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4-*N*-Hydroxy-4-[1-(sulfonyl)piperidin-4-yl]-butyramides as HDAC inhibitors

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ABSTRACT

A series of N-substituted 4-alkylpiperidine hydroxamic acids, corresponding to the basic structure of histone deacetylase (HDAC) inhibitors (zinc binding moiety-linker-capping group) has been previously reported by our group. Linker length and aromatic capping group connection were systematically varied to find the optimal geometric parameters. A new series of submicromolar inhibitors was thus identified, which showed antiproliferative activity on HCT-116 colon carcinoma cells.

We report here the second part of the strategy used in our research group to find a new class of HDAC inhibitors, namely the SAR study for the compounds bearing a sulfonyl group on the piperidine nitrogen. In the present work, we have considered both sulfonamides and sulfonyl ureas.

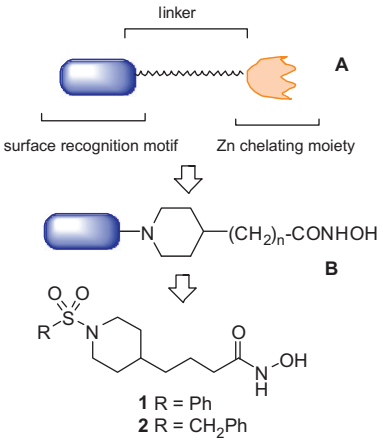
There are two enzyme families that play a crucial role in controlling the acetylation status of histones, histone acetyl transferases (HATs) and histone deacetylases (HDACs).[1,2] Their activity alters the chromatin structure and has consequences on the transcription process. In particular HDACs remove an acetyl group from specific located lysine residues. The inhibition of HDACs results in an increase in the acetylation status of chromatin which has been linked to many pharmacological activities and in particular to the down regulation of genes involved in tumor progression and to the re-expression of aberrantly silenced tumor suppressor genes,[3] although which genes are activated or down-regulated by HDAC inhibitors remain unclear.

We have recently reported how, starting from the basic HDAC inhibitor structure (Fig. 1**A**) and following a minimalist approach, we were able to identify a new class of HDAC inhibitors characterized by a hydroxamic acid as the Zn binding moiety, a 4-propylpiperidine as the linker and variously connected aromatic rings as the capping groups (Fig. 1**B**).[4]

Molecules **1** and **2**, containing an aromatic ring (phenyl and benzyl, respectively) linked to the piperidine nitrogen, were found to be high nanomolar in vitro inhibitors of HDAC and also showed antiproliferative activity on HCT-116 colon carcinoma cells. Thus, we decided that this motif deserved further SAR examination.

We prepared the series of compounds shown in Figure 2. The propyl chain was maintained as linker between the piperidine ring and the hydroxamic acid, since in our previous work we demonstrated, after a systematic study, this to be the optimal length.

The most common aromatic substituents were systematically positioned in the *ortho*, *meta*, and *para* positions (for compounds **3**–**17**, we were, in some cases limited, by the commercial availability of the precursors). Examples of di-substituted (**18**–**20**) and



	IC50 (HDAC) μM	IC50 (HCT-116) μM
1	0.54	2.7
2	0.84	3.2

Figure 1. General strategy that has produced the 4-*N*-hydroxy-4-[1-(sulfonyl) piperidin-4-yl]-butyramides as HDACs inhibitors.

