PELAGO BIOSCIENCE
The home of CETSA®

IS YOUR HIT ON TARGET?

Learn how Pelago Bioscience's CETSA technology enhances your drug discovery projects by improving hit confirmation and lead optimization.

High-throughput screening (HTS) has been widely adopted in small-molecule drug discovery to identify chemical starting points for novel therapeutic targets. However, hit confirmation and structure-activity relationship (SAR) studies demand significant resources.

Using Pelago Bioscience's targeted screening format, you can identify, validate, and optimize hits directly in unmodified cellular systems, with improved success rates and reduced costs in targeted drug discovery. This gives direct confirmation of target engagement of your hits in physiological conditions from the onset.

Identifying Relevant Chemistry with CETSA

CETSA enables robust identification of relevant chemical matter during primary screening. In a study by AstraZeneca, a 0.5 million compound library was screened against the protein kinase CRAF using CETSA. Known CRAF ligands and novel chemistry were identified, increasing the project's likelihood of success. The false positive rate was very low, allowing faster confirmation. In addition, hits demonstrated activity against the related kinase BRAF, validating CETSA's ability to identify chemistry with potential relevance to CRAF.

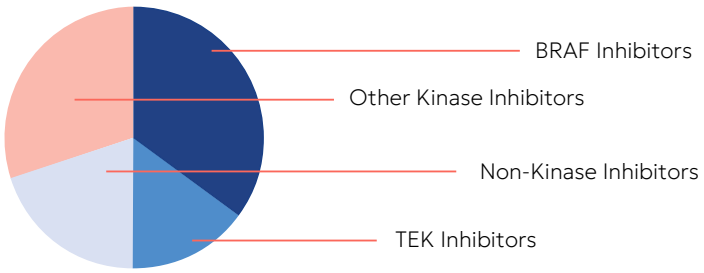

Figure 1: Distribution of hits in the CETSA screening campaign against CRAF.

Pelago Bioscience conducted a primary screening campaign targeting Cyclin Dependent Kinase 4 (CDK4). 11,000 compounds, including lead-like molecules, were screened. CETSA identified known and novel chemical matter, and a selection of hits was further validated in a downstream functional phosphorylation assay, demonstrating CETSA's ability to pinpoint high-quality compounds.

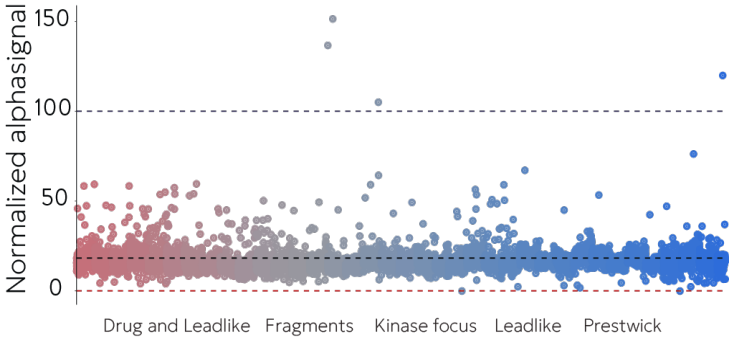

Figure 2: Scatter plot illustrating the results from the CETSA screening campaign against CDK4.

Validating Leads with CETSA

CETSA was performed by AstraZeneca, to confirm hits against PARP1. Initial hits from a biochemical fluorescent polarization (FP) assay were compared with CETSA EC_{50} values and a cellular PARylation assay. CETSA successfully confirmed the activity of potent PARP1 binders, demonstrating immediate permeability and target engagement in cellular systems. Interestingly, CETSA identified "silent binders"—compounds that bind the target but do not inhibit

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its function—highlighting its unique ability to distinguish functional inhibitors from mere binders. By correlating CETSA results with orthogonal assays, a deeper understanding of compound effects in complex models was achieved.

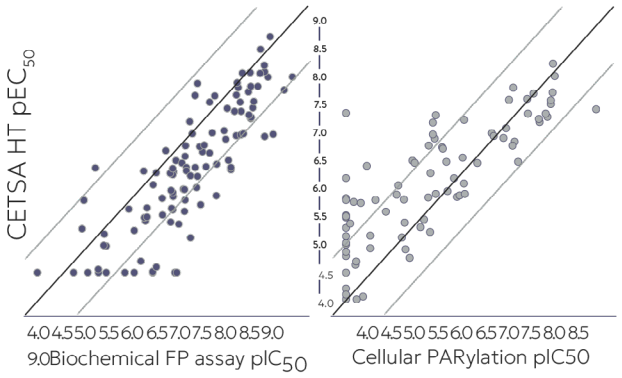

Figure 3: Comparison of PARP1 inhibitor potency values in CETSA with a biochemical FP assay and a cellular PARylation assay.

Leveraging CETSA for SAR and EC_{50} Analysis

A CETSA BRAF assay was applied to identify BRAF inhibitors in a human melanoma cell line containing the clinically relevant V600E mutation. A focused library of 896 kinase inhibitors was screened, yielding 13 hits, including well-characterized BRAF inhibitors and structurally related compounds. CRAF or ERK specific compounds were inactive in the assay, demonstrating the selectivity of the technique. The EC50 values obtained enabled SAR analysis for further lead optimization.

Integrating CETSA-derived ranking information and guiding compound design to support SAR analysis accelerates the transition from lead generation to lead optimization.

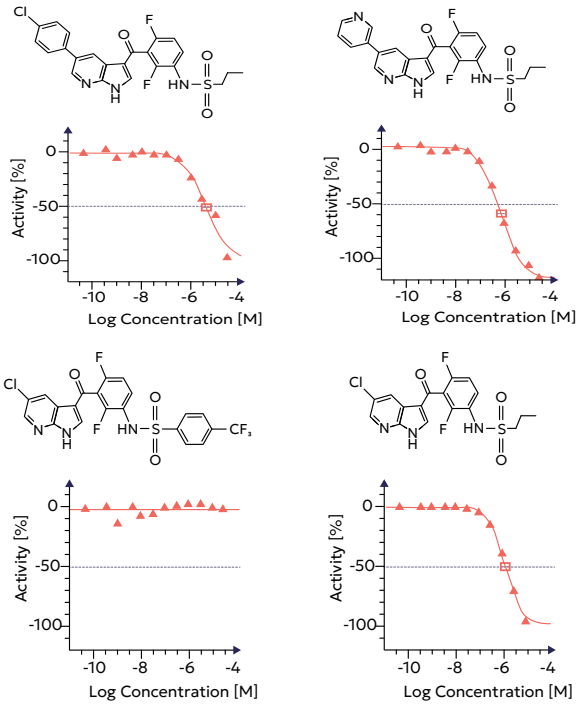

Figure 4: Data for BRAF inhibitor Vemurafenib, a close Vemurafenib analog, PLX556 (a CRAF selective compound) and PLC4720 (a selective BRAF600E inhibitor).

References

Jafari et al. Nature Protocols 2014
Rowlands et al. SLAS Discovery 2023
Shaw et al. SLAS Discovery 2018

Figures in this application note are modified from original.

CETSA's Role in accelerating Drug Discovery

Pelago Bioscience provides services that expedite every stage of your drug discovery journey—from primary screening and hit confirmation to candidate drug selection. Our patented CETSA technology offers deep insights by confirming that compound-mediated effects are driven by target engagement in physiologically relevant systems. CETSA significantly reduces failure rates and costs, ultimately accelerating the delivery of successful clinical drug candidates.

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