Antimalarial Activity of the Anticancer Histone Deacetylase Inhibitor SB939


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Histone deacetylase (HDAC) enzymes posttranslationally modify lysines on histone and nonhistone proteins and play crucial roles in epigenetic regulation and other important cellular processes. HDAC inhibitors (e.g., suberoylanilide hydroxamic acid [SAHA; also known as vorinostat]) are used clinically to treat some cancers and are under investigation for use against many other diseases. Development of new HDAC inhibitors for noncancer indications has the potential to be accelerated by piggybacking onto cancer studies, as several HDAC inhibitors have undergone or are undergoing clinical trials. One such compound, SB939, is a new orally active hydroxamate-based HDAC inhibitor with an improved pharmacokinetic profile compared to that of SAHA. In this study, the in vitro and in vivo antiplasmodial activities of SB939 were investigated. SB939 was found to be a potent inhibitor of the growth of Plasmodium falciparum asexual-stage parasites in vitro (50% inhibitory concentration [IC50], 100 to 200 nM), causing hyperacetylation of parasite histone and nonhistone proteins. In combination with the aspartic protease inhibitor lopinavir, SB939 displayed additive activity. SB939 also potently inhibited the in vitro growth of exoerythrocytic-stage Plasmodium parasites in liver cells (IC50 ~ 150 nM), suggesting that inhibitor targeting to multiple malaria parasite life cycle stages may be possible. In an experimental in vivo murine model of cerebral malaria, orally administered SB939 significantly inhibited P. berghei ANKA parasite growth, preventing development of cerebral malaria-like symptoms. These results identify SB939 as a potent new antimalarial HDAC inhibitor and underscore the potential of investigating next-generation anticancer HDAC inhibitors as prospective new drug leads for treatment of malaria.

Widespread resistance of Plasmodium parasites to commonly available antimalarial drugs (23) and the lack of a licensed vaccine have necessitated increased efforts in the discovery and development of new antimalarial agents. Of particular interest are new antimalarial drug candidates which act on novel parasite targets or mechanisms associated with parasite survival or development. Transcriptional control in malaria parasites is complex, and regulatory mechanisms are the subject of intense investigation. Nevertheless, there is increasing evidence that targeting of transcriptional regulation represents a potential new therapeutic approach for malaria. Histone deacetylase (HDAC) enzymes, which are key regulators of transcription and validated therapeutic targets for some types of cancer, are promising new antimalarial drug targets (7).

Histone acetyltransferases (HATs) posttranslationally modify proteins by catalyzing the transfer of an acetyl group from acetyl-coenzyme A (acetyl-CoA) to the ε-nitrogen on the side chain of lysines, while HDACs catalyze the reverse reaction. These enzymes play crucial roles in modulating the acetylation state of histone proteins, contributing to alterations in chromatin structure and transcription (17). Many nonhistone proteins have also been identified as HDAC substrates (15, 22, 30). As a result, protein lysine acetylation is now considered common and is thought to play critical roles in regulating many important cellular processes, including protein stability, protein-protein interactions, protein localization, and DNA-binding properties of proteins (44).

Mammalian HDACs are classified into four classes (29, 31). Class I, II, and IV HDACs share a zinc cofactor catalytic core (27, 59), while class III HDACs use NAD+ to deacetylate their substrates. Five HDAC-encoding genes have been identified in the Plasmodium falciparum genome: one encoding a homologue of class I HDACs (PfHDAC1), two encoding homologues of class II HDACs (PfHDAC2 and -3), and two encoding class III HDAC homologues (PfSir2A and PfSir2B) (25, 28, 36, 61). PfSir2A and PfSir2B are not essential for asexual, blood-stage growth of P. falciparum in vitro. However, both play roles in regulating the expression of subsets of P. falciparum virulence genes, indicating potentially important roles in immune evasion in vivo (25, 28, 61). Although the functional roles of the class I and II PfHDAC homologues have not yet been elucidated, PfHDAC1 is a likely target of antimalarial HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) (50).

In eukaryotic cells, interfering with HDAC action by using small-molecule inhibitors results in an accumulation of acetylated histones, alterations to transcription, and various cellular responses, such as apoptosis, differentiation, and changes in cell cycle progression. Three HDAC inhibitors are currently in clinical use for treatment of cancer, including two hydroxamate-based compounds (SAHA [Zolinza; Merck & Co.] and 4SC-201 [resminostat; also known as 4SC]) and a cyclic depsipeptide
Can HDM2 protein interact with HDACs to regulate gene expression? A study was conducted to investigate this interaction.

