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Alkyl piperidine and piperazine hydroxamic acids as HDAC inhibitors

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ABSTRACT

We report here the strategy used in our research group to find a new class of histone deacetylase (HDAC) inhibitors.

A series of N-substituted 4-alkylpiperazine and 4-alkylpiperidine hydroxamic acids, corresponding to the basic structure of HDAC inhibitors (zinc binding moiety-linker-capping group) has been designed, prepared, and tested for HDAC inhibition.

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 anti-proliferative activity on HCT-116 colon carcinoma cells.

The last decade has seen histone deacetylase (HDAC) inhibition emerge as an attractive target for the development of new anticancer agents.[1] In addition, more it has been recently demonstrated that HDAC inhibitors may also be used for novel therapeutic applications such as inflammation,[2] neurodegenerative diseases[3] and malaria.[4]

HDACs are a family of enzymes (classes I–IV) that remove the acetyl group from the ε-amino groups of the lysine residues of the core histones. The enzymes of the classes I, II, and IV share many common features in their active sites, which are composed of a long, narrow hydrophobic tunnel leading to a cavity that contains a catalytic Zn^{2+} ion, complexed to two Asp and one His residues. Inhibitors of these HDACs also have a common general structure, consisting of a Zn chelating moiety (often a hydroxamic acid), a linker and a so-called 'surface recognition motif'[1] (Fig. 1).

A number of HDAC inhibitors are at present undergoing clinical trials and two, SAHA and depsipeptide (Fig. 1), are already on the market.

Here we describe the design, the synthesis and the primary in vitro pharmacological characterization of a novel series of HDAC inhibitors.

Capitalizing on the above information, we selected two possible linkers for the design of new classes of HDAC inhibitors, 4-alkyl piperidine and 4-alkyl piperazine (Fig. 2, A). With both these linkers the free heterocyclic nitrogen allows rapid chemical functionalization, useful for explore the space at the entrance of the active site. We also decided to:

- use a hydroxamic acid as Zn binding moiety (Fig. 2, B);
- test alkyl chains spanning from $n = 0$ to $n = 5$;
- follow a minimalist approach, that is, functionalize the terminal nitrogen with simple phenyl rings positioned in different regions of space through the different geometry of the connecting bonds (amide, sulfonamide, urea, Fig. 2, C). This is because the aromatic ring is able to establish a large variety of molecular interactions: aromatic-aromatic interactions, hydrogen bonding, van der Waals, etc. In this first round, functionalization of the aromatic rings was avoided on purpose.

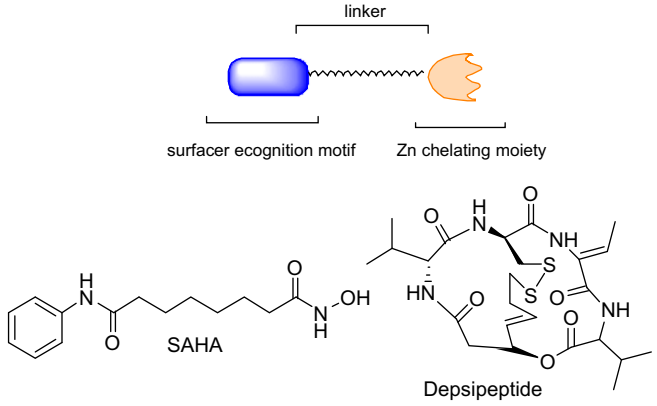

Figure 1. General structure of inhibitors for class I, II and IV HDACs, and the formulae of SAHA and Depsipeptide

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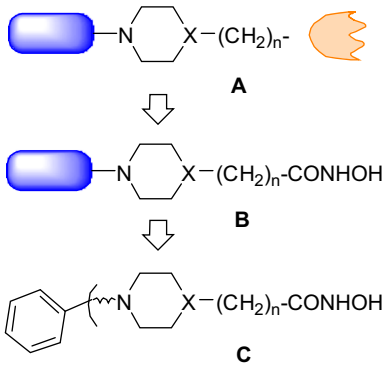


Figure 2. Scheme of the strategy followed for the design of new HDAC inhibitors.

Following this plan, compounds of general formulas shown in Figure 3 were prepared. Representatives with $n = 0–5$ were not synthesized for all the series, since for some chains the length was clearly not appropriate from the very beginning, and one of the two selected scaffolds demonstrated a clear superiority over the other.

