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Design and Synthesis of a Novel Class of Histone Deacetylase Inhibitors

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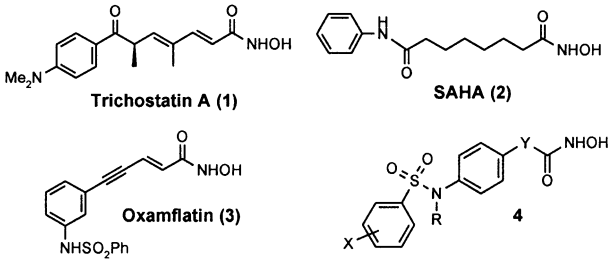
Abstract—Histone deacetylase inhibitors (HDACs) have emerged as a novel class of antiproliferative agents. Utilizing structure-based design, the synthesis of a series of sulfonamide hydroxamic acids is described. Further optimization of this series by substitution of the terminal aromatic ring yielded HDAC inhibitors with good in vitro and in vivo activities.

The identification of potent HDAC inhibitors represents a considerable effort in the development of therapeutics for treatment of cancers. In eukaryotic cells, histone acetylation/deacetylation, which is co-regulated by enzymes called histone acetyltransferases (HATs) and histone deacetylases (HDACs), is essential for chromatin remodeling and the functional regulation of gene transcription.[1] HDACs modulate the deacetylation of ε-amino groups of lysines located near the N-termini of core histone proteins.[2] Deregulation of HDAC activity is implicated in malignant diseases.[3] Small molecules having a hydroxamic acid functional group are highly potent HDAC inhibitors, such as the natural product trichostatin A (TSA)[4] (**1**) or analogues,[5] suberoylanilide hydroxamic acid (SAHA)[6] (**2**), and the ene-yne oxamflatin (**3**).[7]

In the course of our studies to design and develop inhibitors of HDACs, we identified promising lead substances having the general structure **4**.[8]

In this paper, we describe our preliminary results on the synthesis of a series of sulfonamides and their biological evaluation as HDAC inhibitors.

For the first set of structural modifications, we have prepared sulfonamides having a hydroxamate as the zinc chelating moiety. Based on previous work, it has been shown that this function is required for the HDAC activity of TSA and TSA-like hydroxamate analogues.



A synthetic route for preparing sulfonamides having different chain length is outlined in Scheme 1. Esterification of carboxylic acid **5**, followed by the treatment with phenylsulfonyl chloride in the presence of triethylamine and ester saponification afforded the compound **6**. The conversion of acid **6** to the hydroxamic acid **7** was accomplished by coupling with a THP-protected hydroxylamine and the subsequent deprotection under acidic conditions.

A five-step synthesis of compounds **11** and **12** is depicted in Scheme 2. Iodoaniline **8** was treated with an excess of aryl sulfonyl chloride in the presence of triethylamine,

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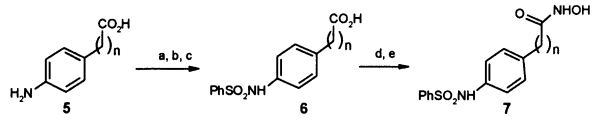
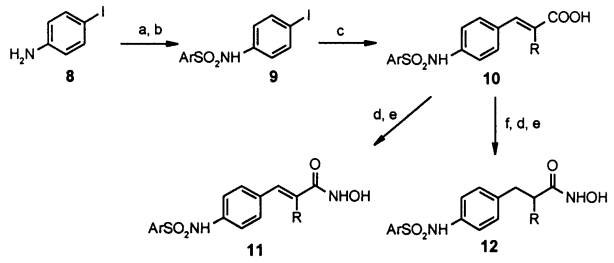


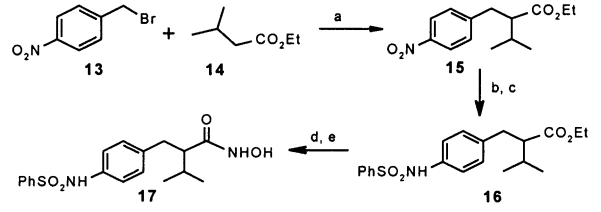
Scheme 1. Conditions: (a) HCl, MeOH; (b) $PhSO_2Cl$, Et_3N, DCM (96–99%); (c) LiOH, H_2O/THF; (d) NH_2OTHP, EDC, HOBt, DMF, 50 °C (50–60%); (e) CSA, MeOH.



