-dependent inhibitor for CYP2B6 & CYP2C19, but not for CYP3A
- An IC_{50} shift value of 3.29 in the dilution method will classify ticlopidine as a CYP3A time-dependent inhibitor with the cut off value of 1.5

Comparison of the IC₅₀ Shift Fold: Non-dilution vs Dilution



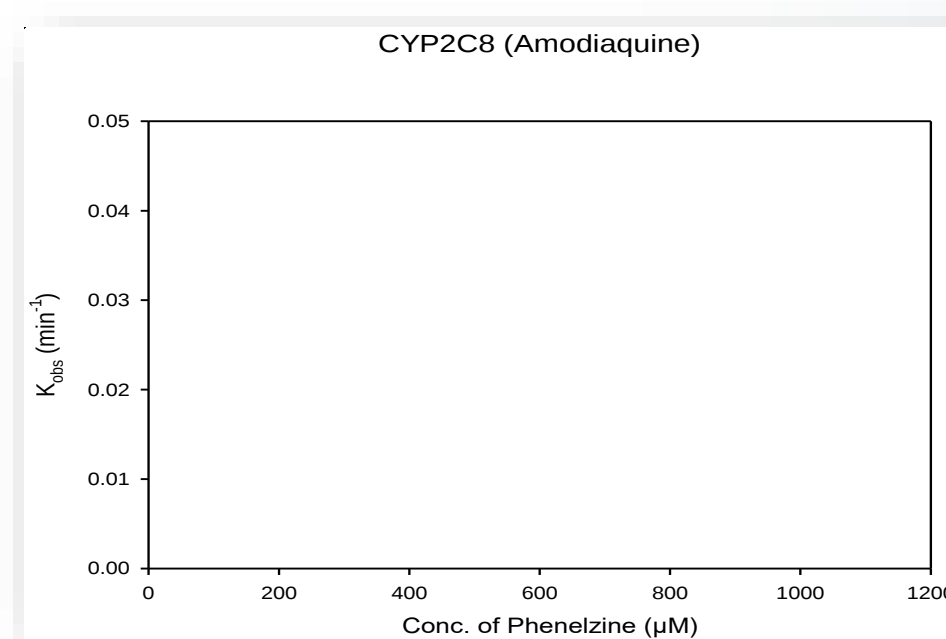
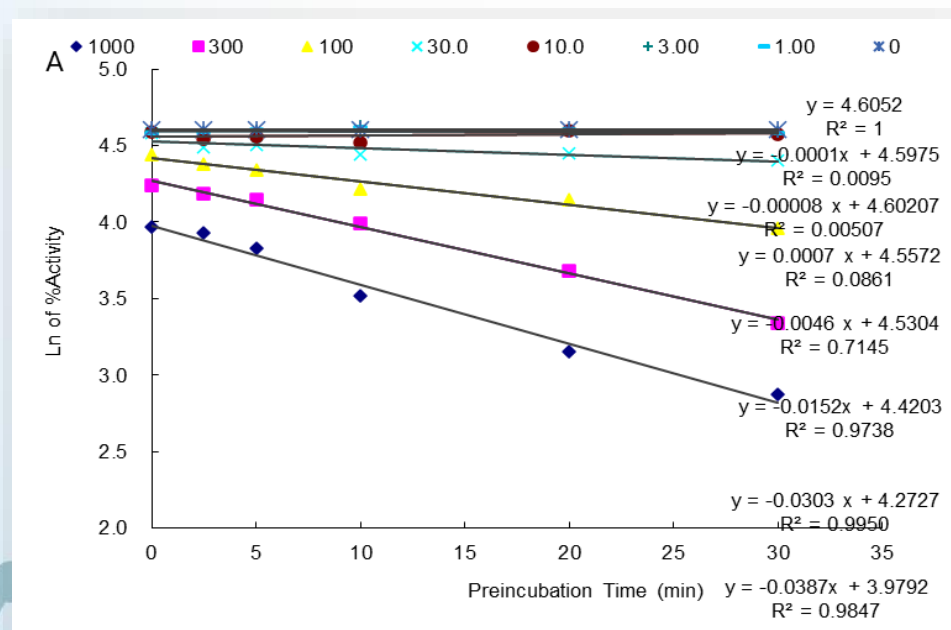
* Potent time-dependent inhibitor ** Moderate/Weak time-dependent inhibitor *** Non time-dependent inhibitor

