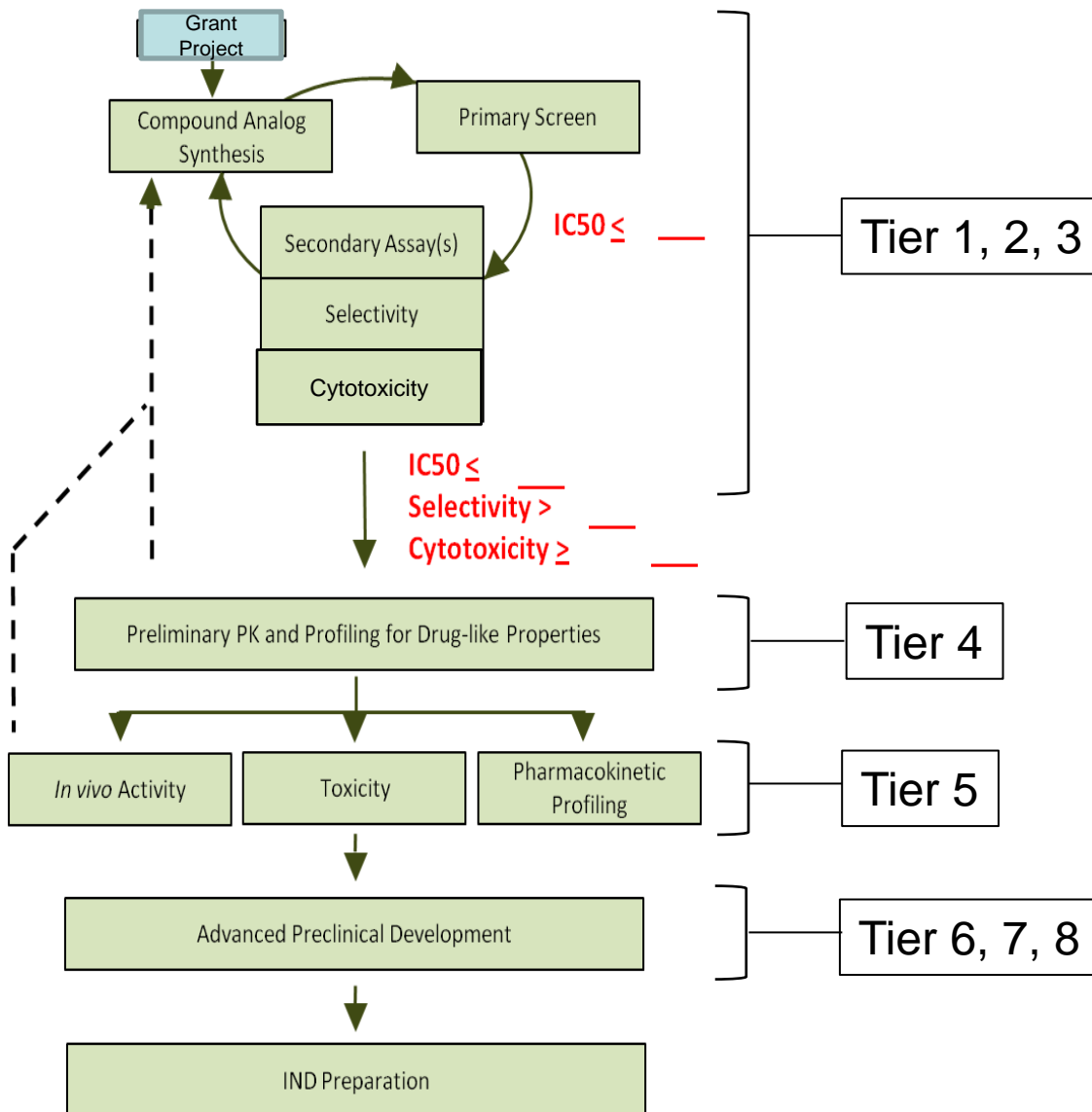


Example Drug Discovery & Development Testing Funnel



Example Drug Discovery & Development Testing Funnel

TIER 1A – Primary Screen

- ◆ Chemical purity and identity of active compounds
- ◆ Primary bioactivity screen
- ◆ Cell viability (When Appropriate)
- ◆ Scaffolds/Moiety Chemical liabilities (*for example: Michael acceptor, GSH reactive*)
- ◆ Calculated properties:
 - ◆ CLogP
 - ◆ PSA
 - ◆ Molecular Weight
 - ◆ rotatable bonds
 - ◆ H-bond donors and acceptors
 - ◆ permeability
 - ◆ pKa
 - ◆ Solubility

TIER 1B

- ◆ Confirm EC50 determinations for actives compounds in primary screen with fresh compounds from the original stock. Confirm EC50 determinations for the lead (most active) compound in primary screen with a new sample either repurchased, purified and characterized in-house, or independently synthesized in-house.



Compounds with IC50s (EC50s) less than **X advance to Tier 2**


Example Drug Discovery & Development Testing Funnel

TIER 2A – Activity Confirmation

- ◆ Secondary screen

TIER 2B

- ◆ Repeat EC₅₀ determinations for actives in secondary screen with fresh compounds from the original stock



Compounds with IC₅₀s (EC₅₀s) less than **X** advance to Tier 3

Example Drug Discovery & Development Testing Funnel

TIER 3 – Drug-like Properties, Specificity

- ◆ IC_{50} selectivity in selectivity screen
- ◆ CYP450 Inhibition competitive and time-dependent if structural alerts exist (spot check illustrative examples from compound series)
- ◆ Measured solubility
- ◆ Measured protein binding (spot check illustrative examples from compound series)
- ◆ Test of Permeability *in vitro* permeability [indicate assay, e.g., Caco2 or/and PAMPA], (spot check illustrative examples from compound series)
