Discovery Research

In-Vitro ADME Capabilities

Discovery Research
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Overview

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Capabilities

Absorption	Solubility (Kinetic), Lipophilicity (LogD7.4), Permeability (PAMPA and Caco-2) and Efflux assays (Caco-2)
Distribution	Protein binding (plasma, brain homogenate, and microsomes), Blood to plasma partitioning (Species: rat, dog, and human)
Metabolism	Metabolic stability and intrinsic Clearance (S9, microsomes, rP450, hepatocytes), Reaction phenotyping (CYP and non-CYP, Enzyme kinetics, Chemical inhibition method and RAF), Metabolite identification and GSH trapping
Drug-Drug Interactions	Direct inhibiton (IC_{50} and Ki), Time dependent inhibition (Single point, IC_{50} shift, KI and Kinact, Dialysis) CYP Induction (mRNA expression and enzyme activity) Uptake transporters: substrate and inhibition assays (OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE-1 and MATE-2K)

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Solubility and Distribution coefficient

Kinetic solubility can help interpret the complication arising from the compound precipitation during biochemical, functional, and cell based assays
Identify poor soluble compounds that reduce productivity in drug discovery and development

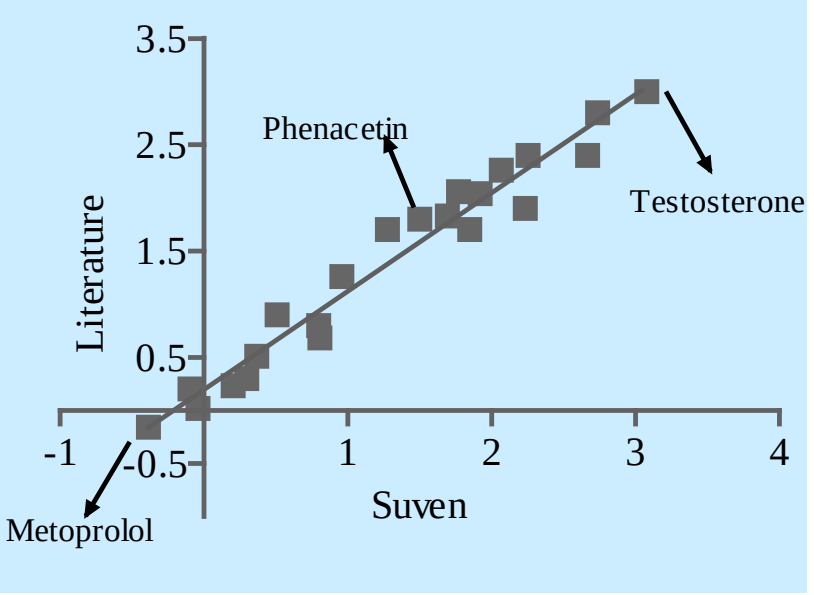
Kinetic solubility

- Phosphate buffer 7.4
- Simulated gastric fluid
- Simulated intestinal fluid

Lipophilicity is a key factor in determining the permeation of physiological membrane, protein binding, and target affinity

Log D 7.4

- Miniaturized shake-flask method
- n-Octanol / Phosphate buffer 7.4
- Cyclohexane / Phosphate buffer 7.4



