A substrate-independent histone deacetylase inhibitor assay

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ABSTRACT

Developing molecularly targeted therapeutics with minimal off-target effects is facilitated by a clear understanding of compound selectivity among related targets. Histone deacetylase (HDAC) inhibitors have been pursued for various indications and are now used clinically, primarily for hematological malignancies. However, a clearer understanding of the specificity of HDAC inhibitors has been challenging. Literature reports of the specificity of HDAC inhibitors have varied substantially, likely due to differences in substrate choice and enzyme sources. In particular, it has been suggested that use of non-specific substrates and the presence of multiple HDAC activities in enzyme preparations may complicate interpretation of inhibitor specificity experiments. To overcome these potential limitations of activity-based assays, we have developed an assay format based on measurement of the binding affinity of HDAC inhibitors rather than measurement of enzyme activity. A key advantage is this format is that it does not require use of a non-substrate and thus ameliorates concerns about lack of specificity of existing substrates. This assay is based on an Alexa Fluor 480-labeled HDAC inhibitor or ‘tracer’ which binds with a high affinity to Class I and Class II HDACs. Binding of the ‘tracer’ to an epitope-tagged HDAC is detected by addition of a europium-labeled anti-epitope tag antibody. Binding of the tracer and antibody to the HDAC results in a high degree of FRET, whereas displacement of the tracer with an inhibitor results in a loss of FRET. Unlike activity assays, which can be affected by the presence of nonspecific untarged endogenous HDACs from the host expression system, the signal in this format is dependent on the presence of an epitope tag on the specific HDAC of interest. We demonstrate utility of this method by determining inhibitor potencies for commonly used HDAC inhibitors for Class I and IIb HDACs.

RESULTS

This assay is based on an Alexa Fluor 480-labeled HDAC inhibitor or ‘tracer’ which binds with a high affinity to Class I and Class IIb HDACs. Binding of the ‘tracer’ to an epitope-tagged HDAC is detected by addition of a europium-labeled anti-epitope tag antibody. Binding of the tracer and antibody to the HDAC results in a high degree of FRET, whereas displacement of the tracer with an inhibitor results in a loss of FRET.

Figure 1. LanthaScreen® HDAC Binding Assay Principle

The apparent dissociation constant (Kd) for the HDAC-tracer binding interaction was measured by incubating 5 nM HDAC, 2 nM antibody, and a tracer of HDAC tracer. 1% DMSO or 10 µM inhibitor A (TSID) was included to represent the total and non-specific TR-FRET signal, respectively. A corrected TR-FRET ratio was obtained from the difference between these two TR-FRET signals.

Table 1. Assay Conditions for Inhibitor Screening

Table 2. Inhibitor Screening Results, IC50 Values (nM)

SUMMARY OF HDAC BINDING ASSAY

• Simple, three step measurement of inhibitor potency
• Coupled with standard HDAC assays
• Substrate-independent and therefore eliminates concerns about substrate specificity
• Binding can be observed continuously or in an end-point fashion
• Signal is specific to the epitope-tagged HDAC, which unlike activity assays, minimizes interference from co-purifying HDACs from a host expression system

REFERENCES

2. Bantscheff et al., Chemical genomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes, Nat Biotechnol 29 (2011) 2265-2272
3. Most of the data presented for knockdowns using this assay format may be found in the references of the HDAC assays and can be found at enverge.com/consulting.

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