Compound Optimization After HTS: From Hit to Lead

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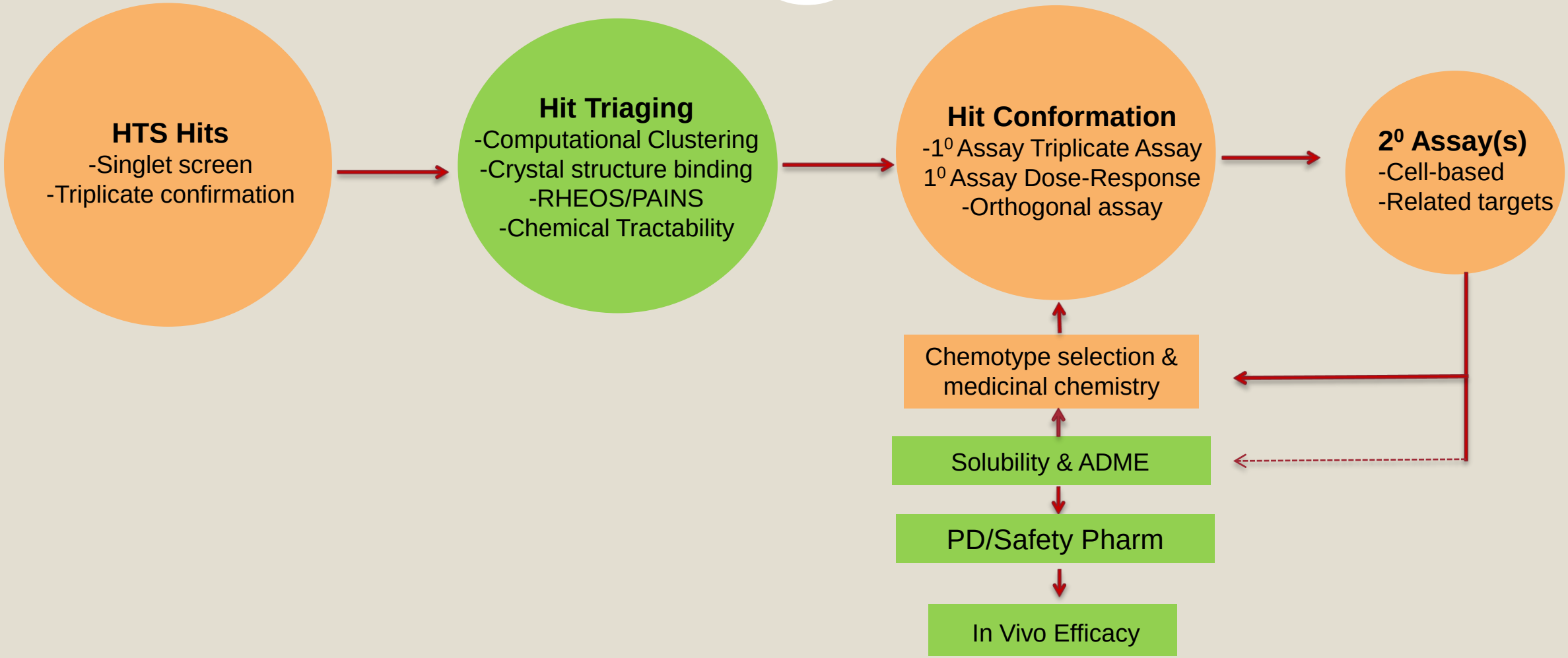
HTS Hit Optimization



Lead Compound Characterization

- The extent of compound characterization/optimization depends on the project objectives.
  - *In Vivo* Efficacy Proof-of-Concept:
    - Adequate ADME properties for valid *in vivo* assessment (solubility, pharmacokinetics)
    - For CNS studies, demonstration of adequate compound levels in brain
    - Ideally, evidence of target engagement/pharmacodynamic effect.
    - Tolerability in animal species of choice at projected efficacy doses
  - IND Candidate : the above, plus;
    - Preliminary *in vitro* safety pharmacology, including hERG and human CYP450 inhibition.
    - Human microsome studies to determine predicted clearance and CYP450 metabolism.
    - IND-enabling studies (CMO/CRO)

