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Optimization of a series of potent and selective ketone histone deacetylase inhibitors

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ABSTRACT

Histone deacetylase (HDAC) inhibitors offer a promising strategy for cancer therapy and the first generation HDAC inhibitors are currently in the clinic. Herein we describe the optimization of a series of ketone small molecule HDAC inhibitors leading to potent and selective class I HDAC inhibitors with good dog PK.

Histone deacetylase (HDAC) and histone acetyltransferase (HAT) are nuclear enzymes involved in regulating gene expression.[1] The HDAC enzyme family, of which eleven isoforms belonging to classes I, II and IV are known, catalyzes deacetylation of the ε-amino group of lysine residues located near the N-terminus of nucleosome histone proteins. Deacetylated histones acquire a net positive charge that interacts strongly with the negatively charged DNA, thereby condensing the chromatin and restricting accessibility to transcription factors, and ultimately changing gene expression.[2] In cancer the acetylation status of these histone tails is aberrantly regulated and many recent studies have shown that HDAC inhibition leads to anticancer effects, as a result of inhibiting cell growth and inducing apoptosis, as well as causing differentiation and inhibition of angiogenesis. Hence, HDAC inhibition represents a novel approach to cancer chemotherapy,[3] and indeed vorinostat (**1**) (Zolinza®, formerly known as SAHA) has been approved for the treatment of the cutaneous T-cell lymphoma (CTCL),[4] while several other HDAC inhibitors (HDACi) along with vorinostat, are in clinical trials showing efficacy in patient with hematological and solid malignancies.[5] Known HDACi's (Fig. 1) cover a wide cross-section of structures[6] including: hydroxamic acids (typically broad-spectrum HDACi) such as **1**,[7] **2**,[8] and **3**[9] as well as aminobenzamides, for instance **4**[10] and **7**[11] together with their *bis*-aryl derivatives recently disclosed as selective HDAC 1 and 2 inhibitors such as **5**.[12] Other classes include short chain fatty acids like **6**[13] and cyclic peptides such as **8**[14] and **9**.[15]

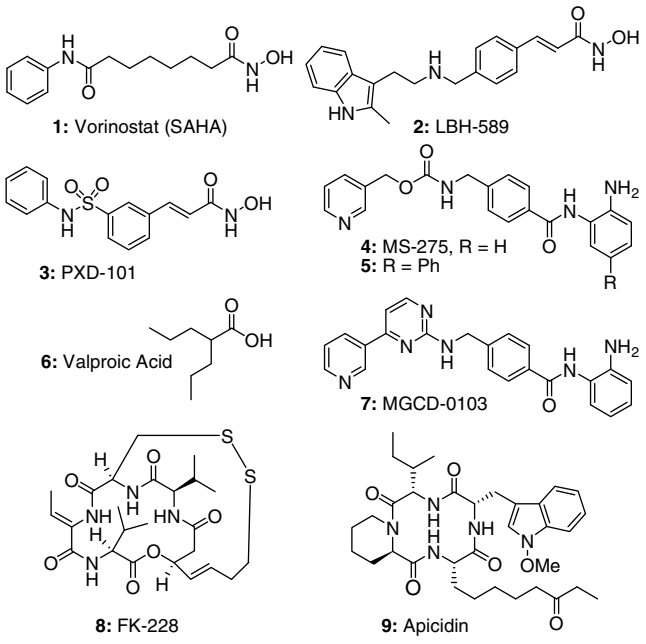

Figure 1. Known HDAC inhibitor.

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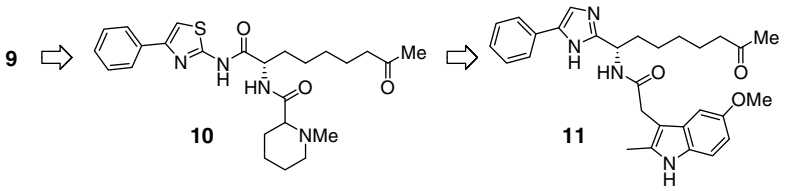


