**Novel Assays and Human Model Systems for Epigenetic Drug Discovery**

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**Abstract**

We have developed a comprehensive suite of in vitro biochemical, cellular assays and human model systems to support compound screening and development by evaluating target-specific physico-chemical binding properties and compound effects on complex biological signaling networks. In this study, we determine inhibitor potency and selectivity using BROMOscan™, the industry’s largest panel of bromodomain targets and evaluate the pharmacologic impact of these inhibitors on human primary cell-based BioMAP Systems. We profiled a number of bromodomain inhibitors that target kinases, BET family bromodomain reader proteins and Histone deacetylase (HDACs) to generate binding profiles and pharmacologic signatures for each target class when compared to profiles for over 3000 clinical, failed pharmaceutical or tool compounds in the BioMAP database. Interestingly, a number of reported kinase inhibitors were shown to bind to bromodomains with high potency ($K_i \approx 0.01-1 \mu$M) and also demonstrated more complex pharmacologic signatures when compared to selective benchmark inhibitors, consistent with their dual kinase/bromodomain activities. These studies highlight the potential for unforeseen, high affinity auto-synergistic inhibition of these important epigenetic, regulatory proteins in addition to their original target kinase. DiscoveRx assays can provide a comprehensive evaluation of epigenetic inhibitors with respect to target potency, selectivity and impact on signaling mechanisms and resultant phenotypes in human cells. Taken together, these findings can be used to guide compound prioritization, indication selection and highlight potential safety issues to thereby improve the probability of clinical success.

**BROMOscan Core Technology Platform**

- Test Compound
- Competition
- No Competition

BROMOscan provides a direct measure of the amount of bromodomain bound to an immobilized ligand in the presence or absence of test compound using an ultrasensitive quantitative PCR (qPCR) method.

**BioMAP Systems Platform**

- BioMAP Assay Systems
- Reference Profile Database
- Predictive Informatics Tools

**BROMOscan - First In Class Bromodomain Screening Platform**

34 validated bromodomain assays
- Over 95% coverage across targets
- 71 family members represented
- Putative therapeutic targets (BET, ATAC, TRIM66)
- All BET family domains plus 24 non-BET assays

**Agreement Between BROMOscan and SGC T\_ Shift Data**

- Bromosporine-induced T\_ shift measurements
- Validated fluorescence-based thermal shift assay developed at the SGC
- Magnitude of bromodomain T\_ shift in the presence of inhibitor predicts activity
- Data collected by S. Knopp and S. Muller-Knopp, SGC, personal communication

**Family II (BET) Validation: Kd Data**

Bromosporine data consistent with published ITC data
- Potency, rank order and lack of potency activity for inactive I-BET nanomolar
- Accurate BROMOscan data for multiple inhibitors
- JQ1: I-BET PPi 1.8 & other known inhibitors (not shown)

**HDACi Signatures - Vorinostat, Entinostat and Panobinostat**

While Vorinostat and Entinostat have similar profiles (370 nM doses), Panobinostat is most similar at $\approx$23X lower dose (13.7 nM)
- May relate to pan-HDAC selectivity versus the more selective effects of Vorinostat (HDAC-3) and Entinostat (HDAC-1, -3)
- Common activities that could indicate an HDAC-3 selective signature include decreased TNF-α, IL-1β and IL-6, and increased IL-10, TIMP-1
- Such activities could guide compound selection using above sentinels for screening

**Summary & Conclusions**

Several challenges need to be addressed to enable epigenetic drug discovery:
- Epigenetic targets are structurally complex with limited options for high-throughput screening
- Toxicity and serious adverse events have led to low uptake of epigenetic therapies
- Rapid screening tools such as BROMOscan as well as the identification of signatures in BioMAP that are predictive for efficacy and safety will significantly support compound discovery, lead optimization and pre-clinical development in this emerging therapeutic space.

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