The researchers isolated HDM2 from mouse liver and used affinity purification to identify its interacting proteins. They found that HDM2 interacted with several HDACs, including HDAC1, HDAC2, and HDAC3.

To further explore the interaction, the researchers used co-immunoprecipitation assays and confirmed the interaction in vivo. They also performed pull-down experiments to demonstrate the interaction in vitro.

The interaction between HDM2 and HDACs is crucial for regulating gene expression and plays a role in various biological processes, including cell cycle progression and apoptosis.

The study provides new insights into the molecular mechanisms underlying the interaction between HDM2 and HDACs, which may have implications for understanding the pathogenesis of various diseases.
mology model; minor side chain geometries and protonation states near the active site Zn atom were corrected manually. This homology model was then used in GOLD for ligand docking studies.

The ligands SB939, TSA, and SAHA were built in Sybyl-X 1.3 and minimized using the MFF94s force field (MMFF94 charges; dielectric constant of 80; distance dependent) to a terminal gradient of 0.05 kcal/mol. GOLD was then used to perform 15 dockings of the ligands in the homology model, using the zinc-bound hydroxamate group of TSA (CONHOH atoms) as a scaffold constraint (10 kcal/mol). Side chains of Thr266, Asp267, Ser146, Arg268, and Leu269 were allowed to rotate freely during the docking experiment, and conformations were scored using Goldscore and rescored using Chemscore (48).

Protein hyperacetylation assays. Protein hyperacetylation assays were carried out by incubating synchronous trophozoite-stage *P. falciparum* 3D7 parasites (3 to 5% parasitemia; 5% hematocrit) in six-well tissue culture plates for 3 h under standard *in vitro* culture conditions with different concentrations of compound or vehicle (0.05% DMSO). Infected erythrocytes were lysed with 0.15% saponin (Sigma), and parasite pellets were obtained by centrifugation. Pellets were washed extensively in cold PBS, pH 7.4, to remove hemoglobin and then resuspended in SDS-PAGE loading dye, heat denatured at 96°C for 3 min, and separated via SDS-PAGE. Proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Roche), and Western blotting was carried out using SDS-PAGE loading dye, heat denatured at 96°C for 3 min, and separated via SDS-PAGE. Proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Roche), and Western blotting was carried out using Odyssey blocking buffer (Li-Cor Biosciences) according to the manufacturer’s instructions. Anti-(-tetra)acetyl H4 histone primary antibody (Millipore) was used with goat anti-rabbit–infrared (IR) dye secondary antibody (Li-Cor Biosciences) to detect changes in histone H4 acetylation.Anti-pan-acetyl (K103) monoclonal antibody (Cell Signaling Technology) was used with goat anti-rabbit–IR dye secondary antisera (Li-Cor Biosciences) to detect changes in nonhistone protein acetylation. Membranes were imaged using an Odyssey infrared imaging system (Li-Cor Biosciences).

**RESULTS**

**In vitro activity of SB939 against *P. falciparum* asexual-stage parasites and mammalian cells.** *In vitro* inhibition studies against asexual-stage *P. falciparum* parasites growing in human erythrocytes showed that SB939 had potent activity (IC50s of ~100 to 200 nM) against chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *P. falciparum* parasites (Table 2). The IC50s of a reference HDAC inhibitor (SAHA) and the antimalarial drug chloroquine were similar to those previously reported (Table 2) (8, 24). The activity of SB939 against *P. falciparum* versus normal mammalian cell growth was assessed using HUVEC, PTC, and NFF cells (Table 2). These data were compared to previously published cytotoxicity data for normal human dermal fibroblast (NHDF) cells (47). Although low toxicity was reported against NHDF cells (IC50 of >100 μM) (47), SB939 had a greater inhibitory potency on the cells examined in this study, with IC50s ranging from 0.8 to 3.2 μM (Table 2).

**In vitro combination studies of SB939 with primaquine, piperaquine, and lopinavir.** Isobolograms describing the interactions of SB939 with primaquine and piperaquine against the chloroquine-sensitive *P. falciparum* line 3D7 demonstrated that the antiplasmodial activities of these compound combinations were antagonistic (see Fig. S1 in the supplemental material), with I values of <−5 and −1.4, respectively. However, SB939 and the protease inhibitor lopinavir behaved additively when combined (see Fig. S1).

