ABSTRACT

The methylation of lysine or arginine residues by histone methyltransferases (HMTs) is an important and complex post-translational modification implicated in the regulation of gene transcription. Abnormal HMT activity plays a role in certain human diseases, and as a result, HMTs have become an important target for the development of inhibitors. HMTs transfer a methyl group from the cofactor S-adenosylmethionine (SAM) to an amine group on lysine or arginine residues of histones in a site- and methylation-state specific manner. The majority of HMTs contain a catalytic SET-domain, which consists of a SAM-binding site located opposite of the histone tail binding site. Since the SET-binding site is heterogeneous across methyltransferases, it is an ideal target to develop selective small-molecule probes to identify novel HMT inhibitors. In this study, we have developed two novel fluorescent small-molecule probes that exhibit high-affinity binding to the SAM cofactor binding site of HMTs. These probes have been characterized by measuring their binding to a panel of methyltransferases using a fluorescent polarization (FP) assay. This assay was validated for high-throughput screening (HTS) with Cayman Chemical’s SAM-binding site probe by screening 14,400 compounds with representative HMTs. The development of these novel probes and screening techniques have provided a valuable HTS-amenable assay to identify SAM binding site inhibitors of HMTs.

Figure 1: Assay Set-up

Schematic diagram of the homogenous, one-step binding assay used to probe the SAM-binding site of SET-domain methyltransferases. This assay is non-kinetic, substrate independent, and suitable for a 384 or 1,536-well format.

Figure 2: Model of the SAM-Screener™ Probe bound to SET7/9 methyltransferase

The SAM-Screener™ probe binds in the SAM-binding site of specific methyltransferase enzymes. Upon binding, the probe will contribute to a fluorescence polarization signal. The FP signal would decrease when the probe is displaced with a competing SAM-site inhibitor.

Figure 3: The SAM-Screener™ Probe binds to several histone methyltransferases

Saturation binding data for A) SET7/9 and B) MLL1 to the SAM-binding site FP probe 1. Data are presented as the mean ± SEM from three independent experiments. C) Probe binding affinities for selected SAM-binding proteins. Shown are the enzymes which bound the probe with an affinity > 2 μM.

Figure 4: Adaptation of the assay to high-throughput format

A) Displacement of the SAM-binding site FP probe from SET7/9. Sinefungin and SAM displace probe binding with IC₅₀ and K₅₀ values indicated. Data are presented as the mean ± SEM. B) SET7/9 assay Z′ score. The probe polarization is increased by > 120 mp by binding to SET7/9 (30 nM). Sixty-four replicates were tested and Z′ score was 0.71. The dashed lines represent the 3 SD line from the mean for each control. C) Assay Z′ scores for SET7/9, GLP, and MLL1.

Table 1: High-throughput screening data for SET7/9

Proteins displacement activity from SET7/9 were tested with 14,400 compounds. The probe polarization is increased by > 120 mp by binding to SET7/9 (30 nM). Sixty-four replicates were tested and Z′ score was 0.71. The dashed lines represent the 3 SD line from the mean for each control. C) Assay Z′ scores for SET7/9, GLP, and MLL1.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Z′</th>
<th>Δ Polarization (mP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SET7/9</td>
<td>0.71</td>
<td>122</td>
</tr>
<tr>
<td>GLP</td>
<td>0.68</td>
<td>113</td>
</tr>
<tr>
<td>MLL1</td>
<td>0.72</td>
<td>147</td>
</tr>
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Table 2: Profiling SET7/9 and GLP

The probe displacement assay was used to profile SET7/9 and GLP against a pre-production version of Cayman Chemical’s exclusive small molecule epigenetics screening library, which contained only one SAM binding site molecule (Sinefungin).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Z′</th>
<th>Hit Rate</th>
<th>Hit Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>SET7/9</td>
<td>0.72</td>
<td>1/45</td>
<td>Sinefungin</td>
</tr>
<tr>
<td>GLP</td>
<td>0.68</td>
<td>1/45</td>
<td>Sinefungin</td>
</tr>
</tbody>
</table>

CONCLUSIONS

- We have developed unique small-molecule fluorescent probes that selectively bind to the SAM-binding site of SET domain methyltransferases with up to nanomolar affinity.
- Using this probe, we have developed a homogeneous, one-step binding assay to investigate the SAM-binding site of SET-domain methyltransferases.
- This assay is non-kinetic, substrate independent, and scalable to 1,536-well format.
- As a proof of concept, we screened a library of 14,400 small molecules in a high-throughput format against the stereotypical SET domain methyltransferase, SET7/9.

References