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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3817–3820

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Novel Series of Histone Deacetylase Inhibitors Incorporating Hetero Aromatic Ring Systems as Connection Units

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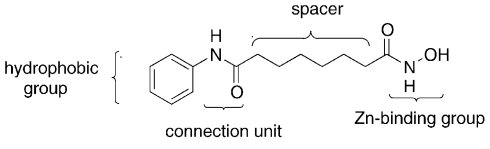
Received 11 June 2003; accepted 25 July 2003

Abstract—A series of structurally novel HDAC inhibitors, in which a hetero aromatic ring connects the spacer with the hydrophobic group, has been designed and synthesized. These new inhibitors are very potent in in vitro enzymatic assays and display antiproliferation activity against two human cancer cell lines.

Reversible acetylation of nuclear histones is essential for chromatin remodeling and the functional regulation of gene transcription. The balance of acetylation is controlled and maintained by the activities of two families of enzymes: histone acetyltranferases (HATs) and histone deacetylases (HDACs). By altering the acetylation status of the N-terminal region of histone proteins packaged with DNA in the nucleosome, histone deacetylases determine the accessibility of DNA within chromatin and consequently influence gene expression.[1] Deregulation of HDAC activity can lead to the repression of a certain subset of genes in favor of excessive proliferation and is implicated in a number of malignant diseases.[2] Identification of potent HDAC inhibitors offers great opportunities in the development of therapeutics for the treatment of cancers.[3]

Most of the HDAC inhibitors reported in the literature to date generally consist of a zinc-binding group and a five- or six-carbon hydrophobic spacer attached to a hydrophobic group via a connection unit. Suberoylanilide hydroxamic acid (SAHA, **1**), a synthetic small molecule HDAC inhibitor,[4] is a well-known example of this design.



SAHA

1

A rationale for this structural design is derived from the X-ray crystal structure of a bacterial HDAC homologue (HDLP) with bound TSA.[5] It has been suggested that the active site consists of a narrow tubular pocket with a zinc atom inside. Comparison of the amino acid sequences around the active site has indicated that the structural features of the active site are well conserved across all the HDACs, with exceptions at the solvent-exposed rim of the pocket. Based on these observations, it has been reasoned that modifications of the connection unit and hydrophobic group, which are assumed to interact with the entrance area of the catalytic pocket, will provide opportunities for discovering potent and possibly selective HDAC inhibitors.[6] However, only a limited diversity of connection units, including amide,[7] sulfonamide,[8] ketone[9] and ether,[10] have been reported. To our knowledge, there have been no reports of utilizing aromatic heterocycles as connection units in HDAC inhibitors.

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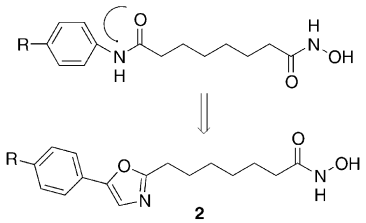
doi:10.1016/j.bmcl.2003.07.012

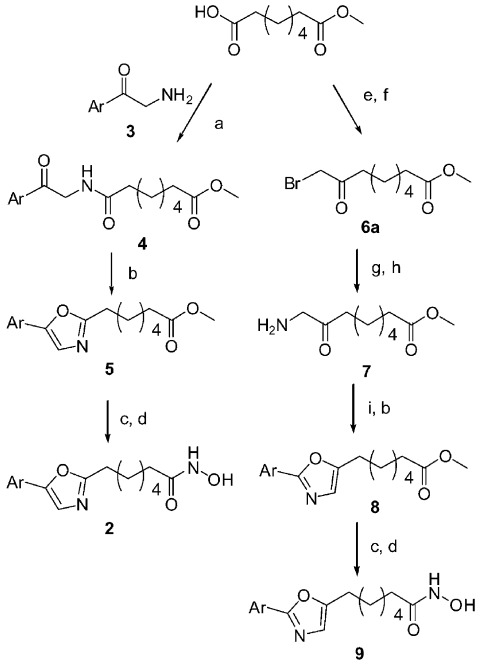


2

As part of our efforts to search for novel HDAC inhibitors, we investigated a series of new hydroxamates represented by structure **2**, in which the spacer and the hydrophobic group are connected via a hetero aromatic ring system. Structure **2** can be regarded as a product derived from SAHA through a 'conformation restriction' approach. In this paper, we report the synthesis of these compounds and their structure–activity relationship for HDAC inhibition.