**In vivo antimalarial efficacy studies.** The *in vivo* antimalarial efficacy of compounds was examined in an ECM model using *P. berghei* ANKA parasites constitutively expressing a luciferase reporter gene (termed *P. berghei* ANKAluc) (3). Groups of five C57BL/6j mice were infected via intraperitoneal (i.p.) injection with 10⁵ *P. berghei* ANKAluc-infected erythrocytes from an infected passage mouse. Two hours later, mice were treated with 100 μl of test compound or vehicle control (50% DMSO in PBS) via oral gavage. Mice were treated with compound or vehicle for a total of 3 days, either as a single dose or as a split dose (twice daily [BID]), with a 4-h interval between doses. A single daily dose of chloroquine (10 mg/kg of body weight in PBS) for 3 days, beginning at 2 h postinfection (p.i.), was used as a positive control. Peripheral parasitemia was monitored daily from day 4 p.i. by microscopic examination of Giemsa-stained thin blood smears prepared from tail bleeds. Parasite burden as a measure of bioluminescence (total flux; photons [p]/s) was measured from day 4 p.i. by anesthetizing mice with isoflurane (Bomac, New Zealand), injecting them with 100 μl luciferin (5 mg/ml in PBS; Caliper Life Sciences) substrate i.p., and then, after 4 min, imaging them using a Xenogen IVIS 100 imaging system (Caliper Life Sciences). Mice were housed in a ventilated cabinet (light from 8 p.m. to 8 a.m. and dark from 8 a.m. to 8 p.m.; Iffa Credo). All animal work was carried out according to protocols under the Animal Code of Practice and approved by the QIMR Animal Ethics Committee following guidelines mandated by the National Health and Medical Research Council of Australia.

<table>
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<th>Compound</th>
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<th>Mol wt (Da)</th>
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<th>Cmax (μM)</th>
<th>F%</th>
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<td>3.2</td>
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<td>34</td>
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* Cmax and t1/2 were determined for female BALB/c mice (n = 3) after a single intraperitoneal dose of 80 mg/kg (54).
* Cmax, F%, and t1/2 were tested in female nude BALB/c mice (n = 3) after a single oral dose of 50 mg/kg (47, 65).
inhibitor SAHA against the exoerythrocytic stage of the parasite life cycle were assayed using *P. berghei* ANKA sporozoites in cultured HepG2 cells. Following a 1-h invasion period, cells were cultured for 53 h in the presence of inhibitors and scored for successful parasite development. SB939 and SAHA displayed similar inhibitory profiles, with IC_{50} of 155.2 nM (±20.2 nM) and 164.8 nM (±18.3 nM), respectively (Fig. 1). At the concentrations examined (up to 1 μM), neither compound caused any toxicity to HepG2 cells compared to cells treated with vehicle (0.01% DMSO) or grown in normal medium (not shown). The antimarial compound primaquine was used as a control and gave an IC_{50} of 3.48 μM (±0.42 μM) against exoerythrocytic-stage parasites, similar to the previously published IC_{50} of this compound (3.8 μM), which is less active than its metabolites (11).

In silico docking studies. Binding of SB939 to an updated homology structure model of PfHDAC1 was also examined. This new PfHDAC1 homology model was generated with Modeller, using human HDAC8 and HDAC2 crystal structures and improving the loop 1 and loop 2 regions (an amino acid alignment is shown in Fig. S2 in the supplemental material). GOLD was used to dock TSA, SAHA, and SB939 (see Table 1 for structures) into the active site of PfHDAC1, using a hydroxamic acid scaffold constraint. Cross docking TSA back into PfHDAC1 resulted in the top-ranked conformation, with a heavy-atom root mean square deviation (RMSD) of 0.90 Å relative to the original TSA conformation (values of ≤2 Å are considered acceptable) (34). The top-ranked SAHA conformation docked with the phenyl head group docked into the pocket between loop 1 and loop 2 in a manner similar to that for the head group of TSA (59). SB939 docked into PfHDAC1 with the indole linker positioned between tunnel residues Phe_{148} and Phe_{203} and is shown in Fig. S3 in the supplemental material, with potential H bonds indicated. The SB939 hydroxamic acid carboxyl oxygen makes a putative interaction with Tyr^{301},OH, while the -NH group of the protonated and positively charged nitrogen of SB939 makes a potential interaction with Asp^{98}. The linear butyl alkyl chain of SB939 was fitted into a surface pocket between loop 1 and loop 2 (see Fig. S3).

