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Histone deacetylase inhibitors with a primary amide zinc binding group display antitumor activity in xenograft model

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ABSTRACT

Histone deacetylase (HDAC) inhibition causes hyperacetylation of histones leading to differentiation, growth arrest and apoptosis of malignant cells, representing a new strategy in cancer therapy. Many of the known HDAC inhibitors (HDACi) that are in clinical trials possess a hydroxamic acid, that is a strong Zn^{2+} binding group, thereby inhibiting some of the class I and class II isoforms. Herein we describe the identification of a selective class I HDAC inhibitor bearing a primary carboxamide moiety as zinc binding group. This HDACi displays good antiproliferative activity against multiple cancer cell lines, and demonstrates efficacy in a xenograft model comparable to vorinostat.

Histone deacetylases (HDACs) are enzymes involved in determining the pattern of acetylation of chromatin proteins (histones) in eukaryotic cells.[1] The acetylation of histones results in transcriptionally active chromatin, increasing the accessibility of nucleosomal DNA; this facilitates gene transcription and leads to differentiation, growth arrest and apoptosis representing a new strategy in cancer therapy.[1,2]

The HDACs belong to four structurally and functionally different classes: class I (HDAC 1–3 and 8), class II (HDAC 4–7, 9 and 10) while class IV (comprising HDAC 11) exhibits properties of both class I and class II HDACs.[3] All the above HDACs are zinc dependent hydrolases, whereas class III HDACs are structurally distinct and require the cofactor NAD^+ for their deacetylase function.

The vast majority of HDAC inhibitors (HDACi) currently undergoing development, such as vorinostat (**1**) (Zolinza®, formerly known as SAHA) approved by the FDA for the treatment of the cutaneous T-cell lymphoma,[4] contain a hydroxamic acid (Fig. 1). Other hydroxamic acid HDACis include panobinostat (**3**),[5] and belinostat (**4**).[6] This functionality binds to a zinc ion in the active site displaying little isoform selectivity between class I, II and IV HDACs.

Hydroxamic acids have some liabilities associated with this functional group including poor oral absorption and they often display poor pharmacokinetics as a result of hydrolysis or rapid glucoronidation. The presence of the strong metal chelating group also can result in inhibition of other metallo-enzymes or sequestration of metal ions. Thus there has been a considerable interest in developing non-hydroxamate HDAC inhibitors.

In the mid 90s researchers at Merck discovered apicidin, an antiprotozoal agent that inhibits parasite histone deacetylase.[7] Apicidin is structurally complex but a targeted screening of the sample collection, looking for compounds containing the L-Aoda amino acid, identified a more tractable lead which was further optimized to **6** (Fig. 2).[8]

This compound displays an interesting level of antiproliferative activity in tumor cells and was demonstrated to function in xenograft studies. Derivatization of this scaffold gave rise to an even more potent compound containing a naphthyl in the 5 position of imidazole.[9] Similarly an ethyl ketone was also shown to improve

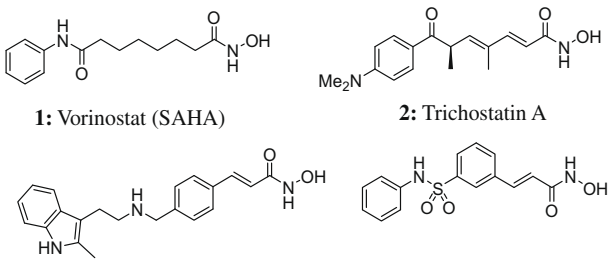

Figure 1. Known HDAC inhibitors containing hydroxamic acid.

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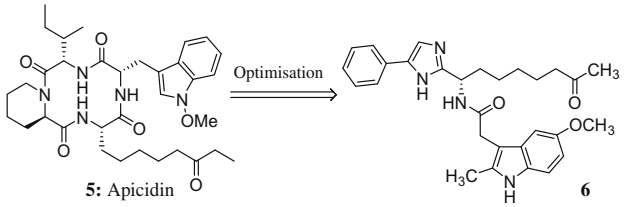


IC_{50} (nM)		
HDAC1	44	27
PRO (HeLa)	290	680

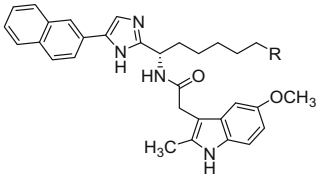
Figure 2. Identification of a lead series from the natural product apicidin.

