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Antimalarial and antileishmanial activities of histone deacetylase inhibitors with triazole-linked cap group

Vishal Patil [a,‡], William Guerrant [a,‡], Po C. Chen [a,†], Berkley Gryder [a], Derek B. Benicewicz [a], Shabana I. Khan [c], Babu L. Tekwani [c,*], Adegboyega K. Oyelere [a,b,*]

[a] School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA
[b] Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA
[c] National Center for Natural Products Research and Department of Pharmacology, School of Pharmacy University of Mississippi, MS 38677-1848, USA

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ABSTRACT

Histone deacetylase inhibitors (HDACi) are endowed with plethora of biological functions including anti-proliferative, anti-inflammatory, anti-parasitic, and cognition-enhancing activities. Parsing the structure–activity relationship (SAR) for each disease condition is vital for long-term therapeutic applications of HDACi. We report in the present study specific cap group substitution patterns and spacer-group chain lengths that enhance the antimalarial and antileishmanial activity of aryltriazolylhydroxamates-based HDACi. We identified many compounds that are several folds selectively cytotoxic to the plasmodium parasites compared to standard HDACi. Also, a few of these compounds have antileishmanial activity that rivals that of miltefosine, the only currently available oral agent against visceral leishmaniasis. The anti-parasite properties of several of these compounds tracked well with their anti-HDAC activities. The results presented here provide further evidence on the suitability of HDAC inhibition as a viable therapeutic option to curb infections caused by apicomplexan protozoans and trypanosomatids.

1. Introduction

Inhibition of histone deacetylase (HDAC) activity has been largely validated as a viable therapeutic strategy for cancer treatment.[1] Of the several mechanisms proposed to explain the molecular basis of the anti-proliferative activity of HDAC inhibitors (HDACi), perturbation of chromatin remodeling and acetylation states of key non-histone proteins have been widely accepted.[2] Consequently, intense research activities are ongoing on the application of HDACi to other diseases where chromatin remodeling and protein acetylation states may play significant roles.[3]

Human pathogenic apicomplexan protozoans and trypanosomatids, such as the causative agents of malaria and leishmaniasis respectively, have been shown to be responsive to HDACi.[4] Malaria is a serious infectious disease that is prevalent in sub- Saharan Africa and Asia.[5] It is caused by members of the genus *Plasmodium*, with an estimated 500 million infections and two million fatalities annually.[6] Similarly, leishmaniasis is a tropical/sub-tropical parasitic disease, caused by the members of the genus *Leishmania*, which infects over two million people annually.[7] These two diseases constitute an emerging serious threat to public health due to the emergence of multi-drug resistant strains and the occurrence of leishmania as opportunistic infective agents in human immunodeficiency virus-infected patients.[8–10] Therefore, there is an urgent need for affordable alternative agents to curtail these diseases.

Plasmodium falciparum, the principal malarial protozoan parasite in humans, has at least five putative HDAC enzymes.[11] Genes encoding two of these putative HDAC—Pf*HDAC-1* and PfSir2—have been partially characterized.[11–13] Because of its homology with the class I family of HDACs from human, chicken, frog and *Saccharomyces cerevisiae*, Pf*HDAC-1* may be one of the intracellular targets whose interaction with HDACi elicits the observed antimalarial activity of HDACi.[4c,f,g,11] Similarly, *Leishmania* parasite's genome contains multiple genes encoding different HDACs isozymes some of which have been shown to be essential for the survival and proliferation of *Leishmania* parasites.[14–16] However, the structure–activity relationship (SAR) of the antimalarial and antileishmanial activities of HDACi is not entirely clear.[4g]

Previously, we and others have reported the synthesis and SAR for aryltriazolylhydroxamates, HDACi incorporating 1,2,3-triazole into the cap group-linking and surface recognition moieties. [4d,17]

* Corresponding authors. Tel.: +1 662 915 7882; fax: +1 662 915 7062 (B.L.T.); tel.: +1 404 894 4047; fax: +1 404 894 2291 (A.K.O.).
E-mail addresses: btekwani@olemiss.edu (B.L. Tekwani), aoyelere@gatech.edu (A.K. Oyelere).
† Present address: School of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, USA.
‡ These authors contributed equally to this manuscript.

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We showed that these compounds displayed cap group- and spacer-length dependent anti-HDAC and whole cell anti-proliferative activities that tracked with the three-motif pharmacophoric model of all HDACi (Fig. 1).[17] In this contribution, we sought to clarify the specific structural attributes that confer antimalarial and antileishmanial activities to aryltriazolylhydroxamates HDACi. We found that for a given cap group, HDACi spacer-group length is a major structural determinant of antimalarial and antileishmanial activities. Specifically, the antimalarial and antileishmanial activities of these aryltriazolylhydroxamates generally peak in analogs having 5 or 6 methylene spacer groups separating the active site zinc binding hydroxamate moiety and the aryltriazolyl group.

2. Chemistry

Our previous protocol for the synthesis of aryltriazolylhydroxamates required the unmasking of the hydroxamic acid moiety of the desired product from a penultimate ester intermediate by treatment with aqueous hydroxylamine in the presence of a catalytic amount of KCN.[17] This reaction sometime gives the carboxylic acid derivative of the desired product as a contaminant, thereby complicating compound isolation. We describe here an alternative route that allows for a facile synthesis of the aryltriazolylhydroxamates of interest while avoiding the formation of carboxylic acid contaminants. Reaction of azido acid **1a–c** with *O*-tritylhydroxylamine under standard activation condition gave O-tritylated hydroxamates **2a–c** in good to excellent yields. Subsequent Cu(I) catalyzed cycloaddition reaction between **2a–c** and terminal alkynes **3a–g** resulted in *O*-trityl protected aryltriazolylhydroxamates **4–14**.[17] The unmasking of the *O*-trityl protection group was facilitated by $BF_3{\cdot}OEt_2$ or TFA treatment to furnish the requisite compounds, **19**, **20**, **23**, **24**, **26**, **27**, **34**, **42**, **43**, **47** and **50**, all new aryltriazolylhydroxamates having longer methylene spacer-groups (n = 6–9) and varied HDAC surface recognition cap groups (Scheme 1).

3. Results and discussion

3.1. In vitro assays

Our goal in the present study is to elucidate the structural determinants that confer antimalarial and antileishmanial activities to aryltriazolylhydroxamates HDACi. To complement our previous studies on the anti-HDAC activities of this class of compounds and also establish a SAR of their anti-protozoan activities, we synthesized additional aryltriazolylhydroxamates having longer methylene spacer-groups (n = 6–9) and varied HDAC surface recognition cap groups. We first evaluated the anti-HDAC activity of the new compounds using the *Fluor de Lys* assay[18] as described previously.[17,19a]