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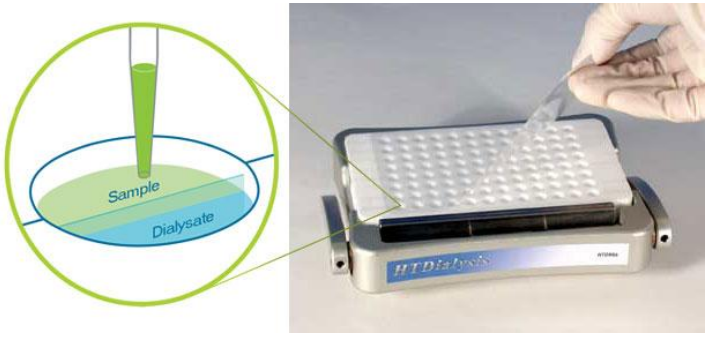
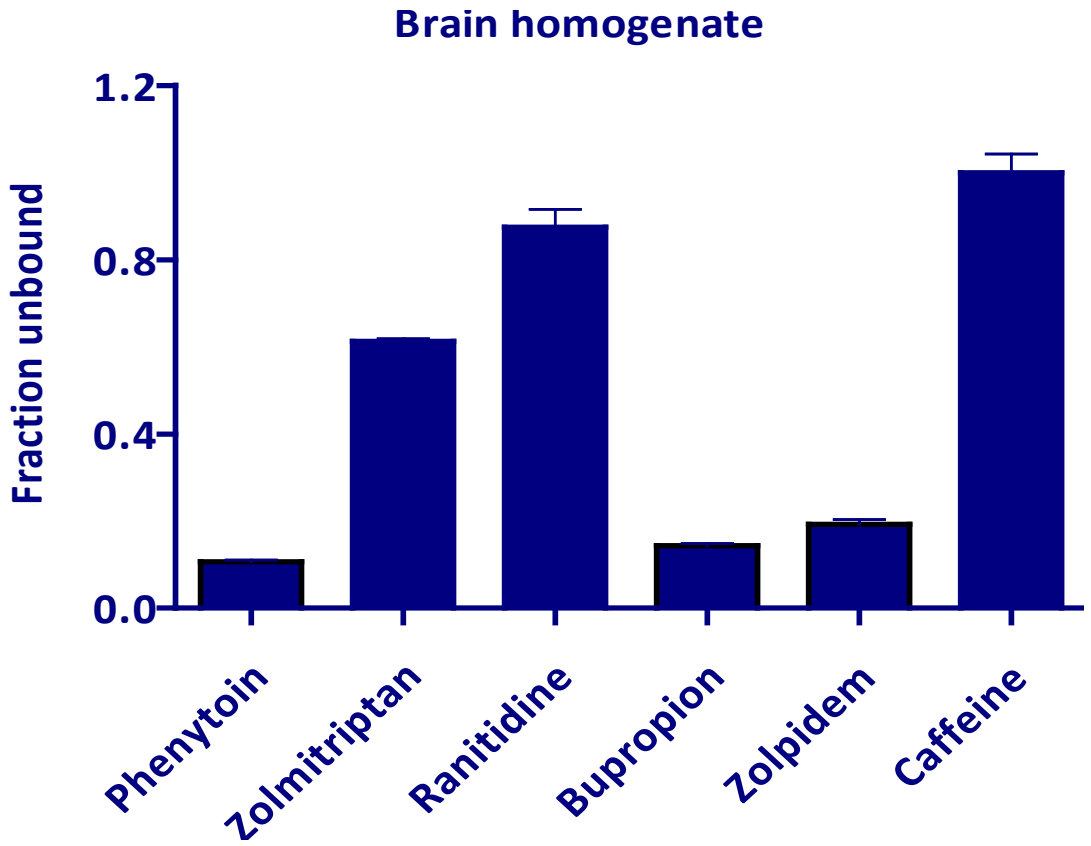
Unbound fraction (plasma/brain homogenate/microsomes)

Knowledge of Unbound fraction is essential to correct the total plasma concentration and correlate with efficacy





