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Probing the Cancer Epigenome – Empowering Target Validation by Open Innovation

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CONCEPT SUMMARY

The challenge

- Low reproducibility of published target validation studies has been recognized as a roadblock for cancer drug discovery[1]
- Widespread use of inappropriately characterized or unspecific chemical entities (**Figure 1**) as tools for cancer target validation limits the translation of basic research findings into successful drug discovery[2, 3]

The solution

- Academic and industrial institutions have started to address this issue by providing access to high-quality small molecular probes for novel targets of interest[4]
- Here, we present probe quality criteria and three probes for epigenetic targets of interest, all of which are available to academic labs for advancing the understanding of SMYD2, BRPF2, and ATAD2

Polyphenolic natural products such as resveratrol, quercetin, and curcumin pose an especially daunting challenge for target validation work. While it has been recognized for years that these compounds show nonspecific behavior, are unstable under many assay conditions, and may contain impurities, target validation studies using these and other similarly problematic substances remain common in the scientific literature.

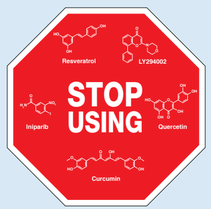
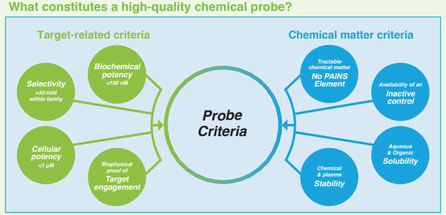


Figure 1. Examples of widely used, low quality tool compounds.

What constitutes a high-quality chemical probe?



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Disclosures

I.V.H., L.B., C.A., V.B., N.B., M.B., C.D.C., E.E., U.E., A.E.F.-M., M.G., A.H., B.H., R.H., S.H.H., S.J.K., A.L., A.M.-F., C.S., S.S., T.S., D.S., C.S., J.W., and H.W. are employees of Bayer AG. C.A., P.J.B., O.F., K.V.M.H., S.M., and M.V. are employees of the Structural Genomics Consortium, which has received finding from Bayer AG. This study was funded by Bayer AG.

Acknowledgments

We thank A. Chaikuad, L. Diaz-Saez, J. Bennett, P. Centrella, M. Clark, C. Dumelin, E. Sigel, H. Soutter, D. Troast, Y. Zhang, J. Cuozzo, A. Keffe, D. Roche, and V. Rodeschini for their support in identification of ATAD2 inhibitors and their further optimization; C. Tallant and J. Meier for their support in profiling BRPF2 inhibitors; F. Hübner, M. Geyer, N. Dittmar, and R. Karmaus for their technical assistance; and J. Kahmann and S. Fuellerton for their expert advice with protein-observed NMR and MST measurements. Under the authors' conceptual direction, editorial and typesetting assistance was provided by Bernard Kerr, PGDipSci, CMPP™, and Maryam Vahdat, PGDip, of Scion (London, UK), and was funded by Bayer AG.

BAY-598 (SMYD2)[5]

Biology

- SMYD2 was postulated to play a key role in regulation of p53 (**Figure 2**)
  - SMYD2 monomethylates p53 at lysine 370
  - Recruitment of methylated p53 to target genes is impaired
  - Increased SMYD2 activity could lead to apoptosis resistance in cancer cells
- SMYD2 is highly expressed in many cancers, and is a prognostic indicator for reduced overall survival
  - Esophageal squamous-cell carcinoma
  - Bladder cancer
  - Gastric cancer

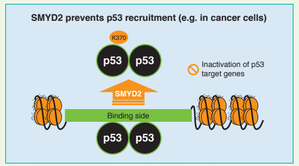


Figure 2. Role of SMYD2 in prevention of p53 recruitment.

Lead finding

- Screening of approximately 3 million compounds followed by hit validation and cluster prioritization using biophysical assays, selectivity assays, and chemical inspection identified a series of pyrazoline SMYD2 inhibitors (**Figure 3**)
- Systematic and X-ray-guided structure-activity relationship exploration yielded a >100-fold increase in potency (**Figure 4**)

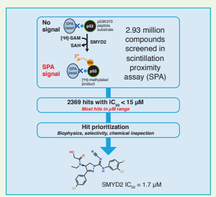


Figure 3. Lead finding process for the SMYD2 inhibitor BAY-598.

