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A series of novel, potent, and selective 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 clinical trials. A structurally novel series of HDAC inhibitors based on the natural cyclic tetrapeptide Apicidin is described. Selected screening of the sample collection looking for *L*-2-amino-8-oxodecanoic acid (*L*-Aoda) derivatives identified a small acyclic lead molecule **1** with the unusual ketone zinc binding group. SAR studies around this lead resulted in optimization to potent, low molecular weight, selective, non-hydroxamic acid HDAC inhibitors, equipotent to current clinical candidates.

Aberrant gene regulation plays an important role in tumor initiation and progression, and methods to correct this dysfunction represent a new anti-cancer strategy.[1] The acetylation status of lysine residues in nucleosomal histone proteins is known to play a crucial role in chromatin structure and hence gene transcription.[2] The presence of acetyl groups on these lysine residues neutralizes the positive charges of the histone tails, therefore decreasing their interactions with DNA, relaxing the chromatin, and allowing access to transcription factors. In contrast, removal of the acetyl groups from these Ac-lysines facilitates the interactions of ε-amino group with the DNA, condensing the chromatin. This acetylation status is controlled by the opposing actions of histone acetyl transferases and HDACs; eleven class I and II HDACs are known.[3] Histone deacetylase inhibitors (HDACi) are emerging as a new class of anti-cancer agents and have been shown to alter gene transcription and exert various anti-tumor effects such as: growth arrest, differentiation, apoptosis and inhibition of tumor angiogenesis.[4–6] However, the precise anti-tumor mechanism of these HDACis remains unclear.[4] Currently, there are several HDACis in clinical trials and showing efficacy in patients with hematological and solid malignancies.[7,8]

HDACis have been widely studied, X-ray crystal structures with bound inhibitors exist,[9] and they can be characterized by a common pharmacophore consisting of: a zinc binding group (ZBG), a linker domain, and a surface recognition domain. To date they belong to several distinct structural classes (Fig. 1): hydroxamic acids: such as **SAHA** (Suberoyl anilide hydroxamic acid),[10] aminobenzamides: for instance, **MS-275**,[11] electrophilic ketones,[12] short chain fatty acids like **Valproic acid**[13] and cyclic peptides: such as **FK-228**[14] and **Apicidin**.[15] Each class has its own limitations: hydroxamic acids are rapidly metabolized, non-selective inhibitors of most HDAC isoforms; aminobenzamides and fatty acids have limited potency; electrophilic ketones are reduced in plasma; whilst cyclic peptides offer issues of chemical intractability and **FK-228** contains an undesirable thiol ZBG.

As part of a project to identify a second generation HDACi the natural product **Apicidin** was selected as a suitable starting point. This molecule contains an ethyl ketone as potential ZBG, a long alkyl chain as linker, and the cyclic tetrapeptide that interacts with the surface

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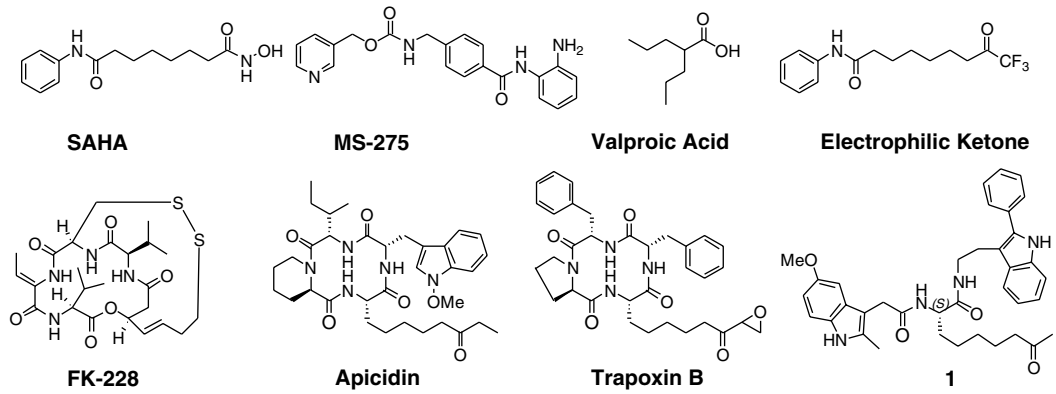


Figure 1. Known HDAC inhibitors and the structure of novel lead **1**.