Rapid Equilibrium Dialysis (RED) and High Throughput (HT) Dialysis

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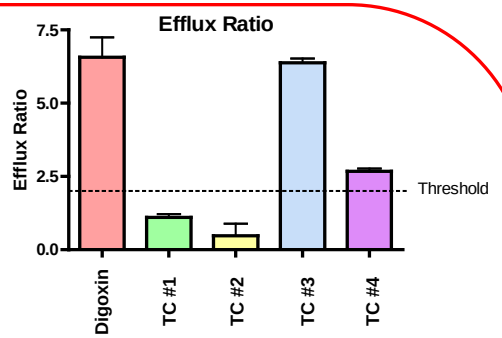
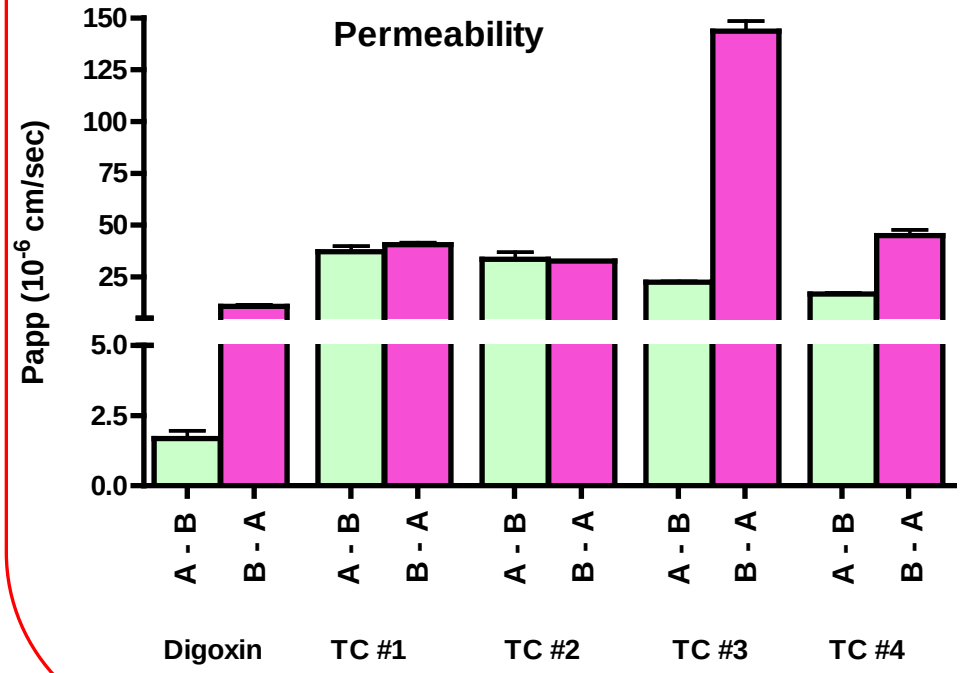
P-glycoprotein (P-gp) Substrate assessment

A net flux ratio (or efflux ratio (ER)) of ≥ 2 in cells that express P-gp (e.g., Caco-2 cells) suggest that New chemical entity is a P-gp substrate

Test System:	Caco-2
Test Conc.:	10 µM
Incubation:	60 min, 37 °C
Positive control	Digoxin
Membrane	TEER & Lucifer Yellow
Analysis	LC-MS/MS