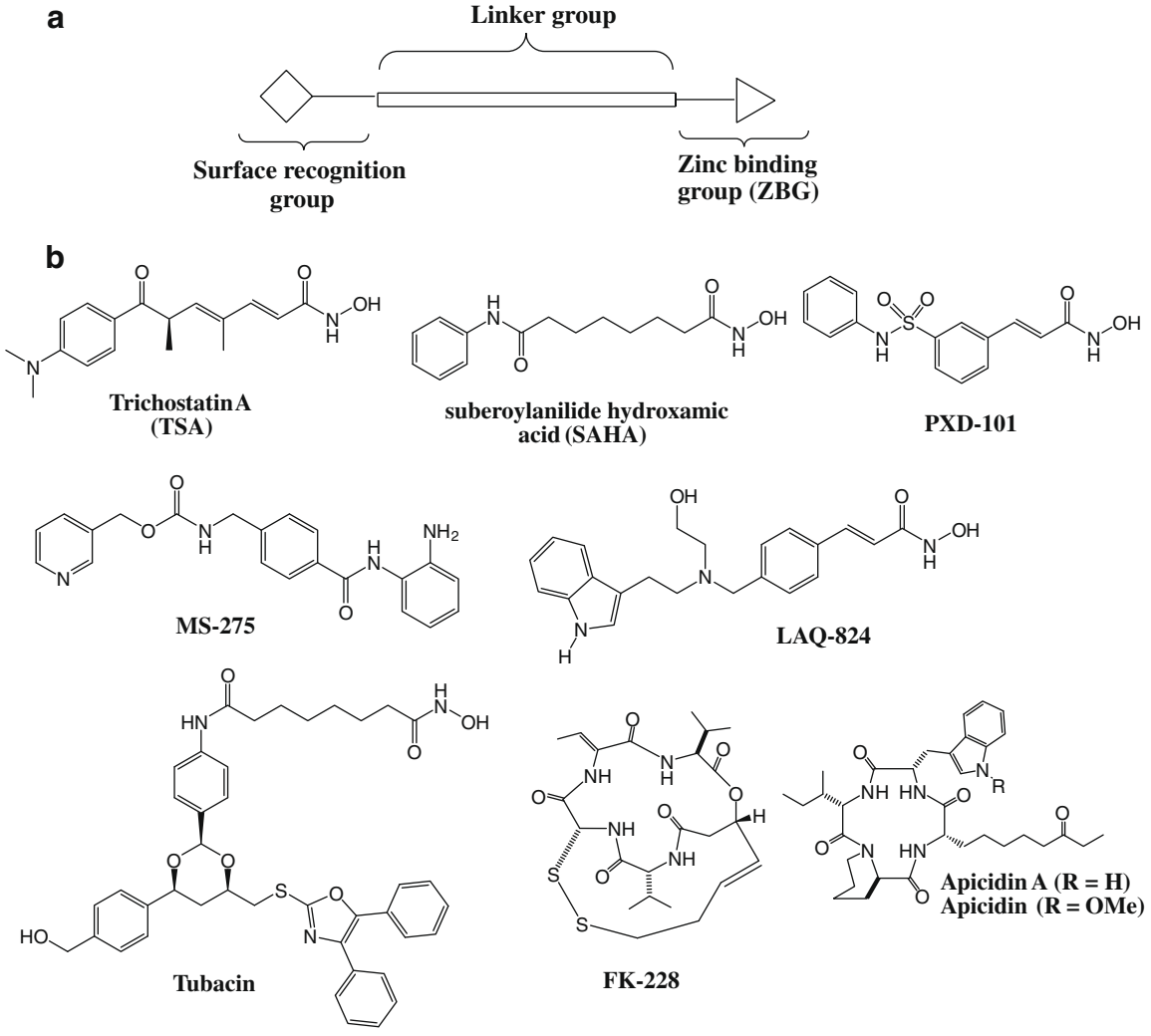
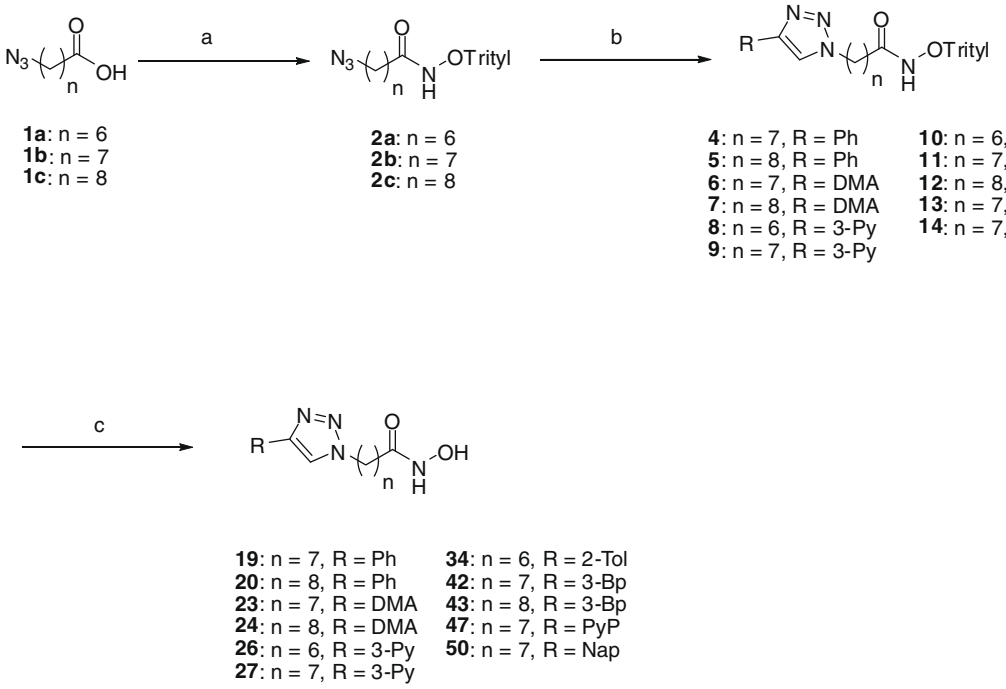


Figure 1. (a) A three-motif pharmacophoric model of HDAC inhibitors. (b) Selected examples of small molecule HDAC inhibitors.



Scheme 1. Synthesis of aryltriazolylhydroxamates for SAR studies. Reagents and conditions: (a) NH_2–*O*–trityl, IBCF, THF, −15 °C, 2 h; (b) **3a**–**g**, CuI, Hunig's base, THF, rt; (c) $BF_3{\cdot}OEt_2$, THF, rt, 20 min or CH_2Cl_2, TFA/thioanisole (1:1), 0 °C, 3 h. Abbreviations: 3-Bp, 3-biphenyl; DMA, *p*-*N*,*N*-dimethylanilyl; Nap, 6-methoxynapthyl; Ph, phenyl; 3-Py, 3-pyridyl; PyP, 4-pyridylphenyl; 2-Tol, 2-tolyl.

All aryltriazolylhydroxamates were tested for their ability to induce in vitro inhibition of the proliferation of chloroquine-sensitive (D6, Sierra Leone) and chloroquine-resistant (W2, Indochina) strains of *P. falciparum*. The in vitro antileishmanial activities of compounds were tested against the promastigote stage of *Leishmania donovani*, the causative agent of visceral leishmaniasis. Plasmodium growth inhibition was determined by a parasite lactate dehydrogenase assay using Malstat reagent,[20] while inhibition of viability of the promastigote stage of *L. donovani* was determined using standard Alamar blue assay, modified to a fluorometric assay.[21] Amphotericin B and pentamidine, standard antileishmanial agents; chloroquine and artemisinin, standard antimalarials; and suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA), standard HDACi were all used as positive controls. To determine selective toxicity index, all compounds were simultaneously tested against a non-transformed mammalian cell line namely, monkey kidney epithelial (Vero) using Neutral Red assay.[22]

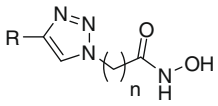
3.2. Structure–activity relationship

The HDAC inhibition profiles of the newly synthesized aryltriazolylhydroxamates showed a trend that paralleled those of the previously reported compounds.[17] In general; anti-HDAC activities are dependent on two key features of these molecules, namely—the length of the methylene spacer-group (n) and the identity of the cap group. Again, for a given cap group, the optimum n is either 5 or 6 (Table 1).

The anti-protozoan activities of several of these aryltriazolylhydroxamates are also dependent on the two structural features described above. For example, homologous compounds **15**–**20**, derived from unsubstituted phenyl ring, have spacer length-dependent antimalarial activities that peaked when $n = 6$ (i.e., 6 methylene spacers separating the triazole ring from the zinc binding hydroxamic acid group) (Table 1). This antimalarial trend is in close agreement with the anti-HDAC activities of these compounds against HDACs 1 and 2 from HeLa cell nuclear fraction.[17] Additionally, a similar spacer-length restriction in antimalarial activity has been recently observed for acyl arylhydrazone based compounds.[4f] Introduction of a *N*,*N*-dimethylamino moiety to the *para* position of the cap group, similar to the substitution pattern on TSA, had no effect on the methylene spacer length dependence of the antimalarial activities. However, these *N*,*N*-dimethylamino compounds have a relatively relaxed preference for either 5 or 6 methylene spacers (Table 1, comparing compounds **21** and **22**). Incorporation of nitrogen into the phenyl ring did not improve the potency of the simple phenyl-substituted compounds. Nevertheless, the resulting pyridine derivatives **25**–**30** possessed antimalarial activity profile that was dependent on the ring location of the nitrogen atom. For compounds with 5 methylene spacers, the antimalarial activity was intolerant of nitrogen substitution at the *ortho* position. This observation is contrary to the trend of the anti-HDAC activities of these pyridyl compounds. However, the *ortho* N-substitution intolerance was relieved with one additional methylene group (Table 1, comparing compounds **29** and **30**). For compounds with the same pyridyl group, we observed spacer length-dependent antimalarial activities in similar manner to those of the unsubstituted phenyl analogs.