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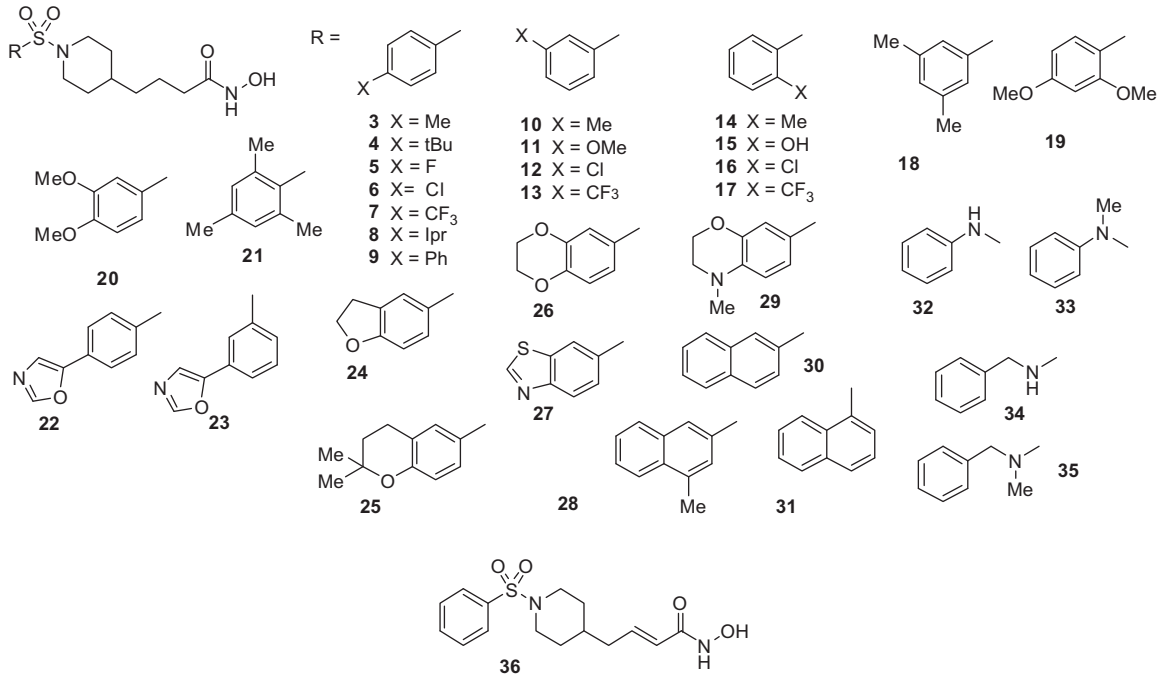
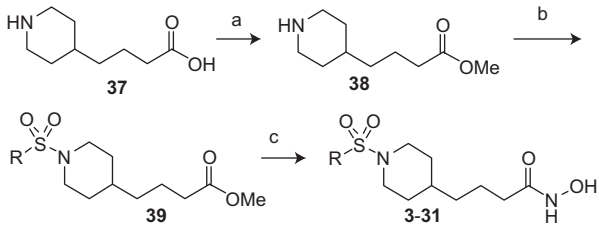
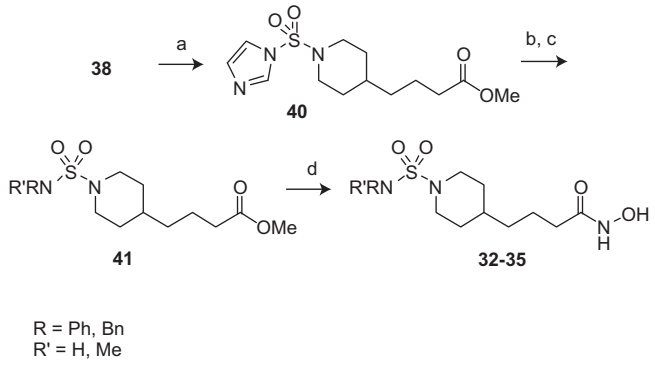


Figure 2. General structure for sulfonylpiperazine HDAC inhibitors, substitutions at *R* and the structure of compound **36**.



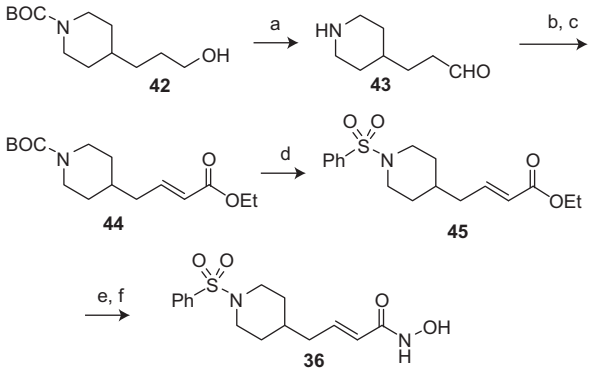
Scheme 1. (a) $SOCl_2$, MeOH; (b) RSO_2Cl, DIPEA, THF; (c) NH_2OH–HCl, KOH, MeOH.



Scheme 2. (a) Sulfonyldiimidazole; (b) methyl triflate; (c) RR'NH.

trisubstituted (**21**) analogs were also prepared. Compounds **22** and **23** are examples of *meta* and *para* hetero-biphenyls, while **24**–**31** represent saturated and unsaturated benzofused molecules. Compounds **32**–**35** were prepared to examine the effect of phenyl and benzyl sulfonylureas, (both methylated and not). Finally, we prepared **36**, where a double bond was inserted into the linker to limit its flexibility.