TC: Test compound

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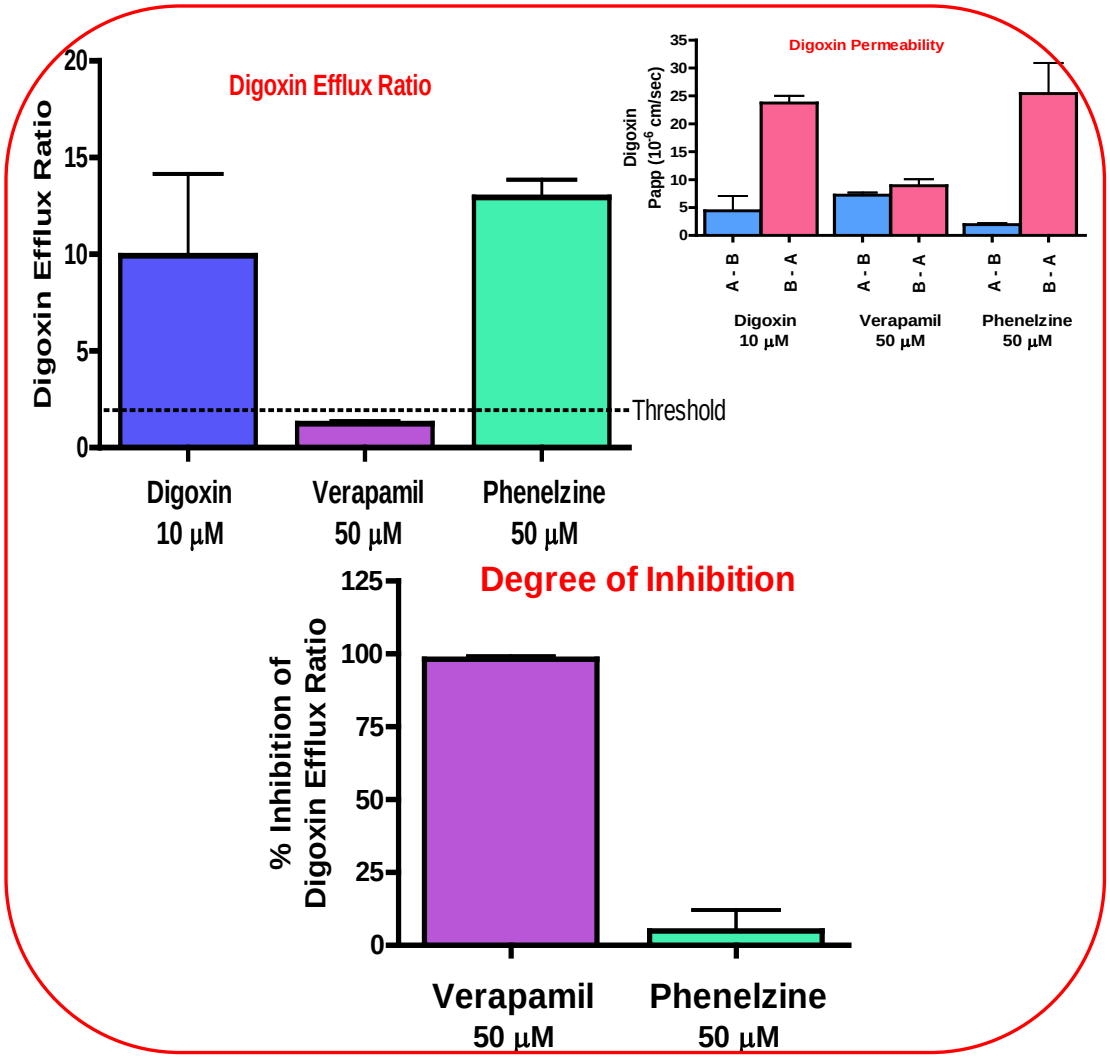
P-glycoprotein (P-gp) Inhibitor assessment

Test System	Caco-2
Test Concentration	50 µM
Positive control	Verapamil
Negative Control	Phenelzine
P-gp Substrate	Digoxin
Incubation	1 hour
Membrane Integrity	TEER & Lucifer Yellow
Analysis	LC-MS/MS



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Phenotyping (Chemical inhibition method and RAF)

Contribution ≥ 25% by an enzyme is considered significant based on in vitro phenotyping studies and Human Pharmacokinetic study