Scheme 2. Conditions: (a) $ArSO_2Cl$, Et_3N, DCM (96–99%); (b) NaOMe, MeOH; (c) $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, CH_2=CRCOOH, Et_3N, DMF, 100 °C (97–99%); (d) NH_2OTHP, EDC, HOBt, DMF, 50 °C (55–65%); (e) CSA, MeOH; (f) H_2, Pd/C.

followed by the treatment with sodium methoxide to provide the aryl sulfonamide (**9**). Compound **10** was prepared using a Heck coupling reaction of iodide **9** with acrylic acid in the presence of $Pd_2(dba)_3$ and tri(*o*-tolyl) phosphine. Subsequently, acid **10** was converted to the hydroxamic acid **11**. Hydrogenation of alkene **10** yielded the acid which was converted to the hydroxamic acid (**12**).

The α-substituted hydroxamic acid (**17**) was synthetized as shown in Scheme 3. The condensation of 4-nitro-bromo-benzyl **13** and ethyl isobutyrate **14** in the presence of LDA afforded ester **15**. Reduction of the nitro group, followed by treatment with phenylsulfonyl chloride in the presence of triethylamine afforded compound **16**. Saponification of **16** under alkaline conditions afforded the acid which was coupled with hydroxylamine to give the target hydroxamic acid (**17**).



Scheme 3. Conditions: (a) LDA, THF; (b) H_2, Pd/C; (c) (i) $PhSO_2Cl$, Et_3N, DCM; (ii) MeONa, MeOH (80%); (d) LiOH, H_2O/THF; (e) NH_2OH·HCl, Et_3N, EDC, HOBt, rt, 12 h (88%).

The SAR of representative chain lengths for the template **18** is presented in Table 1.

In vitro activity was measured through the inhibition of partially purified recombinant human HDAC-1.[9] The antiproliferative activity of these compounds was analyzed by MTT assays (spectrophotometric quantification of viable cells by cleavage of tetrazolium salt). HCT 116 cells were chosen for MTT assays because they have a high level of HDAC expression. To discover whether the treatment with these compounds induced histone acetylation, the human bladder carcinoma T24 cells were chosen.

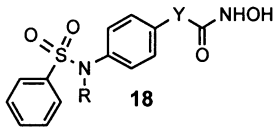
For HDAC inhibitory activity, the optimal chain length between the aryl and the hydroxamate was found to be two carbons which was comparable to the unsaturated chain (**12a**, **11a**, IC_{50} = 0.1, 0.2 μM, respectively, in HDAC-1). The benzamide (**7a**), the methylene (**7b**), and the propyl (**7c**) decreased the potency by 10-fold.

By substituting adjacent to the amide bond, **11b** was found to be 10-fold less potent than the corresponding unsubstituted compound (**11a**). A dramatic decrease in potency (170-fold) was observed when the saturated analogue (**12b**) was substituted with a methyl group. The more hindered isopropyl was also detrimental for HDAC inhibition (**17**, inactive). These results suggest that steric hindrance is not acceptable near the zinc binding site and is in agreement with the proposed narrowness of the binding site based on the co-crystal structure of the bacterial HDAC-1 enzyme with the hydroxamic acid TSA.[10]

N-Methylation of the sulfonamide (**18a**) resulted in a decrease in inhibitory activity. None of the parent carboxylic acid analogues was active.

