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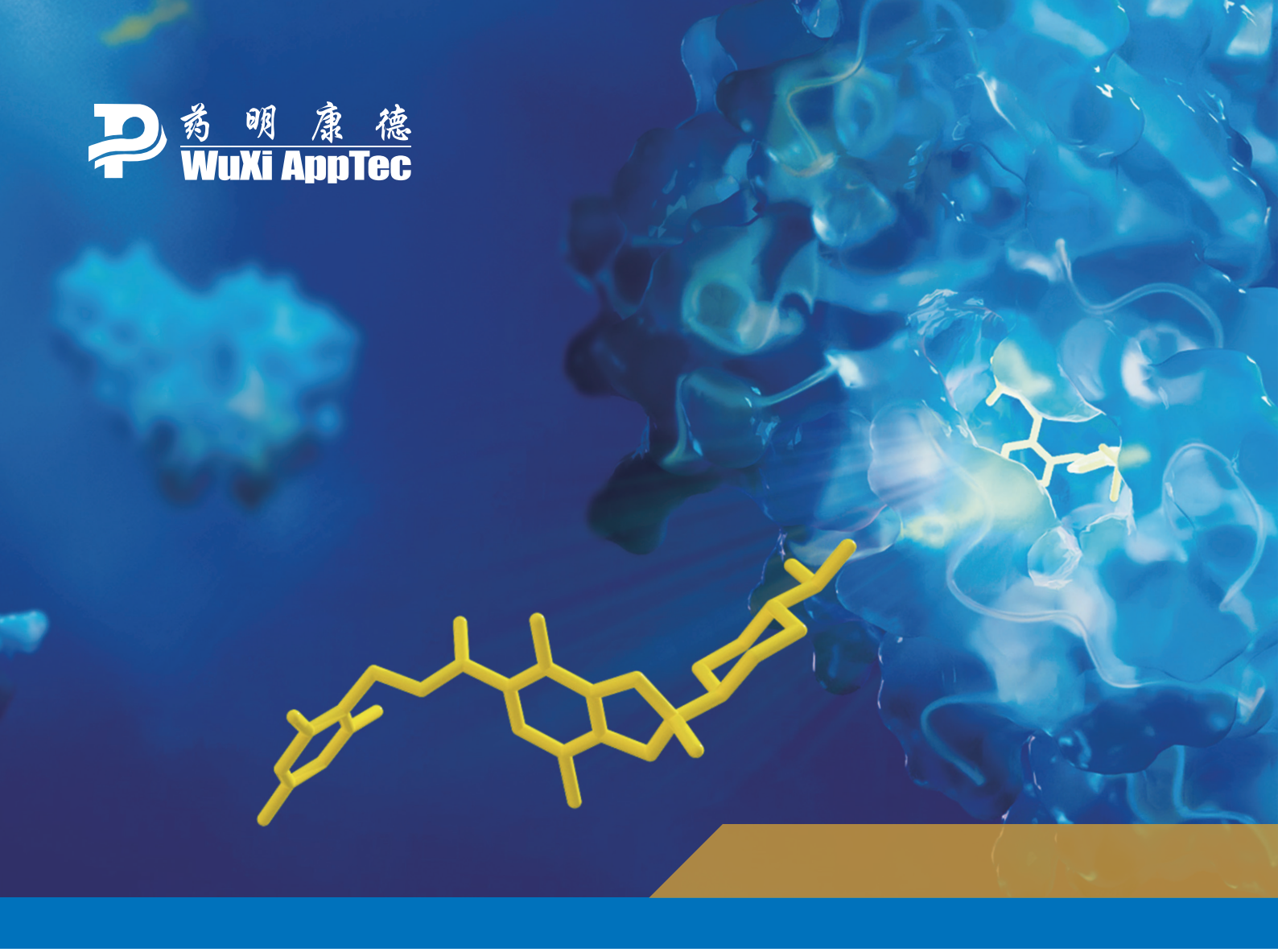