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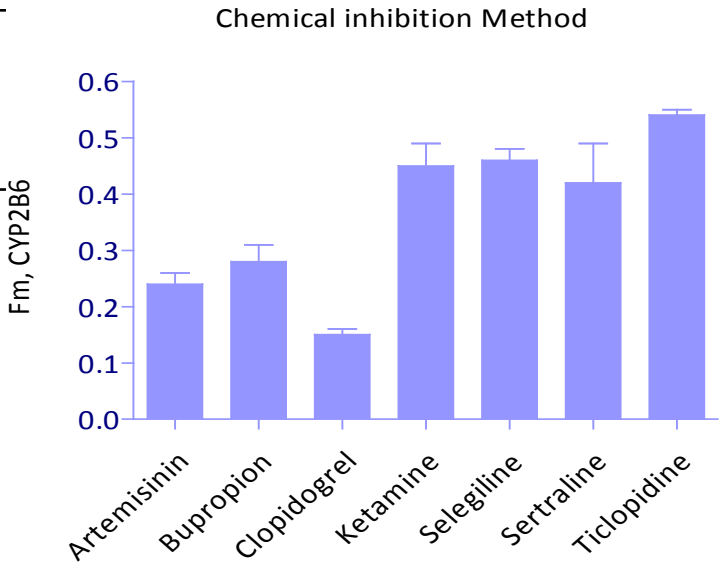
Evaluate the role of CYP1A2, CYP2B6, 121 CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 and additional enzymes including MAO, FMO, and UGT

↓

In vitro Phenotyping studies include chemical inhibition and Metabolism in recombinant enzymes (RAF approach)

P450	HLM	rCYP	CL-RAF (pmol.mg^{-1})	Mean CYP abundance (pmol.mg^{-1})	CL-ISEF
	CL_{int} (uL.min^{-1}.mg^{-1})	CL_{int} (uL.min^{-1}.pmol CYP^{-1})			
1A2	7 ± 0.3	1.2 ± 0.05[a]	5.6 ± 0.5	39	0.14 ± 0.01
2A6	1298 ± 58	23 ± 0.5	56.9 ± 3	27	2.11 ± 0.1
2B6	3 ± 0.2	0.13 ± 0.01	19.8 ± 1	16	1.24 ± 0.07
2C8	777 ± 38	13 ± 2	58.8 ± 6	22.4	2.62 ± 0.3
2C9	96 ± 6	4.3 ± 0.1	21.2 ± 1	61	0.35 ± 0.01
2C19	0.4 ± 0.1	0.15 ± 0.01	2.8 ± 0.4	11	0.25 ± 0.04
2D6	33 ± 0.3	32 ± 1	1.0 ± 0.03	12.6	0.08 ± 0.002
2 E1	8 ± 0.3	0.13 ± 0.001	62.0 ± 2	64.5	0.96 ± 0.03
3A4	399 ± 33	18 ± 1.3	22 ± 1	93	0.24 ± 0.01



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Enzyme Kinetics and CYP Inhibition

CYP Inhibition (IC_{50} and Ki)
CYP1A2, CYP2B6, 121 CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5

↓

Basic Model (I/Ki) or Mechanistic Static Model to predict drug interaction risk

Validated marker activities for major CYP isoforms in HLM and rCYP

P450	HLM			rCYP		
	K_m (uM)	Vmax (pmol.min^{-1}.mg^{-1})	Kinetic Mechanism	K_m uM	Vmax (pmol.min^{-1}.pmolCYP^{-1})	Kinetic Mechanism
1A2	59 ± 3	395 ± 10	MM	82 ± 5.9[a]	31 ± 2.7	NC
2A6	0.5 ± 0.07	649 ± 10	MM	0.5 ± 0.004	11.4 ± 0.3	MM
2B6	70 ± 3	187 ± 7	SI	88.4 ± 10.6	11.9 ± 0.5	SI
2C8	2.7 ± 0.2	2097 ± 51	MM	0.5 ± 0.1	6.6 ± 0.1	MM
2C9	9.7 ± 0.5	933 ± 17	MM	1.1 ± 0.04	5.0 ± 0.1	MM
2C19	92 ± 8	38 ± 2	MM	13.4 ± 0.7	2.0 ± 0.01	MM
2D6	4.6 ± 0.1	152 ± 4	MM	0.2 ± 0.1	6.3 ± 0.1	MM
2 E1	177 ± 9	1384 ± 61	MM	101.5 ± 2.2	12.8 ± 0.1	MM
3A4	3.0 ± 0.2	1197 ± 63	MM	3.1 ± 0.2	56.1 ± 0.9	SI