Although compounds **11a** and **12a** have comparable biological activity, for simplicity, only the unsaturated compound was explored for further chemistry. The sul-

Table 1. HDAC-1 inhibition, cell proliferation quantification, and histone acetylation for **7a**–**18**



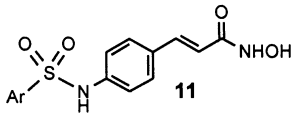
Compd	Y	R	HDAC-1 IC_{50} (μM)[a]	MTT EC_{50} (μM)[b]	H4-Ac EC_{50} (μM)[c]
(±)**TSA**			0.005	1	1
7a	—	H	0.9	2	5
7b	CH_2	H	1	22	>25
12a	$(CH_2)_2$	H	0.1	2	5
7c	$(CH_2)_3$	H	1	8	>25
11a		H	0.2	3	5
11b		H	2	15	>25
12b		H	17	>50	>25
17		H	>25	30	>25
18a		CH_3	0.6	4	>25

[a]Values are means of three experiments.
[b]In vitro antiproliferative activity against human colon cancer HCT 116 cells. Values are means of two experiments.
[c]Concentration of inhibitor for acetylation of histone-4 in human T24 cancer cells.

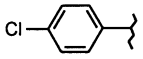
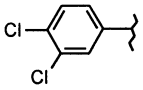
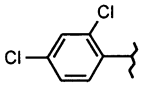
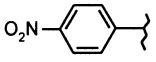
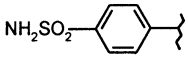
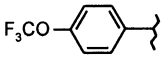
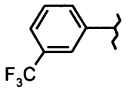
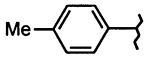
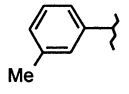
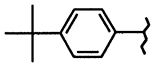
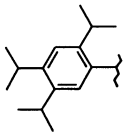
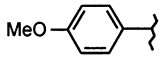
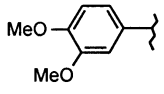
fonamides **11** were prepared with various substitution on the left phenyl ring (Table 2).

The first analogue synthesized was the *p*-chlorophenyl (**11c**), which showed a greater potency for HDAC inhibition and MTT than the parent compound **11a**. The potency of the 3,4-dichloro (**11d**) and 2,4-dichlorophenyl (**11e**) did not cause the desired effect and turned out to be less active than the 4-chloro analogue. This might be ascribed to either an unfavorable steric effect of *meta*- and *ortho*- substitution or to exceeding the optimum lipophilic value of the substituents. This result seems to be confirmed by the *m*-trifluoromethyl analogue (**11i**). A more electron withdrawing group such as a nitro or a sulfonamide in the *para* position seem to be deleterious for potency since **11f** and **11g** are 10-fold less active than **11c**. Whereas the *para*-methyl (**11j**), *meta*-methyl (**11k**) and *para*-*t*butyl (**11l**) analogues display almost the same potency as the parent compound, the activity seems to depend on the steric effect since the triisopropyl substituted analogue (**11m**) is less potent. The *para*-methoxy (**11n**) and the 3,4-dimethoxy (**11o**) analogues are equipotent to **11c** in HDAC-1, but show more activity in MTT. Compounds **11n** and **11o** have significant antiproliferative activity and can induce core histone acetylation at doses as low as 1–5 μM. The ability of these compounds to induce histone acetylation correlates well with their ability to inhibit HDAC-1 in vitro. Compound **11o** was selected for further in vivo antitumor efficacy studies and was injected intraperitoneally daily into nude mice bearing subcutaneous non-small cell lung carcinoma A549 tumors, for 21 days, at doses of 50, 40, and 20 mg/kg. Tumor growth of the treated mice was inhibited by 57, 50, and 41%, respectively, compared to that of mice in the control group. Gross toxicity, as measured by loss of body weight, was not observed.

Table 2. HDAC-1 inhibition, cell proliferation quantification, and histone acetylation for **11c**–**11o**



11

Compd	Ar	HDAC-1 IC_{50} (μM)[a]	MTT EC_{50} (μM)[b]	H4-Ac EC_{50} (μM)[c]
11c		0.075	1	1.5
11d		0.1	2	5
11e		0.3	4	>25
11f		0.7	6	20
11g		0.8	>42	>25
11h		0.6	0.9	2
11i		0.1	1	22
11j		0.3	0.7	10
11k		0.1	0.3	12
11l		0.1	0.8	5
11m		0.6	4	>25
11n		0.06	0.4	5
11o		0.09	0.2	1

[a,b,c]See footnotes in Table 1.