Entry	HDAC1 IC_{50} (nM)	PRO (HeLa) IC_{50} (nM)
9	44	290
10	55	430
11	59	880

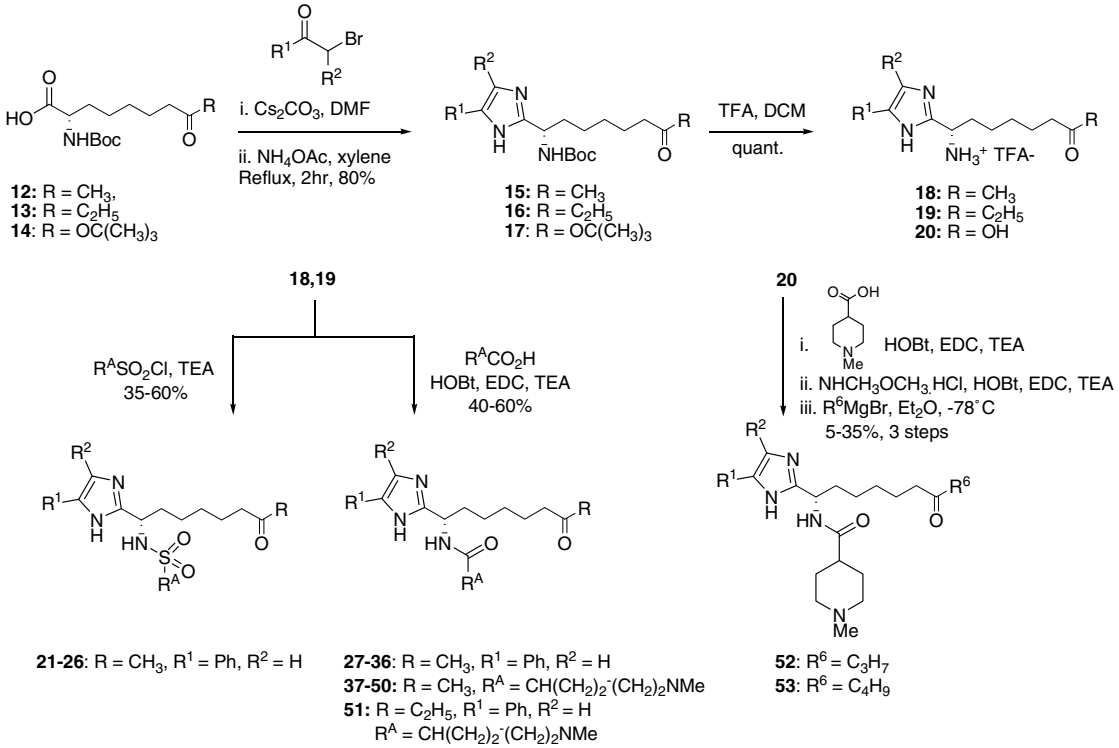
Figure 2. Development of a series of small molecule alkyl ketone HDACi's and the identification of lead **11**. [16]

Recently we have reported the discovery of a structurally novel series of HDAC inhibitors based on the natural compound Apicidin.[16] This natural product contains the unusual ethyl ketone as a zinc binding group (ZBG) yet it is a potent HDAC1 inhibitor (IC_{50} = 44 nM) and displays good anti-proliferative activity (HeLa cells IC_{50} = 290 nM). A related series of simplified acyclic derivatives was identified and optimized to potent and selective HDAC inhibitors such as **10** showing good enzymatic activity against HDAC1 (IC_{50} = 55 nM) and submicromolar activity in the anti-proliferative assay (HeLa cells IC_{50} = 430 nM) (Fig. 2). Unfortunately the heterocyclic acylamino bond was shown to be unstable in rodent plasma, and SAR studies were conducted to identify a replacement to this labile bond by a suitable bioisostere, culminating with the identification of imidazole derivatives exemplified by **11**. [16] Compound **11** displays class I HDAC subtype selectivity and levels of cellular activity in different cancer cell lines comparable to existing clinical candidates. Furthermore in an in vivo efficacy study **11** was shown to cause tumour growth inhibition in a colon HCT-116 carcinoma xenograft model comparable to that achieved with vorinostat and MS-275; to our knowledge this is the first example of an unactivated small molecule alkyl ketone HDACi to be efficacious in vivo. Unfortunately **11** was highly cleared in rats (Cl = 80 mL/min/kg, Rat microsomes Cl_{int} = >300 μL/min/mg) and showed only modest oral bioavailability (F = 15%).