[a]- S50 instead of Km; [b] - Negative co-operativity, clearance read from plot of v/[S] vs [S];

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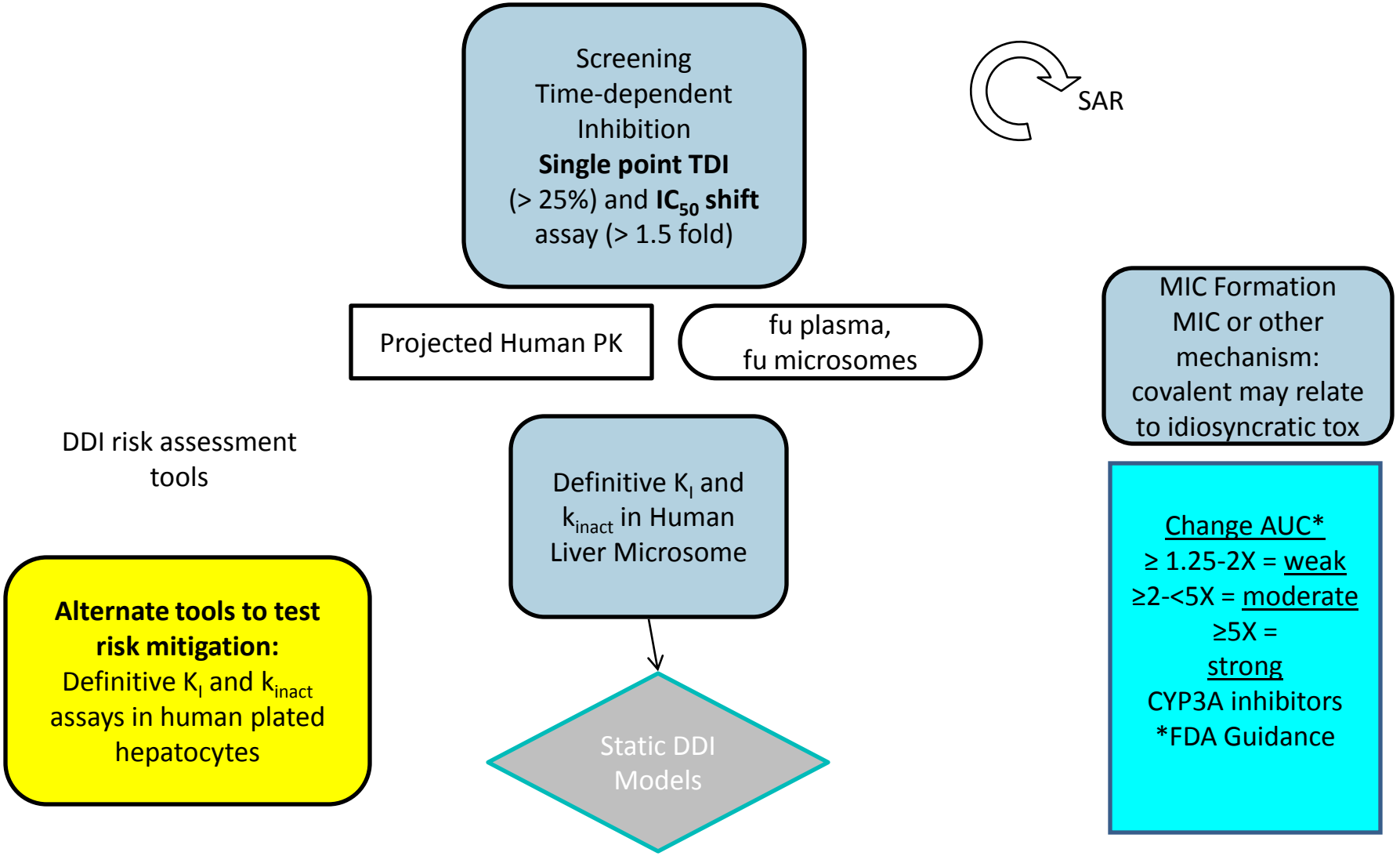
Time Dependent Inhibition