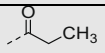
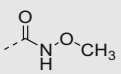
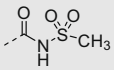
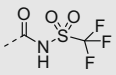
activity. Combining the two pieces gives **7** that displays IC_{50} = 27 nM against HDAC1 and inhibits proliferation of HeLa cells with IC_{50} = 220 nM (Table 1). Knowing that an alkyl ketone is a relatively weak zinc chelator we rationalized that significant binding affinity was due to the two capping groups: the methyl indolyl and substituted imidazole. Having such a potent HDACi we were interested in further exploring other weak zinc chelating groups in the hope of further refining the isoform selectivity. Apart from amino benzamides, sulfides and ketones few other HDACi have been reported.[10]

Trying to identify a selective class I HDAC inhibitor we changed the ketone functionality into different possible metal binding moieties (Table 1).

The hydroxamic acid **9** was confirmed to be a potent HDACi (IC_{50} = 3 nM) and displayed IC_{50} = 180 nM in a 72 h anti-proliferation assay in HeLa cervical cancer cells.[8] Another two derivatives which displayed interesting cellular activity were *N*-methoxy amide **10** and the methylamide **13** (HDAC1 IC_{50} = 34 and 120 nM, HeLa IC_{50} = 0.66 and 1.7 μM, respectively). Interestingly the methyl amide although showing weak enzyme inhibition displayed low micromolar antiproliferative activity. We were pleased to observe

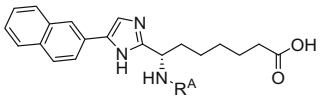
Table 1
SAR of zinc binding group

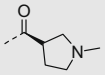
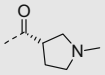
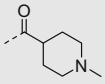
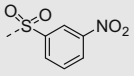


Compound	R	HDAC1 IC_{50} (nM)[a]	PRO (HeLa) IC_{50} (nM)[a]
7		27	200
8		28	390
9		3	180
10		34	660
11		47	8000
12		94	33,500
13		120	1700

[a] Values are means of two or more experiments.

Table 2
SAR of the capping group



Compound	R[a]	HDAC1 IC_{50} (nM)[a]	PRO (HeLa) IC_{50} (nM)[a]
14		23	5800
15		73	>25,000
16		40	>25,000
17		220	26,400
18		35	35,000
19		19	6860

[a] Values are means of two or more experiments.

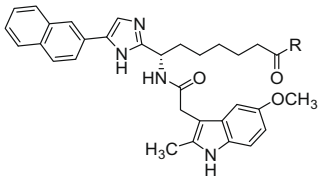
that simple functionality such as a carboxylic acid **8** show promising levels of anti-proliferation activity in the cell based assay. Acylsulfonamides are described as lipophilic and more permeable bioisosters of carboxylic acids, however **11** showed a significant loss of antiproliferative activity in cells (PRO HeLa IC_{50} = 8 μM). The more acidic analogue **12** led to an even further loss of activity both in enzyme and in cells (HDAC1 IC_{50} = 94 nM, PRO HeLa IC_{50} = 33.5 μM). Given the interesting cellular activity we started to profile further compound **8**. It suffered from extensive metabolism in rat liver microsomes in the presence of NADPH (Cl_{int} = 177 μL/min/mg P) while more stability was detected in human and dog liver microsomes (Cl_{int} = 9 and 3 μL/min/mg P, respectively). Moreover, the compound was glucoronidated in the presence of UDPGA particularly in human microsomes (Cl_{int} = 24 μL/min/mg P). Nevertheless we decided to conduct further SAR to try to improve its pharmacokinetics properties (Table 2).

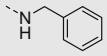
The fragments selected were those which had previously been demonstrated to improve microsomal stability in the ketone series.[9] Unfortunately in the carboxylic acid series although maintaining enzyme inhibitory activity, **14–19** lost cellular activity. The best derivatives, **14**, showed only 6 μM anti-proliferation activity, one order of magnitude weaker than **8**.

Considering the promising smaller shift in cells observed for methyl amide **13** (Table 1), we decided to perform a further SAR around the amide group (Table 3).

The simple primary amide **20** showed the same level of antiproliferative activity of the carboxylic acid **8** (HDAC1 IC_{50} = 30 nM, PRO HeLa IC_{50} = 0.44 μM). The ethyl substitution although losing only twofold on the enzyme (IC_{50} = 66 nM) was sixfold less active in cells (IC_{50} = 2.7 μM). Similarly the tertiary dimethylamide **25** displayed HDAC1 IC_{50} = 170 nM and weak HeLa anti-proliferation activity (IC_{50} = 3.8 μM). While the introduction of a branched residue such as the isopropyl group present in **22** causes a substantial loss of activity (PRO HeLa IC_{50} = 12 μM). As predicted the aminobenzamide **27** showed an anti-proliferative activity in the low micromolar range (HeLa IC_{50} = 1.34 μM) despite showing only weak enzyme activity. Micromolar anti-proliferation activity in

Table 3
SAR of carboxamide



Entry	R	HDAC1 IC_{50} (nM)[a]	PRO (HeLa) IC_{50} (nM)[a]
20		30	440
21		66	2700
22		480	12,000
23		1000	6800
24		200	5900
25		170	3800
26		350	4700
27		110	1340

[a] Values are means of two or more experiments.