The synthesis of the simple sulfonamides followed the sequence shown in Scheme 1. The commercially available aminoacid **37** was converted into the methyl ester by classical treatment with $SOCl_2$/methanol; the free piperidino nitrogen was then sulfonylated via the reaction with the appropriate sulfonyl chloride, in the presence of ethyldiisopropylamine (DIPEA) in tetrahydrofuran (THF). The ester **39** was converted into the hydroxamic acid by aminolysis with hydroxylamine in the presence of a base.[5]



Scheme 3. (a) $(COCl)_2$, DMSO, CH_2Cl_2, −80 °C; (b) $Ph_3P = CHCO_2Et$, THF; (c) 4 N HCl in dioxane; (d) $PhSO_2Cl$; (e) LiOH 8 equiv, THF/MeOH/H_2O 4:1:0.1; (f) EDAC, HOAt, DMF and then NH_2OH·HCl DIPEA.

The preparation of sulfonyl ureas is outlined in Scheme 2. Amine **38** was treated with sulfonyldiimidazole to obtain the stable intermediate **40**, which was activated by treatment with methyltriflate and reacted with an amine, aniline, *N*-methyl aniline, benzylamine or *N*-benzylamine to obtain the corresponding sulfonyl ureas.[6] The conversion of the esters **41** to the corresponding hydroxamic acids was done according to Ref. 5.

Compound **36**, was synthesized starting from alcohol **32** (Scheme 3). A Swern oxidation gave aldehyde **33**, which was than submitted to a Wittig reaction with (ethoxycarbonylmethylene) triphenylphosphorane. Boc deprotection of the amine with 4 N HCl in dioxane and reaction with phenylsulfonyl chloride gave the desired compound. In this case it was not possible to perform the direct aminolysis with hydroxylamine in basic media, because this led to a degradation of the product. Instead, it was necessary to carefully hydrolyze the ester and the form the hydroxamic acid using a classical coupling method (Scheme 3).[7]

Table 1
Pharmacological activity of SAHA and compounds **1**–**36**

Cmpnd	% Of inhibition			IC_{50}[a] HDAC (μM)	GI_{50}[a] HCT-116 (μM)
	0.1 μM	1.0 μM	10 μM		
SAHA	55	81	86	0.079	0.6
1	23	63	84	0.54	2.7
2	17	58	71	0.84	3.2
3	28	71	nt	nt	nt
4	9	51	nt	nt	nt
5	21	60	nt	nt	nt
6	25	62	nt	nt	nt
7	15	55	nt	nt	nt
8	18	66	84	0.537	nt
9	20	44	80	nt	nt
10	24	61	83	nt	nt
11	28	67	83	nt	nt
12	4	40	nt	nt	nt
13	13	57	nt	nt	nt
14	29	69	nt	nt	nt
15	37	75	84	nt	nt
16	42	77	86	0.173	2.8
17	30	69	nt	nt	nt
18	22	65	86	nt	nt
19	22	62	83	nt	nt
20	31	71	84	0.317	nt
21	32	75	nt	nt	nt
22	35	74	nt	nt	nt
23	38	75	85	0.213	nt
24	36	72	nt	nt	nt
25	30	75	86	nt	nt
26	29	68	nt	nt	nt
27	26	69	84	nt	nt
28	48	82	86	0.124	17.3
29	32	71	nt	nt	nt
30	53	68	86	0.128	1.9
31	40	80	74	0.181	1.04
32	40	70	nt	nt	2.0
33	19	48	nt	nt	nt
34	29	65	nt	nt	nt
35	29	56	nt	nt	nt
36	19	48	78	nt	nt

nt, not tested.
[a] Variability is maintained within ±10.

All final compounds were characterized by ^{1}H NMR and LC–MS analyses, and showed a purity >97%.[8] When necessary they were purified either by flash chromatography on silica gel or by reverse phase preparative HPLC.

Compounds were initially screened at two concentrations (1.0 and 0.1 μM), using an enzymatic assay measuring total HDAC activity in HeLa cell extracts (Table 1).[9]

On the basis of these results a group of compounds was selected for the evaluation of their IC_{50}s. However, for some of them the solubility in the test medium, at high concentrations, was found to be so low (often in the order of a few micrograms/mL) that the resulting data were unreliable.

A first observation is that rigidifying the chain with a double bond, (**36** vs **1**), gave no advantage in terms of inhibition; the same was true for the *para*-substituted phenyl sulfonamides **3**–**9**, which, with the exception of **8**, were all worse than the unsubstituted phenyl compound **1**. The best results, in terms of inhibition, were obtained with the naphthyl derivatives **28**, **30**, and **31**.