Other aryl group substitutions resulted in distinct antimalarial activities that may give further indications of the electronic and steric environment of the intracellular drug target(s) within the parasite. Methyl substituted compounds **31**–**33** ($n = 5$) showed a ring *meta*-position preference despite anti-HDAC activity that favored a ring *ortho*-position substitution. Extension of the linker length by one methylene group drastically relieved the *ortho*-substitution intolerance of these series of methyl substituted compounds (Table 1, comparing compounds **33** and **34**). Conversely, *meta*-substitution with a stronger electron-donating methoxy moiety (relative to the methyl group) eliminated anti-parasite activity while there was no clear preference between *ortho*- and *para*-substitution (Table 1, comparing compounds **35**–**37**). This lack of preference is surprising considering the fact that these compounds had anti-HDAC activity that was about 40-fold in favor of the *para*-

Table 1
In vitro HDAC Inhibition (nM), antilieshmanial (μg/mL) and antimalarial (ng/mL) activities of aryltriazolylhydroxamates HDACi



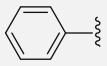
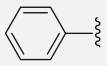
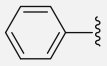
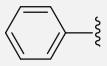
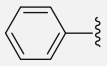
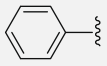
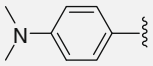
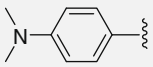
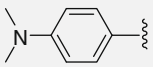
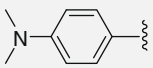
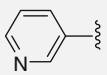
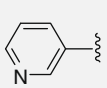
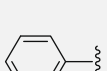
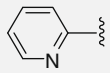
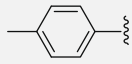
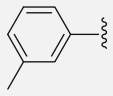
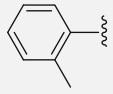
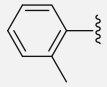
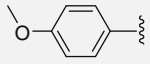
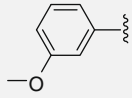
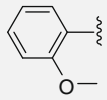
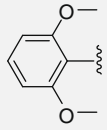
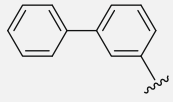
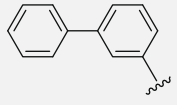
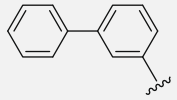
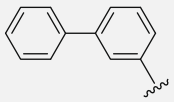
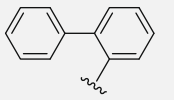
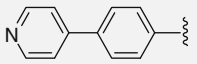
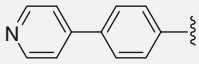
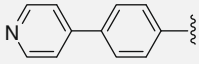
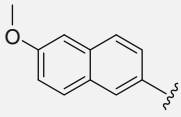
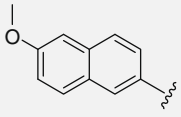
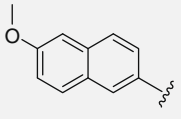
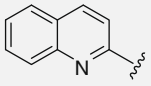
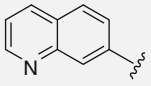
Compd	R	*n*	HDAC inhibition IC_{50} (nM)[a]	Antileshmanial activity[c]		Antimalarial activity[c]		Cytotoxicity (VERO) IC_{50} (ng/mL)	S. I. D6 (W2)
				IC_{50} (μg/mL) ±SD	IC_{90} (μg/mL) ±SD	*Plasmodium falciparum* (D6 clone) IC_{50} (ng/mL)	*Plasmodium falciparum* (W2 clone) IC_{50} (ng/mL)		
15		3	N.D.[b]	NA	NA	2850 ± 50	3500 ± 800	NC	>1.7 (>1.4)
16		4	110.0[b]	NA	NA	125 ± 5	120 ± 10	NC	>38.08 (>39.6)
17		5	14.2[b]	17.5 ± 0.71	>40	88 ± 2	130 ± 30	3950 ± 550	44.9 (30.4)
18		6	9.6[b]	4.55 ± 0.35	40.0 ± 0.0	38 ± 5	24.5 ± 1.5	430 ± 20	11.3 (17.6)
19		7	363.6	17.75 ± 0.35	>40	645 ± 55	695 ± 115	NC	>7.4 (>6.8)
20		8	55.4	18.5 ± 0.71	>40	2650 ± 450	2750 ± 50	3350 ± 550	1.9 (1.2)
21		5	4.3[b]	15.5 ± 0.71	>40	68 ± 8	38.5 ± 1.5	3400 ± 200	50.0 (88.3)
22		6	106.1[b]	5.35 ± 0.35	36.0 ± 1.41	24.5 ± 5.5	32 ± 13	765 ± 35	31.2 (23.9)
23		7	15.9	24.0 ± 2.83	>40	175 ± 105	185 ± 15	970 ± 130	3.8 (3.6)
24		8	596.6	20.75 ± 0.35	>40	1800 ± 500	1650 ± 50	NC	>2.6 (>2.9)
25		5	287.2[b]	11.45 ± 3.61	>40	175 ± 45	205 ± 55	NC	>27.2 (>23.2)
26		6	365.8	15.5 ± 0.71	40	75.5 ± 21.5	81 ± 49	3780 ± 980	50.1 (46.7)
27		7	425.8	19.75 ± 0.35	>40	1495 ± 253	1687 ± 321	NC	>3.2 (>2.8)
28		5	112.5[b]	17.75 ± 0.35	>40	195 ± 65	132.5 ± 57.5	NC	>24.4 (>35.9)
29		5	67.6[b]	20.5 ± 0.71	>40	985 ± 15	625 ± 95	2900 ± 300	2.9 (4.6)

Table 1 (*continued*)

Compd	R	*n*	HDAC inhibition IC_{50} (nM)[a]	Antileshmanial activity[c]		Antimalarial activity[c]		Cytotoxicity (VERO) IC_{50} (ng/mL)	S. I. D6 (W2)
				IC_{50} (μg/mL) ±SD	IC_{90} (μg/mL) ±SD	*Plasmodium falciparum* (D6 clone) IC_{50} (ng/mL)	*Plasmodium falciparum* (W2 clone) IC_{50} (ng/mL)		
30		6	23.9[b]	5.75 ± 0.35	40.0 ± 0.0	69 ± 1	40 ± 5	950 ± 150	13.8 (23.8)
31		5	43.4[b]	21.5 ± 2.12	>40	130 ± 40	140 ± 40	3850 ± 650	29.6 (27.5)
32		5	31.9[b]	17.0 ± 1.41	>40	79.5 ± 0.5	68 ± 8	2700 ± 300	34.0 (39.7)
33		5	17.4[b]	23.0 ± 1.41	>40	1250 ± 50	1950 ± 750	3300 ± 700	2.6 (1.7)
34		6	2.8	26.0 ± 0.0	40	120 ± 20	140 ± 20	710 ± 210	5.9 (5.1)
35		5	2.1[b]	18.5 ± 0.71	>40	130 ± 6	90 ± 10	2350 ± 850	18.1 (26.1)
36		5	13.9[b]	NA	NA	NA	NA	NC	—
37		5	76.0[b]	18.75 ± 0.35	>40	150 ± 10	97.5 ± 2.5	2300 ± 500	15.3 (23.6)
38		5	315.9[b]	40	>40	2000 ± 300	2600 ± 200	NC	>2.4 (>1.8)
39		5	31.7[b]	11.5 ± 0.71	>40	46 ± 6	36.5 ± 13.5	NC	>103 (>130)
40		5	1.9[b]	11.25 ± 0.35	32.5 ± 0.71	37.5 ± 17.5	26 ±4	NC	>127 (>183.1)
41		6	5.4[b]	20.5 ± 0.71	>40	25 ± 5	25 ± 5	NC	>190.4 (>190.4)
42		7	108.9	20.25 ± 0.35	40	840 ± 40	650 ± 50	NC	>5.6 (>7.3)

(*continued on next page*)

Table 1 (*continued*)