Hit Triaging

- It is highly recommended that an experienced medicinal chemist assist in the triaging of HTS hits.
- A variety of molecular filters are available to remove hits with undesirable chemical features:
  - Lipinski-like filters (MW, H-bond acceptors/donors, rotatable bonds, PSA)
  - PAINS (**P**an **A**ssay **In**terfering Compounds)
  - REOS (**R**apid **E**limination **O**f **S**will)
  - CNS MPO Score (**M**ulti-**P**arameter **O**ptimization)

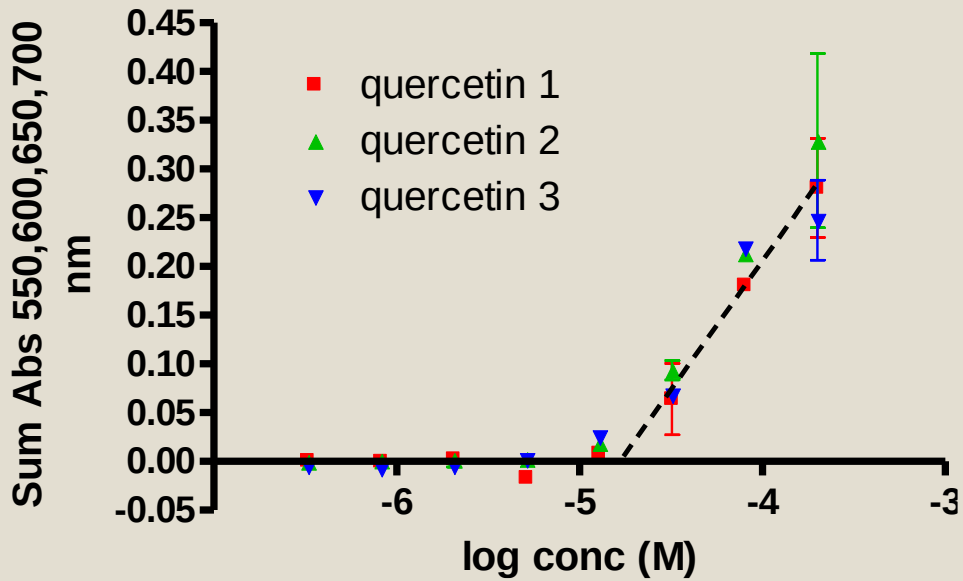
- See Dahlins & Walters (2014) Fut Med Chem 6:1265-90; Bruns & Watson (2012) J Med Chem 55:9763-72; Wager et al. (2010) ACS Chem Neurosci 6:435-49.

Compound Solubility

- Compounds must be sufficiently soluble in assay buffer systems to allow interpretable results.
- For animal studies, need sufficient solubility to allow accurate dosing.
- Compound solubility is affected by many factors (salt forms, pH, buffer systems, etc.)
- Generally want solubility >60 µg/ml or >100 µM (Lipinski et al., Adv. Drug Disc. Rev. 23:3-25).
- Two basic types of solubility determinations
  - DMSO stock dilutions: measure compound precipitation after addition to aqueous solution (typically a kinetic measurement)
  - Solid compound: measure compound dissolution in aqueous solution (typically an equilibrium measurement)
- Kinetic solubility measurements from DMSO stocks are quicker, but not as accurate.

Compound Solubility

- Multiple simple kinetic solubility methods exist (e.g., see Pan et al., J. Pharm. Sci. 90:521-29 and Hoelke et al., Anal. Chem. 81:3165-72).
- One method accessible to most labs is solubility determination based on light scatter of precipitates using a UV-Vis plate reader.



Pharmacokinetics/ADME

- Key aspects of **A**bsorption**D**istribution**M**etabolism**E**xcretion are enabled with a LC-MS/MS
  - Compound plasma and pharmacokinetics, including clearance and half-life determinations.
  - Estimation of free drug levels in plasma and brain through equilibrium dialysis studies.
  - Approximation of metabolism using liver microsomes, including identification of major CYP450 isozymes involved in compound metabolism (for IND candidate development).