Table 4
Activity of compound **20** on HDAC isoforms and various cell line[a], IC_{50} (nM)[b]

Compound	1	2	3	4	6	8
Vorinostat	30	82	57	>10 μM	43	1700
20	30	160	250	>10 μM	120	>5 μM
	Cervical HeLa		Colon HCT116		Lung A549	
Vorinostat	460		1000		1800	
20	440		860		3560	

[a] Assay condition as described in Ref. 8.
[b] Values are means of two or more experiments.

the cell based assay was also detected either with benzyl (**23**) and phenyl (**24**) amides (HeLa IC_{50} = 6.8 and 5.9 μM, respectively).

Compound **20** was profiled on individual HDAC isoforms, where it was demonstrated to show fivefold selectivity for HDAC1 over HDAC2 and nearly 10-fold selective over HDAC3 (IC_{50} = 30, 160 and 250 nM, respectively). This is in contrast either to vorinostat, which displayed similar level of activity on HDAC 1, 2 and 3, and to ketone **7** almost equipotent respect to these 3 HDACs.[8] Excellent selectivity was also seen over HDAC 4 and 8 (Table 4).

Compound **20** was also evaluated on several other cell lines where it showed comparable activity to vorinostat. With the encouraging cellular activity it was decided to evaluate **20** in an in vivo efficacy study, a human colon HCT-116 carcinoma xenograft model. The compound was profiled alongside vorinostat. In preliminary studies, the maximum tolerated dose (MTD) of both compounds was determined following intraperitoneal (IP) once daily dosing for 2 weeks, as defined by no mortality and body weight loss less than 5%. The amide **20** showed good exposure at the MTD dose (100 mpk) in mice, and the plasma concentration remained above IC_{50} for inhibition of HCT116 proliferation for nearly 12 h (Fig. 3).

Compound **20** was then evaluated in a HCT116 xenograft study, it was dosed at 100 mpk for 18 days once a day, and efficacy was compared to that seen with vorinostat which was dosed at 150 mpk using the same dosing regime. Interestingly the amide **20** displayed similar efficacy to **1** (29% tumor growth inhibition compared with the vehicle group was observed) with less than 5% body weight loss (Fig 4).

To our knowledge this is the first example of a small molecule HDACi bearing a primary amide zinc binding motif demonstrating efficacy in xenograft studies.

In parallel, target engagement was measured by assessing the level of histone acetylation seen in tumor biopsies of HCT116 xenograft mice (4 per group). At efficacious doses of 100 mpk ip an

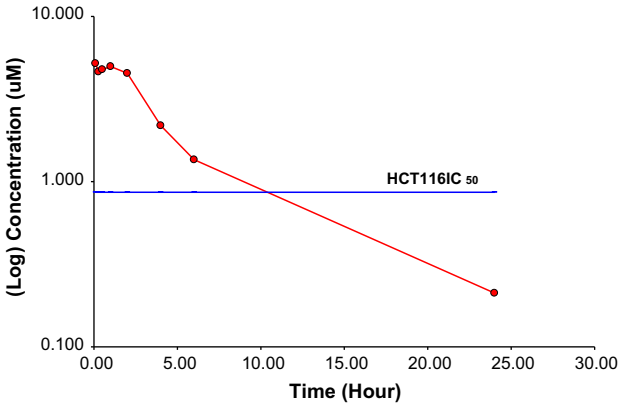


Figure 3. Mouse PK (100 mpk, ip).

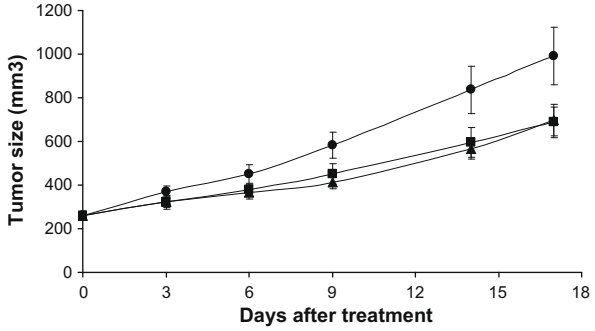


Figure 4. Antitumor activity of **1** (■) and **20** (▲) in a human HCT116 colon cancer xenograft model in nude mice (12 per group) following ip dosing in vehicle (●) (error bars represent mean ± SEM values).

