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Hydroxamic acid based histone deacetylase inhibitors with confirmed activity against the malaria parasite

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ABSTRACT

Recent studies have highlighted a key role in regulating gene transcription, in both eukaryotes and prokaryotes, by enzymes that control the acetylation and deacetylation of histones. In particular, inhibitors of histone deacetylases (HDAC-Is) have been shown effective in controlling the development of many parasites, such as the plasmodium of malaria. Here we report the results of a study aimed at evaluating antiparasitic effect of two classes of HDAC-Is bearing different zinc binding group (hydroxamic acid vs thiol). The study showed that only the hydroxamic acid based HDAC inhibitors were active, with *Plasmodium falciparum* being the most sensitive parasite, having from low double-digit to single-digit nanomolar range in vitro activities. Among three derivatives evaluated also in vivo, ST8086AA1 (**8**) effectively inhibited 88% of the development of *Plasmodium falciparum*.

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The problem of parasitic infections is far from being defeated, so diseases caused by protozoan parasites are still an important health problem with remarkable social and economic impact on human societies. Some parasites, if untreated, could cause a wide spectrum of diseases. Problem of drug resistance is also always lurking.[1] Researchers around the world, working on this front, are facing the problem following two different approaches: screening on the protozoa old molecules already approved for other therapeutic indications, for drug repurposing,[1] or trying to investigate on new biological targets such as histone deacetylases (HDACs) (Fig. 1).[2–4]

HDACs are a group of Zn-dependent enzymes found in several organisms such as bacteria, fungi, plants, and animals. In the latter group they play crucial roles in modulating mammalian cell chromatin structure, transcription, and gene expression. They belong to the huge class of so-called 'lysine-deacetylase', a class of enzymes that work removing acetyl groups from ε-amino-lysine residues on many different substrates, not only histones but non-histone nuclear and cytoplasmic proteins too.[5]

Inhibition of histone deacetylase is one of the last biological targets investigated successfully. Two HDAC inhibitor drugs, the first-in-class synthetic hydroxamic acid derivative (Vorinostat) and a natural thiol derivative (Romidepsin), have been approved by the US Food and Drug Administration (FDA) to treat cutaneous T-cell lymphoma (CTCL). The latter was also approved for additional indication, for peripheral T-cell lymphoma (PTCL) together with Belinostat, the third HDAC-I recently approved by FDA.

From a medicinal chemistry point of view, most of the HDAC inhibitors falls into a pharmacophoric model widely accepted, which consists of a capping group (CAP), able to interact with the rim of the catalytic tunnel of the enzyme, opposite of a zinc-binding group (ZBG), able to complex the Zn^{2+} ion at the bottom of the catalytic cavity, and hydrophobic linker connecting the two parts (Fig. 2). Results from our researches in this field, along with others, have highlighted the positive contribution that some substituents on the kink atom—connection unit—(C.U.) may give to the activity of this class of compounds.[6,7]

Beyond the well-known cellular effects in various human cancer cells line, like growth arrest, pro-differentiation and pro-apoptosis, other fields of application, such as the antiprotozoan role, are possible for HDAC inhibitors according to recent studies.

In 2008 Andrews, Fairlie et al. reported the results of a screening, with different classes of HDAC inhibitors, on the malaria parasite *Plasmodium falciparum*, showing that hydroxamate-based HDAC-Is (i.e., TSA and SAHA) were the most powerful as antimalarial agents. Benzamide analogues (i.e., MS-275) were less potent and, even less were the thiol-based HDAC inhibitor derivatives.[8] Recently, other researchers have re-proposed these data enriched with other examples, such as the activity of the Pracinostat (SB939).[9a]

Over the last years, our research group has carried out an important project aimed at identifying HDAC inhibitors,[5] leading to the selection of a drug candidate, ST7612AA1 (**3**), currently in

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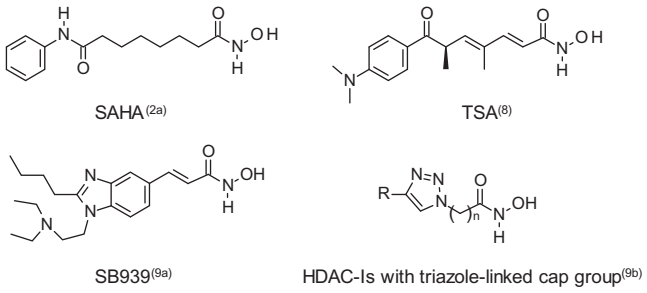

Figure 1. Examples of HDAC inhibitors with antimalarial activity.