CNS Candidate 1h PK

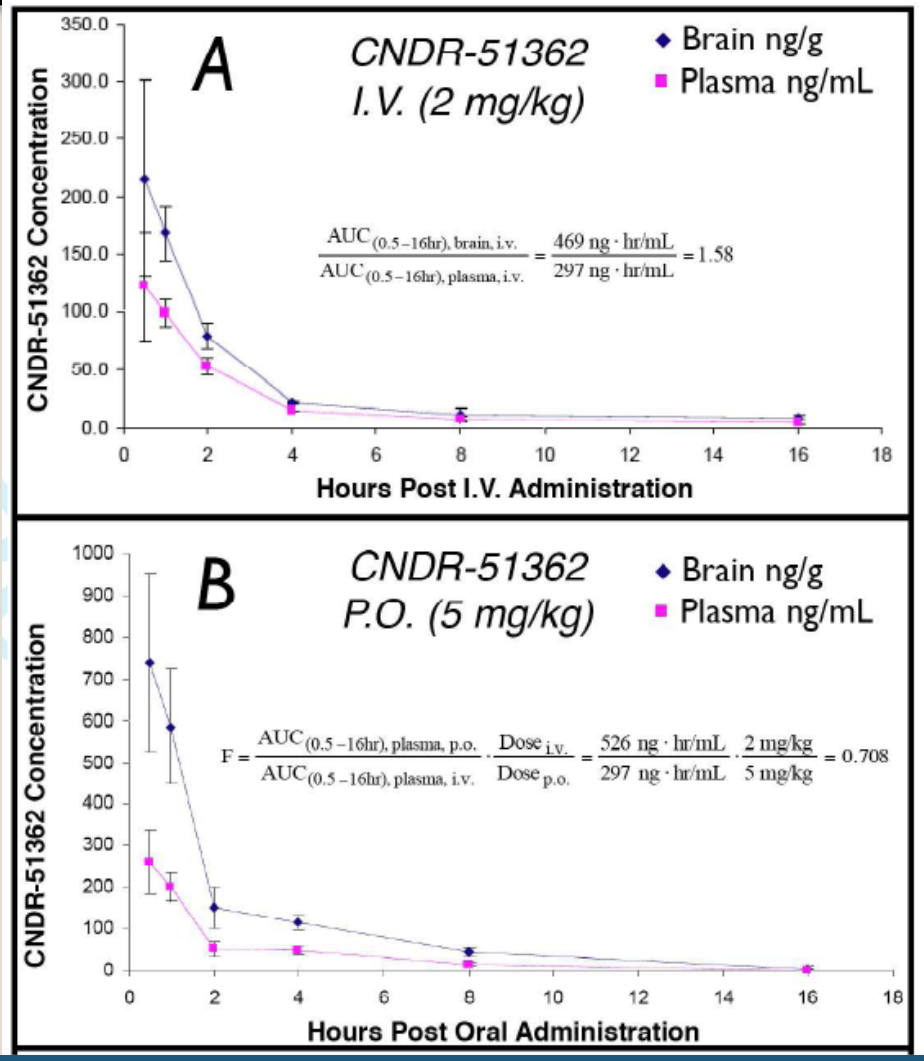
- In vitro assays such as PAMPA/MDCK, can be used to predict BBB penetration, but are not always accurate. We thus typically go directly to “probe” PK.
- Dose mice (n=3) at 1-5 mg/kg i.p. (~0.5 mg compound needed).
- Assess compound concentration in plasma and brain 1 h post-dosing.

Mouse	Brain (ng/g)	Plasma (ng/ml)	B/P
1	1179	806	1.46
2	1312	680	1.93
3	1404	983	1.43
Mean	**1298**	**823**	**1.61**
SD	113	152	0.28

CNDR-51362

- Can obtain preliminary information on compound clearance and brain-penetration.
- Many universities have LC/MS core services; can also utilize CROs.
  ~$3200 CRO cost for analysis of 3 cassette-dosed cmpds in triplicate after dosing & tissue collection.

Full IV and PO PK



$B_{AUC}/P_{AUC} = 1.58$

$AUC_{oral}/AUC_{iv} = F = 71\%$

Brain Clearance = 66 ml/hour

Estimated CRO cost of $6000 or $3200 for iv/ip only

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Target Engagement/Pharmacodynamics

- Although PK defines how the compound is metabolized, it does not reveal how the compound affects biological activity.
- To adequately evaluate compound efficacy in a disease model, one must have a sense of the appropriate dose range to test.
- Ideally, an *in vivo* readout of compound target activity is available to allow determination of appropriate compound doses and to evaluate the duration of compound effect.
- Example brain target engagement markers include:
  - Enzyme activity in brain homogenates
  - Receptor occupancy studies in brain homogenates
  - Secondary markers of target engagement, such as changes in post-translational phosphorylation of downstream targets.