The synthesis of **2** started with the coupling of α-amino ketone **3** and suberic acid monomethyl ester mediated by HOBT and EDC (Scheme 1). Treatment of the amide **4** with phosphorus pentoxide in refluxing chloroform afforded oxazole ester **5** in 84% yield. The ester was then saponified. The resulting acid was first converted to the corresponding mixed anhydride and then coupled with hydroxylamine to yield the desired hydroxamate **2**.

Suberic acid monomethyl ester also served as the starting material for the synthesis of oxazoles **9**. It was first converted to its acid chloride by reacting with oxalyl chloride and a catalytic amount of DMF, and then treated with diazomethane and concentrated HBr to provide α-bromo ketone **6a**. Reaction of **6a** with potassium bis(*t*-butoxycarbonyl)aminide followed by BOC cleavage gave rise to α-amino ketone **7**. Compound **7** was coupled with aromatic acids to afford the corresponding amides, which were converted to oxazoles **8** again by reaction with phosphorus pentoxide in refluxing chloroform. Methyl esters **8** were cleaved by saponification to generate the corresponding acids, which were then converted to the hydroxamates **9** as before.



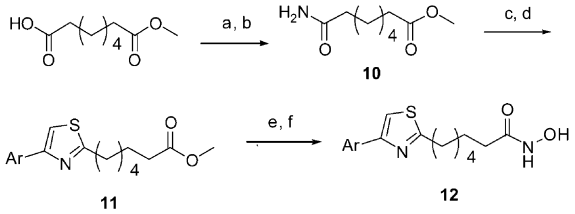
Scheme 1. Synthesis of oxazole hydroxamates: (a) HOBt, EDC, NMM, DMF; (b) P_2O_5, $CHCl_3$, reflux; (c) NaOH, MeOH, H_2O; (d) *i*-PrOCOCl, Et_3N, DMF; then 50% aq NH_2OH; (e) $(COCl)_2$, cat DMF, CH_2Cl_2; (f) CH_2N_2, HBr, Et_2O; (g) $(BOC)_2NK$, DMF, 90 °C; (h) HCl solution in dioxane; (i) $ArCO_2H$, HOBt, EDC, NMM, DMF.

The synthesis of thiazole analogues **12** was achieved by following the synthetic route shown in Scheme 2. Suberic acid monomethyl ester was converted to amide **10** by treatment with $SOCl_2$ and subsequently with aqueous ammonia solution. Lawesson's Reagent was used to transform amide **10** to its corresponding thiamide, which was reacted with commercially available amino ketones to give rise to thiazoles **11** in excellent yields. Again, the methyl esters **11** were hydrolyzed and converted to hydroxamic acids **12** via reaction of hydroxylamine with the corresponding mixed anhydrides.

The HDAC inhibitory activity of the synthesized compounds was measured using a peptide substrate and a mixture of HDACs, predominantly HDAC1 and HDAC2, prepared from nuclear extraction of K562 erythroleukemia cells followed by partial purification.[11]

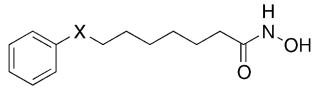
We first evaluated the effect of replacing the amide in SAHA (**1**) with alternate connection moieties (Table 1). Gratifyingly, the oxazole and thiazole-linked compound **2a**, **9a** and **12a** turned out to be very potent HDAC inhibitors. With IC_{50} values of 9.8, 13 and 19 nM, **2a**, **9a**, and **12a** are 14-, 11-, and 7-fold more potent, respectively, than SAHA (**1**), which exhibited a 140 nM activity in the same assay. Compound **13**, a reversed amide analogue of SAHA with an IC_{50} value of over 1 μM, is a much weaker inhibitor. Replacing the amide residue of SAHA with a methylene (**14**) or an ether linkage (**15**) also led to deterioration in HDAC inhibitory activity. Compound **16**, which has a benzene ring inserted in the same location, is more potent than SAHA, but still less active than **2a**, **9a** and **12a**.