For the $n = 0$ compounds, only piperidine derivatives were prepared, using the sequence shown in Scheme 1. Commercially available acid **11** was coupled with the desired acyl or sulfonyl chloride by treatment with bis-trimethylsilylacetamide (BSA) in tetrahydrofuran (THF), in the presence of diisopropylethylamine (DIPEA), to obtain, after workup, the free acids **12**. These were converted into the corresponding acyl chlorides using oxalyl chloride with a catalytic amount of dimethylformamide (DMF); subsequent reaction with hydroxylamine hydrochloride afforded the desired hydroxamic acids **1a**–**4a**.

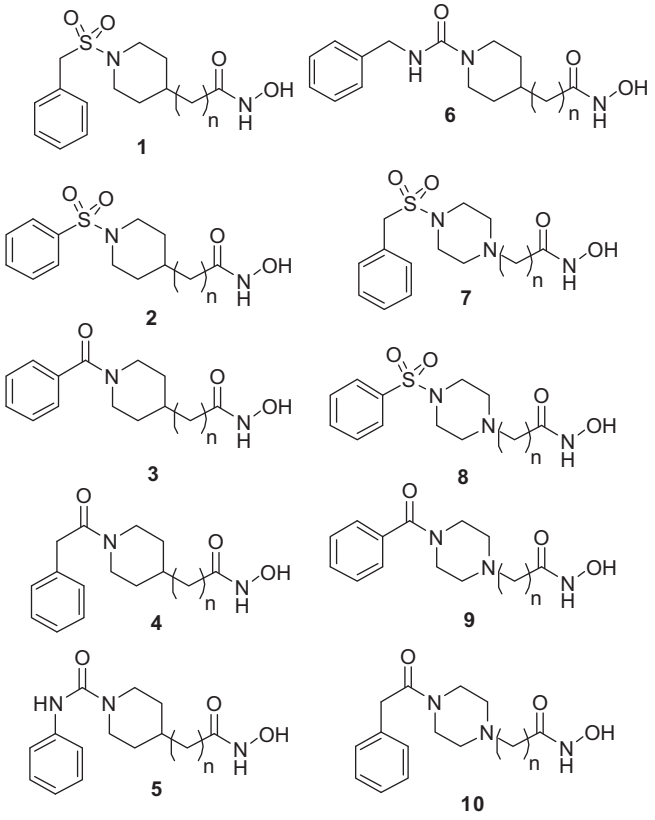
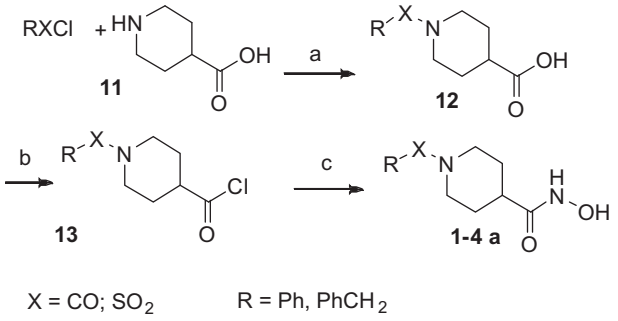


Figure 3. For $n = 0$: **a**; $n = 1$: **b**; $n = 2$: **c**; $n = 3$: **d**; $n = 4$: **e**; $n = 5$: **f**.



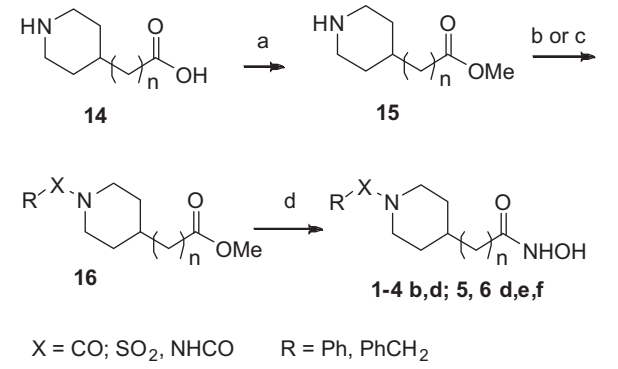
Scheme 1. Reagents: (a) BSA, DIPEA, THF; (b) $(COCl)_2$, DMF, CH_2Cl_2; (c) NH_2OH–HCl, $NaHCO_3$, CH_2Cl_2/H_2O.