Projected Dosing

If No Target Engagement Readout

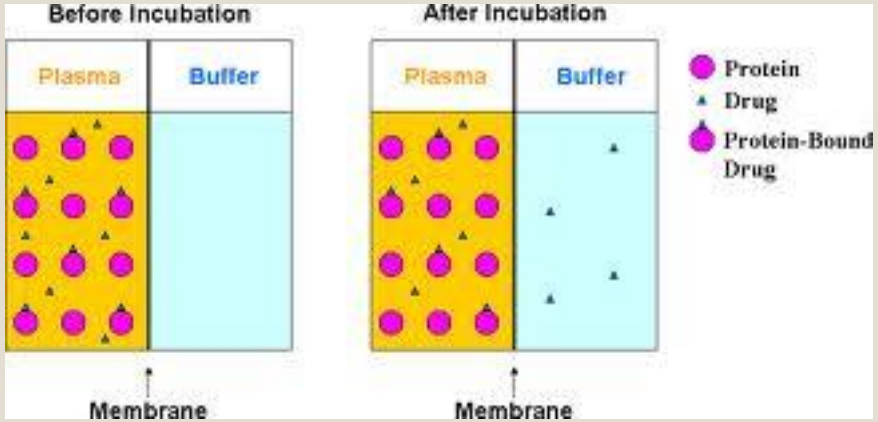
Dose = $[(Cl \times Ct \times T)/F]/f_u$

Where:
Cl = Clearance (mL/h)
Ct = Target drug level
T = Dosing interval (hours)
F = Oral Bioavailability
Fu = Fraction unbound

Assuming:
Ct = 10 nM (3.5 ug/L)
T = 24 hour
Mouse Weight = 25 g

Compound	51362	51397
CL,brain (mL/hr)	**66**	**50**
CL, plasma (mL/hr)	108	78.6
fu,brain	**0.011**	**0.043**
fu,plasma	0.019	0.069
F	0.71	0.63
Predicted Daily Dose (mg/kg)	***28.4***	***6.2***

Equilibrium Dialysis



CNDR-51362; B/P = 1.61

f_u, brain homogenate = 0.011
f_u, plasma = 0.019

$B/P_{free} \div f_{u(brain)})/f_{u(plasma)} = B/P$

$B/P_{free} = 0.93$

Requires LC-MS capabilities, and thus may only want to check priority compounds

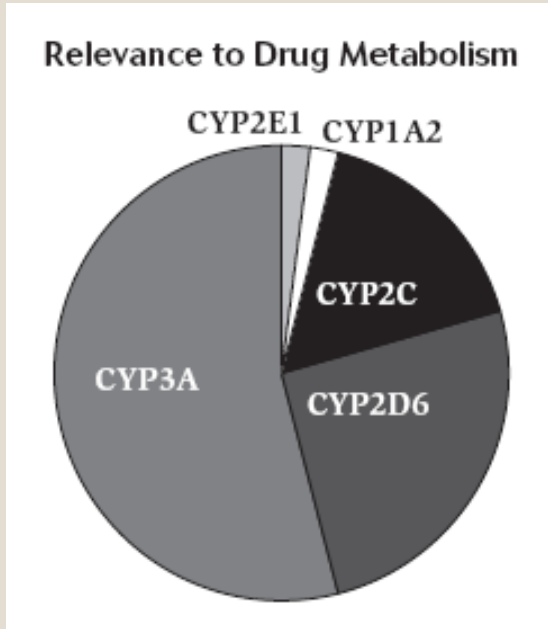
Rodent Tolerability Studies

- **MTD**
  - Normal mice (n=4) are dosed via oral gavage (0.5% methylcellulose) starting near projected efficacy dose, with 3X dose-escalation.
  - Dosed every two days until at least 10X projected efficacy dose is reached, or two or more mice show signs of intolerance (altered locomotor activity, sedation, ataxia, hypo- or hypertonia, salivation or excitation).
- **2-Week Tolerability**
  - Normal mice (n=6/dose) are dosed at 0.1x-, 0.3x- and 1x-MTD via oral gavage for 2 weeks.
  - Assessments include
    - Behavioral observations & body weights
    - Organ weights at study completion
    - Complete blood counts at study completion
    - Plasma and brain compound levels at study completion (compared to separate group receiving drug for 3 days)

