Historically, deacetylases (HDACs) are a class of enzymes that remove acetyl groups from ε-N-acetyl lysine amino acids on histones and other proteins. The removal of acetyl groups serves to increase the positive charge of histone tails, encouraging binding between histones and the DNA backbone, and preventing transcription. Because of this role, HDACs have been implicated in a variety of human diseases including cancer; making them an increasingly popular target for drug discovery research. Here we demonstrate an application to monochromator-based detection activity of multiple Class I and II HDAC enzymes in an automated fashion. Validation and pharmacology results demonstrate the ability to fluorometrically detect deacetylase activity and technology and instrumentation can be used to provide a relevant system to assay enzyme kinetics and inhibition.

Overview

Materials

Table 1 – Inhibitor IC50 Values. The IC50 values for BML-281 and Tabacin with HDAC3, 6, 8, and 10 can be explored by the concentration and type of substrate used in the experiment. Substrate concentrations above the IC50 value can lead to an inhibitory shift in the IC50 values.

Conclusions

Figure 7 – Z′-factor validation data. Results for all compounds with HDAC3, 6, 8, and 10. BML-281 and Tabacin inhibition curves demonstrate specificity for HDAC6.

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Figure 6 – Fluorescent assay technology and instrumentation can be used to perform a relevant system to assay enzyme kinetics and inhibition.

Figure 5 – The results show that a concentration of 15, 35, 0.03, and 0.5 ng/well lead to a shift in fluorescence for BML-281 for Class I and II HDACs, 6, and 10 while still within the linear range of the curve. This was further tested using a Z'-factor test to determine the robustness of the assay using the optimized conditions.

Figure 4 – Data reduction and detection instrumentation to be used to perform a relevant system to assay enzyme kinetics and inhibition.

Figure 3 – HDAC enzyme titration. The results demonstrate the linear increase in fluorescence. The optimized enzyme concentrations for each substrate demonstrated an IC50 value of 0.5 ng/well for HDAC3, 6, 8, and 10 with Z'-factor values of 0.76.

A Flexible and Robust Solution for Automated 384-well HDAC Profiling

Brad Larson1, Peter Banks1, Diana Hulbo2, Kara Cannon1, Wayne Patton2

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Figure 2 – Representation of Fluorescent Assay Technology and Instrumentation. The delta RFU value for each well was calculated using the following equation:

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Figure 1 – The Precision™ Microplate Pippetting System contains an 8-channel dispensing head and an 8-channel bulk reagent dispenser in one instrument. The instrument was designed to easily transfer uL to mL volumes across a 96-well polypropylene plate, transfer the compound to the 384-well assay plate, as well as dispense all assay components to 384-well format.

B. Synergy™ Hi with Hybrid Technology and the Synergy™ Multi-Mode Detector System that includes both filters and a 488 nm excitation filter.

Inhibitor sensitivity of the Synergy H4 and Fluor de Lys®-Quenched HDAC assay allow for assay miniaturization to 384-well format. A 1:2 dilution scheme was used to perform a relevant system to assay enzyme kinetics and inhibition.

4. Excellent assay robustness, using low concentrations of enzyme and substrate concentrations, with Z'-factor values of 0.74.

5. Profiling of lead compounds against multiple HDAC enzymes can be easily carried out using the automated Fluorescent assay technology and instrumentation can be used to perform a relevant system to assay enzyme kinetics and inhibition.