Unbiased Selectivity Profiling, CETSA with MS detection

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INTRODUCTION

Assessment of target engagement between a compound and its intended primary target versus interactions with off-target proteins helps ensure reduced liabilities in drug discovery projects. The ability to prioritize compounds based on off-target interactions help move the project forward.

Traditional selectivity assays, with panels limited to about 200 proteins, are available but result in a biased selectivity assessment with limited information about selectivity of the hit matter.

Pelago Bioscience has established an unbiased standardized selectivity profiling assay, enabling discrimination between selective and more promiscuous binding molecules at the proteome level. High throughput sample preparation and superior data quality, with assessment of more than 5,000 proteins, enables large compound batches to be assayed during early phases of drug discovery. This speeds up the process and increases the confidence in lead series and future lead candidates.

METHOD

We have applied the proprietary Cellular Thermal Shift Assay (CETSA®)[1,2] coupled to mass spectrometric readout to profile selectivity of a library of well known probes and marketed drugs. The use of compressed, integrated melt curve format (also known as PISA[3]) ensures a high throughput protocol.

The compounds were assayed at 30 µM in human cell line lysate followed by a heat challenge and isolation of soluble proteins. A semi-automated sample handling platform in combination with DIA analysis allowed fast, precise and reproducible profiling of compound induced thermal stability changes with a protein coverage of over 5,000 proteins.

RESULTS

Figure 1 shows selectivity patterns for the two histone deacetylase (HDAC) inhibitors Panobinostat and Abexinostat. As apparent from the volcano plots, there is a clear difference in the selectivity between the two, where all shifts induced by Abexinostat are identified also following Panobinostat treatment. However, Panobinostat displayed additional hits, comprising both additional HDAC proteins as well as non-HDAC proteins. Intriguingly, Abexinostat induced a destabilization of TTC38, whereas Panobinostat induced a stabilization of this protein.

Another example is shown in Figure 2 where target engagement profiles of the cyclin dependent kinase (CDK) inhibitors Abemaciclib and Roniciclib were partly overlapping. However, Roniciclib, well known as a pan-CDK inhibitor showed a much less selective profile. In total Roniciclib induced thermal shifts of 38 proteins. Out of those, 33 were kinases, with eight of them being CDKs. In contrast, Abemaciclib induced thermal shifts of 20 proteins in total, where 15 were kinases, four of them CDKs.

SUMMARY

A new standardized selectivity profiling method, based on CETSA with MS detection, allows for target engagement assessment of >5,000 proteins.

In contrast to other selectivity panels, this standardized selectivity profiling assay is unbiased, as all detected proteins are probed for compound interactions.

As the results in Figure 1 and Figure 2 clearly display, the method is well suited to compare selectivity of compounds with similar mode of action and/or target. The method can also be used for comparison of selectivity of a compound at different concentrations.

The method is well suited to be used in the lead generation/lead optimization phase to assess selectivity of different compounds or compound series.

The standardized selectivity profiling method enables early prioritization and assessment of compound promiscuity, potential liabilities, and identification of critical off-target proteins.