IND Candidate Safety/Toxicology

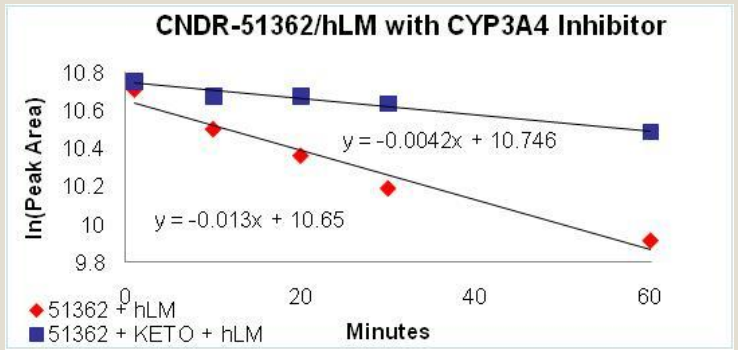
CYP450 Inhibition

- Significant inhibition of major CYP450 isozymes can result in drug-drug interactions. ~80% of drugs are metabolized by CYP450 enzymes.
- Although there are >50 CYP450 isozymes, most drugs are metabolized by just 7 isozymes (3A4/5, 2C8, 2C9, 2C19, 2D6, 1A2, and 2E1).
- CYP450 inhibition profiling can be readily assessed using:
  - Commercial assay kits (e.g. Invitrogen Vivid fluorescent kits).
  - LC-MS/MS (measure inhibition of known *CYP450 substrates using baculozomes).*
- *FDA guidance suggests that in vivo CYP450 inhibition studies are needed if plasma C_{max} is >10% of CYP450 K_i.*
- We flag compounds if CYP450 inhibition is >50% at 10 μM.



IND Candidate Safety/Toxicology
CYP450 Metabolism

- Drug metabolism:
  - Phase I (oxidation) - CYP450 isozymes and FMOs
  - Phase II (conjugation) - UDP-glycosyltranferases, glutathione transferases and sulfotransferases.
- Human metabolic rate can be estimated through use of liver microsomes (contain CYP450s, FMOs and UGTs).



<30% HBF = low clearance
30-70% HBF= moderate clearance
>70% HBF= high clearance

$Cl_{int} = k_e$ (-slope) X (20 mg microsome/g liver) x (45 g liver/kg BW) x (1 ml/mg microsomal protein) = 11.7

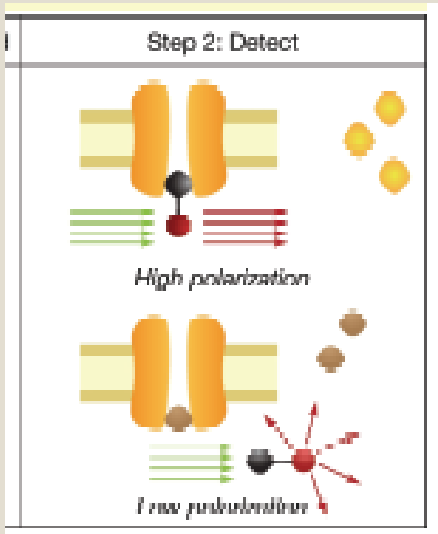
$Cl_{hep} = Cl_{int}$ x HBF/ Cl_{int} + HBF; HBF=20 ml/min/kg Cl_{hep}= 7.4 ml/min/kg (37% HBF)

- Compound-metabolizing CYP450 isozymes can be indentified with liver microsomes.
- Significant CYP2D6 metabolism is a flag due to allelic variation in humans (5-10% of Caucasians are poor 2D6 metabolizers).

IND Candidate Safety/Toxicology

hERG Binding

- Many drugs have been withdrawn from the U.S. as a result of their likely hERG cardiac channel inhibition (long QT intervals).
- Compound interaction with the hERG channel can be readily estimated with commercial ligand binding kits (e.g., ThermoFisher Predictor hERG FP assay kit).
- hERG Binding assays have generally good correlation with more definitive patch-clamp analyses.



Want ~100-fold window between effective plasma drug concentrations and hERG K_i.

We flag compounds that show >50% inhibition at 30 μM or <500-fold difference in Ki with drug target (if known).

IND Candidate Safety/Toxicology

Selectivity Analysis

- Academics can contact the NIMH Psychoactive Drug Screening Program for possible compound evaluation against CNS receptors.
- More comprehensive off-target screens for receptor and enzyme interaction are commercially available, although expensive.

Example Workflow