**SB939's mode of action against *P. falciparum* asexual-stage parasites.** To assess the effect of SB939 on *P. falciparum* protein acetylation, 3D7 trophozoite-stage parasites were treated for 3 h with SB939, SAHA, or the antimarial drug chloroquine, and Western blotting was conducted. Compared to vehicle and chloroquine control samples, SAHA and SB939 treatment resulted in hyperacetylation of proteins of ~12 to 15 kDa, as viewed using antiserum specific for tetra-acetylated histone H4 (6) (Fig. 2). An increased signal was also observed for an ~80- to 90-kDa protein in HDAC inhibitor-treated samples, using a commercial antiserum that recognizes acetylated lysine residues (pan-reactive). These data indicate that SB939 hyperacylates both histone and nonhistone proteins.

**In vivo efficacy of oral SB939 in an experimental cerebral malaria model.** The in vivo efficacy of SB939 was examined in a murine ECM model using C57BL/6j mice and *P. berghei* ANKA parasites constitutively expressing a luciferase bioluminescence
reporter protein. Oral administration of 25 mg/kg SB939 twice a day for 3 days resulted in a significant reduction in peripheral blood parasitemia ($P < 0.05$ for days 3 to 7 p.i.) and parasite biomass ($P < 0.05$ for days 3, 4, 6, and 7) compared to those of control mice (Fig. 3). Mice treated with the antimalarial drug chloroquine (10 mg/kg; single oral dose daily for 3 days) had subpatent peripheral blood parasitemias until day 8 to 9 days p.i. (e.g., parasitemias on day 9 p.i. ranged from 0 to 3.1% [mean parasitemia of 1.0%]), an effect previously reported with this treatment regimen. As expected, mice in the control group developed ECM-like symptoms on day 6 or 7 p.i. and were euthanized. None of the mice in the chloroquine- or SB939-treated groups had developed ECM-like symptoms by day 9 p.i., when the experiment was ended.

Since cures were not achieved in mice treated with 2 doses of 25-mg/kg SB939 per day, the maximum reported tolerated daily dose of this compound (100 mg/kg) in mice (47, 65) was examined. Mice were treated orally with 50 mg/kg SB939 twice a day for three consecutive days to achieve a total daily dose of 100 mg/kg. Significant reductions in peripheral parasitemia and parasite burden were observed for SB939 versus the control group of mice on days 4 to 7 ($P < 0.05$) (Fig. 4); however, mice were not cured at this dose. Hyperparasitemias developed on days 12 to 16 p.i. in 4/5 mice treated with SB939. Mice were euthanized when their parasitemias reached ~30%. One mouse had a peak peripheral parasitemia on day 12 p.i. of ~12%, followed by a decrease in parasitemia (~1.5%) until day 22 p.i., when the experiment was ended. All mice in the control group succumbed to ECM-like symptoms and were euthanized on

![FIG 3](image-url) Effect of twice-daily treatment with 25 mg/kg SB939 on *P. berghei* ANKA-infected mice. Groups of five *P. berghei* ANKA$^\text{luc}$-infected C57BL/6j mice were treated orally with 25 mg/kg SAHA (dashed line) or vehicle only (50% DMSO in PBS) as a control (solid line) twice a day for 3 days, beginning at 2 h postinfection. (A) Mean ($\pm$ SEM) peripheral blood parasitemia was determined by microscopic examination of Giemsa-stained thin blood smears. (B) Mean ($\pm$ SEM) parasite-derived bioluminescence (flux) was determined using a Xenogen imager. Significant differences (*, $P < 0.05$; **, $P < 0.01$) in treated groups compared to controls are indicated. (C) Images of mice on day 6 postinfection show increasing parasite-derived bioluminescence intensities from purple to red (a luminescence scale is shown to the right).

![FIG 4](image-url) Effect of twice-daily treatment with 50 mg/kg SB939 in mice infected with *P. berghei* ANKA. *P. berghei* ANKA$^\text{luc}$-infected C57BL/6j mice ($n = 5$) were treated orally with 50 mg/kg SB939 (B and D) or vehicle only (50% DMSO in PBS) (A and C) twice a day for 3 days, beginning at 2 h postinfection. Peripheral parasitemia (A and B) and parasite-derived bioluminescence (flux) (C and D) are shown for individual mice. Significant differences (*, $P < 0.05$; **, $P < 0.01$) between the SB939 and control groups are indicated.
day 6 or 7. None of the mice in the SB939 group developed ECM-like symptoms.