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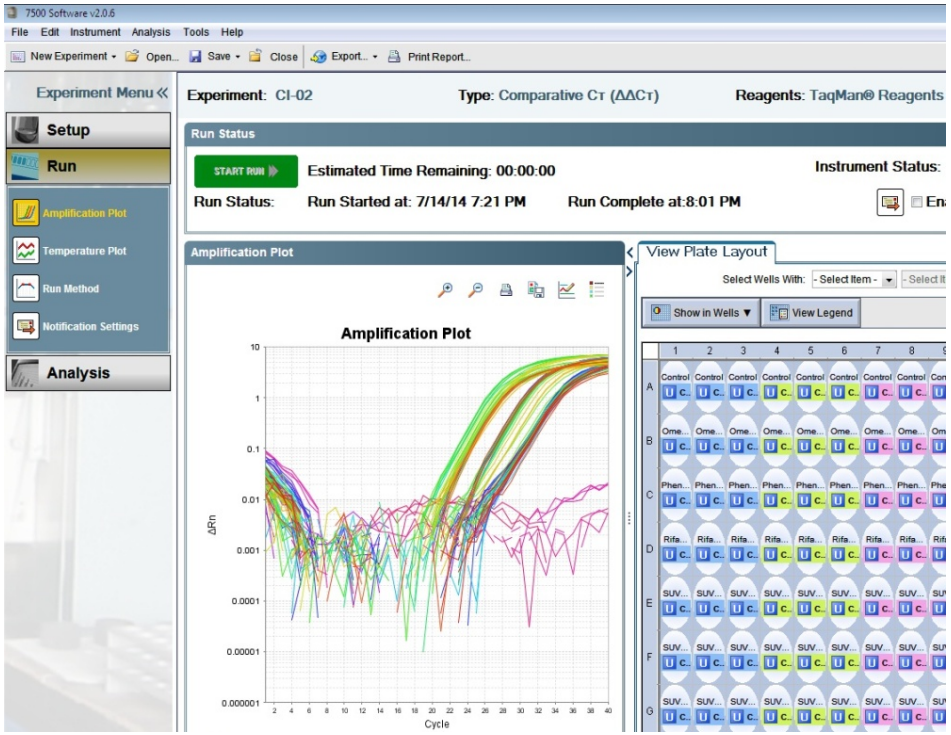
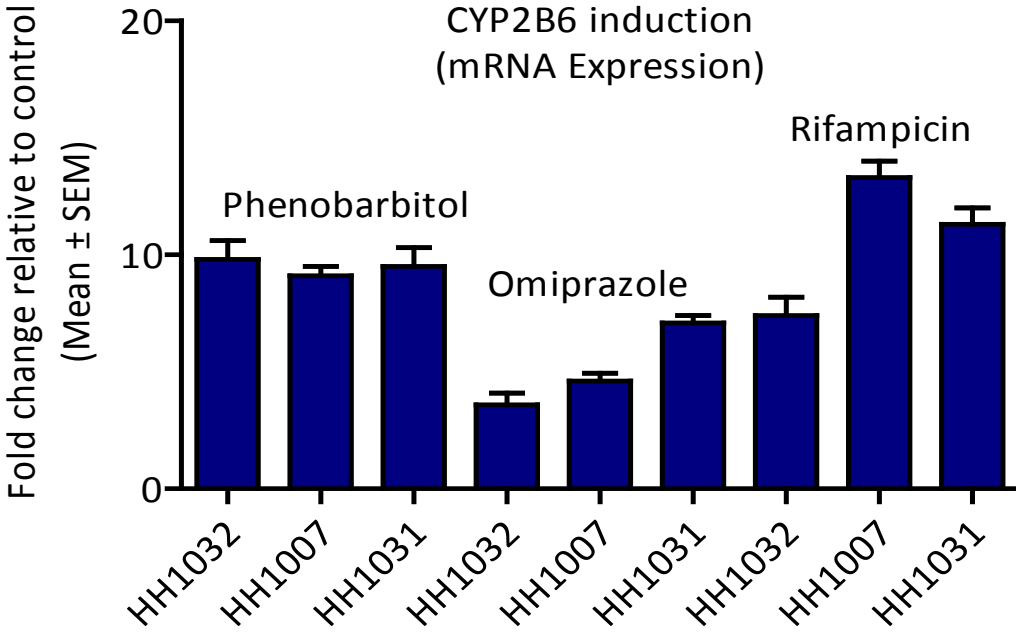
CYP Induction