The synthetic sequence followed for the synthesis of piperidines with $n = 1$, 3, 4, and 5 is shown in Scheme 2.

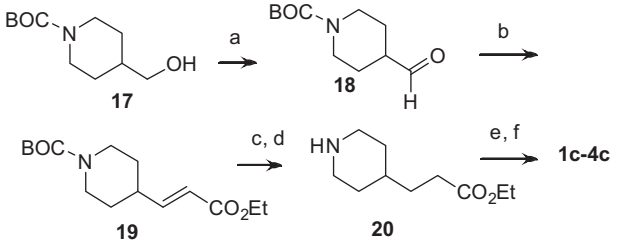
Commercially available piperidines 4-alkylcarboxylic acids were converted into the methyl esters **15** in acidic methanol, submitted to base catalyzed reaction with the opportune acyl chloride, sulfonyl chloride or isocyanate (**16**) and then treated with hydroxylamine in basic media for the preparation of the hydroxamates **1**–**4b**, **d** and **5**, **6d**, **e**, **f**.

Compounds **1c–4c** were prepared according to the synthetic pathway of Scheme 3.

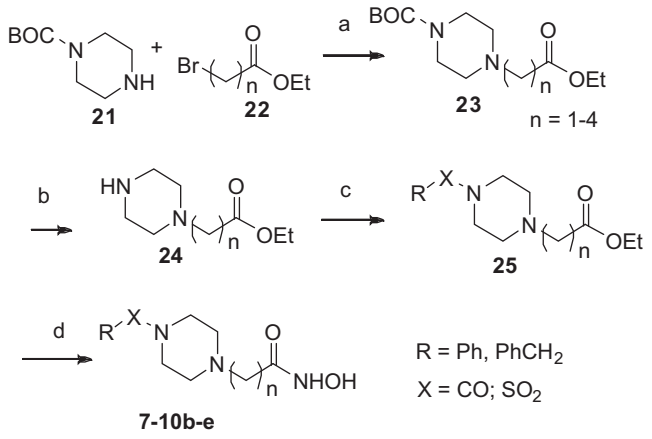
Boc protected piperidinyl alcohol **17** was oxidized to the corresponding aldehyde (**18**) and then submitted to a Wittig reaction with (ethoxycarbonylmethylene)triphenylphosphorane (**19**), followed by catalytic hydrogenation of the double bond, Boc deprotection afforded the free amine (**20**), which was reacted with selected acyl and sulfonyl chlorides. Aminolysis of the ethyl ester with hydroxylamine in basic media afforded the desired hydroxamic acids **1c**–**4c**.



Scheme 2. Reagents: (a) $SOCl_2$, MeOH; (b) RXCl, DIPEA, THF; (c) RNCO; (d) NH_2OH–HCl, KOH, MeOH.



Scheme 3. Reagents: (a) $(COCl)_2$, DMSO, CH_2Cl_2; (b) $Ph_3P{=}CHCO_2Et$, THF; (c) H_2, Pd/C 10%, MeOH; (d) 4 N HCl in dioxane; (e) RXCl (R = Ph, CH_2Ph; X = CO, SO_2), DIPEA, CH_2Cl_2; (f) NH_2OH–HCl, NaOH, MeOH.



Scheme 4. Reagents: (a) DIPEA or Et_3N, THF; (b) 4 N HCl/dioxane; (c) RXCl, DIPEA, CH_2Cl_2; (d) NH_2OH–HCl, NaOH, MeOH.

For the piperazine derivatives **7**–**10** the synthesis is shown in Scheme 4.

Boc-protected piperazine **21** was alkylated with the ω-bromoalkyl esters **22** to obtain the ethyl ω-piperazinecarboxylates **23**. Boc deprotection in acidic media was followed by acylation or sulfonylation **(25)**, and finally ethanol aminolysis gave the desired hydroxamic acids **1–4 b–d**.