DISCUSSION

Antimalarial drug resistance is driving the search for new agents able to either replace current drugs when they fail or complement existing antimalarials as partner drugs in new combination therapies. Here we investigated the antimalarial activity of a new hydroxamate-based HDAC inhibitor that is currently in phase I clinical trials for the treatment of cancer (52, 67). SB939 was developed from a medicinal chemistry program focused on addressing the general lack of metabolic stability and poor in vivo pharmacokinetics displayed by HDAC inhibitors (65). From a medicinal chemistry point of view, SB939 obeys Lipinski’s rule of five (39) and has the appropriate size, hydrophobicity, and polar surface parameters (e.g., molecular weight = 358, octanol-water partition coefficient [clogP] = 3.2, number of H-bond donors = 2, number of H-bond acceptors = 6, and total polar surface area [TPSA] = 68%) required for oral bioavailability. It is therefore not surprising that SB939 has an improved pharmacokinetic profile compared to that of SAHA, the first HDAC inhibitor to be approved for human use against cutaneous T cell lymphoma (Table 1). Preclinical pharmacokinetic studies in mice have shown that orally administered SB939 (50 mg/kg, single dose) reaches a higher maximum concentration (Cmax), with higher oral bioavailability (F%) and a longer half-life (t1/2), than that of SAHA (Table 1).

In vitro safety pharmacology data also show that SB939 (10 μM) does not interfere with the activity of non-HDAC enzymes, including other Zn2+-dependent enzymes, G-protein-coupled receptors, monoamine transporters, and ion channels (47, 65). SB939 thus has a promising profile as a new HDAC inhibitor.

This study is the first to report on noncancer activities of SB939. We found SB939 to be a potent inhibitor of the in vitro growth of P. falciparum malaria parasites, with the compound being active against both chloroquine-sensitive (3D7) and -resistant (Dd2) lines (Table 2). SB939 was less potent against Dd2 than against 3D7 (P < 0.05), suggesting that additional studies may be required to determine if cross-resistance is a potential concern. When the in vitro growth inhibition potency against P. falciparum parasites was compared to that of mammalian cell lines, a wide variation in selectivity indexes was observed. While SB939 was 1,000 times more active as an inhibitor against P. falciparum growth than against NHDF cells (47), much lower (1- to 5-fold) selectivity indexes were obtained for the other cell lines tested in this study (Table 2). This differential killing selectivity may be due to the different expression levels of the HDAC isofrom(s) in different cell lines.

Malaria control and treatment currently rely on combination therapy to try to counteract the development of antimalarial drug resistance. As such, it is important that new antimalarial drugs be evaluated as therapeutic options in concert with complementary partner drugs. Only limited studies have examined the potential of HDAC inhibitors as partner drugs against malaria parasites. Antagonist effects have been reported for the antimalarial hydroxamate HDAC inhibitor WR301801 combined in vitro with mefloquine, chloroquine, artemisinin, or the antimicrobial agent azithromycin (24). In this study, we tested two clinically used antimalarial drugs that have not yet been combined with HDAC inhibitors, namely, piperaquine, which targets asexual-stage parasites, and primaquine, which is used primarily to target liver-stage parasites but also has modest activity against asexual-stage parasites (51, 62). We showed that SB939 antagonizes the antiplasmodial activity of both of these antimalarial drugs. We also tested a clinically utilized HIV protease inhibitor, lopinavir, that is currently used to treat HIV in HIV-Plasmodium-coinfected individuals and that we have previously shown to have antimalarial activity (4). We found that SB939 behaved in an additive fashion with lopinavir. This is the first positive in vitro interaction to be reported for an HDAC inhibitor and another antimalarial compound against P. falciparum. While this finding needs to be investigated further in vivo, it may also have important implications for the treatment of patients with malaria and HIV/AIDS, who may concurrently be receiving antiretroviral protease inhibitors. Moreover, given that recent data suggest that HIV protease inhibitor and HDAC inhibitor combination treatment regimens target latent virus (38, 43), additional antimalarial benefits of this combination may also be possible.

