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Discovery of HDAC inhibitors with potent activity against multiple malaria parasite life cycle stages

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ABSTRACT

In this work we investigated the antiplasmodial activity of a series of HDAC inhibitors containing an alkoxyamide connecting-unit linker region. HDAC inhibitor **1a** (LMK235), previously shown to be a novel and specific inhibitor of human HDAC4 and 5, was used as a starting point to rapidly construct a mini-library of HDAC inhibitors using a straightforward solid-phase supported synthesis. Several of these novel HDAC inhibitors were found to have potent *in vitro* activity against asexual stage *Plasmodium falciparum* malaria parasites. Representative compounds were shown to hyperacetylate *P. falciparum* histones and to inhibit deacetylase activity of recombinant *Pf*HDAC1 and *P. falciparum* nuclear extracts. All compounds were also screened *in vitro* for activity against *Plasmodium berghei* exo-erythrocytic stages and selected compounds were further tested against late stage (IV and V) *P. falciparum* gametocytes. Of note, some compounds showed nanomolar activity against all three life cycle stages tested (asexual, exo-erythrocytic and gametocyte stages) and several compounds displayed significantly increased parasite selectivity compared to the reference HDAC inhibitor suberoylanilide hydroxamic acid (SAHA). These data suggest that it may be possible to develop HDAC inhibitors that target multiple malaria parasite life cycle stages.

1. Introduction

Despite decades of research on its prevention and treatment, malaria remains a significant disease in tropical and subtropical regions of the world. As reported by the World Health Organization (WHO), 3.3 billion people were at risk of malaria in 2011, which is approximately half of the world's population [1]. In 2010 alone it is estimated that there were ~1.2 million malaria related deaths [2], the vast majority of which were due to infection with *Plasmodium falciparum* parasites. There is currently no licensed malaria vaccine and recent clinical trials in African children with the most advanced candidate, RTS,S/AS02D, were disappointing, with only ~30% protection being achieved over 18 months follow-up [3,4]. Hence, antimalarial drugs currently remain the most effective tool for malaria treatment and, together with vector control strategies, for malaria prophylaxis. Unfortunately, the rapid spread of drug-resistant *P. falciparum* parasites is compromising antimalarial drug efficacy in a clinical setting [5]. Alarming signs of emerging resistance to artemisinin derivatives [6,7] could threaten the now widely-used artemisinin combination therapies (ACTs) and highlight the urgent need to discover and develop new antimalarials with novel modes of action. Drugs that target different, or

Abbreviations: DIC, *N,N′*-diisopropylcarbodiimide; DMF, *N,N*-dimethylformamide; EEF, exo-erythrocytic form; HDAC, histone deacetylase; *Pf*, *Plasmodium falciparum*; *Pf* LSG, *P. falciparum* NF54 late stage gametocytes (IV–V); *Pb*, *Plasmodium berghei*; RT, room temperature; SAHA, suberoylanilide hydroxamic acid; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TSA, trichostatin A.

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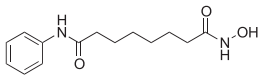
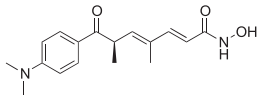
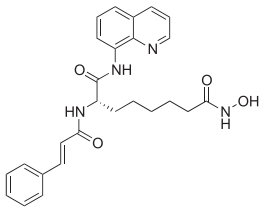
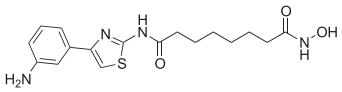
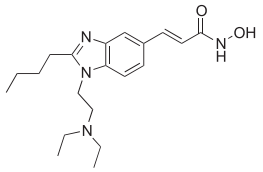
Table 1
Structures and properties of selected hydroxamate-based HDAC inhibitors.

