Hit-to-Lead Compound Checklist

With confirmed hits in hand, the goal at this stage is to prioritize hits based on their potential drug-likeness determined by pre-specified structural, physiochemical, and activity criteria. This process filters out the least promising hits and advances the most promising molecules to lead compound status for subsequent optimization.

Chemical Structure

An experienced medicinal chemist can visually inspect a molecular structure and identify defined structural classes or functional groups that are known to cause toxicity, form reactive metabolites, or show promiscuity (specific compounds or compound classes that repeatedly appear to show activity in assays, but are artifacts that interfere with assay readouts). Hits with these structural liabilities tend to make very poor drugs and should be discarded as early as possible. Although free *in silico* tools are available to identify structural liabilities, we recommend engaging a qualified medicinal chemist at this stage.

Setting Criteria

Establishing goal properties both for the hit-to-lead and the lead optimization stages is vital. Pre-specified criteria can serve as go-no/go or decision points for advancing viable compounds to subsequent stages of development. The lists below, adapted from the Alzheimer's Drug Discovery Foundation's Compound Report Card, outlines some of the key properties to assess during the hit-to-lead stage.

Physiochemical properties

The properties listed below can affect absorption, distribution, metabolism, and excretion (ADME), as well as toxicity. Lipinski et. al developed the "Rule of 5" to describe the ideal set of physiochemical criteria for orally bioavailable drugs [1]. Keep in mind that hits are starting points that tend to grow in size and lipophilicity as they're developed into leads and then drugs [2]. For CNS drugs, passive brain penetration generally requires more restricted physiochemical properties than the standard "Rule of 5" [3].

- **Molecular weight** – The size of a molecule can affect how well it is absorbed into the bloodstream and its ability to penetrate the blood brain barrier (BBB).

- **cLogP** – The calculated (or predicted) partition coefficient of a molecule determines its lipophilicity. Higher cLogP values predict that a molecule will be poorly absorbed and will have low solubility.

- **H-bond donors** – The number of H-bonds with O or N atoms (H-bond donors) will affect the solubility and permeability.

- **H-bond acceptors** – The sum of O and N atoms in a molecule. The number of H-bond acceptors can impact its cell membrane permeability and accumulation in the brain.

- **PSA** – Polar surface area, which is the sum of the surface area of polar atoms (i.e. O and N atoms). Molecules with higher PSA's are generally less likely to cross the BBB.

- **Rotatable bonds** – Rotatable bonds are single chemical bonds, outside of ring structures that are bound to non-terminal, non-hydrogen atoms. C-N bonds are excluded from this consideration, due to their high rotational energy. Compounds with fewer rotatable bonds are more likely to be orally bioavailable and BBB penetrant.

- **Number of chiral centers** – Chiral centers are carbon atoms bound to 4 different substituents, meaning a molecule can exist in different enantiomers (or mirror images). Enantiomeric isomers can have different potencies, selectivity, and toxicities.

In vitro activity

- **Activity in primary assay** (potency) – Before beginning your screening campaign, a minimum requirement for activity should be set. Ideally, this should be in single-digit micromolar range for hits and nanomolar range for leads.

- **Activity in secondary and tertiary assays** – Activity in these assays will confirm that results from primary assays are due to desired activity and not assay interference or other off-target effects.

- **Selectivity (related family)** – Hits should be tested for activity against biological target isoforms or closely related proteins (i.e. test JNK3 kinase inhibitor activity on related isoforms, JNK1 and JNK2).

- **Selectivity (unrelated family)** – Test hits against unrelated families of molecules to assess whether the compound has potential off-target toxicities (i.e. test JNK3 kinase inhibitor against a large panel of diverse kinases).

Other parameters

- **Solubility (vehicle)** – Solubility is a function of a molecule's physiochemical properties and will greatly impact bioavailability and other properties.

- **Scale-up feasibility** – At the earliest stages, compounds will be synthesized on a smaller scale. If a compound is to be taken further in pre-clinical development (*in vivo* pharmacokinetics and preclinical efficacy studies) it will need to be synthesized

in larger amounts while maintaining purity and affordability. An important consideration in scale-up feasibility is the number of synthetic steps required to synthesize the molecule.

- **Patent protection** – Hits should be assessed for their patentability. Even if a molecule has a host of drug-like qualities, outside investors will be hesitant to support a program if it will not produce novel, patentable material.

References

1. Lipinski, C.A., et. al, *Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings*. Adv Drug Delivery Revs, 2001; 46:3-26.
2. Teague, S.J., et. al., *The Design of Leadlike Combinatorial Libraries*. Angew Chem Int Ed Engl, 1999; 38(24): 3743-3748.
3. Pajouhesh, H. and Lenz, G.R. *Medicinal Chemical Properties of Successful Central Nervous System Drugs*. NeuroRx, 2005; 2(4): 541–553.