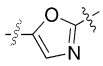
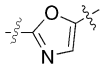
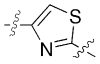
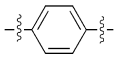
Interestingly, compounds **2a** and **9a** are equipotent although they are regioisomers in terms of the nitrogen atom's position on the oxazole rings. Compound **12a**, with a thiazole ring as connection unit, also exhibited a very comparable activity. This along with the potent activity of **16** suggests that the position and the type of hetero atoms on the five-membered aromatic ring are



Scheme 2. Synthesis of thiazole hydroxamates: (a) $SOCl_2$, benzene, reflux; (b) THF, aq NH_4OH; (c) Lawesson's Reagent, benzene, 70 °C, 1.5 h; (d) $ArCOCH_2Br$, MeOH, rt; (e) NaOH, MeOH, H_2O; (f) *i*-PrOCOCl, Et_3N, DMF; then 50% aq NH_2OH.

Table 1. HDAC inhibition of hydroxamic acids



Compd	X	HDAC[11] IC_{50}, nM	Compd	X	HDAC[11] IC_{50}, nM
2a		9.8	**13**		1010
9a		13	**14**	CH_2	560
12a		19	**15**	O	330
1		140	**16**		58

not important for retaining enzymatic potency. It is unclear what role the hetero aromatic systems may play in the interaction between the inhibitors and HDAC enzymes. However, the results in Table 1 indicate that: (1) there is no critical hydrogen bond interaction between the connection units and the enzymes, and (2) a flat or sp^2 configuration of the connection unit is crucial for potent HDAC inhibition. These observations are consistent with a recent report[9b] that hydroxamates with an olefinic moiety as the connection unit exhibited potent HDAC activity.

The influence on HDAC activity of substitution at the terminal phenyl ring in **2a**, **9a** and **12a** was then examined (Table 2). Not surprisingly, similar trends are observed across the oxazole and thiazole series. Introduction of a bromide at the *p*-positions of the phenyl rings in **2a**, **9a** and **12a** enhanced their activity to 2.7, 4.4 and 8.5 nM, respectively. Improved potency was also seen when a methoxy group was introduced to the same position (**2b**: 2.1 nM; **12b**: 2.5 nM). However, incorporating a CF_3O at the same position caused a drop in their activity (**9c**: 89 nM and **12f**: 74 nM). The position of substitution seems to have little impact on activity. This is exemplified by **12g** (5.8 nM) and **12h** (4.3 nM). Their activities are comparable to those of **12c** and **12b**, respectively. Introduction of a second pendant phenyl ring did not have an effect on potency (**2d**, **9d** and **12d**). However, replacement of the phenyl with a naphthyl group led to a 4-fold increase in potency (**12i**).

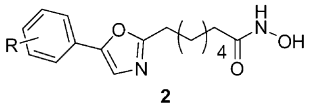
The compounds in Table 2 were also evaluated for antiproliferative activity against human HT1080 fibrosarcoma and human MDA435 breast carcinoma cell lines. Most of these compounds displayed a significant inhibitory effect against the growth of both cell lines, comparable to the activity shown by SAHA (HT1080: 2.4 μM; MDA435: 1.9 μM).

Encouraged by the results from these hydroxamate HDAC inhibitors, we also applied the hetero aromatic connection units to keto amides, a new class of HDAC inhibitors discovered recently, in which a keto amide moiety functions as the zinc-binding group.[13]

The strategy for the synthesis of compounds **17**–**20** (Table 3) is represented by the preparation of compound **20** in Scheme 3. Reaction of α-bromo ketone **6b** with thiobenzamide afforded thiazole **21**, which was then reduced to aldehyde **22** using DIABAL-H. The aldehyde was treated with Horner–Wadsworth–Emmons reagent **23** to give rise to enol ether **24**. Removal of the silyl group with CsF generated the corresponding α-keto ester, which was finally converted to the amide **20** by treatment with methylamine. Exhibiting double-digit nanomolar IC_{50} values, compounds **17**–**20** are much more potent than compound **25**,[13] in which an amide linker served as connection unit.

