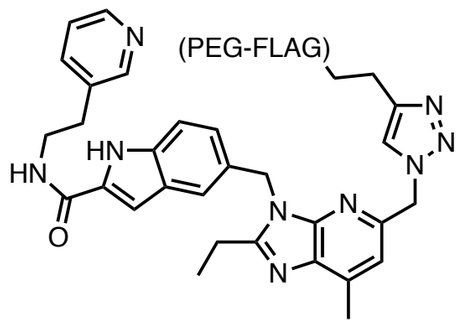
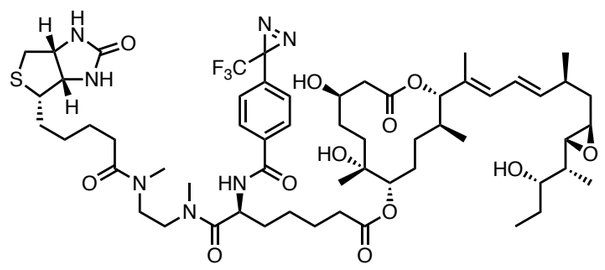
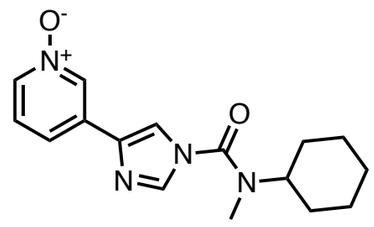
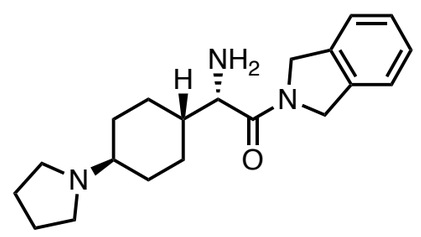
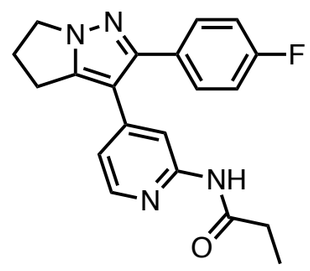
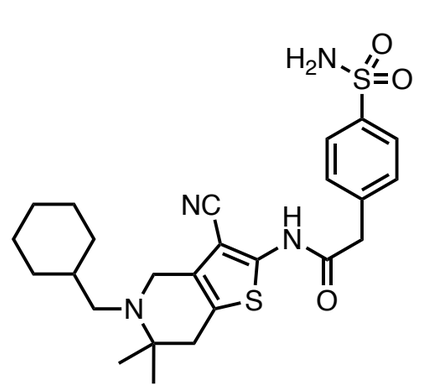
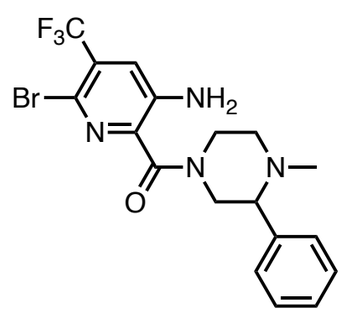
Target Identification Methods in Phenotypic Drug Discovery

	METHOD	STRENGTHS	LIMITATIONS	INDUSTRY APPLICATION	EXAMPLE REFERENCE
Chemical Probe-Based	**Affinity-Based Pulldown** (e.g., kinobeads, SILAC, click, iTRAQ)	• Probes can be reversible • Relatively high probability of success	• Requires probe linker modification • Potent reversible probe needed (K_D = nM) • Requires cell lysis	 KYOWA KIRIN, NAMPT	Yamaguchi, D. et al. *Sci. Rep.* **2019**, *9*, 7742.
	Photoaffinity Labeling (e.g., diazirines, competitive labeling)	• Irreversible binding, can provide binding site information • Can be done in living cells and for insoluble proteins	• Requires photoreactive group and purification tag group • Relatively lower probability of success	 EISAI, SF3b	Kotake, Y. et al. *Nat. Chem. Biol.* **2007**, *3*, 570.
	Activity-Based Protein Profiling (e.g., sulfonyl fluorides, beta-lactones)	• Irreversible binding, can provide binding site information • Can be done in living cells and for insoluble proteins	• Requires reactive probe for target class • Limited to specific classes of enzymes	 PFIZER, ALDH2+	Huang, Z. et al. *ACS Chem. Biol.* **2019**, *14*, 192.
Label-Free Methods	**Thermal Stability Profiling** (CETSA® Explore / TPP / PISA)	• No modification of compound needed • Reversible molecules can be used • Can be done in living cells • High protein coverage among label-free methods	• Does not provide binding site information • May have lower sensitivity than probe-based methods • Requires soluble target	 MERCK, DPP9	Moore, K. P. et al. *ACS Chem. Biol.* **2022**, *17*, 2595.
	Proteolytic or Chemical Stability Profiling (e.g., PP, DARTS, LiP-MS, SPROX)	• No modification of compound needed • Reversible molecules can be used • Provides alternative methods of detection	• Can theoretically localize binding site to peptide regions • Cannot be conducted in live cells • May be limited in protein coverage (e.g., SPROX requires methionines)	 BAYER, FUNGAL CK1	Piazza, I. et al. *Nat. Commun.* **2020**, *11*, 4200.
Indirect Methods	**Resistance Screening** (e.g. anti-infectives, cytotoxics)	• No modification of compound needed • Reversible molecules can be used • Provides relatively strong evidence for target	• Only applicable in certain indications (e.g., anti-infectives, cancer) • Can be time and biology resource-intensive	 NOVARTIS, DENV NS4B	Moquin, S. A. et al. *Sci. Transl. Med.* **2021**, *13*, issue 579.
	Chemogenomic Profiling (e.g. HIP-HOP, CRISPR mutagenesis scanning)	• No modification of compound needed • Reversible molecules can be used • Unbiased for target abundance	• Only applicable in certain settings (e.g., antifungals, cancer) • Can be time and biology resource-intensive	 NOVARTIS, SEC14P	Pries, V. et al. *Cell Chem. Biol.* **2018**, *25*, 279.

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