CHOOSING & USING CHEMICAL PROBES

What are Chemical Probes?

Chemical probes are well-characterized small molecules that potently and selectively modulate the activity of a target protein. These probes have a defined mechanism of action and can be used to elucidate the roles of the target proteins in cellular and disease phenotypes for target validation or phenotypic profiling.

Why Use a Chemical Probe?

Chemical probes allow for sensitive modulation of protein function without necessarily reducing protein levels, unlike certain biological approaches such as CRISPR and RNAi. This allows scientists to determine concentration- and time-dependent effects of target protein inhibition to better explore the nuances of a protein's function within a biological system.

The Ideal Probe

IC_{50} K_d

- **Potent:** *In vitro* IC_{50} or K_d <100 nM
- **Selective:** >30-Fold selective for the target over proteins in the same family
- **Active in Cells:** Significant on-target cellular activity at 1 µM

Also look for...

- Concentration-dependent effects
- Cell permeability (for intracellular targets)
- Solubility
- Stability

To strengthen your probe set, include...

An **orthogonal control** (a potent, selective probe for the same target with a different chemotype than the primary probe)

At least one **negative control** (an inactive analog of the primary probe)

View Cayman's ***Small Molecule Inhibitors Selection Guide*** for in-depth information on parameters to be considered during chemical probe selection, including chemistry, potency, selectivity, and mechanisms of action.

QUESTION:
How do you evaluate whether an inhibitor is a suitable chemical probe for your target in cell-based assays?

1. Is the compound sufficiently potent *in vitro*? ✓ → 2. ✗ → COMPOUND NOT RECOMMENDED
2. Is the compound sufficiently active in cells? ✓ → 3. ✗ → COMPOUND NOT RECOMMENDED
3. Is the compound sufficiently selective? ✓ → 4. ✗ → 5.
4. Are orthogonal and/or negative controls available for the probe? ✓ → Use probe with available chemical controls. ✗ → 5.
5. Is there an additional probe with a complementary selectivity profile that can be used to control for off-target effects? ✓ → Use both probes in parallel with appropriate chemical controls. ✗ → Use first compound as a chemical tool/chemogenomic compound in dose response and complement with biological controls

Explore these expert-curated resources to find information and recommendations on high-quality probes for your target protein.

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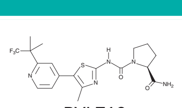
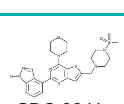
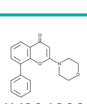
RUN THE COMPARISON:
Potential Chemical Probes for PI3Kα

	In vitro potency (IC_{50})	Selectivity (IC_{50})	Target engagement in cells	Recommendation
 BYL719	✓ 4.0-4.8 nM (wild-type and mutant PI3Kα)	✓ 250 nM (PI3Kγ) 290 nM (PI3Kδ) 1,156 nM (PI3Kβ)	✓ IC_{50} = 74 nM for inhibition of substrate phosphorylation in Rat1-myr-p110α cells	Use to study PI3Kα. Complement with orthogonal and negative controls.
 GDC-0941	✓ 3 nM (PI3Kα)	✗ 3 nM (PI3Kδ) 33 nM (PI3Kβ) 75 nM (PI3Kγ)	✓ IC_{50}s = 28-46 nM for inhibition of substrate phosphorylation in MDA-MB-361, PC3, and U87MG cells	Use in parallel with a secondary probe to ensure that effects are due to modulation of PI3Kα specifically. Complement with orthogonal and negative controls.
 LY294002	✗ 1.4 µM (PI3K)	✗ Non-selective compound		Historic compound: While this compound has been used to interrogate PI3K, it does not meet the criteria to be considered a chemical probe.

Explore these and more than 4,500 additional small molecule inhibitors at **www.caymanchem.com**

DON'T	Underestimate the importance of probe selection. Using a suboptimal chemical probe can produce misleading results. This can lead to serious consequences in biomedical research and drug development.	Use a probe just because it's common in the literature. Just because a probe has been widely used in the past, it does not necessarily mean that it is the best probe for the job.	Rely on a single chemical probe for your experiments.	Assume that phenotypes observed at high concentrations of your probe, such as cell death, are directly related to the specific protein target.
DO	Be selective and rigorous in choosing the right chemical probe for your application.		Use orthogonal controls, negative controls, and complementary biological approaches to validate findings.	Identify and use appropriate probe concentrations to get the most relevant experimental results.

REFERENCES

1. https://www.chemicalprobes.org/
2. https://www.thesgc.org/chemical-probes
2. Barsyte-Lovejoy, D., Szewczyk, M.M., Prinos, P., *et al.* Chemical biology approaches for characterization of epigenetic regulators. *Methods in Enzymology.* Marmorstein, R., editor, *Academic Press* (2016).
3. Antolin, A.A., Workman, P., and Al-Lazikani, B. Public resources for chemical probes: The journey so far and the road ahead. *Future Med. Chem.* **13(8)**, 731-747 (2019).
4. Blagg, J. and Workman, P. Choose and use your chemical probe wisely to explore cancer biology. *Cancer Cell* **32(1)**, 9-25 (2017).