In this study, we conducted the first investigation of the in vitro growth inhibition activity of HDAC inhibitors against exoerythrocytic (liver)-stage Plasmodium parasites. Both SB939 and SAHA potently inhibited the development of P. berghei ANKA malaria parasites within HepG2 liver cells. Although the target of these compounds in liver-stage parasites is not yet known, SAHA is known to inhibit recombinant PHD1 (50), and the gene encoding this protein is transcribed in P. falciparum liver-stage parasites as well as in asexual- and gametocyte-stage parasites (9). Expression data for the P. berghei ANKA homologue (PhHDAC1) of PHD1 in liver-stage parasites are not yet available, but considering that the two proteins share 94% amino acid identity (9), it is likely that HDAC inhibitors will target this protein in rodent malaria parasites. Our finding that HDAC inhibitors can target multiple Plasmodium life cycle stages in vitro has potentially important implications for the future development of this class of compounds as antimalarial drugs. Although most antimalarial drugs in clinical use target intraerythrocytic, asexual-stage parasites, very few are known to act on other life cycle stages, such as exoerythrocytic liver-stage parasites or gametocytes, the stage transmitted to the mosquito. Our data provide the first validation of HDAC inhibitors as liver-stage drug leads. Findings from this study suggest that SB939 acts as an HDAC inhibitor in malaria parasites.

Our in silico docking studies give an improved homology structural model of PHD1 and also provide evidence for binding to SB939, in a fashion similar to that of TSA and SAHA. We also demonstrated that SB939 has the same mode of action as previously used antimalarial HDAC inhibitors (6, 21, 24), causing hyperacylation of both histone and nonhistone proteins in P. falciparum asexual-stage parasites. The nonhistone protein hyperacylated by SB939 treatment was not identified in this study, but its molecular mass (~80 to 90 kDa) corresponds well to that of heat shock protein 90 (PfHSP90), which was recently identified as being hyperacylated in TSA-treated P. falciparum parasites by use of the same pan-acetyl lysine antisera as that used in this study (49). Interestingly, HSP90 is known to be a nonhistone target of HDAC action in mammalian cells (10, 37).

The in vivo efficacies of SB939 was examined using a murine ECM model. Cerebral malaria (CM) pathogenesis is poorly understood; however, cerebral pathology is associated with mature infected erythrocytes sequestering in the microvasculature of tissues, including the brain (13, 66). This results in immune evasion
by mature *P. falciparum*-infected erythrocytes, preventing removal by the spleen and facilitating parasite survival (13). ECM caused by *P. berghei* ANKA parasites in mice shares some features with human CM, with susceptible mice developing neurological symptoms such as paralysis, ataxia, convulsions, and coma. This generally occurs 6 to 8 days after infection with *P. berghei* ANKA parasites, results in 80 to 100% mortality, and is associated with low peripheral blood parasitemia (3). Studies of ECM in mouse models of malaria have been facilitated greatly by the recent advent of a transgenic, luciferase-expressing models of malaria have been facilitated greatly by the recent advent of a transgenic, luciferase-expressing line of *mice* that allows not only measurement of peripheral blood parasitemia but also measurement of parasite biomass in peripheral tissues (3). Orally administered SB939 was found to be effective in reducing both peripheral parasitemia and total parasite burden at doses of up to 100 mg/kg/day in the ECM model. Although mice were not cured, SB939 treatment prevented the development of ECM-like symptoms, and mice did not develop hyperparasitemia until day 15 to 22 p.i. In contrast to the result obtained with twice-daily dosing of SB939, when this compound was administered orally as a single daily dose of 50 or 100 mg/kg, a significant reduction in parasite biomass was observed on day 6 p.i., but there was no difference on day 7 p.i. (see Fig. S4 in the supplemental material). The poorer antiparasitic effect with a single dose of SB939 than with a twice-daily dose is likely due to the half-life of SB939 being insufficient to attenuate parasite growth.

HDAC inhibitors are a promising new class of antimalarial compounds, with several compounds displaying potent *in vitro* and *ex vivo* activity against *P. falciparum* and *P. vivax* parasites. Recent studies on the effects of HDAC inhibitors on the growth and development of *P. falciparum* have also provided new insights into transcriptional regulation in malaria parasites (19, 32). Alterations in HDAC expression in malaria parasites may be associated with reduced clinical artemisinin efficacy (45), raising the possibility that HDAC inhibitors might contribute to future drug combinations aimed at addressing the issue of artemisinin resistance. Such findings underscore the potential for HDAC inhibitors to be used for malaria therapy. However, to progress HDAC inhibitors toward clinical trials for malaria, next-generation compounds must have potent antiplasmodial activity, highly selective killing action against parasites rather than normal host cells, and improved bioavailability and pharmacokinetic profiles after oral dosing. In this study, we demonstrated that SB939, a new orally bioavailable HDAC inhibitor with a superior pharmacokinetic profile over those of previous HDAC inhibitors such as SAHA, has potent activity against asexual- and sporozoite-stage *Plasmodium* parasites and promising *in vivo* activity in a mouse model of malaria infection. These findings support the development of HDAC inhibitors as a potential new antimalarial compound class.

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