Encouraged by these results we herein describe the further development of this novel class of HDACi and report our work optimizing enzymatic and anti-proliferative activities, together with the efforts to address pharmacokinetic liabilities.

Guided by X-ray crystallographic analysis of a related hydroxamic acid inhibitor bound to HDAC8,[17] SAR investigations focused primarily on the optimisation of the two surface recognition domains, the imidazole substituent and the amino capping group.

The synthesis of these derivatives starts from the readily prepared α-amino acid derivatives **12, 13** (scheme 1) which were first alkylated with the suitable α-bromo ketone and then cyclized to the corresponding imidazoles in refluxing xylene in the presence of a large excess of NH_4OAc. Deprotection and standard coupling conditions yielded either the desired amides **27–51** or the corresponding sulfonamides **21–26**. A small exploration of the ZBG was also conduced starting from the (2*S*)-8-*tert*-butoxy-2-[(*tert*-butoxycarbonyl)amino]-8-oxooctanoic acid **14**. Cyclization as previously yielded the imidazole intermediate **17** which was fully deprotected by treatment with TFA. The resulting amino acid **20** was then coupled with 1-methylpiperidine-4-carboxylic acid and the acid moiety converted into the Weinreb amide. Reaction with the corresponding Grignard reagent provided the homologated *n*-propyl and *n*-butyl derivatives **52** and **53**.



Scheme 1. Synthesis of compounds **21–53**.

These compounds were screened in our primary assays for the ability to inhibit recombinant human HDAC-1 and in 72 h anti-proliferation against cervical HeLa cancer cell line.[16]

Previous work in the *bis*-amide series based on lead **10** had shown that the electron rich 2-methyl-5-methoxy indolylacetamide could be replaced by smaller neutral and basic substituents.[18] Indeed in the imidazole series these groups offered the optimum combination of reduced molecular weight, cellular activity and in vitro stability (Tables 1 and 2).

The indolyl group could be replaced by a wide range of substituted aryl amides and sulfonamides (Table 1) although none offered any significant advantages in terms of activity compared to lead **11**. Little difference in activity was seen between amides and the corresponding sulfonamides (e.g. **22** and **27**). Electron deficient aromatic rings like **22**, **24** and **26** appeared to give greater HDAC1 inhibition compared to their electron rich counterparts like **25** and **28**. As previously seen in the *bis*-amide series one of the most interesting derivatives was the thiazole moiety **29** which despite slightly reduced potency against HDAC1 (IC_{50} = 140 nM) showed better cellular activity than **11**, with IC_{50} = 530 nM on HeLa cervical cancer cells.

Having seen that none of these neutral derivatives offered significant advantages in activity compared to **11**, attention focussed on the incorporation of basic fragments (Table 2). Small cyclic amino groups, such as *N*-methyl pyrrolidine **30** and *N*-methyl piperidines **31–33**, as well more elaborated amines were evaluated.

Despite having only modest activity in the enzymatic assay, almost all these compounds showed little shift between enzyme and cellular activities, typically 3- to 4-fold compared to 15-fold for **11**.

Of the three piperidine analogs, the 2-substituted derivative (**31**) was the least active and was rapidly degraded in rat liver microsomes; the 3-derivative (**32**) was 3-fold more potent against HDAC1 (IC_{50} = 220 nM) but still had only modest metabolic stability, while the 4-derivative (**33**) showed similar enzyme activity and comparable cell-based activity to **11** (HeLa IC_{50} = 980 vs 880 nM) but significantly improved microsomal stability (Cl_{int} = 68 μL/min/mg) with respect to both the other piperidine isomers and also to **11**.

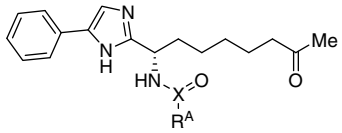
More sterically demanding amino derivatives were also tolerated, such as the [2,2,1]-bicyclohexylamine (**36**) indicating that the surface of the enzyme is very spacious in that region.