CYP Induction evaluated by “Gold standard method” using cryopreserved plateable hepatocytes from three donors at three concentrations of new chemical entity with both mRNA expression and enzyme activities monitored using RT-PCR and LC-MS/MS respectively. Vehicle control, Positive control, and Negative controls are included in the assay

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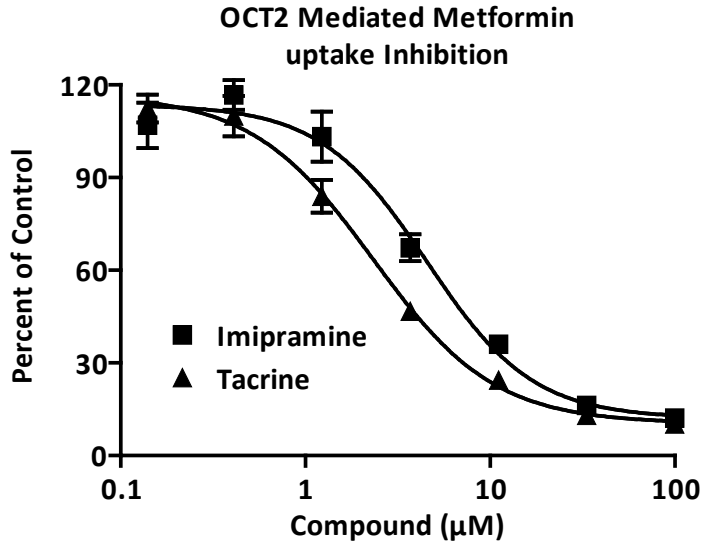
Uptake Transporter (IC_{50} assay)

Transporter	Substrate	Positive control	Absolute IC_{50} (uM)
OAT1	Para-amino hippuric acid (PAH)	Flufenamic acid	0.5
OAT3	Estrone 3-sulfate (E3S)	Indomethacin	0.7
O CT1	Tetraethyl ammonium (TEA)	Verapamil	5.0
O CT2	Metformin	Imipramine	6.4
OATP1B1	Estradiol β-D Glucuronide	Sulfasalazine	3.4
OATP1B3	Estradiol β-D Glucuronide	Rifampicin	2.3



Compound ID	OCT2 Abs IC_{50} (µM)	Literature Reported
Imipramine	6.4	3.3
Tacrine	3.4	3.1

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Quality Assurance

- Independent Quality Assurance team
- Quality System Procedures (QSP's) for Quality System Management and Standard Operating Procedures (SOP's) for Operation, Calibration, Maintenance of Equipment's
- Document and Data Control, Conducting Internal Audits, Study Specific Audits
- Dedicated Archive facility for the retention of the records
- Facility audited and approved by many global pharmaceutical companies and majority of Indian Pharma Companies

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