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Sulfamides as novel histone deacetylase inhibitors

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ABSTRACT

The sulfamide moiety has been utilized to design novel HDAC inhibitors. The potency and selectivity of these inhibitors were influenced both by the nature of the scaffold, and the capping group. Linear long-chain-based analogs were primarily HDAC6-selective, while analogs based on the lysine scaffold resulted in potent HDAC1 and HDAC6 inhibitors.

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Histone deacetylases (HDACs) are a family of 18 enzymes that play an important role in the regulation of gene expression, cell growth, and proliferation by regulating the deacetylation of ε -*N*acetyl groups on the L-lysine residues at the N-terminal tails of core histones, tubulin, and other proteins.^{1a} These enzymes are divided into two categories: zinc-dependent (HDAC1-11) and NAD⁺dependent enzymes (known as sirtuins, Sirt1-7, or HDAC class III). The zinc-dependent enzymes are divided into class I (HDAC1, 2, 3, and 8), class IIa (HDAC4, 5, 7, and 9), class IIb (HDAC6 and 10), and class IV (HDAC11), which exhibits properties of both class I and class II HDACs.^{1b,c}

The majority of the known HDAC inhibitors (HDACis) are hydroxamic acids.^{2a,2b} SAHA (Zolinza[®], Vorinostat) was approved in 2006 to treat cutaneous T-cell lymphoma (CTCL),^{3a} and a number of other HDACis are in different stages of clinical development.^{3b-d} The quest for HDACis resulted in a number of diverse structures,^{4a,b} such as aliphatic acids,^{4c} hydroxamic acids,^{2a,b,4d,e} *o*-aminoanilides,^{4f} cyclic peptides,^{4g} electrophilic ketones,^{4h} and thiols^{4i-k} (Fig. 1). These structures all share a common pharmacophore composed of a zinc-binding group (ZBG), a linker (scaffold), and a surface recognition domain (cap).⁵ The crystal structures of both the histone deacetylase-like protein (HDLP) complexed with SAHA and TSA^{6a}, and that of HDAC8^{6b,c} suggest that the carbonyl

and hydroxyl groups of the hydroxamic acid chelate the zinc ion in the active site in a bidentate fashion.

SAHA^{3a} is reported to be a pan-HDACi while MGCD0103, an *o*-aminobenzanilide currently in Phase I/II cancer clinical trials, is an isotype-selective inhibitor.^{4f}

The nature of the ZBG seems to confer both the activity and selectivity of these inhibitors. Search for ZBGs that could serve as a suitable pharmacophore in the design of novel HDACis is the subject of our current research. In this letter, we detail the identification of a new class of HDACis bearing the sulfamide moiety as the ZBG. Sulfamides are well-documented inhibitors of carbonic anhydrase, among other zinc-dependent enzymes.^{7a-c}

To test our hypothesis that the sulfamide moiety could act as an efficient ZBG for HDACis, we fixed the 'cap region' using biphenylamide and varied both the sulfamide parent structure and the length of the linker.

Sulfamides **4a–c** were prepared according to Scheme 1 starting from the Boc-protected amino acids utilizing standard coupling conditions. The one-step procedure for the synthesis of sulfamide **4a** from amine **3a** utilizing sulfuric diamide⁸ resulted in low yields and required high temperatures. We therefore opted for the twostep procedure, making the benzyl carbamates^{9a,b} **4b** and **4c**, followed by hydrogenation to furnish sulfamides **5a** and **5b**. The reaction of **3b** with ClSO₂NCO and *t*-BuOH gave the *t*-butyl carbamate intermediate which was methylated with Mel to produce **6**. Deprotection with TFA furnished the mono-methylated sulfamide **7a**.

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Figure 1. HDAC inhibitors.



Scheme 1. Reagents and conditions: (a) 3-phenylaniline, BOP, Et₃N, DMF; (b) TFA, DCM, or 4 N HCl, dioxane; (c) **3a**, H₂NSO₂NH₂, Et₃N, toluene, 120 °C; (d) **3b** or **3c**, ClSO₂NCO, benzyl alcohol, DCM, Et₃N; (e) H₂/10%Pd–C, MeOH; (f) i–**3b**, ClSO₂NCO, *t*-BuOH, DCM, Et₃N, ii–Mel, DBU, CH₃CN, 0 °C; (g) TFA, DCM; (h) **3b**, ClSO₂NMe₂, Et₃N, THF, rt, 18 h; (i) **5a**, Ac₂O, DBU, DMF.



Scheme 2. Reagents and conditions: (a) 3-phenylaniline, BOP, Et₃N, DMF; (b) LiAlH₄, THF; (c) Dess-Martin periodinane, DCM; (d) MeNH₂, NaBH₄, MeOH; (e) $H_2NSO_2NH_2$, Et₃N, toluene, 120 °C; (f) ClSO₂NCO/formic acid/CH₃CN.

The dimethylated sulfamide **7b** was obtained from the reaction of **3b** with a preformed solution of ClSO₂NMe₂ (Scheme 1). Sulfamoyl **8** was obtained by the reaction of **4b** with acetic anhydride and DBU in DMF.