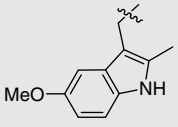
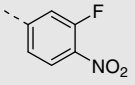
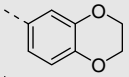
Based on these results it was decided to use **33** as the lead for a further round of optimization, this time focusing on the imidazole substituent (Table 3).

It was found that by varying the substituent on the imidazole ring, both enzymatic and anti-proliferative activities could be affected and a number of potent compounds were identified. Substitution in the *ortho* position was poorly tolerated compared to the *meta* and *para* positions; these latter two positions provided a 2- to 3-fold boost in enzyme and cellular activities (e.g. **38** and **39** vs **40**). Lypophilic substituents such as –Br, –Cl and –Ph (**37**, **38**, and **42**) increased activity, whereas polar substituents such as –F, –CN (**41** and **44**) and polar heterocycles such as 2-pyridyl (**46**) lowered the activity. Replacement of the phenyl group with naphthyl gave compound **43** which showed a dramatic improvement both in enzymatic and cell-based assays; it was 20-fold more potent than **33** on HDAC1 and 3-fold more potent in HeLa cells.

Unfortunately the opposite trend in terms of metabolic stability was observed with the more polar compound being the more metabolically stable in rat liver microsomes as observed for **37**, **38** and **41**. Replacement of the phenyl with polar heterocycles such as the 2-pyridyl group (**46**) were not tolerated both in enzymatic and anti-proliferative assays. In contrast the 2-thiophene-derivative (**47**) was equipotent on the enzyme although it proved to be 3-fold less active than **33** in proliferation assay. By increasing the lypophilicity, cellular activity could be regained as the benzo-fused ana-

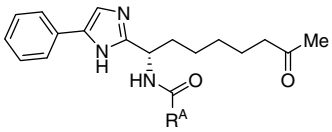
Table 1
Preliminary SAR on the 2-methyl-5methoxyindole group

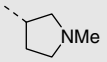
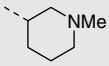
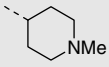
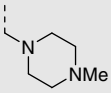
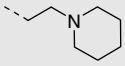
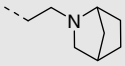


Entry	X	R^A	HDAC 1 IC_{50}[a] (nM)	PRO (HeLa) IC_{50}[a] (nm)
11	C		59	880
21	SO	*p*-CN-Ph–	180	1200
22	SO	*m*-CN-Ph–	110	1100
23	SO	*o*-CN-Ph–	270	7300
24	SO	*m*-NO_2-Ph–	55	1300
25	SO	*p*-OMe-Ph–	250	1400
26	SO		130	1000
27	C	*m*-CN-Ph–	160	1100
28	C		300	1100
29	C		140	530

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

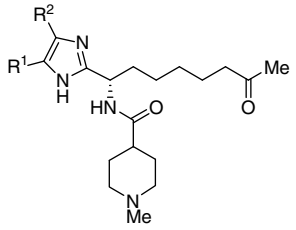
Table 2
SAR of replacements of the 2-methyl-5methoxyindole group: amine derivatives

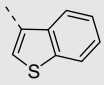


Entry	R^A	HDAC 1 IC_{50}[a] (nM)	PRO (HeLa) IC_{50}[a] (nm)	Cl_{int} rat microsomes (μl/min/mg)
30		280	1100	501
31		710	2700	>300
32		220	400	154
33		250	980	68
34		500	2000	64
35		190	1300	>300
36		220	1700	143

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

Table 3
SAR of replacements of the phenyl group



Entry	Rt	R^2	HDAC 1 IC_{50}[a] (nM)	PRO (HeLa) IC_{50}[a] (nM)	Cl_{int} rat microsomes (μl/min/mg)
37	*p*-Br-Ph	H	100	550	>300
38	*p*-Cl-Ph	H	75	680	246
39	*m*-Cl-Ph	H	90	400	
40	*o*-Cl-Ph	H	180	1300	
41	*p*-F-Ph	H	380	2200	99
42	*p*-Ph-Ph	H	50	390	170
43	2-naphthyl	H	11	300	194
44	*p*-CN-Ph	H	120	1500	330
45	*m*-CN-Ph	H	480	1540	
46		H	>500	9700	
47		H	240	3200	
48		H	180	1300	
49	*tert*-Bu	H	>5000	>25000	
50	Ph	Me	>1500	14700	

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

logue **48** displayed IC_{50} = 1.3 μM in HeLa cells. The addition of excessive steric bulk, such as *tert*-butyl (**49**) produced a significant loss of activity, as did disubstitution on the imidazole, as 4-methyl-5-phenyl analogue (**50**) was 20-fold less active. We hypothesized this could be due to a steric clash between the two substituents forcing the phenyl ring to adopt a more out of plane orientation.