The three-dimensional (3–D) structural model of human histone deacetylase class I (HDAC1, residues 9–329) was obtained by homology modeling using the COMPOSER program as part of SYBYL 6.6. The 1.8 Å-resolution crystal structure of *A. aeolicus* histone deacetylase-like protein (HDLP),[9] which shares 35.2% sequence identity with human HDAC1, was used as template.

In order to model the HDAC1–Zn–TSA complex, the TSA molecule was manually positioned into the binding site of the HDAC1–Zn–water model after removal of the catalytic water molecule. The TSA-like inhibitor **11o** was positioned into the HDAC1 binding site with the hydroxamic acid anchor moiety as in the HDAC1–Zn–TSA complex, and the rotatable bonds of the inhibitor were conformationally sampled. The generated computational model in the HDLP catalytic core of TSA (Fig. 1) and compound **11o** (Fig. 2) show hydrogen bonding with key amino acids.

The hydroxamic acid function of TSA is located in a polar pocket formed by several residues capable of forming hydrogen bonds (Tyr303, His140, and His141). Compound **11o** seems to form only one hydrogen bond interaction (His140) in the binding pocket. However, it seems that His178 interacts with the methoxy group of the aromatic ring located outside of the binding pocket.

While substitution on the sulfonamide or on the double bond α of the hydroxamic acid function was insufficient to confer measurable activity, the substitution on the aryl group provided an increase in potency. The results

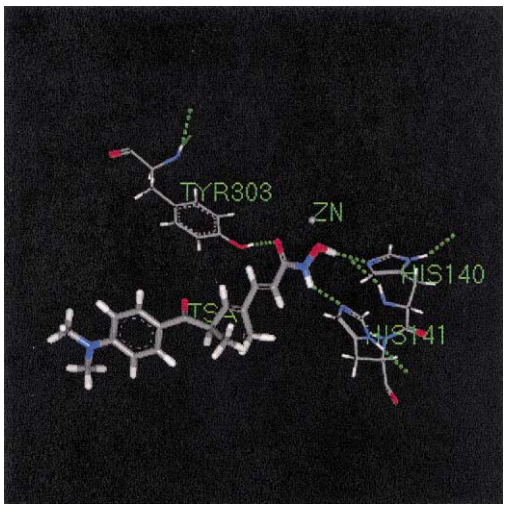


Figure 1. Model of TSA docked into the HDLP catalytic core. Possible hydrogen bonding between TSA and key amino acids can be seen.

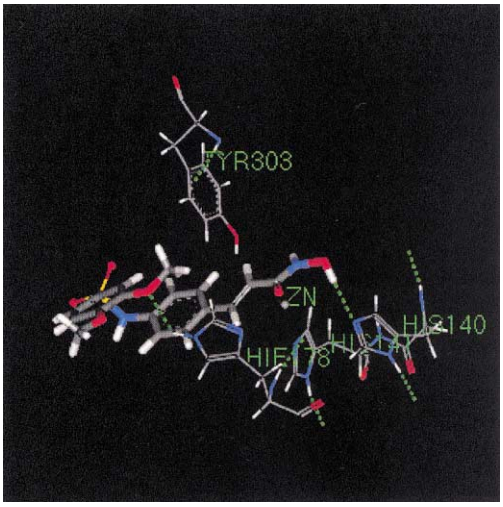


Figure 2. Model of compound **11o** docked into the HDLP catalytic core. Possible hydrogen bonding between **11o** and key amino acids exists.

of the docking experiment can explain the lower potency of **11o** in comparison with TSA but also the significant influence of the *m*-methoxy substituent on the aromatic ring. Further optimization of **4** is in progress, including new zinc chelating moieties and further changes to the aromatic groups.

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