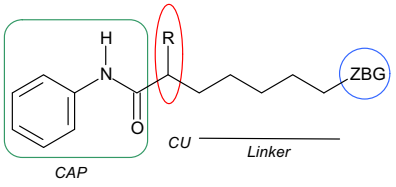

Figure 2. Pharmacophoric model of HDAC inhibitors.

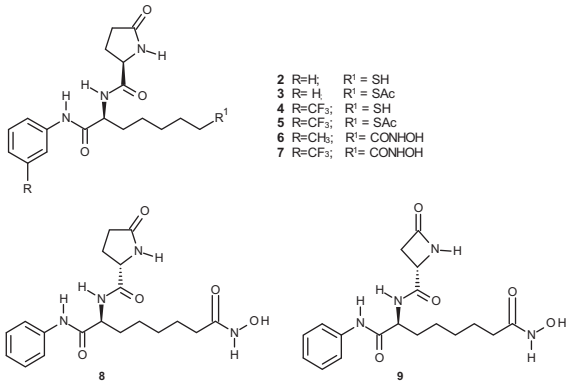

Figure 3. Lactam-carboxyamides thiol- and hydroxamate-based HDAC inhibitors.

preclinical phase.[6] According to the above anticipation, it is known that HDAC inhibitors are effective on various protozoa, in particular against *Plasmodium falciparum*.[9] Other authors have also associated to HDAC inhibitors a potent activity against the African protozoa of the *Trypanosoma brucei*.[10] This was the context when this study was inspired (Fig. 3).

Influence of various groups (i.e., substitution in position α to the amide CAP, and nature of ZBG) on the antiparasitic activity of a few SAHA analogues has been looked at. After screening each single derivative on all know HDAC isoform, and further experiments on H460 tumor cell line, a few derivatives were selected for their antiparasitic activity. Thio derivatives were investigated as such in binding experiments, meanwhile their thio-acetyl prodrugs were used in cell cytotoxicity assays (Table 1).

Trypanosoma brucei rhodesiense, *Trypanosoma cruzi*, *Plasmodium falciparum* and *Giardia lamblia* were the four protozoan parasites selected for this study. Cytotoxicity was evaluated on L6 rat skeletal muscle derived myoblasts. A set of compounds tested against *Leishmania donovani*, showed weak activity.

Under these experimental conditions, thio derivatives either as free drug (**2**, **4**) or as their corresponding prodrugs (**3**, **5**) displayed poor antiparasitic activity. Then, we investigated the hydroxamic acid analogous (drug **6**–**9**), which showed to be very powerful with a favorable range of activity compared to the cytotoxicity of L6 (Table 2).

Excluding the β-lactam derivative (**9**), slightly soluble and less stable than γ-lactam analogs, these compounds have been studied in an in vivo model, with a non-optimized schedule. The results in Table 3, demonstrated that compound ST8086AA1 (**8**) is a good inhibitor of *Plasmodium falciparum*, with a 87.5% activity versus 99.8% of DHA (DiHydroArtemisinine).

According to the 2013 World Health Organization report,[12] malaria is responsible for over 627 thousand deaths a year, especially among young children and pregnant women, on 207 million people affected worldwide. The disease can be caused by 5 different species of the protozoan *Plasmodium* parasite but of these *P. falciparum* is the most lethal and the most prevalent in Sub-Saharan Africa.

Our preliminary data of **8** on *Plasmodium* represent a very interesting starting point for an in-depth analysis. Since the most critical aspect of the traditional antimalarial drugs is rapid development of resistance, it would be interesting to assess this parameter on **8**.

This study confirmed that hydroxamic derivatives are active on *Plasmodium* but it also showed their higher activity towards the corresponding thiols, contrary to what was observed in terms of cytotoxicity. Moreover, placing a methyl or a trifluoromethyl group as substituent in *meta* position of phenyl moiety of CAP, was associated with the well-known enhancement of the cytotoxic activity[13] but also with an enhanced antiparasitic activity.

Although several compounds are currently in preclinical and clinical phase of development, it is noteworthy that successful development of these compounds is not guaranteed. Discovery and development of new molecules with novel mechanisms of action able to circumvent antimalarial drug resistance is strongly needed.