- ◆ hERG
- ◆ Cytotoxicity assays



All compounds with no significant issues (**Define Minimum Conditions for Advancement**) to advance to Tier 4

Example Drug Discovery & Development Testing Funnel

TIER 4 – Scale-up Synthesis and Preliminary PK

- ◆ Scale-up synthesis
- ◆ Purity determination: >98% with no single impurity >1%
- ◆ Rodent bioavailability and PK (define target delivery route):
 - ◆ T_{max}
 - ◆ C_{max},
 - ◆ AUC,
 - ◆ Bioavailability,
 - ◆ V_{ss}, CL, T_{1/2}, MRT
 - ◆ Brain to Plasma ratios
 - ◆ P-glycoprotein transport MDCK-MDR1 and MDCK-mdr1a
 - ◆ Plasma Protein Binding (species),
 - ◆ Microsomal Stability – rodent and human
- ◆ Define/plan Patent Protection Strategy



All compounds with no significant issues (Define Minimum Conditions for Advancement**) advance in parallel to Tiers 5A&B**

Example Drug Discovery & Development Testing Funnel

TIER 5A – In Vivo Bioactivity

- ◆ Animal efficacy
- ◆ Validate Biomarker
- ◆ Target engagement

TIER 5B – Advanced Drug-like Properties

- ◆ Microsomal stability in multiple species
- ◆ Chemical Stability
- ◆ CYP450 induction
- ◆ CYP reaction phenotyping
- ◆ Metabolism – human hepatocytes/microsomes
- ◆ Metab ID:
 - ◆ define major human, rat, dog and non-human primates (NHP) metabolites
- ◆ *In vitro* Tox:
 - ◆ Ames
 - ◆ Chromosome Aberration
 - ◆ CNS effects



Advance to Tier 6 if **(Define Minimum Conditions for advancement)**

Example Drug Discovery & Development Testing Funnel

TIER 6 – Liability Assessment

- ◆ Broad Pharmacological Profile and Toxicology
- ◆ PK in second species

TIER 7

- ◆ Non-GLP exposure studies, single and multiple dose

Advance to late stage pre-clinical development (**Define Minimum Conditions for advancement**)