Tiered DMPK Research Strategy for Small Molecules

From Screening to IND

Tiered Approaches for Screening and Prioritizing Compounds

Candidate Profiling for DMPK/ADME Properties

Evaluation of Drug-Drug Interaction Potential

In Vivo PK and Radiolabeled Studies

Tiered DMPK Assay List

Assay* / Category	Tier I (Hit to Lead)	Tier II (Lead Optimization)	Tier III (Preclinical Candidate)	Tier IV (IND)
Physicochemical Properties	• Kinetic solubility • LogD/LogP • pKa		• Thermodynamic solubility	
Absorption	• PAMPA or MDCK II (wild type or MDR1-transfected)	• MDR1-MDCK II • Rodent PK–IV cassette dosing • Rodent single dose PK–IV, PO/SC/IP	• Caco-2 • Dosing vehicle screening • Rodent PK (dose escalation) • Large animal PK (single dose and escalation)	• Rodent PK (single & repeat dose) • Large animal PK (single & repeat dose) • Bioanalytical method qualification
Distribution	• Plasma protein binding (multiple methods including dialysis, UC, UF)	• Blood/plasma ratio (optional)	• Target tissue PK in efficacy model animals • Protein binding in tissue homogenates	• Plasma protein binding • Tissue distribution in rodent species
Metabolism	• Liver microsome stability	• Hepatocyte stability • MetID in liver microsomes (soft-spot) • Reactive metabolites trapping • Plasma stability (optional)	• Cross-species MetID (liver microsomes and/or hepatocytes)	• Liver microsome or hepatocyte stability • MetID (liver microsome and hepatocyte) • MetID of *in vivo* samples
Excretion			• Excretion study in rodent species (feces, urine, bile)	• Mass balance in rodent species (cold or radiolabeled)
Drug-Drug Interaction	• CYP reversible inhibition (discrete/ cocktail)		• CYP time-dependent inhibition (IC_{50} or AUC shift, K_{inact}/K_I)	• CYP inhibition (reversible and time-dependent) • CYP induction in hepatocytes (enzyme and/or mRNA) • Reaction phenotyping (CYP, UGT, etc.) • P-gp, BCRP (inhibition and substrate) • 7 SLC transporters (inhibition and substrate) assessment

Notes:

*The assay tiers can be adjusted according to the project requirements.
MetID: metabolite identification
UC: ultracentrifugation
UF: ultrafiltration

Assay Description, Compound Requirement, and Turnaround Time

Stage	Assay	Assay Description	Compound Requirement (Powder or 10 mM Stock in DMSO)	Turnaround Time (Working Days)
Tier I (Hit to Lead)	Kinetic solubility	pH 7.4 or other pH conditions in aqueous matrix, 4 or 24 hour incubation	50 µL for one matrix (10 µL for each additional matrix)	3-5 days
	LogD/LogP	pH 7.4 or other pH conditions	20 µL (LogD) 60 µL (LogP)	3-5 days
	pKa	Spectrometric titration Potentiometric titration	20 µL (spectrometric) 3 mg (potentiometric)	5-7 days
	Liver microsome stability	Human and one rodent species	20 µL	3-5 days
	PAMPA or MDCK II (wild type or MDR1-transfected)	Egg-PAMPA, 4-hour incubation Bidirectional permeability in MDCK-II, 150 min incubation	20 µL of each	5-10 days
	CYP reversible inhibition	Discrete isozymes or 5-in-1 or 7-in-1 cocktails, single or multiple concentrations	20 µL for single concentration, 60 µL for multiple concentrations	3-5 days
	Plasma protein binding	Human and relevant species	30 µL	3-5 days (Dialysis, UF) 5-10 days (UC)
Tier II (Lead Optimization)	Rodent PK–IV cassette	Rat/mouse IV cassette dosing (up to 5 compounds), n=3 animals/group	6 mg for 1 mg/kg	5 days
	Rodent PK–IV, PO/SC/IP	Rat/mouse Single dose, n=3 animals/group, two groups	For a dose up to 10 mg/kg, up to 7 mg for mouse and up to 20 mg for rat	5 days
	Blood/plasma ratio (optional)	Freshly prepared human and animal whole blood; Direct method	30 µL	10 days
	Hepatocyte stability	Human and relevant species	30 µL	3-5 days
	Plasma stability (optional)	Human and relevant species	30 µL	3-5 days
	Metabolite identification in liver microsomes (soft-spot)	Human and relevant species Major metabolites (≤ 3/species, and ≤ 8 for all species)	40 µL	5-10 days
	Reactive metabolites trapping	Human liver microsomes with NADPH and GSH GSH adducts with relative abundance and structure elucidation	40 µL	7-10 days
	MDR1-MDCK II	Bidirectional permeability and efflux evaluation	10 µL	3-5 days
Tier III (Preclinical candidate)	Thermodynamic solubility	pH 7.4 or other pH conditions in aqueous matrix, 24 hours incubation	3 mg for one matrix (2 mg for each additional matrix)	3-5 days
	Cross-species metabolite identification (liver microsomes or hepatocytes)	Human, mouse, rat, dog, and monkey Metabolic pathway and structural elucidation	40 µL	10 days
	Caco-2	Bidirectional permeability and efflux evaluation	10 µL	5 days
	CYP time-dependent inhibition	CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 (2 substrates), IC_{50} or AUC shift, K_{inact}/K_I	100 µL/substrate for IC_{50} or AUC shift, 2 mg/substrate for K_{inact}/K_I	5 days for IC_{50} or AUC shift, 10 days for K_{inact}/K_I
	Dosing vehicle screening	In support of poorly soluble compounds and high-dose PK and toxicity studies Test up to 10 feasible formulation recipes	50 mg (assuming 10 mg/mL), adjusted by dosing concentration	5-7 days
	Rodent PK (dose escalation)	PO/SC/others, 3 dose levels, n=3/group	200 mg for up to 100 mg/kg	5-7 days
	Large animal PK (single dose)	Dog/monkey/rabbit/pig IV, PO/SC/others, n=3/group	400 mg for up to 10 mg/kg	5-7 days
	Large animal PK (dose escalation)	IV, PO/SC/others, 3 dose levels, n=3/group	5 g for up to 100 mg/kg	5-7 days
	Excretion study in rodent species	PO/SC/others, n=3/group Collect urine and feces for up to 72 hours	50-100 mg, depending on dosage	7-9 days