In conclusion, although not being exhaustive but a contribution to the advancement of knowledge in this field, this study showed that: (a) thio-based HDAC inhibitors showed only weak antiparasitic activity; (b) hydroxamate-based histone deacetylase inhibitors, having an amide-lactam moiety on kink atom are more powerful compared to linear counterpart (i.e., suberoylanilide hydroxamic acid; SAHA). Among the protozoa studied, *Plasmodium falciparum* was the most sensitive to this class of drugs.

Table 1
In vitro screening on class I, IIb and IV HDAC isoforms, and cytotoxic activity against non-small cell lung cancer cell line (NCI-H460)[6,7]

ID	Name or lab. code	Class I (1-3,8), IIb (6,10) and IV HDAC isoforms; IC_{50} (nM)							Cytotoxicity; IC_{50} (nM)
		HDAC1	HDAC2	HDAC3	HDAC6	HDAC8	HDAC10	HDAC11	NCI-H460
1	SAHA	258	921	350	29	243	456	362	3400
2	ST7464AA1	13	78	5	3	281	11	14	
3	ST7612AA1								66
4	ST8081AA1	5	12	11	5	316	7	44	
5	ST8039AA1								30
6	ST8074AA1	7	27	11	3	52	30	15	500
7	ST8078AA1	2	8	2	2	33	11	55	500
8	ST8086AA1	34	89	62	3	93	113	13	2200
9	ST8087AA1	59	143	88	6	137	125	37	5400

Table 2
Antiprotozoan activities against different protozoa as well as cytotoxicity against L6 cells, in vitro

ID	Name or lab. code	*Trypanosoma brucei rhodesiense* IC_{50} (nM)	*Trypanosoma cruzi* IC_{50} (nM)	*Leishmania donovani* IC_{50} (nM)	*Plasmodium falciparum* IC_{50} (nM)	*Giardia lamblia* IC_{50} (nM)	Cytotoxicity L6 cells IC_{50} (nM)
	Melarsoprol	6					
	Benznidazole		2130				
	Miltefosine			3150			
	Chloroquine				6		
	Metronidazole					580	
	Podophyllotoxin						19
	Reference HDAC inhibitor						
1	SAHA (Vorinostat)	3170	10,100	[a]	25	56	266
	Thiol derivatives						
2	ST7464AA1	3230	25,800	>10,000	9660	12,300	5250
3	ST7612AA1	2510	2100	[a]	6570	8110	189
4	ST8081AA1	4750	19,100	>3000	3260	64,800	2670
5	ST8039AA1	2380	3980	[a]	3240	40,900	91
	Hydroxamic acid derivatives						
6	ST8074AA1	5515	849	N.D.	6	4640	1232
7	ST8078AA1	4155	972	N.D.	3	4270	638
8	ST8086AA1	19,950	48,350	N.D.	19	10,720	75,100
9	ST8087AA1	6045	32,950	N.D.	23	12,750	7460

Reference drugs commonly used against each parasite were compared to these novel inhibitors. (see Table1-S.I.). SAHA was used as a further reference in all tests.[11]
[a] Tox at 3 μM, inactive at 1 μM. N.D: not determined.

Table 3
Activity of HDAC inhibitors versus DHA, in the *Plasmodium F. berghei* mouse model

ID	Name or lab. code	% Activity vs. control 50 mg/kg; DMSO/water; i.p.; qd; 4 days (after 4 days)
6	ST8074AA1	33.75
7	ST8078AA1	63.25
8	ST8086AA1	87.53
	DHA	99.78

In the same model, Artemisinin cures it at 4 × 50 mg/kg (activity 99.9% and survival >30 days).[14]

Interestingly, all hydroxamic acid derivatives displayed in vitro activity in the same range of chloroquine (IC_{50}: 3–23 nM) and slightly more potent than SAHA (IC_{50}: 25 nM). However, it should be emphasized that this class of amide-lactam hydroxamate-based HDAC inhibitors showed varied degrees of selectivity versus other parasites as well as cytotoxicity against L6 cells.

Taken together, these results demonstrated that this class of HDAC inhibitor could be a new tool against malaria; ST8086AA1 (**8**) proved to be an effective antimalarial and is therefore a useful hit compound for further medicinal chemistry optimization.

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Supplementary data

Supplementary data (a table with information on parasites and on reference drugs used in the screening and two schemes with synthesis of tested product) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.12.051.

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