Benzyl and phenyl sulfonylureas, both simple (**34** and **32**) and methylated (**35** and **33**) gave disappointing results: methylation was very detrimental to inhibition, while the simple ones were almost equiactive with the corresponding sulfonamides, in spite of the different geometry of this functional group. Very likely all the modifications we introduced, when not detrimental, gave no additional productive binding interactions with the enzyme, and limited their influence essentially to the physicochemical properties of the molecules.

To complete the evaluation of this series of compounds the antiproliferative activity of a selected panel of sulfonamide derivatives was determined in HCT-116 colon carcinoma cells.[10]

With the exception of **28**, the IC_{50}s were all found to be in the low micromolar range.

Some in vitro PK parameters were evaluated for compounds **30** and **31**. In protein binding experiments they were found to be only 2% and 3% free, respectively, while after incubation with the S9 fraction of rat, only 2% of **30** and 14% of **31** remained.

In conclusion, we have prepared a series of sulfonamide and sulfonyl urea analogs starting from compounds **1** and **2** published previously.(Ref. 4) A slight improvement was seen in the IC_{50} values for HDAC inhibition for compounds **16**, **23**, **28**, **30**, and **31**, although these did not always translate into an improvement in the HCT-116 activity.

The work also brought to light problems with the solubility of the compounds in the test medium, and the metabolic stability of certain analogs in the presence of rat liver S9 fraction homogenates. Further studies are planned, with a view to improving the enzyme inhibition activity, and the physicochemical and PK properties of this class of compounds.

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7. General procedure for the synthesis of hydroxamic acids from carboxylic acids. A solution of carboxylic acid, EDAC (1.2 equiv) and HOAt (1.2 equiv) in DMF (5 V) was stirred at room temperature for 30 min, then hydroxylamine hydrochloride (1.2 equiv) and DIPEA (3 equiv) were added and stirring was continued at room temperature for 12 h. Five percent aqueous $KHSO_4$ was added to have an acidic solution, followed by Et_2O and the mixture was transferred in a separatory funnel. The organic layer was washed with H_2O, brine and Na_2SO_4 then concentrated under reduced pressure. The crude compound obtained in this way was purified by preparative HPLC to afford the corresponding hydroxamic acid derivative.
8. NMR experiments were recorded on a Brucker Avance 400 MHz spectrometer equipped with a 5 mm inverse probe and processed using Xwin-NMR version 3.5. Mass spectra were recorded using a WATERS Alliance 2795 HPLC system fitted with a UV-PDA 996 diode array detector, a ZMD mass spectrometer and a GL Science Inertsil ODS-3 column (50 × 3 mm. 3 **μ**m). Generally, the gradient used was 20–80% B in 8 min at a flow rate of 0.8 mL/min (the eluents used were A: H_2O + 0.1% TFA and B MeCN + 0.1% TFA), the sample concentrations were 0.1 mg/mL and the injection volume 10 **μ**L. Part of the eluent (20 **μ**L/min.) was diverted to the mass spectrometer and subjected to ESI^+ ionization (cone voltages 20 and 50 V, source temperature 105 °C).
9. The compounds were dissolved in DMSO and stored at −80 °C. HDAC enzymatic activity was tested using a commercially available assay kit (HDAC Fluorescent Activity Assay/Drug Discovery Kit AK-500, Biomol, International LP, Plymouth Meeting PA) based on the Fluor de LysTM substrate and developer combination. Enzymatic reactions (50 μL) were run in 96-well plates. The release of the fluorophore was monitored with a Victor 1420 fluorescent plate reader set at excitation/emission wavelength of 335/460 nm. The activity of compounds was expressed as IC_{50} (drug concentration causing a 50% inhibition of enzymatic activity) and calculated with Easy-fit software application. All the experiments were carried out in triplicate.
10. HTT-116 cells were plated in a 96-well tissue culture plates containing 200 μL of complete medium. After 24 h HFAC inhibitors were added at different concentrations, ranging from 10 to 0.1 μM in quadruplicate. After 5 days, 20 μL of Alamar blue were added to each well and the plates were further incubated for 4 h. The chemical reduction of Alamar Blue in the growth medium is a fluorometric/colorimetric indicator of cellular growth based on the detection of metabolic activity. Fluorescence was monitored in a multilabel counter Victor 1420 at 530 nm excitation and 590 nm emission wave length. All the results were expressed as IC_{50} calculated using the Easy-fit software.