Confirmation of the Time-dependent Inhibition

Inhibitor	IC ₅₀ Shift Fold	
	Non-Dilution	Dilution
Phenelzine	14.1 (+)	1.21 (-)

k_{inact}/K_i determination

The kinetic study indicated that the non-dilution method yields more accurate results



- Phenelzine: a known time-dependent inhibitor for CYP2C8

Results of TDI Evaluation: Non-dilution vs Dilution

Poster#: P129

Kappa Coefficient

Non-dilution		<i>In Vivo</i>		
	Classification	+	-	Total
<i>In Vitro</i>	+	18	0	18
	-	0	11	11
	Total	18	11	29
Kappa Value	1			
	Note: "+" means "TDI", "-" means "No TDI"			

Dilution		<i>In Vivo</i>		
	Classification	+	-	Total
<i>In Vitro</i>	+	17	2	19
	-	1	9	10
	Total	18	11	29
Kappa Value	0.897			
	Note: "+" means "TDI", "-" means "No TDI"			

The non-dilution method could generate higher accuracy with less cost.

For example, TDI can be detected by assessing a difference in IC_{50} curves generated with and without a pre-incubation with nicotinamide adenine dinucleotide phosphate (NADPH) (i.e., IC_{50} shift), decreases in enzyme activity (measurement of the pseudo first-order rate constant, k_{obs}) or percent activity loss with the inactivator over time. **Dilution and non-dilution** methods can be used for the IC_{50} shift assay.

UGT Inhibition Evaluation in HLMs

Poster#: P130

UGT Enzyme	Substrate	Inhibitor	IC ₅₀ (μM)	HLM Conc. (mg/mL)
UGT1A1	Bilirubin	Nilotinib	0.0809-0.122	0.01
	β-Estradiol	Nilotinib	0.0571-0.0627	0.1
UGT1A3	Telmisartan	Zafirlukast	0.651-0.993	0.1
UGT1A4	Trifluoperazine	Hecogenin	0.180-0.194	0.05
UGT1A6	Serotonin	Zafirlukast	1.04-1.33	0.05
UGT1A9	Propofol	Magnolol	0.0111-0.0261	0.025
UGT2B7	Zidovudine	Zafirlukast	21.2-23.7	0.1
UGT2B10	Cotinine	Desloratadine	3.17-4.16	0.1

Reduced costs compared with using human recombinant UGTs.
An excellent tool to evaluate UGT inhibition in early discovery stage.

Capabilities for Evaluating Enzyme-Mediated DDIs

Enzyme Phenotyping

- CYP enzymes
 - Common CYP enzymes
 - Other CYP enzymes,
CYP2A6, CYP2E1, CYP2J2, and CYP4F2
- Other Phase 1 enzymes
ADH/ALDH, AO, CES, FMO, MAO, and XO
- Potential UGTs
 - UGT1A1, 1A3, 1A4, 1A6,
1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, 2B15, and 2B17

Inhibition Evaluation

Reversible inhibition

- CYP inhibition (15 isoforms)
 - 5 in 1 cocktail
 - 7 in 1 cocktail**
 - Discrete incubation
 - K_i value determination
- UGT Inhibition
 - using rhUGTs (**13 isoforms**)
 - using HLM (7 isoforms)
- Other enzyme inhibition

Time-dependent inhibition

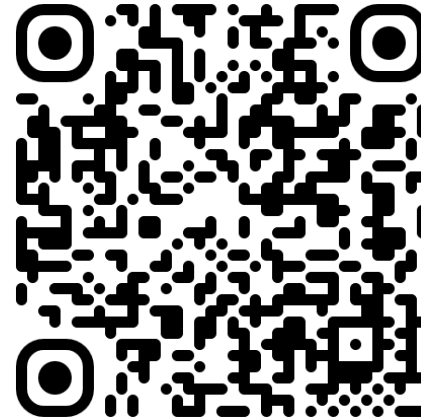
- IC_{50} shift/AUC shift
- k_{inact}/K_i Determination

Induction Evaluation

- CYP enzymes
 - CYP1A2, CYP2B6, CYP3A4
 - CYP2C8, CYP2C9, CYP2C19
- Potential UGTs
 - UGT1A1 UGT2B7
- Mechanistic static Models
 - Basic 'mRNA Fold-Change' Method
 - Correlation Methods (**RIS score**)
 - Basic Kinetic Model

Questions

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<https://dmpkservice.wuxiapptec.com/>



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