Name	Structure	MW	*P. falciparum* IC_{50} [μM]	Mammalian cell cytotoxicity IC_{50} [μM]	SI[a]
Vorinostat (SAHA)[b]		264	0.109–0.309	2.200–>20	7–>183
Trichostatin A (TSA)[b]		302	0.008–0.011	0.200	18–25
2-ASA-9[b]		461	0.015–0.039	1.240	32–82
WR301801 (YC-II-88)[c]		362	0.0006–0.0018	0.600	333–1000
SB939[d]		358	0.080–0.150	0.800–>100	4–>1250

[a] SI = (mammalian cell IC_{50})/(*P. falciparum* IC_{50}) – larger values indicate greater malaria parasite selectivity.
[b] Data from Ref. [25].
[c] Data from Ref. [28].
[d] Data from Ref. [29].

preferably multiple, parasite life cycle stages are also a high priority. Most current antimalarials are active against the asexual blood stages of the parasite, which are responsible for the clinical symptoms of malaria [1]. However, recent drug discovery efforts have moved towards eradication of malaria [8], and seek to additionally target exo-eryothrocytic liver stages and gametocyte (transmission) stage parasites [9]. *Plasmodium* liver stages are clinically silent pre-erythrocytic life cycle stages that are promising targets for new drugs as inhibition of this stage leads to a true causal prophylaxis [10]. The transmission of malaria parasites to the female *Anopheles* mosquito vector occurs when sexual stage gametocytes are taken up in the blood of an infected individual during a blood meal. Following fertilization, meiosis and sporogony in the mosquito, progeny parasites can then be transmitted to another host when the female mosquito feeds again. A considerable number of drugs, which kill asexual parasites and alleviate symptoms, do not kill late stage gametocytes, allowing the infected individual to continue to spread the disease even after symptoms have disappeared [11]. Therefore, therapeutically blocking transmission is also a high priority for the malaria elimination agenda [11,12].

One promising strategy to identify new antimalarial agents is the "piggyback" approach, which focuses on drug targets that have been validated for other diseases. Using this approach, we, and others, have previously investigated the antimalarial potential of compounds that target histone deacetylase (HDAC) enzymes [13,14]. While no HDAC inhibitor has yet been used clinically for malaria, this class of compound has been progressed to clinical use for cancer. Both the hydroxamate-based pan-HDAC inhibitor, vorinostat (suberoylanilide hydroxamic acid (SAHA)), and the class I selective prodrug, romidepsin (FK228), have been approved for treatment of cutaneous T-cell lymphoma (CTCL) [15–21]. The *P. falciparum* genome contains at least five putative HDACs [22] and the enzyme *P. falciparum* histone deacetylase 1 (*Pf*HDAC1) has been identified as a target of antimalarial HDAC inhibitors [23]. Treatment of *P. falciparum* parasites with HDAC inhibitors results in genome wide transcriptional alterations [24–26] and altered *Pf*HDAC1 expression has been found in *P. falciparum* parasite lines with reduced clinical susceptibility to artemisinin [27]. Together these findings underscore *Pf*HDACs' potential as novel parasite drug targets. The structures and properties of selected hydroxamate-based HDAC inhibitors with antimalarial activity are summarized in Table 1.

Despite some progress in recent years, there are still a number of challenges in the rational development of HDAC inhibitors as antimalarial drug leads. Next generation compounds should retain potent antiplasmodial activity and low host cell toxicity, but they also require improved pharmacokinetic properties relative to current generation compounds. In addition, while most work to date has focused on asexual stage parasites [13], we recently showed that two HDAC inhibitors (SAHA and SB939; see Table 1 for structures) have potent activity (IC_{50} ~150 nM) against exo-erythrocytic stage *Plasmodium* parasites [29]. This raises the possibility that HDAC inhibitors could be developed as causal prophylactic and/or transmission blocking agents.

In this work we investigated the antimalarial activity of a new type of HDAC inhibitor, containing an alkoxyamide connecting-unit linker region [30], against different parasite life cycle stages. Previous work on the cytotoxicity and HDAC inhibitory activity of these alkoxyamide-based HDAC inhibitors against different human cisplatin sensitive and