Compd	R	*n*	HDAC inhibition IC_{50} (nM)[a]	Antileshmanial activity[c]		Antimalarial activity[c]		Cytotoxicity (VERO) IC_{50} (ng/mL)	S. I. D6 (W2)
				IC_{50} (μg/mL) ±SD	IC_{90} (μg/mL) ±SD	*Plasmodium falciparum* (D6 clone) IC_{50} (ng/mL)	*Plasmodium falciparum* (W2 clone) IC_{50} (ng/mL)		
43		8	147.7	20.0 ± 0.0	40.0 ± 0.0	3350 ± 150	3200 ± 200	NC	>1.4 (>1.5)
44		5	162.6[b]	13.0 ± 2.83	38.0 ± 0.0	2100 ± 800	2600 ± 100	2700 ± 200	1.3 (1.0)
45		5	2.3[b]	28.0 ± 2.83	>40	45.5 ± 9.5	47.5 ± 22.5	NC	>104.6 (>100.2)
46		6	16.6[b]	NA	NA	1150 ± 150	1250 ± 50	NC	>4.1 (>3.8)
47		7	212.9	NA	NA	88.5 ± 6.5	127.5 ± 2.5	3850 ± 650	43.5 (30.2)
48		5	1.8[b]	18.0 ± 2.83	>40	35 ± 5	31 ± 11	2850 ± 50	>81.4 (>91.9)
49		6	15.3[b]	18.5 ± 2.12	>40	47.5 ± 4.5	53 ± 13	2650 ± 350	55.8 (50.0)
50		7	226.1	NA	NA	112 ± 13	165 ± 35	650 ± 250	5.8 (3.9)
51		5	2.1[b]	9.5 ± 0.71	39.0 ± 1.41	33 ± 3	34 ± 16	2400 ± 400	72.7 (70.6)
52		5	151.5[b]	18.5 ± 0.71	>40	52.5 ± 12.5	33 ± 3	NC	>90.7 (>144.2)
	Chloroquine	—	NT	NT	NT	17	125	NT	NT
	Artemisinin	—	NT	NT	NT	4	6	NT	NT
	Pentamidine	—	NT	0.90 ± 0.9	1.80 ± 0.0	NT	NT	NT	NT
	Amphotericine B	—	NT	0.14 ± 0.01	0.31 ± 0.01	NT	NT	NT	NT
	SAHA	—	65	29.7 ± 5.4	57.9 ± 6.8	261.6 ± 39.5	478.9 ± 33.4	1200	4.8 (2.5)
	TSA	—	5	1.37 ± 0.16	20.5 ± 0.71	41.2 ± 6.1	49.5 ± 7.4	95	2.6 (2.2)

NA = not active up to the highest concentration tested.
NC = no cytotoxic up to highest concentration tested.
NT = not tested.
[a] Each value is obtained from three independent experiments.
[b] Cited from Ref. 17.
[c] Values are mean ± SD of triplicates.

methoxy substitution (Table 1, comparing compounds **35** with **37**). However, bisortho methoxy-substitution, incorporated into compound **38**, led to a reduction of the antimalarial activity, an observation that may suggest that the parasite's intracellular target(s) may have similar steric constraints as the mammalian HDAC1.

Larger cap groups such as biphenyls, naphthalenes and quinolines also furnished compounds with potent antimalarial activities. We observed that this class of compounds displayed spacer length-dependent antimalarial activities in similar manner to their simple aryl counterparts. Moreover, compounds' antimalarial potency was

dependent on the substitution pattern on these larger cap group moieties. Similar to their anti-HDAC activities, the biphenyl compounds **40**–**47** displayed varying antimalarial activities that were dependent on the relative position of the triazole ring.[17] Specifically, the *meta*-placement of the triazole ring was about 65–100-fold preferred over an *ortho*-substitution by either strains of *P. falciparum* (Table 1, comparing compounds **40** and **44**). While there was no strong preference for either 5 or 6 methylene spacer group among the *meta*-biphenyl compounds **40** and **41**, 5 methylene-linked 4(4-pyridyl) compound **45** was surprisingly 25-times more potent compared to the 6 methylene congener **46**. The antimalarial activities of the 5 and 6 methylene spacer, six–six fused ring naphthalenes **48**–**49** and quinolines **51**–**52** were virtually indistinguishable. These compounds showed potent antimalarial activities with IC_{50} values that were comparable to those of TSA and 5–15-fold lower than those of SAHA against either strain of *P. falciparum* (Table 1).

We also investigated the effects of these aryltriazolylhydroxamates on the viability of the promastigote stage of *L. donovani* and found that they have modest to moderate in vitro antileishmanial activities. Similar to their antimalarial effects, the antileishmanial activities of these compounds were generally maximal in analogs with 5 or 6 methylene spacer groups. Worthy of specific note are compounds **18**, **22**, **30**, **39**, **40** and **51** which inhibited the proliferation of the promastigote stage of *L. donovani* with IC_{50} values that were 2–4-fold lower than that of SAHA and comparable to that of miltefosine, the only clinically approved oral drug against visceral leishmaniasis.[23,24] It is imperative to point out that the antileishmanial activities of some of these compounds may be primarily due to their general cytotoxicity at the measured IC_{50} values, in similar manner to that of SAHA.

Finally, several of the compounds that potently inhibited parasite proliferation were found to be less cytotoxic to normal mammalian Vero cells. For example, 18 compounds—**16**, **17**, **21**, **22**, **25**, **26**, **28**, **31**, **32**, **39**–**41**, **45**, **47**–**49**, **51** and **52**—were several folds selectively toxic to plasmodium parasite compared to the two standard HDAC inhibitors—SAHA and TSA (Table 1).

3.3. Conclusion

We have identified in the present study specific cap group substitution patterns and spacer-group chain lengths that enhanced the antimalarial and antileishmanial activities to aryltriazolylhydroxamates HDACi. The anti-parasite properties of several of these compounds tracked well with their anti-HDAC activities. Many of the compounds herein disclosed are several folds selectively cytotoxic to the *Plasmodium* parasite compared to standard HDACi. Also, a few of these compounds have antileishmanial activities that rivaled the only currently available oral agent against visceral leishmaniasis. Our results provide additional evidence on the suitability of HDAC inhibition as a viable therapeutic approach to curtail infections caused by apicomplexan protozoans and trypanosomatids. The remarkable and selective anti-parasitic properties of several of these aryltriazolylhydroxamates warrant further investigation. A few lead compounds are being advanced further for in vivo evaluation in murine malarial models.

4. Experimental

4.1. General

ω-Bromoalkanoic acids and 7-bromoheptane nitrile were purchased from Sigma–Aldrich. Anhydrous solvents and other reagents were purchased and used without further purification. Analtech silica gel plates (60 F254) were used for analytical TLC, and Analtech preparative TLC plates (UV 254, 2000 μm) were used for purification. UV light was used to examine the spots. Silica gel (200–400 Mesh) was used in column chromatography. NMR spectra were recorded on a Varian-Gemini 400 magnetic resonance spectrometer. ^{1}H NMR spectra were recorded in parts per million (ppm) relative to the peak of $CDCl_3$, (7.24 ppm), CD_3OD (3.31 ppm), or DMSO-d_6 (2.49 ppm). ^{13}C spectra were recorded relative to the central peak of the $CDCl_3$ triplet (77.0 ppm), CD_3OD (49.0 ppm), or the DMSO-d_6 septet (39.7 ppm), and were recorded with complete heterodecoupling. Multiplicities are described using the abbreviation s, singlet; d, doublet, t, triplet; q, quartet; m, multiplet; and app, apparent. High-resolution mass spectra were recorded at the Georgia Institute of Technology mass spectrometry facility in Atlanta. Melting points (uncorrected) were recorded on a Mel-Temp II apparatus. 7-Azidoheptanoic acid **1a** was synthesized starting from 7-bromoheptane nitrile while 8-azidooctanoic acid **1b** and 9-azidononanoic acid **1c** were synthesized from the corresponding ω-bromo acids as described previously.[19a–c]

4.2. Analogue synthesis

4.2.1. Representative procedure for conversion of ω-azidoalkanoic acid to *O*-trityl protected hydroxamates. 7-Azido-*O*-tritylheptahydroxamate (2a)