All the final compounds were characterized by ^{1}H NMR and LC–MS analyses, showing a purity >97%.[5]

Compounds were initially screened at two or three concentrations, selected from 50, 10, 1.0, and 0.1 μM, using an enzymatic assay measuring total HDAC activity in HeLa cell extracts (Table 1).[6,7] From the results obtained it was clear that the optimal linker length was $n = 3$, and this was true for all the 'external recognition motives' (**1d**–**8d**). Another observation was that the binding of the compounds with the piperazine linker was very poor. This is probably due to its basic character, which requires a desolvation energy gap to be overcome prior to entering the hydrophobic tunnel. From the inhibitory activity data six molecules (**1d**–**6d**), all belonging to the piperidine class with $n = 3$, were selected and the IC_{50} for HDAC inhibition evaluated. Both the benzyl (**1d**) and phenyl (**3d**) sulfonamides had comparable submicromolar activity, while differences were seen between the phenyl (**3d**) and the benzyl (**4d**) amides, with the former being the least active of the four analyses (IC_{50} 1.39 μM). However, the most active compounds in the series were the urea analogs, with the phenyl (**5d**) and the benzyl (**6d**) compounds showing inhibition values comparable to that of SAHA.

To determine whether HDAC inhibition in vitro was paralleled by similar effects in cultured cells, the antiproliferative activity of the selected compounds was determined in HCT-116 colon carcinoma cells.[8,9] Again the urea derivatives (**5d**, **6d**) showed the best values (IC_{50} 0.7 and 0.3 μM, respectively), comparable with the values for SAHA obtained in parallel.

In conclusion, applying a minimalist and systematic approach we were able to find a new series of HDAC inhibitors with a molecular weight around 300 and activity comparable to that of SAHA. This series is characterized by having a 4-propylpiperidine as the linker, a hydroxamic acid as the zinc binding moiety and can tolerate a phenyl ring linked through a sulfonamide, an urea and an amide with or without a methylene as an additional spacer. We believe that all the three classes of compounds (sulfonamide, urea, and amide) deserve further exploration to improve inhibitory activity and PK properties with the goal of producing an orally active clinical candidate. These studies and the subsequent findings, will be the subject of future communications.

Table 1
Pharmacological activity of the HDAC inhibitors **1**–**10**[a]

Compound	% of inhibition				IC_{50} (μM)	IC_{50} HCT-116 (μM)
	0.1 μM	1 μM	10 μM	50 μM		
SAHA	55	81	86		**0.079**	**0.6**
1a	–	–	1.0	33	–	–
2a	–	–	29	50	–	–
3a	–	–	0	16	–	–
4a	–	–	33	65	–	–
1b	–	–	21	22	–	–
2b	–	–	41	68	–	–
3b	–	–	7	41	–	–
4b	–	–	39	61	–	–
7b	–	–	16	51	–	–
8b	–	–	3.0	10	–	–
10b	–	–	6.0	19	–	–
1c	8	33	–	–	–	–
2c	9	33	–	–	–	–
3c	11	30	–	–	–	–
4c	2	24	–	–	–	–
7c	–	–	1.0	38	–	–
8c	–	–	14	44	–	–
9c	3	3	–	–	–	–
10c	–	–	0	2	–	–
1d	12	58	71	77	**0.84**	**3.2**
2d	23	63	84	87	**0.54**	**2.7**
3d	11	–	69	77	**1.39**	**17.2**
4d	36	72	80	85	**0.26**	**5.1**
5d	53	81	86	–	**0.10**	**0.7**
6d	59	69	86	–	**0.09**	**0.3**
7d	–	–	<10	19	–	–
8d	–	–	30	50	–	–
9d	–	–	13	30	–	–
10d	–	–	28	52	–	–
2e	13	45	–	–	–	–
3e	12	44	–	–	–	–
4e	11	54	–	–	–	–
5e	23	63	–	–	–	–
6e	18	49		–	–	–
7e	4	9	–	–	–	–
8e	9	12	–	–	–	–
9e	3	9	–	–	–	–
10e	19	40	–	–	–	–
5f	16	54	–	–	–	–
6f	20	62	–	–	–	–

[a] For details see the References and notes section.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.085.

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4. Wells, T. N. C.; Alonso, P. L.; Gutteridge, W. E. *Nat. Rev. Drug Disc.* **2009**, *8*, 879.
5. NMR experiments were recorded on a Bruker 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 μm). 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_20 + 0.1% TFA and B MeCN + 0.1% TFA), the sample concentrations were 0.1 mg/mL and the injection volume 10 μL. Part of the eluent (20 μL/min) was diverted to the mass spectrometer and subjected to ESI+ ionisation (cone voltages 20 and 50 V, source temperature 105 °C).
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7. 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.
8. Page, B.; Page, M.; Noel, C. *Int. J. Oncol.* **1993**, *3*, 473.
9. 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 μM 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 wavelength. All the results were expressed as IC_{50} calculated using the Easy-fit software.