Based on its improved potency, **43** was further profiled; it displayed excellent inhibitory activity on HDACs 1, 2, 3 and 6 while no or weak activity was found on the other isoforms (Table 4). Comparison of **43** with vorinostat and our lead **11** demonstrated that it was from 3- to 8-fold more potent in the enzyme assay and had 2-fold improvement in the antiproliferative activity across a panel of cancer cell lines. **43** was also evaluated in terms of microsomal stability in three different species: it was found to be unstable in rat liver microsomes with a Cl_{int} of 194 μL/min/mg whereas it showed lower turnover in human and dog liver microsomes (12 and 10 μL/min/mg, respectively).

The plasma protein binding in the three species was also measured showing the compound 89% bound to rat protein, 96% bound to both human and dog plasma protein. When dosed in dog **43**, as expected, showed a good PK profile with a low clearance, a long half-life and acceptable oral bioavailability (Cl = 7 mL/min/kg, F = 28%, $t_{1/2}$ = 11.8 h); however the compound was cleared rapidly in rat and unsurprisingly no oral bioavailability was found (Cl = 133 mL/min/kg, F = 0%) (Table 5). Based on intrinsic clearance **43** might have human PK similar to dog.

Table 4
Activity of compound **43** on HDAC isoforms and various cell line, IC_{50} (nM)

Entry	1	2	3	6	4,5,7,8 (μM)
43	11	29	17	10	>1
11	59	110	120	340	>1
Vorinostat(1)	30	82	57	43	>1

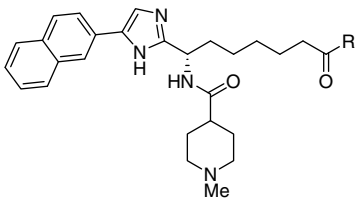
	Cervical HeLa	Colon HCT-116	Lung A-549
43	300	590	720
11	880	500	6300
Vorinostat(1)	460	1000	1800

Values are means of two or more experiments.

Table 5
PK profile for compound **43**

	Cl_{int} μL/min/mg	$t_{1/2}$ (h) *iv*	V_{dss} (L/Kg) *iv*	Cl (mL/min/Kg) *iv*	*F* (%) *po*
Rat	194	2.4	6.9	133	0
Dog	10	11.8	5.2	7	28
Hum	12				

Table 6
SAR on the zinc binding group



Entry	R	HDAC IC_{50}[a] (nM)			PRO (HeLa) IC_{50}[a] (nM)
		1	3	6	
43	Me	11	17	10	300
51	Et	12	12	800	210
52	Pr	16	27	1400	200
53	Bu	74	180	3500	690

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

Following the results from our previous studies, a rapid investigation into the ZBG was conducted and the higher homologs of **43** containing the ethyl (**51**), *n*-propyl (**52**) and *n*-butyl (**53**) ketone were profiled (Table 6). The ethyl analogue (**51**) proved to be the optimal chain length gaining both enzymatic and cellular activities, and moreover it displayed only a weak activity on HDAC 6 and consequently good HDACs class I selectivity. The *n*-propyl derivative (**52**) showed equivalent activity in the cell-based assay while a slightly reduced HDAC activity was observed, still maintaining class I selectivity. Further elongation of the chain (**53**) resulted in an erosion of the inhibitory activity probably as a result of a steric clash at the bottom of the binding cavity.

In summary we have reported the optimization work of a series of ketone small molecule HDAC inhibitors which have led to compounds such as **43** and **51** displaying low nanomolar activities against HDAC 1,2 and 3 and good selectivity over the other class I and II HDACs. These compounds showed anti-proliferative activities equivalent to existing clinical candidates despite their lack of a well established ZBG. Moreover compound **43** demonstrated to have good PK properties in dogs, with acceptable oral bioavailability and a long half-life.

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