7-Azidoheptanoic acid **1a** (434 mg, 2.54 mmol) was dissolved in anhydrous THF. *N*-Methylmorpholine (257 mg, 2.54 mmol) was added to the solution. The reaction mixture was then cooled down to −15 °C and stirred for 5 min. Isobutylchloroformate (346 mg, 2.55 mmol) was added and the mixture was stirred for 10 min at −15 °C. *O*-Tritylhydroxylamine (700 mg, 2.55 mmol) was added followed by 2 more equivalents of *N*-methylmorpholine. Stirring continued for 15 min at −15 °C and 2 h at room temperature. Afterwards the mixture was poured into 2 M HCl and extracted three times in each case with water, sodium bicarbonate solution (5%) and water. After washing with brine and drying over Na_2SO_4, solvent was evaporated in vacuo. Column chromatography using 25% EtOAc in hexanes as the eluent system gave compound **2** 837 mg (77%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91–0.99 (2H, m), 1.11–1.21 (4H, m), 1.44 (2H, p, J = 7.6 Hz), 1.77 (2H, t, J = 7.2 Hz), 3.24 (2H, t, J = 6.8 Hz), 7.29–7.38 (15H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.0, 26.1, 28.3, 30.8, 51.0, 93.0, 127.3, 127.9, 128.8, 140.9, 176.9.

4.2.2. 8-Azido-*O*-trityloctahydroxamate (2b)

Reaction of 8-azidooctanoic acid **1b** (1.71 g, 9.21 mmol) and *O*-tritylhydroxylamine (2.55 g, 9.27 mmol) as described for the synthesis of **2a**, followed by flash chromatography (eluent 2:1 hexanes/EtOAc) gave 2.59 g (88%) of **2b** as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88–1.39 (8H, m), 1.39–1.54 (4H, m), 3.12 (2H, t, J = 6.9 Hz), 7.10–7.49 (15H, m), 7.67 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.2, 24.9, 26.4, 28.7, 31.1, 33.2, 51.3, 93.1, 127.1, 128.0, 128.9, 141.0, 141.8, 146.8, 177.1.

4.2.3. 9-Azido-*O*-tritylnonahydroxamate (2c)

Reaction of 9-azidononanoic acid **1c** (724 mg, 3.63 mmol) and *O*-tritylhydroxylamine (1.00 g, 3.63 mmol) overnight as described for the synthesis of **2a**, followed by flash chromatography (eluent 2:1 hexanes/EtOAc) gave 940 mg (56%) of **2c** as a sticky white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (10H, m), 1.57 (4H, m), 3.24 (2H, t, J = 6.8 Hz), 7.34 (15H, m), 7.74 (1H, s).

4.2.4. Representative procedure for Cu(I)-catalyzed cycloaddition reaction. *O*-Trityl-3-pyridyltriazolylheptahydroxamate (8)

7-Azido-*O*-tritylheptahydroxamate **2a** (207 mg, 0.48 mmol) and 3-ethynylpyridine (50 mg, 0.48 mmol) were dissolved in anhydrous THF (10 mL) and stirred under argon at room temperature.

Copper(I) iodide (9 mg, 0.05 mmol) and Hunig's base (0.1 mL) were then added to the reaction mixture, and stirring continued for 24 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with 1:4 NH_4OH/saturated NH_4Cl (3 × 30 mL) and saturated NH_4Cl (30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography (gradient 3:1 CH_2Cl_2/acetone, then CH_2Cl_2/MeOH (5%)) to give 206 mg (81%) of **8** as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.94–1.01 (2H, m), 1.06–1.19 (4H, m), 1.71–1.78 (4H, m), 3.59 (1H, s), 4.34 (2H, t, J = 6.8 Hz), 7.25–7.36 (16H, m), 7.44–7.47 (1H, m), 8.07 (1H, s), 8.17–8.20 (1H, m), 8.51–8.52 (1H, m), 8.68 (1H, s), 9.03–9.04 (1H, m), 10.16 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.8, 29.7, 30.7, 46.9, 50.1, 53.8, 119.8, 123.5, 126.6, 127.7, 128.4, 128.8, 132.7, 144.0, 144.4, 146.7, 148.9, 176.8.

4.2.5. *O*-Tritylphenyltriazolyloctahydroxamate (4)

Reaction of 8-azido-*O*-trityloctahydroxamate **2b** (150 mg, 0.34 mmol) and phenylacetylene (35.9 mg, 0.35 mmol) as described for the synthesis of **8**, followed by purification using preperative TLC (eluent 1:1 hexanes/EtOAc) gave 90 mg (48.6%) of **4** as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.94–1.12 (2H, m), 1.12–1.46 (6H, m), 1.49–1.65 (2H, m), 1.75–1.97 (2H, m), 4.33 (2H, t, J = 7.3 Hz), 7.17–7.64 (18H, m), 7.72 (1H, s), 7.75 (1H, s), 7.81 (2H, d, J = 7.5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.2, 24.7, 26.1, 28.7, 31.0, 33.1, 50.3, 93.0, 119.3, 125.7, 128.0, 128.9, 130.6, 141.0, 141.7, 147.7, 178.2.

4.2.6. *O*-Tritylphenyltriazolylnonahydroxamate (5)

Reaction of 9-azido-*O*-tritylnonahydroxamate **2c** (80.4 mg, 0.18 mmol) and phenylacetylene (23.5 mg, 0.23 mmol) as described for the synthesis of **8**, followed by purification using preperative TLC (eluent 11:10 hexanes/EtOAc) gave 72.0 mg (73.2%) of **5** as a sticky white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88–1.44 (12H, m), 1.89 (2H, m), 4.34 (2H, t, J = 7.1 Hz), 7.15–7.6 (18H, m), 7.72 (1H, s), 7.79–7.81 (2H, d, J = 8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.6, 26.6, 28.9, 29.2, 30.5, 31.2, 31.4, 50.6, 119.7, 125.9, 128.3, 129.0, 129.3, 141.3, 147.9, 177.5.

4.2.7. *O*-Trityl-*p*-*N*,*N*-dimethylanilyltriazolyloctahydroxamate (6)

Reaction of 8-azido-*O*-trityloctahydroxamate **2b** (514 mg, 1.16 mmol) and 4-ethynyl-*N*,*N*-dimethylanaline (169 mg, 1.16 mmol) as described for the synthesis of **8**, followed by purification using preperative TLC (eluent 8:1 CH_2Cl_2/acetone) gave 560 mg (82.1%) of **6** as a white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 0.93–1.06 (2H, m), 1.11–1.36 (6H, m), 1.76–1.92 (4H, m), 2.92 (6H, s), 4.31 (2H, t, J = 7.0 Hz), 6.76 (2H, d, J = 9.0 Hz), 7.18–7.43 (15H, m), 7.62 (2H, d, J = 9.0 Hz), 8.05 (1H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ 24.5, 26.2, 27.3, 29.6, 31.1, 33.4, 40.8, 51.3, 94.3, 113.8, 119.8, 120.5, 127.6, 128.6, 130.4, 143.6, 149.4, 152.1, 173.2.

4.2.8. *O*-Trityl-*p*-*N*,*N*-dimethylanilyltriazolylnonahydroxamate (7)

Reaction of 9-azido-*O*-tritylnonahydroxamate **2c** (100 mg, 0.22 mmol) and 4-ethynyl-*N*,*N*-dimethylanaline (49 mg, 0.34 mmol) as described for the synthesis of **8**, followed by purification using preperative TLC (eluent 40:1:0.1 CH_2Cl_2/MeOH/NH_4OH) gave 72.5 mg (55%) of **7** as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.04–1.60 (12H, m), 1.88 (2H, m), 4.32 (2H, t, J = 7.2 Hz), 6.74–6.76 (2H, d, J = 8.8 Hz), 7.25–7.53 (16H, m), 7.58 (1H, s), 7.66–7.69 (2H, d, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.6, 26.6, 29.0, 29.2, 29.9, 30.5, 31.4, 40.7, 50.5, 112.7, 118.2, 119.2, 126.9, 127.0, 128.3, 129.3, 141.3, 142.1, 148.4, 149.5, 150.6, 177.5.