Compound **10** (Scheme 2), was obtained starting from 6-methoxy-6-oxohexanoic acid by an amide coupling reaction with 3phenylaniline followed by reduction of the intermediate ester (not shown in Scheme 2) with LiAlH₄ to furnish alcohol **9**. Oxidation of **9** with Dess–Martin periodinane to form the corresponding aldehyde (not shown in Scheme 2) followed by reductive amination with methylamine yielded the *N*-methylamino-intermediate which was then converted into sulfamide **10** using sulfuric diamide. To obtain sulfamate **11**, alcohol **9** was reacted with ClSO₂N-CO and formic acid in acetonitrile.¹⁰

Sulfamoyl **12** was obtained by the reaction of 7-ethoxy-7-oxoheptanoic acid with 3-phenylaniline followed by hydrolysis and standard CDI coupling with sulfuric diamide (Scheme 3).

Analogs **13a–f** with diverse capping groups were prepared according to Scheme 4.

The synthesized inhibitors **4a**, **5a**, **5b**, **7a**, **7b**, **8**, **10**, **11**, and **12**^{11a} were screened against a panel of recombinant human HDACs



Scheme 3. Reagents and conditions: (a) 3-phenylaniline, BOP, Et₃N, DMF; (b) LiOH, THF, H₂O; (c) CDI, DBU, NH₂SO₂NH₂, DMF.

Table 1

Effect of zinc-binding group and chain length on HDAC1 and 6 inhibitory activity^a \mathbf{r}

0

Ph N H n										
Compound	п	R	HDAC1 IC ₅₀ (μ M)	HDAC6 IC50 (µM)						
SAHA ^b			0.1	0.2						
1 ^b	6	,₅, H N_OH O	0.009	0.009						
2 ^b	6	O NH NH2	0.49	>50						
4a	4	Q, ,O , ^{,,,C} N, S, NH ₂ H	>10	>10						
5a	5	Q N N H NH ₂	>10	1.1						
5b	6	Q, ,O , ^{,,,,} N ,S NH ₂ H	>10	>10						
7a	5	Q S N N H H H	>10	>10						
7b	5	³ ² ^N	>10	>10						
10	5	5 ⁵ NH₂	>10	>10						
11	5	0, 0 , 0 NH ₂	>10	>10						
12	5	Q Q Q N ^S NH₂	>10	>10						
8	5		>10	>10						

^a Values are means of at least two experiments.

^b HDAC inhibitory activities for SAHA and for compounds 1 and 2 were determined in our laboratories

1-8.^{11b} The IC₅₀s of the inhibition of HDAC1 (representative of class I HDACs) and HDAC6 (representative of class IIb) are summarized in Table 1, while the results for the other HDACs were omitted for clarity.

Unlike hydroxamic acid **1**,^{11c} which is a low-nanomolar inhibitor of both HDAC1 and HDAC6, or o-aminobenzamide 2, a submicromolar inhibitor of HDAC1,^{11d}none of the compounds **4a**, **5a**, 5b, 7a, 7b, 8, 10, 11, or 12 showed activity against HDAC1.^{11e} The same compounds were also inactive against HDAC6, with the exception of sulfamide **5a**, which showed a low micromolar activity. Similar to other HDACis, the inhibitory activity of the sulfamide 'ZBG' was directly affected by the length of the linker tying it to the capping region. Optimal activity was observed with the 5-methylene linker, **5a**, while **4a** and **5b** were devoid of activity. In addition, methyl or acetyl substitution at the proximal or distal nitrogen of sulfamide 5a was deleterious to the activity (7a, 7b, 8, 10, and **12**), as was the replacement of the proximal sulfamide nitrogen by an oxygen (compound **11**). To study the SAR of the capping region, compounds **13a-f** were synthesized using standard coupling methodology replacing the 3-aminobiphenyl with other aryl and heteroaryl amines (Scheme 4). Compounds 13b-f turned out to be more potent against HDAC6 than both 13a and 5a (Table 2). However, these linear sulfamides 13b-f (Table 2), were 40- to 50-fold less active than the corresponding hydroxamic acids and



Scheme 4. Reagents and conditions: (a) R'-NH₂, BOP, Et₃N, DMF; or R'-NH₂, PS-CDI, HOBt, DCM; or Vilsmeier reagent, R'-NH₂, Et₃N, DCM; (b) TFA, DCM, or 4 N HCl in dioxane; (c) H₂NSO₂NH₂, Et₃N, toluene, 120 °C; or ClSO₂NCO, benzyl alcohol, DCM, Et₃N, followed by H₂/10%Pd-C, MeOH; or CISO₂NCO, t-BuOH, DCM, Et₃N, followed by TFA. DCM.

Table 2 Effect of cap on the HDAC activity

Compound	R′	HDAC1 IC ₅₀ (μ M)	HDAC6 IC_{50} (μM)
13a	***	>10	1.5
13b	N	>10	0.39
13c	, St. N	>10	0.49
13d	N	>10	0.71
13e	MeO	>10	0.44
13f	N N	>10	0.9

^a Values are means of at least two experiments.



