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Accessibility
Identification and Characterization of Small Molecule Inhibitors of a Class I Histone Deacetylase from Plasmodium falciparum


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Abstract: A library of approximately 2000 small molecules biased toward inhibition of histone deacetylases was assayed for antimalarial activity in a high-throughput P. falciparum viability assay. Active compounds were cross-analyzed for induction of histone hyperacetylation in a human myeloma cell line to identify HDAC inhibitors with selectivity for P. falciparum over the human host. To verify on-target selectivity, pHDAC-1 was expressed and purified and a biochemical assay for pHDAC-1 activity was established.

The overwhelming majority of morbidity and mortality associated with human malaria is caused by the apicomplexan parasite Plasmodium falciparum. Widespread resistance to mainstay therapies such as chloroquine,1 atovaquone,2 pyrimethamine,3 and sulfadoxine4 has highlighted the need for new mainstay therapies such as chloroquine,1 atovaquone,2 pyrimethamine,3 and sulfadoxine4 has highlighted the need for new

Figure 1. HDAC-biased chemical library. The archetypal HDAC inhibitor, and the basis for this focused library, consists of a capping element joined to a metal chelating moiety by a hydrophobic linker. Diversity was introduced at all three of these motifs with the final library consisting of ~2000 chemical members.

Table 1. Substrate-Specificity of pHDAC-1

<table>
<thead>
<tr>
<th>substrate</th>
<th>K_M (µM)</th>
<th>k_cat (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac-Leu-Gly-Lys(Ac)-AMC</td>
<td>30 ± 2</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Boc-Lys(trifluoroacetyl)-AMC</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*No activity observed with 2 in the presence of excess substrate and pHDAC-1.

Table 2. Inhibition of pHDAC-1 Activity and P. falciparum 3D7 Proliferation by Known HDAC Inhibitors

<table>
<thead>
<tr>
<th>compd</th>
<th>pHDAC-1 IC₅₀ (nM)</th>
<th>P. falciparum 3D7 IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.6 ± 0.1</td>
<td>16 ± 7</td>
</tr>
<tr>
<td>4</td>
<td>59 ± 6</td>
<td>210 ± 30</td>
</tr>
<tr>
<td>5</td>
<td>1.8 ± 0.2</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>940 ± 90</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>7</td>
<td>1 ± 0.1</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>splitomicin</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
</tr>
</tbody>
</table>

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# Abbreviations: pHDAC-1, P. falciparum histone deacetylase-1; AMC, 4-methyl-7-aminocoumarin; HDAC, histone deacetylase; HAT, histone acetyltransferase; TSA, trichostatin A; SAHA, suberoylanilide hydroxamic acid.

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is certain to be vital for parasite survival. The HDAC inhibitor 7, which elicits an increase in *P. falciparum* histone acetylation concomitant with reduced parasite proliferation, provided the initial proof of concept for the essentiality of HDAC function in the parasite.\(^{16}\) Unfortunately, unfavorable pharmacological properties limited the further development of 7 as an antimalarial agent.

Genome sequencing of *Plasmodium falciparum* uncovered one class I HDAC, two class II HDACs, and two class III sirtuins. Only one of the class III enzymes, *P. falciparum* silent information regulator 2 (pfSir2; PlasmoDB gene ID, PFI13_0152), has been definitively shown to possess HDAC activity.\(^{17,18}\) The putative class I and II HDACs have not yet been examined in sufficient detail to confirm actual HDAC activity. Expression and purification of class I HDACs have generally afforded greater success than the class II enzymes, and thus, we focused our study on the sole class I HDAC, pfHDAC-1 (PlasmoDB gene ID, PFI1260c). The enzyme is a 51 kDa nuclear protein that is expressed in gametocytes and mature blood stages of the malaria parasite life cycle and shares significant homology to all of the class I human HDACs.\(^{19}\)

I. For expression and purification of pfHDAC-1, pfHDAC-1 was recombinantly expressed and purified from S2 insect cells. The cDNA encoding the pfHDAC-1 was shuttled into the pAc5.1 expression vector using Gateway cloning (Invitrogen) for antimalarial efficacy and inhibition of pfHDAC-1 activity.