4.2.9. *O*-Trityl-3-pyridyltriazolyloctahydroxamate (9)

Reaction of 8-azido-*O*-trityloctahydroxamate **2b** (165 mg, 0.37 mmol) and 3-ethynylpyridine (38.3 mg, 0.37 mmol) as described for the synthesis of **8**, followed by purification using preperative TLC (eluent 5:3 CH_2Cl_2/acetone) gave 170 mg (83.3%) of **9** as a white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 0.90–1.06 (2H, m), 1.09–1.38 (6H, m), 1.77–2.00 (4H, m), 4.38 (2H, t, J = 7.3 Hz), 7.10–7.59 (15H, m), 8.22 (1H, d, J = 8.3 Hz), 8.44 (1H, s), 8.46 (1H, d, J = 4.8 Hz), 8.99 (1H, d, J = 2.3 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 24.5, 26.2, 27.2, 29.6, 31.1, 33.4, 51.6, 94.2, 123.0, 125.6, 128.7, 130.4, 134.8, 143.5, 145.2, 147.2, 149.5, 173.0.

4.2.10. *O*-Trityl-2-tolyltriazolylheptahydroxamate (10)

Reaction of 7-azido-*O*-tritylheptahydroxamate **2a** (147 mg, 0.34 mmol) and 2-ethynyltoluene (40 mg, 0.34 mmol) as described for synthesis of **8**, followed by flash chromatography (gradient CH_2Cl_2/acetone 7:1, 6:1, 5:1) gave 160 mg (85%) of compound **10** as white solid.. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.94–1.01 (2H, m), 1.07–1.19 (4H, m), 1.72–1.79 (4H, m), 2.40 (3H, s), 4.33 (2H, t, J = 7.2 Hz), 7.22–7.30 (17H, m), 7.70–7.74 (1H, m), 8.35 (1H, s), 10.16 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 21.1, 25.5, 27.6, 29.5, 31.8, 49.3, 54.8, 91.6, 123.0, 125.9, 127.3, 127.4, 127.6, 128.1, 128.9, 130.1, 130.8, 134.8, 142.4, 145.4, 170.2.

4.2.11. *O*-Trityl-3-biphenyltriazolyloctahydroxamate (11)

Reaction of 8-azido-*O*-trityloctahydroxamate **2b** (137 mg, 0.31 mmol) and 1,1′-biphenyl-3-ethynyl (60 mg, 0.34 mmol) as described for the synthesis of **8**, followed by purification using preperative TLC (eluent 18:1 CH_2Cl_2/acetone) gave 170 mg (88.5%) of **11** as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.92–1.12 (2H, m), 1.12–1.47 (6H, m), 1.75–1.97 (4H, m), 4.34 (2H, t, J = 7.1 Hz), 7.20–7.39 (13H, m), 7.40–7.52 (5H, m), 7.54 (2H, d, J = 7.7 Hz), 7.64 (2H, d, J = 7.4 Hz), 7.77 (1H, s), 7.78 (2H, m), 8.06 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.2, 24.9, 26.1, 28.5, 30.9, 33.0, 50.4, 93.0, 119.6, 124.5, 126.8, 127.1, 127.4, 128.1, 128.7, 129.0, 129.2, 131.1, 140.7, 141.0, 141.7, 147.6, 177.4.

4.2.12. *O*-Trityl-3-biphenyltriazolylnonahydroxamate (12)

Reaction of 9-azido-*O*-tritylnonahydroxamate **2c** (130 mg, 0.29 mmol) and 1,1′-biphenyl-3-ethynyl (65 mg, 0.37 mmol) as described for the synthesis of **8**, followed by purification using preperative TLC (eluent 30:1 CH_2Cl_2/MeOH) gave 144.2 mg (81.5%) of **11** as a red-brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.9–1.2 (6H, m), 1.21–1.42 (6H, m), 1.86 (2H, m), 4.31 (2H, m), 7.16–7.47 (19H, m), 7.51 (1H, d, J = 8.0 Hz), 7.61 (2H, d, J = 7.2 Hz), 7.76 (3H, m), 8.05 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.6, 26.6, 29.0, 29.2, 30.0, 30.5, 31.4, 50.6, 119.9, 124.7, 124.8, 127.1, 127.4, 128.1, 128.2, 128.4, 129.0, 129.3, 129.5, 131.5, 141.0, 142.0, 147.8, 177.6.

4.2.13. *O*-Trityl-4-pyridylphenyltriazolyloctahydroxamate (13)

Reaction of 8-azido-*O*-trityloctahydroxamate **2b** (150 mg, 0.34 mmol) and 4-(4-ethynylphenyl)-pyridine (61 mg, 0.34 mmol) as described for synthesis of **8**, followed by purification using preparative TLC (eluent 10:1 Et_2O/EtOH) gave 133 mg (63%) of compound **13** as white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.86–0.92 (2H, m), 1.10–1.20 (6H, m), 1.73–1.81 (4H, m), 4.37 (2H, t, J = 6.8 Hz), 7.26–7.34 (15H, m), 7.74 (2H, d, J = 6.0 Hz), 7.89 (2H, d, J = 8.4 Hz), 7.97 (2H, d, J = 8.4 Hz), 8.62–8.64 (2H, m), 8.67 (1H, s), 10.1 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.1, 26.1, 28.5, 28.7, 29.6, 30.1, 50.3, 119.7, 121.2, 123.6, 126.2, 127.3, 127.8, 128.5, 128.9, 131.4, 137.3, 140.9, 144.1, 146.7, 147.5, 150.1, 177.1.

4.2.14. *O*-Trityl-6-methoxynapthyltriazolyloctahydroxamate (14)

Reaction of 8-azido-*O*-trityloctahydroxamate **2b** (135 mg, 0.30 mmol) and 2-ethynyl-6-methoxynapthalene (56 mg, 0.30 mmol) as described for synthesis of **8**, followed by purification using preparative TLC (eluent 25:1 Et_2O/EtOH) gave 142 mg (76%) of compound **14** as white solid. ^{1}H NMR (DMSO-d_6, 400 MHz)

δ 0.86–0.95 (2H, m), 1.10–1.21 (3H, m), 1.73–1.81 (2H, m), 3.86 (3H, s), 4.37 (2H, t, *J* = 6.8 Hz), 7.15–7.32 (17H, m), 7.83–7.92 (3H, m), 8.29 (1H, m), 8.61–8.62 (1H, m), 10.12 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.1, 26.1, 28.5, 28.7, 29.6, 30.1, 50.3, 55.2, 93.6, 105.7, 119.1, 119.3, 124.1, 124.3, 125.8, 127.2, 127.8, 127.8, 128.0, 128.9, 128.9, 129.6, 134.2, 140.9 147.8, 157.8, 177.1.

4.2.15. Representative procedure for conversion of *O*-tritylhydroxamate to hydroxamic acid via TFA deprotection. 7-(3-Pyridyl)-triazolylheptahydroxamic acid (26)

To a solution of *O*-trityl-3-pyridyltriazolylheptahydroxamate **8** (90 mg, 0.16 mmol) in CH_2Cl_2 (7 mL) was added trifluoroacetic acid (TFA) (0.2 mL) and thioanisole (0.2 mL) dropwise at 0 °C. Reaction mixture stirred for next 3 h at 0 °C. Solvent evaporated off in vacuo. The crude mixture was loaded on preparative TLC, eluted with 12:1:0.2 CH_2Cl_2/MeOH/NH_4OH to give 39 mg (84%) of **26** as a white solid; mp 146–148 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.21–1.29 (4H, m), 1.46 (2H, m), 1.84 (2H, m), 1.91 (2H, m), 4.39 (2H, t, *J* = 7.2 Hz), 7.45–7.48 (1H, m), 8.18 (1H, m), 8.52 (1H, s), 8.65 (1H, s), 8.70 (1H, s), 9.03 (1H, s), 10.31 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.9, 25.5, 27.9, 29.4, 32.1, 49.5, 121.9, 124.0, 132.4, 143.4, 146.2, 148.7, 169.0. HRMS (FAB, thioglycerol) calcd for $[C_{14}H_{19}N_5O_2+H]^+$ 290.1617, found 290.1615.