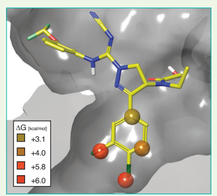
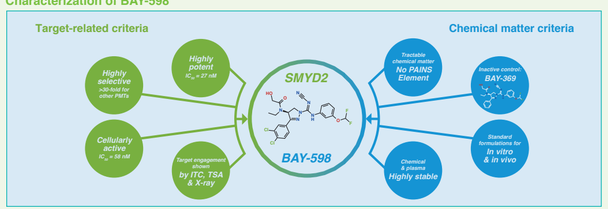


Figure 4. Optimal positioning of substituents, guided by Watermap calculations, boosted target potency and binding efficiency.

Characterization of BAY-598



- BAY-598 is highly active in cells, and can be dosed orally to mice for target validation studies **(Figure 5)**

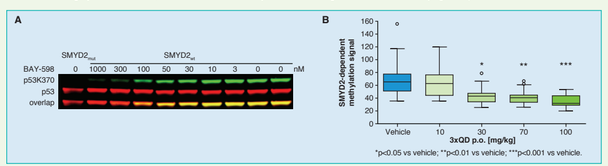
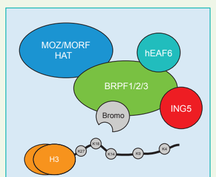


Figure 5. (**A**) Cellular activity of BAY-598: Inhibition of cellular p53 methylation. HEK 293 cells co-transfected with p53-FLAG and SMYD2-FLAG or catalytically inactive SMYD2^{mutant}-FLAG were treated with BAY-598 for 24 hours. (**B**) Inhibition of SMYD2-dependent methylation was evaluated *ex vivo* by treating mice bearing subcutaneous tumor xenografts (SMYD2 overexpressing KYSE-150 cell line) with oral doses of BAY-598 and subsequent analysis of tumors for methylation signals by dot-blotting.

BAY-299 (BRPF2-TAF1)[6]

Biology

- BRPF proteins are a subgroup of the bromodomain protein reader family with three members: BRPF1, BRPF2, and BRPF3
- BRPFs are transcriptional regulators and scaffold proteins forming a quaternary complex with the histone acetyltransferase MOZ/MORF (**Figure 6**)
- BRPF1 plays an essential role in AML bearing the MOZ-TIF2 fusion, and its knockdown reduces transformation ability[7]



Adapted from Poplawski A, et al. *J Med Biol.* 2014; 426:1661–1676.

Figure 6. Role of BRPF proteins.

Lead finding

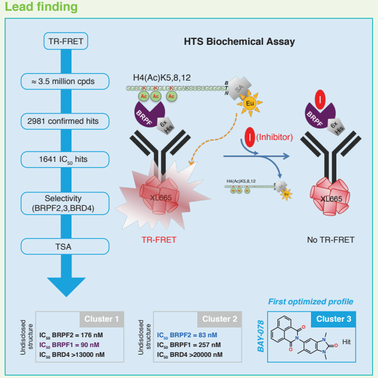
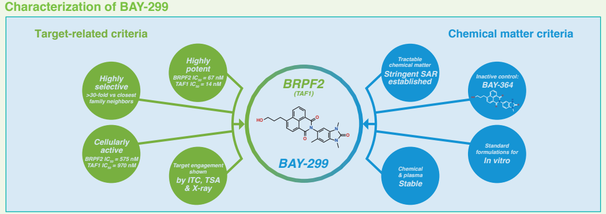


Figure 7. Lead finding process for the BRPF2 inhibitor BAY-078. Systematic SAR studies gave subsequently rise to the more potent and selective probe BAY-299.