Scheme 5. Reagents and conditions: (a) CISO₂NCO tBuOH, Et₃N, DCM: (b) LiOH/THF MeOH/H₂O; (c) Ar-NH₂, BOP, Et₃N, DMF; or Ar-NH₂, PS-CDI, HOBt, DCM; or Vilsmeier reagent, Et₃N, DCM, Ar-NH₂; or POCl₃/Py, Ar-NH₂; or iso-BuOCOCI, Et₃N, DCM, Ar-NH₂; (d) TFA, DCM, or 4 N HCI in dioxane.





Compound	Ar	R″	HDAC-1 IC ₅₀ (μ M)	HDAC-6 IC ₅₀ (μ M)	293TV cells IC_{50} (µM)	H3Ac ^b EC ₅₀ (µM)	TubAc ^c EC ₅₀ (µM)
SAHA			0.1	0.2	0.6	0.12	0.25
13f	N S	Н	>10	0.9	>50	20	3.5
14a	N S	NH-Cbz	0.16	0.18	2.3	0.7	0.6
13e	MeO	Н	>10	0.44	>50	>25	1
14b	MeO	NH-Cbz	0.46	0.06	1.8	0.35	0.2

^a Values are means of at least two experiments.

^b HAc, histone H3 acetylation.

 $^{\rm c}\,$ Tub Ac, $\alpha\text{-tubulin}$ acetylation.

they were still not active against the other HDACs (data not shown). Attempts to improve the HDAC inhibitory activity by using a more rigid scaffold or by introducing a phenyl ring in the chain resulted in loss of all activity (data not shown).

Jones et al., reported potent HDACis bearing a methylketone ZBG utilizing L-Aoda (L-2-amino-8-oxodecanoic acid) as a simplified apicidin scaffold.¹² We investigated whether we could achieve similar success utilizing L-lysine bearing the sulfamide moiety at the ε -nitrogen.

Scheme 5 depicts the synthesis of these sulfamides from the commercially available Z-Lys-OMe. Interestingly, we observed a new activity profile when the linear scaffold (as in **5a**) was changed to a lysine scaffold (**14a** and **14b**). Branching at the α -amino-position resulted in beneficial interactions and conferred activity to these sulfamides against HDAC1 in addition to HDAC6¹³ (Table 3).

Compounds **13e**, **13f**, **14a**, and **14b** were further profiled in cellular assays measuring their ability to inhibit total HDAC class I activity in the whole cell (293TV).^{14a-c} Compounds **13f** and **13e**, which are specific inhibitors of HDAC6, showed no measurable decrease in HDAC cellular activity (Table 3), whereas sulfamides **14a**

and **14b**, which inhibited HDAC1, demonstrated a clear ability to abrogate HDAC activity in the cell with $IC_{50} = 2.3$ and $1.8 \,\mu$ M, respectively, in line with the observed enzymatic profile.^{14d} By comparison, SAHA, brought down cellular HDAC activity with $IC_{50} = 0.6 \,\mu$ M. These results suggest that the contribution of HDAC6 to total cellular HDAC activity is minimal.

The observed enzymatic profile and cellular activities were further corroborated in functional cellular end points, namely, histone (H3Ac) and α -tubulin acetylation (TubAc) in T24 bladder cancer cells.¹⁵

As expected, compounds **13e** and **13f** had no effect on H3Ac, nevertheless caused a measurable induction of TubAc with EC_{50} of 1.0 and 3.5 μ M, respectively, in line with their observed enzymatic and cellular potencies. This is in agreement with literature reports implicating HDAC6 in tubulin deacetylation.¹⁶ On the other hand, **14a** and **14b**, which were potent inhibitors of both HDAC1 and HDAC6 displayed increased levels of both H3Ac and TubAc acetylation. Compound **14b** showed H3Ac and TubAc EC₅₀s of 0.35 and 0.2 μ M, respectively, comparable to the observed potencies of SAHA (Table 3).

In conclusion, we have identified the sulfamide moiety for the design of novel HDACis with bona fide HDAC cellular activities in accordance with their observed enzymatic potencies. In addition, we were able to manipulate the selectivity of these inhibitors. The lysine-based inhibitors were HDAC1 and HDAC6 active, while the long-chain compounds were selective toward HDAC6 and did not show activity against the other HDACs tested. These compounds are novel HDACis worthy of further investigation and optimization.

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- 13. In addition to the observed HDAC1 and HDAC6 activity, this class of compounds also showed activity against HDAC2 and HDAC3 but not against HDAC8 or class IIa namely HDAC4, 5, and 7 enzymes ($IC_{50} > 20 \mu M$). For example **14a** IC_{50} is 0.24 and 0.11 μM on HDAC2 and HDAC3, respectively.
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- 15. The cell-based ELISA assays for tubulin and histone acetylation were conducted according to the following protocol: cells were seeded the day before the treatment in 96-well plates. The following day, the cells were incubated for 3 h with the HDAC inhibitors at different concentrations. The cells were fixed on the plate and the appropriated acetylated histone H3 and tubulin antibody was added. The reaction was developed by Amplex Red. The cell number was normalized by Alamar blue.
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