Stage	Assay	Assay Description	Compound Requirement (Powder)	Turnaround Time
Tier IV (IND)	Liver microsome or hepatocyte stability	Human, mouse, rat, dog, and monkey	10 mg	30 days
	Metabolite identification (liver microsomes and hepatocytes)	Human, mouse, rat, dog, and monkey Metabolic pathway and structural elucidation	10 mg	20 days
	Plasma protein binding	Human, mouse, rat, dog, and monkey	15 mg	40 days
	CYP inhibition (reversible and time-dependent)	7 CYP enzymes (2 substrates for CYP3A4), three curves for 8 concentrations Optional: K_{inact}/K_I evaluation if positive for certain CYP isoform	55 mg Optional: 40 mg/CYP isoform (adjusted by IC_{50} from TDI)	30 days Optional: 30 days
	CYP induction in hepatocytes	CYP1A2, 2B6 and 3A4, 5 concentrations, mRNA and enzyme activity detection Optional: CYP2C evaluation if induction potential for CYP3A	25 mg Optional: 20 mg	45 days Optional: 45 days
	CYP phenotyping	7 CYP enzymes, chemical inhibitors and recombinant CYP isoforms	30 mg	40 days
	P-gp inhibition	MDR1-MDCK II cells, 7-8 concentrations	20 mg	30 days
	BCRP inhibition	Caco-2 cells, 7-8 concentrations	20 mg	30 days
	Bioanalytical method qualification	Rodent and large animal plasma Rodent tissues, excretions	*In vivo* assays: 10-20 g (adjusted by dosage)	3-4 months
	Rodent PK (single dose & repeat dose)	1 IV group, 3 PO/SC/other groups for single dose 1 group for repeat dose		
	Large animal PK (single dose & repeat dose)	1 IV group, 3 PO/SC/other groups for single dose 1 group for repeat dose		
	Mass balance in rodent species	2 groups, one for urine and feces, one for bile		
	Tissue distribution in rodent species	4 time points, 13 tissues for each sex		
	Metabolite identification of *in vivo* samples	Plasma from rodent and large animal PK, excreta from mass balance study Metabolic pathway and structural elucidation		
Optional (IND)	P-gp substrate, concentration dependency	MDR1-MDCK II and wild-type MDCK II cells, 3 concentrations	20 mg	40 days
	BCRP substrate, concentration dependency	Caco-2 cells, 3 concentrations	20 mg	40 days
	SLC transporter inhibition and substrate	OATP1B1/ OATP1B3/ OCT2/ OAT1/ OAT3/ MATE1/ MATE2-k transfected and mock HEK293 cells, 3 concentrations	80 mg	45 days
	$^{14}C/^{3}H$ radiolabeled studies	$^{14}C/^{3}H$ radiolabeled compound synthesis, PK, mass balance, tissue distribution, and QWBA	200 mg (variable by dosage) 2 mCi radiolabeled compound	3-4 months

* Saving of DMSO stock normally happens when multiple assays are ordered together.

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