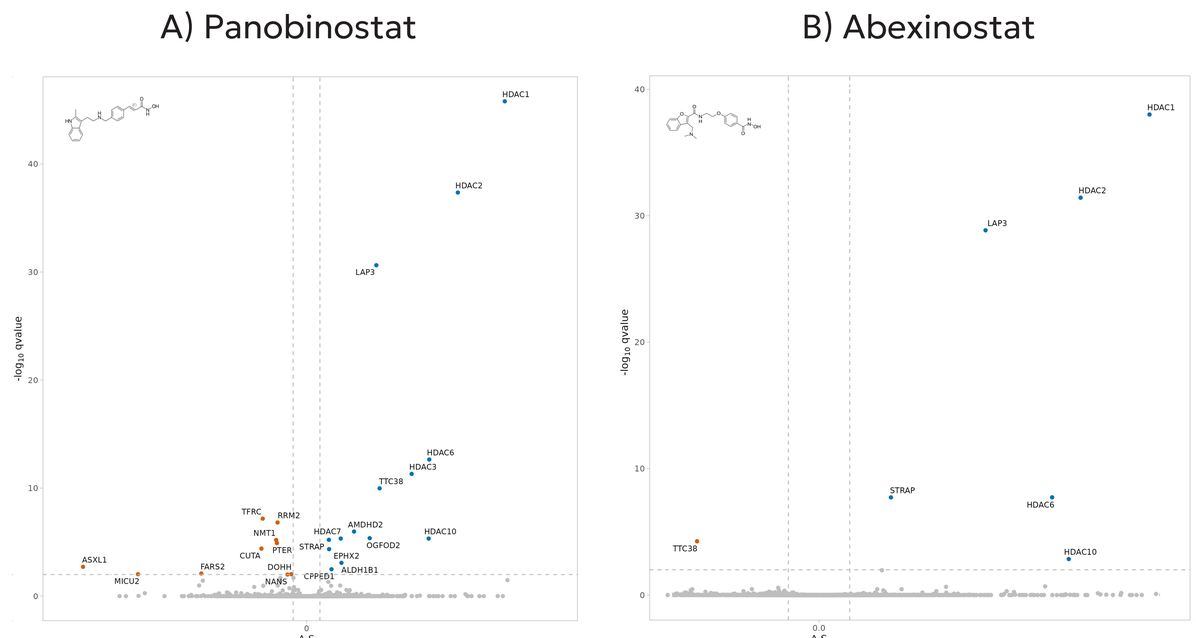

Figure 1. Examples of Selectivity profiling of two different HDAC inhibitors, Panobinostat (A) and Abexinostat (B). Here a difference in selectivity can be observed for both HDAC protein engangement and non-HDAC protein off-targets. At the investigated compound concentration (30 µM), Panobinostat induced thermal shifts of more off-targets than Abexinostat.

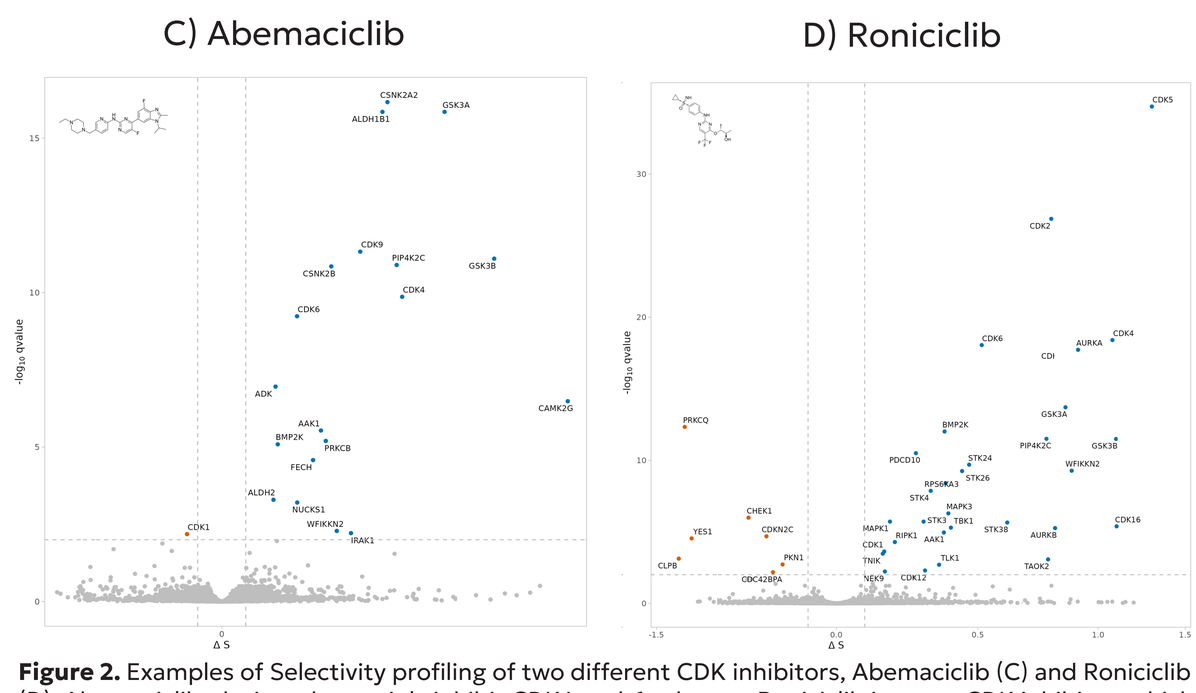

Figure 2. Examples of Selectivity profiling of two different CDK inhibitors, Abemaciclib (C) and Roniciclib (D). Abemaciclib, designed to mainly inhibit CDK4 and 6, whereas Roniciclib is a pan-CDK inhibitor, which was confirmed here.

References

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3. Gaetani, M. et al. Proteome Integral Solubility Alteration: A High-Throughput Proteomics Assay for Target Deconvolution. J of Prot. Res. 18, (2019)