In summary, we have synthesized a series of structurally novel and potent HDAC inhibitors, in which a five-membered hetero aromatic ring connects the spacer to the hydrophobic group. These compounds are sig-

Table 2. HDAC inhibition of hydroxamic acids



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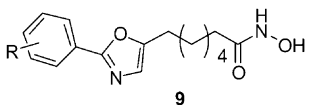

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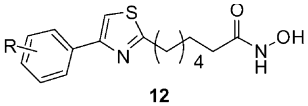
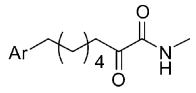
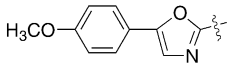
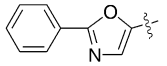
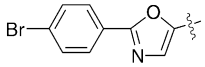
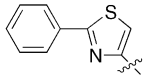
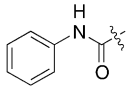


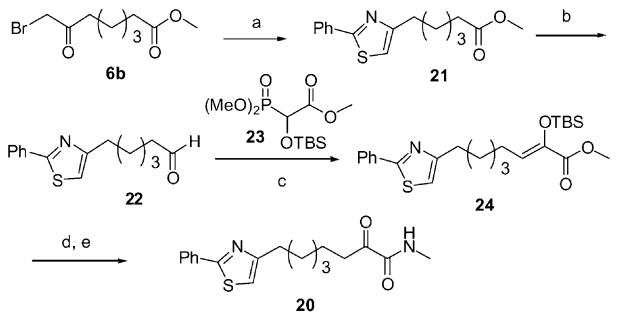
12

Compd	R	HDAC[11] IC_{50} (nM)	HT1080[12] proliferation IC_{50} (μM)	MDA435[12] proliferation IC_{50} (μM)	Compd	R	HDAC[11] IC_{50} (nM)	HT1080[12] proliferation IC_{50} (μM)	MDA435[12] proliferation IC_{50} (μM)
2a	H	9.8	8.41	2.2	**12a**	H	19	5.07	99.7%
2b	*p*-CH_3O	2.1	0.31	2.52	**12b**	*p*-CH_3O	2.5	3.63	2.97
2c	*p*-Br	2.7	7.7	6.7	**12c**	*p*-Br	8.5	5.68	8.11
2d	*p*-(*p*-ClC_6H_4)	6.8	0.66	0.95	**12d**	*p*-Ph	11	1.32	1.95
9a	H	13	23%@50	6.12%@50	**12e**	*p*-CF_3	42	2.39	0.85
9b	*p*-Br	4.4	27	97%	**12f**	*p*-CF_3O	74	100%@50	2.04
9c	*p*-CF_3O	89	5.14	20.4	**12g**	*m*-Br	5.8	10.1	98.6%@50
9d	*p*-Ph	12	15.3	98.7	**12h**	2-CH_3O-6-CH_3O	4.3	2.0	2.91
9e	*p*-F	23	76%	7.94	**12i**	β-Naphthyl	4.3	2.39	0.85

Table 3. HDAC inhibition of keto amides



Compd	Ar	HDAC[11] IC_{50}, nM
17		11
18		21
19		30
20		47
25		340



Scheme 3. Synthesis of keto amide **20**: (a) $PhCSNH_2$, MeOH, rt, 78%; (b) DIBAL-H, CH_2Cl_2, −78 °C; (c) LiCl, DBU, THF, 95%; (d) CsF, HOAc, CH_3CN, 64%; (e) CH_3NH_2, Et_3N, THF, rt, 25%.

nificantly more potent than SAHA in the HDAC enzymatic assay and exhibit significant antiproliferative activity against the growth of human HT1080 fibrosarcoma and human MDA435 breast carcinoma cell lines. We have shown that these hetero aromatic ring systems provide new opportunities for the identification of potent and possibly selective HDAC inhibitors as potential antitumor agents.

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