II. For biochemical characterization of recombinant pfHDAC-1, the endogenous histone substrate from *P. falciparum* is not conveniently available to perform a detailed kinetic analysis of pfHDAC-1. Therefore, we investigated the possibility of measuring enzyme activity using a series of artificial substrates that resemble an N-acetylated lysine residue and that have been demonstrated to be processed by mammalian and bacterial class I or class II HDACs.\(^{20,21}\)

Of the two substrates that were examined for recognition by pfHDAC-1 only 1 was efficiently catalyzed (Table 1). The Michaelis–Menten model was fitted to the data which afforded the kinetic constants \(k_{cat} = 0.19 \pm 0.01 \text{ s}^{-1}\) and \(K_{m,app} = 30 \pm 2 \mu\text{M}\). The binding affinity and catalytic efficiency of 1 to pfHDAC-1 are comparable to those observed with class I HDACs from other organisms. The known HDAC inhibitors 3–7 were tested for the inhibition of pfHDAC-1 activity and parasite proliferation (Table 2). 3–5 and 7 were found to be inhibitory in both assays, whereas 6 did not demonstrate significant inhibition of either pfHDAC-1 activity or parasite growth. Interestingly, \(\alpha\)-aminoanilide based compounds such as 6 have been documented to exhibit selectivity for human class I over class II enzymes.\(^{22}\) The class III specific HDAC inhibitor splitomicin was also determined to be inactive in enzyme and parasite inhibition assays.

III. For inhibition of pfHDAC-1 and parasite proliferation, the archetypal chemical structure for a HDAC inhibitor consists of a metal chelating group joined by a carbon chain linker to a hydrophobic capping element that confers potency and selectivity (Figure 1).\(^{23}\)

Given the effectiveness of hydroxamate-based HDAC inhibitors, it was hypothesized that selectivity could be engineered for pfHDAC-1 and the malaria parasite with a core scaffold containing a hydroxamate metal chelating unit. Although not species selective, the spectrum of activity already observed for 3–5 supported this model (Table 2). For this reason, a ~2000 member HDAC-biased chemical library was sequentially screened for antimalarial efficacy and inhibition of pfHDAC-1 activity. Chemical diversity within the library was introduced at the recognition cap or metal chelating portion of the molecule, and the linker length between these two groups was varied from four to six carbons (Figure 1).\(^{24}\)

We have previously analyzed this library for the alteration of in vivo bulk histone acetylation in mammalian cells (Supporting Information Figure 1). Many compounds were found to potently inhibit parasite proliferation and pfHDAC-1 activity; however, only 17 displayed antiparasitic activity coupled with minimal perturbation of mammalian histone acetylation (Table 3). Inhibition of *P. falciparum* proliferation was biased toward compounds with ortho-substitution (bromine, hydroxyl) in the cap region of the core scaffold.

The presence of a hydroxamic acid as a metal chelating ligand was necessary for activity. The ideal linker length for selective compounds was found to be five methylene units in contrast to the six methylene units preferred for compounds inducing histone hyperacetylation in mammalian cells.
Transcriptional regulation orchestrates the multiple morphological transformations that occur throughout the complex developmental stages of the *P. falciparum* life cycle. HDACs participate in the reversible acetylation of lysine residues within histone tails to control chromatin unwinding and DNA transcription. Inhibition of HDAC activity has already proven useful for the treatment of various cancers and may also be valuable for the development of antimalarial chemotherapies. Here, we have expressed recombinant pfHDAC-1, the sole class I isoform identified by homology in *P. falciparum*, and confirmed that the protein has in vitro HDAC activity. The known HDAC inhibitor 4 elicits hyperacetylation of *P. falciparum* histones and retards parasite proliferation. Unfortunately 4 is neither species nor HDAC-isofrom selective, and yet specificity for pfHDAC-1 and *P. falciparum* was obtained herein by modifying the core scaffold. Seventeen compounds were found to significantly inhibit parasite proliferation and pfHDAC-1 activity, and yet these compounds did not perturb histone acetylation or inhibit whole-cell growth of mammalian MM.1S. These compounds generally contained bromine and hydroxyl substituted phenol derivatives as recognition caps and a hydroxamic acid metallochelate substituent as probes of intracellular histone and tubulin acetylation. Chem. Biol. 2003, 10, 397–410.