4.2.16. 8-(Phenyl)triazolyloctahydroxamic acid (19)

Reaction of *O*-trityl-phenyltriazolyloctahydroxamate **4** (70 mg, 0.13 mmol) with TFA (0.1 mL) and thioanisole (0.1 mL) in CH_2Cl_2 (10 mL) at 0 °C within 3 h as described for the synthesis of **26**, followed by purification using preparative TLC (eluent 8:1 CH_2Cl_2/MeOH) gave 20 mg (51%) of **19** as a white solid; mp 118–121 °C. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.22–1.48 (6H, m), 1.52–1.70 (2H, m), 1.87–2.01 (2H, m), 2.02–2.18 (2H, m), 4.44 (2H, t, *J* = 7.1 Hz), 7.34 (1H, t, *J* = 7.2 Hz), 7.43 (2H, t, *J* = 7.9 Hz), 7.81 (2H, d, *J* = 7.2 Hz), 8.32 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 26.5, 27.2, 29.6, 29.8, 31.1, 33.6, 51.4, 122.2, 126.6, 129.3, 130.0, 131.7, 148.8, 169.1. HRMS (FAB, thioglycerol) calcd for $[C_{16}H_{22}N_4O_2+H]^+$ 303.1821, found 303.1806.

4.2.17. 9-(Phenyl)triazolylnonahydroxamic acid (20)

Reaction of *O*-trityl-phenyltriazolylnonahydroxamate **5** (57 mg, 0.10 mmol) with TFA (0.1 mL) and thioanisole (0.1 mL) in CH_2Cl_2 (10 mL) at 0 °C within 3 h as described for the synthesis of **26**, followed by purification using preparative TLC (eluent 18:1:0.1 CH_2Cl_2/MeOH/NH_4OH) gave 9.9 mg (27.5%) of **20** as a white solid; mp 135–137 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.12–1.3 (8H, m), 1.42 (2H, m), 1.81 (2H, m), 1.87 (2H, t, *J* = 7.2 Hz), 4.34 (2H, t, *J* = 7.0 Hz), 7.28 (1H, t, *J* = 7.4 Hz), 7.40 (2H, t, *J* = 7.4 Hz), 7.80 (2H, d, *J* = 8.2 Hz), 8.54 (1H, s), 8.61 (1H, s), 10.28 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.7, 26.5, 29.0, 29.15, 29.22, 30.3, 32.9, 50.2, 121.9, 125.8, 128.4, 129.6, 131.5, 146.9, 169.8. HRMS (FAB, thioglycerol) calcd for $[C_{17}H_{24}N_4O_2+H]^+$ 317.1933, found 317.1984.

4.2.18. 9-(*p*-*N*,*N*-Dimethylanilyl)triazolylnonahydroxamic acid (24)

Reaction of *O*-trityl-(*p*-*N*,*N*-dimethylanilyl)triazolylnonahydroxamate **7** (65.3 mg, 0.11 mmol) with TFA (0.1 mL) and thioanisole (0.1 mL) in CH_2Cl_2 (10 mL) at 0 °C within 3 h as described for the synthesis of **26**, followed by purification using preparative TLC (eluent 40:1 CH_2Cl_2/MeOH) gave 28.1 mg (72%) of **24** as a white solid; mp 106–108 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.21 (8H, m), 1.44 (2H, m), 1.81 (2H, m), 1.90 (2H, t, *J* = 7.2 Hz), 4.31 (2H, t, *J* = 7.2 Hz), 6.75 (2H, d, *J* = 8.8 Hz), 7.62 (2H, d, *J* = 8.8 Hz), 8.33 (1H, s), 8.64 (1H, s), 10.31 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.7, 26.6, 29.0, 29.16, 29.24, 30.3, 32.9, 40.7, 50.0, 113.0, 119.5, 120.0, 126.7, 147.5, 150.6, 169.8. HRMS (FAB, thioglycerol) calcd for $[C_{19}H_{29}N_5O_2+H]^+$ 360.2400, found 360.2402.

4.2.19. 7-(2-Tolyl)triazolylheptahydroxamamic acid (34)

Reaction of *O*-trityl-2-tolyltriazolylheptahydroxamate **10** (90 mg, 0.16 mmol) with TFA (0.2 mL) and thioanisole (0.2 mL) in CH_2Cl_2 (7 mL) at 0 °C within 3 h as described for the synthesis of **26**, followed by purification using preparative TLC (eluent 12:1:0.2 CH_2Cl_2/MeOH/NH_4OH) gave 46 mg (93%) of **34** as an off-white semi-solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26–1.34 (4H, m), 1.53–1.61 (2H, m), 1.84–1.91 (2H, m), 2.05–2.14 (2H, m), 2.40 (3H, s), 4.34 (2H, t, *J* = 6.8 Hz), 7.20–7.22 (3H, m), 7.64 (1H, s), 7.67–7.70 (1H, m), 9.72 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 21.3, 24.9, 25.6, 27.8, 29.6, 29.8, 50.0, 121.9, 126.0, 128.1, 128.7, 129.7, 130.8, 135.4, 146.9, 171.3. HRMS (FAB, thioglycerol) calcd for $[C_{16}H_{22}N_4O_2+H]^+$ 303.1821, found 303.1810.

4.2.20. 8-(3-Biphenyl)triazolyloctahydroxamic acid (42)

Reaction of *O*-trityl-3-biphenyltriazolyloctahydroxamate **11** (170 mg, 0.23 mmol) with TFA (0.1 mL) and thioanisole (0.1 mL) in CH_2Cl_2 (10 mL) at 0 °C within 3 h as described for the synthesis of **26**, followed by purification using preparative TLC (eluent 12:1 CH_2Cl_2/MeOH) gave 80 mg (77.2%) of **42** as a white solid; mp 110–112 °C. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.14–1.44 (6H, m), 1.45–1.66 (2H, m), 1.78–1.96 (2H, m), 1.96–2.13 (2H, m), 4.35 (2H, t, *J* = 6.9 Hz), 7.31 (1H, t, *J* = 6.7 Hz), 7.41 (2H, t, *J* = 7.6 Hz), 7.46 (1H, d, *J* = 7.3 Hz), 7.55 (1H, d, *J* = 7.3 Hz), 7.63 (2H, d, *J* = 7.3 Hz), 7.75 (1H, d, *J* = 7.3 Hz), 8.07 (1H, s), 8.33 (1H, s) ^{13}C NMR (CD_3OD, 100 MHz) δ 26.5, 27.3, 29.6, 29.8, 31.1, 33.7, 51.4, 122.4, 125.1, 125.5, 127.8, 128.0, 128.6, 129.9, 130.5, 132.2, 141.8, 143.1, 148.6, 172.9. HRMS (FAB, thioglycerol) calcd for $[C_{22}H_{26}N_4O_2+H]^+$ 379.2134, found 379.2139.

4.2.21. 9-(3-Biphenyl)triazolylnonahydroxamic acid (43)

Reaction of *O*-trityl-3-biphenyltriazolylnonahydroxamate **12** (114 mg, 0.18 mmol) with TFA (0.25 mL) and thioanisole (0.25 mL) in CH_2Cl_2 (10 mL) at 0 °C within 3 h as described for the synthesis of **26**, followed by purification using preparative TLC (eluent 20:1 CH_2Cl_2/MeOH) gave 63.4 mg (91%) of **43** as a white solid; mp 122–125 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.23 (8H, m), 1.42 (2H, m), 1.88 (4H, m), 4.36 (2H, m), 7.36 (1H, t, *J* = 7.6 Hz), 7.42–7.54 (3H, m), 7.59 (1H, d, *J* = 7.2 Hz), 7.69 (2H, d, *J* = 7.6 Hz), 7.82 (1H, d, *J* = 7.6 Hz), 8.08 (1H, s), 8.62 (1H, s), 8.68 (1H, s), 10.29 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.8, 26.5, 29.0, 29.1, 29.2, 30.3, 50.2, 122.3, 124.0, 124.7, 126.8, 127.4, 128.3, 129.6, 130.2, 132.1, 140.5, 141.4, 146.9, 167.9. HRMS (FAB, thioglycerol) calcd for $[C_{23}H_{28}N_4O_2+H]^+$ 393.2246, found 393.2238.