Characterization of BAY-299



- BAY-299 binds to BRPF2 (K_d = 45 nM) and TAF1 (K_d = 17 nM) with high affinity and selectivity (**Figure 8**)

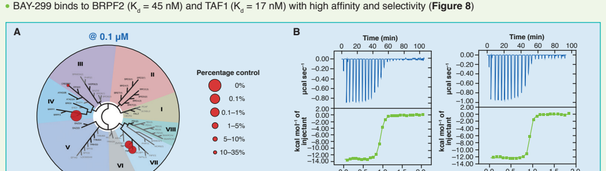


Figure 8. (**A**) Selectivity of BAY-299 using the BROMO*scan*™ panel. BROMO*scan*™ tree generated using the TREE*spot*™ software tool and is reprinted with permission from DiscoverX®. (**B**) Isothermal calorimetry measurements for BAY-299 with BRPF2 and TAF1 BD2.

BAY-850 (ATAD2)[8]

Biology

- ATAD2 (ATPase family AAA-domain containing protein 2) is an epigenetic regulator that binds to chromatin through its bromodomain (BD), a motif specialized for acetyl-lysine recognition
- ATAD2 directly associates with multiple transcription factors, and has thus been proposed to act as a co-factor for oncogenic transcription factors (**Figure 9**)
- High expression of ATAD2 is strongly correlated with poor prognosis in a wide range of tumor types, including breast, lung, gastric, endometrial, hepatocellular, and ovarian cancers

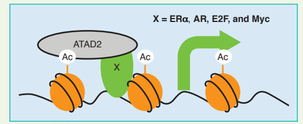


Figure 9. ATAD2 acts as a regulator of transcription through its interaction with various transcription factors.

Lead finding

- Screening of **11 DNA-encoded libraries** comprising a total of **65 billion compounds** provided a structurally unprecedented bromodomain inhibitor scaffold
- The hit was derived from a 110 million-membered library based on central formyl acid building blocks
- Systematic SAR exploration has led to BAY-850, a potent, cellularly active and exquisitely selective ATAD2 BD inhibitor (**Figure 10**)

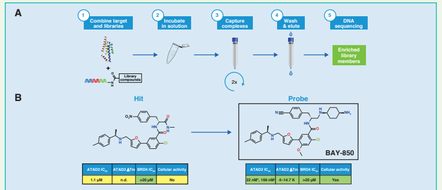
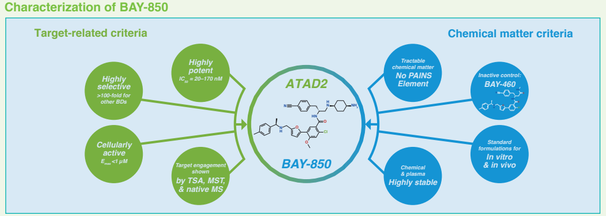


Figure 10. Lead finding process for BAY-850. (**A**) A hit was identified from a 65 billion compound library. (**B**) Systematic SAR exploration led to identification of BAY-850, a potent, cellularly active, and selective ATAD2 BD inhibitor.

Characterization of BAY-850



- BAY-850 engages ATAD2 in cells, as shown by FRAP experiments (**Figure 11**), and is highly selective (BROMO*scan*™, **Figure 12**)

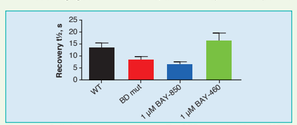


Figure 11. Cellular target engagement. TagGFP-tagged wild type (WT), BD mutant, and ATPase domain mutant ATAD2 proteins were expressed in MCF7 breast cancer cells and their binding to chromatin was measured by fluorescence recovery after photobleaching (FRAP). The recovery t½ of tagGFP-tagged WT ATAD2 protein was significantly faster in MCF7 cells treated with 1 µM BAY-850 than in untreated cells, and was comparable with the tagGFP-tagged BD mut ATAD2. Treatment of MCF7 cells with BAY-460 control compound had no major effect on the recovery t½.

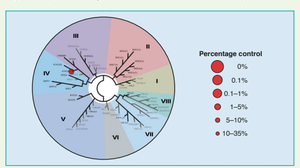


Figure 12. TREEspot™ plots of BROMOscan™ data for BAY-850 (test concentration: 10 µM) showing monoselectivity of the compound for ATAD2. The image was generated using the TREE*spot*™ software tool and reprinted with permission from KINOME*scan*®.