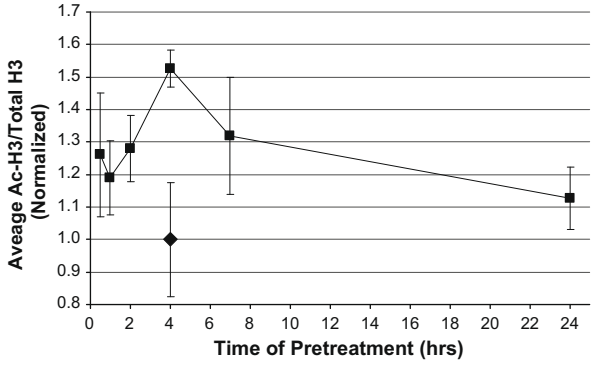
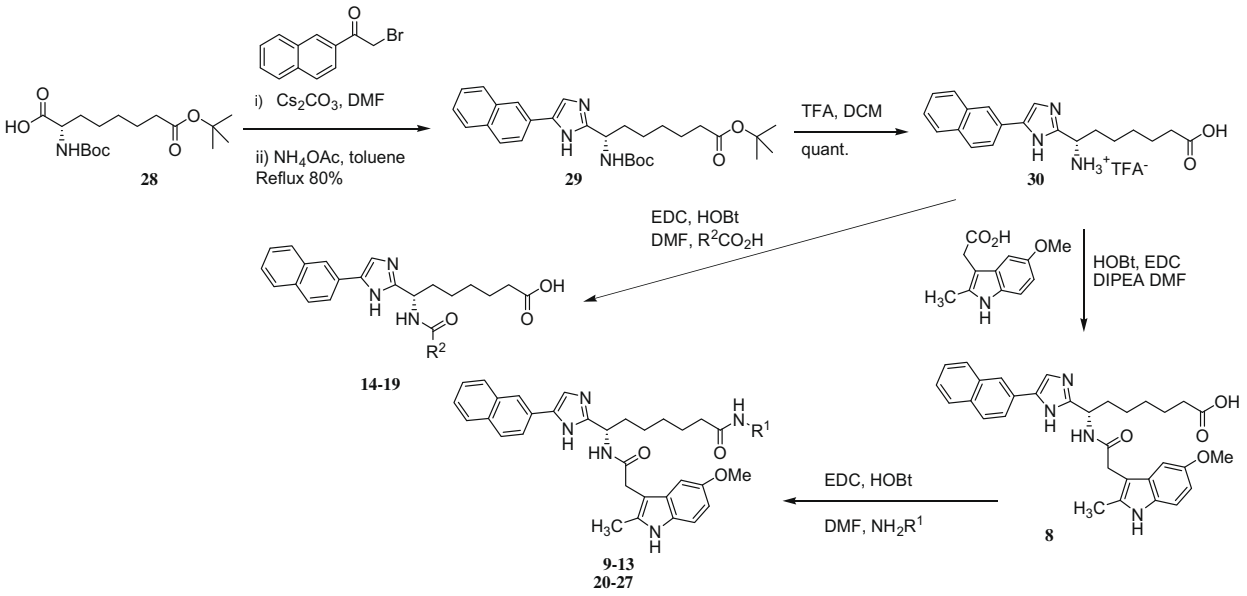


Figure 5. Histone acetylation in HCT116 tumor cells in xenografts after ip dosing of **20** at 100 mpk (■) and vehicle (♦) (error bars represent mean ± SEM values).



Scheme 1. Synthesis of compound **11**–**29**.

increase of acetylated histone was seen for more than 8 h with maximal activity at 4 h post administration (Fig. 5).

The synthesis of these derivatives starts from the readily prepared α-amino acid **28** (Scheme 1) which was first alkylated with 2-(bromoacetyl)naphthalene and then cyclized to the corresponding imidazole **29** in refluxing toluene in the presence of an excess of NH_4OAc. Double deprotection gave intermediate **30** which was added to a dichloromethane solution of 5-methoxy-2-methyl-3-indoleacetic acid or the other carboxylic acids, under standard coupling conditions, to get the free acid **8** or compounds 14–**19**. Derivatives **9–13** and **20–27** were obtained after the reaction of **8** with the suitable coupling reagents.

In conclusion, we have described the first example of a selective class I HDAC inhibitor with a primary carboxamide moiety as zinc binding group. Furthermore **20** displays selectivity for HDAC 1 and demonstrated efficacy in an HCT116 xenograft model comparable with vorinostat.

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