4.2.22. 8-(4-Pyridylphenyl)triazolyloctahydroxamamic acid (47)

Reaction of *O*-trityl-4-pyridylphenyltriazolyloctahydroxamate **13** (80 mg, 0.12 mmol) with TFA (0.2 mL) and thioanisole (0.2 mL) in CH_2Cl_2 (7 mL) at 0 °C within 3 h as described for the synthesis of **26**, followed by purification using preparative TLC (eluent 12:1:0.2 CH_2Cl_2/MeOH/NH_4OH) gave 29 mg (61%) of **47** as a white solid; mp 152–154 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.21–1.26 (6H, m), 1.45 (2H, p, *J* = 7.2 Hz), 1.83–1.93 (4H, m), 3.86 (3H, s), 4.39 (2H, t, *J* = 7.2 Hz), 7.75–7.81 (2H, m), 7.91–7.93 (2H, m), 7.98–8.00 (2H, m), 8.62–8.70 (3H, s), 10.34 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.0, 25.7, 28.0, 28.3, 29.5, 32.2, 49.5, 121.1, 121.8, 125.7, 127.4, 131.8, 136.1, 145.6, 146.6, 150.0, 169.1. HRMS (FAB, thioglycerol) calcd for $[C_{21}H_{26}N_5O_2+H]^+$ 380.2086, found 380.2102.

4.2.23. 8-(6-Methoxynapthyl)triazolylheptaoctahydroxamamic acid (50)

Reaction of 6-methoxynapthyltriazolyloctahydroxamate **14** (90 mg, 0.14 mmol) with TFA (0.2 mL) and thioanisole (0.2 mL) in CH_2Cl_2 (7 mL) at 0 °C within 3 h as described for the synthesis of

26, followed by purification using preparative TLC (eluent 12:1:0.2 CH_2Cl_2/MeOH/NH_4OH) gave 34 mg (64%) of **50** as a white solid; mp 166–168 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.20–1.26 (6H, m), 1.45 (2H, p, J = 7.2 Hz), 1.83–1.92 (4H, m), 3.86 (3H, s), 4.38 (2H, t, J = 7.2 Hz), 7.16 (1H, dd, J = 2.3 Hz, 9.0 Hz), 7.31 (1H, d, J = 2.3 Hz), 7.84–7.92 (2H, m), 8.29 (1H, s), 8.62 (1H, s), 8.64 (1H, s), 10.31 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.9, 25.7, 28.0, 28.3, 29.6, 32.1, 49.4, 55.2, 105.9, 119.1, 121.1, 123.3, 124.1, 126.0, 127.3, 133.8, 146.4, 157.3, 169.0. HRMS (FAB, thioglycerol) calcd for $[C_{21}H_{26}N_4O_3+H]^+$ 383.2083, found 383.2104.

4.2.24. Representative procedure for conversion of *O*-tritylhydroxamate to hydroxamic acid via $BF_3{\cdot}OEt_2$ deprotection. 8-(3-Pyridyl)triazolyloctahydroxamic acid (27)

To a solution of *O*-trityl-3-pyridyltriazolyloctahydroxamate **9** (121 mg, 0.22 mmol) in just enough THF to dissolve (ca. 1 mL) was added thioanisole (0.31 mL) followed by $BF_3{\cdot}OEt_2$ (0.34 mL) dropwise at rt. The reaction mixture was stirred for 20 min during which a quantitative deprotection was observed bt TLC. The mixture was poured into 20% MeOH in EtOAc (40 mL) and washed with water (10 mL). The pH of the aqueous layer was adjusted to ~7 and extracted with 20% MeOH in EtOAc (3 × 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the crude product using preparative TLC (eluent 10:1 CH_2Cl_2/MeOH) gave 62.1 mg (92.3%) of **27** as a white solid; mp 110–112 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.98–1.36 (6H, m), 1.38–1.62 (2H, m), 1.72–2.06 (4H, m), 4.39 (2H, t, J = 6.8 Hz), 7.46 (1H, t, J = 6.3 Hz), 8.19 (1H, d, J = 8.0 Hz), 8.52 (1H, d, J = 4.6 Hz), 8.64 (1H, s), 8.70 (1H, s), 9.02 (1H, s), 10.31 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.0, 25.7, 28.1, 28.4, 29.6, 32.2, 49.8, 121.9, 124.0, 126.8, 132.2, 143.5, 146.3, 148.8, 169.1. HRMS (FAB, thioglycerol) calcd for $[C_{15}H_{21}N_5O_2+H]^+$ 304.1773, found 304.1765.

4.2.25. 8-(*p*-*N*,*N*-Dimethylanilyl)triazolyloctahydroxamic acid (23)

Deprotection of *O*-trityl-*p*-*N*,*N*-dimethylanilyltriazolyloctahydroxamate **6** (186 mg, 0.32 mmol) under unoptimized conditions[25] used $BF_3{\cdot}OEt_2$ (0.08 mL) without thioanisole in 4:1 $CHCl_3$/MeOH (15 mL) at rt for 3 h. The reaction was poured into 1:1 $H_2O/CHCl_3$ (100 mL) and the aqueous layer extracted with $CHCl_3$ (50 mL). The combined organic layer was washed with brine (50 mL), then dried over Na_2SO_4 and concentrated in vacuo. Purification using preparative TLC (eluent 12:1 CH_2Cl_2/MeOH) gave 43 mg (40%) of **23** as a white solid; mp 108–110 °C. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.24–1.45 (6H, m), 1.49–1.68 (2H, m), 1.84–2.00 (2H, m), 2.00–2.18 (2H, m), 2.97 (6H, s), 4.39 (2H, t, J = 7.1 Hz), 6.83 (2H, d, J = 8.1 Hz), 7.65 (2H, d, J = 8.7 Hz), 8.12 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.1, 25.8, 28.1, 28.4, 29.7, 32.2, 40.0, 49.4, 112.3, 118.8, 119.3, 126.0, 146.8, 149.9, 169.1. HRMS (FAB, thioglycerol) calcd for $[C_{18}H_{27}N_5O_2+H]^+$ 346.2243, found 346.2242.

4.2.26. HDAC activity assay

In vitro HDAC inhibition was assayed using the HDAC Fluorimetric Assay/Drug Discovery Kit as previously described.[17–19] Briefly, 15 μL of HeLa nuclear extract was mixed with 5 μL of 10× compound and 5 μL of assay buffer. Fluorogenic substrate (25 μL) was added, and reaction was allowed to proceed for 15 min at room temperature and then stopped by addition of a developer containing TSA. Fluorescence was monitored after 15 min at excitation and emission wavelengths of 360 and 460 nm, respectively. IC_{50} values were determined using logit plots.

4.2.27. In vitro antimalarial and antileishmanial assays

Antimalarial activity of the compounds was determined in vitro on chloroquine-sensitive (D6, Sierra Leone) and resistant (W2, IndoChina) strains of *P. falciparum*. The 96 well microplate assay is based on evaluation of the effect of the compounds on growth of asynchronous cultures of *P. falciparum*, determined by the assay of parasite lactate dehydrogenase (pLDH) activity.[20] The appropriate dilutions of the compounds were prepared in DMSO or RPMI-1640 medium and added to the cultures of *P. falciparum* (2% hematocrit, 2% parasitemia) set-up in clear flat bottomed 96 well plates. The plates were placed into the humidified chamber and flushed with a gas mixture of 90% N_2, 5% CO_2 & 5% O_2. The cultures were incubated at 37 °C for 72 h. Growth of the parasite in each well was determined by pLDH assay using Malstat® reagent.[20] The medium and RBC controls were also set-up in each plates. The standard antimalarial agents, chloroquine and artemisinin, were used as the positive controls while DMSO was tested as the negative control. Antileishmanial activity of the compounds was tested in vitro on a culture of *L. donovani* promastigotes. In a 96 well microplate assay compounds with appropriate dilution were added to the leishmania promastigotes culture (2×10^6 cell/mL). The plates were incubated at 26 °C for 72 h and growth of leishmania promastigotes was determined by Alamar blue assay.[21] Pentamidine and Amphotericin B were tested as standard antileishmanial agents. All the compounds were simultaneously tested for cytotoxicity on VERO (monkey kidney epithelial) cells by Neutral Red assay.[22] IC_{50} value for each compound was computed from the growth inhibition curve.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.10.042.

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