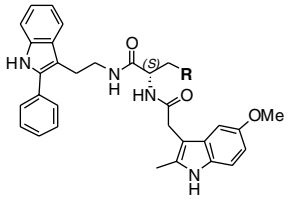
of the HDAC. This compound, despite the unusual ZBG, is a relatively potent HDAC 1 inhibitor,[16] IC_{50} = 44 nM, and displays good anti-proliferation activity against cervical cancer HeLa cells in a 72 h growth inhibition assay, IC_{50} = 290 nM.[17] Apicidin is unique in that it lacks the epoxide whose presence is integral for biological activity in closely related cyclic tetrapeptide HDACi such as **Trapoxin**,[18] suggesting that Apicidin's macrocycle contributes significantly to activity.[19]

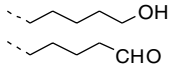
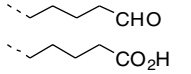
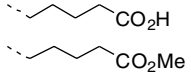
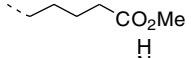
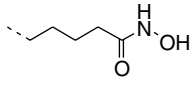
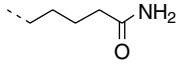
Directed screening of the sample collection looking for compounds containing the unusual *L*-Aoda amino acid identified **1** as a more tractable lead. This compound maintains the ketone motif and pentyl chain, whilst exerting HDAC inhibitory activity, IC_{50} = 590 nM (albeit with a 12-fold loss compared to Apicidin), and good cellular activity remained as characterized in a 72 h cellular proliferation assay in HeLa cervical cancer cells,[17] PRO(HeLa) IC_{50} = 730 nM. This loss of enzyme activity was deemed tolerable as the compound is amenable to rapid analogue synthesis, although it suffers from: modest potency; high MWt; many rotatable bonds; and high liver microsome turnover.

Naturally, the SAR focused on three regions of the molecule: the ZBG, the linker domain, and surface recognition domain. At an early stage of the program it was demonstrated that the chirality of these molecules was critically important as the enantiomer, (***R***)-**1**, lost significant HDAC inhibitory activity. Subsequently all analogues were prepared homochirally in the *S*-series.

Investigations into the ZBG revealed that the ketone **1** was one of the most promising compounds as some alternative structures either lost HDAC activity, such as alcohol **2** or amide **7** (Table 1) or lacked cell-based activity despite being more potent enzyme inhibitors, for instance acid **4**. The aldehyde **3** and methyl ester **5**, although showing activities comparable to that of the lead, were not followed up further due to potential liabilities associated with these functionalities in vivo. Furthermore, the necessity for the ketone functionality was demonstrated by compound **8** lacking the carbonyl. This compound lost all activity, demonstrating the probable binding interaction made by this weak zinc chelator.

Table 1. Activity of **1** and related compounds with alternative ZBGs



Compound	R	HDAC 1 IC_{50}[a] (nM)	PRO(HeLa) IC_{50}[a] (nM)
SAHA		27	460
MS-275		110	1800
Apicidin		44	290
1		590	730
(***R***)-**1**		3200	>25,000
2		>5000	23,700
3		350	5700
4		220	>25,000
5		150	1300
6		0.8	12
7		1760	>25,000
8		>5000	>25,000

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

Interestingly, when the ketone was replaced by the strong zinc chelator, a hydroxamic acid, a sub-nanomolar enzyme inhibitor was identified. In fact, compound **6** is one of the most potent HDACis currently known and shows excellent anti-proliferation activity, PRO(HeLa) IC_{50} = 12 nM.

Further profiling of compound **6** demonstrated excellent inhibitory activity on HDACs 1–3 whilst the other HDAC isoforms were inhibited at higher concentrations (Table 2).[16] Potent anti-proliferative activity was also seen against a wide panel of cancer cell lines and the compound showed good selectivity over normal cells, such as renal epithelial cells.[17] This anti-proliferative activity correlated an increased level of histone H3 acetylation. Despite the potency of this compound work continued to focus on the ketone series.

Table 2. Activity of **6** on the HDAC isoforms and various cell lines

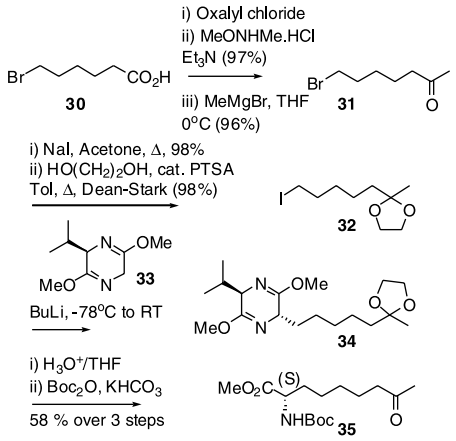
HDAC	1	2	3	4	5	6	7	8
IC$_{50}$[a] (nM)	0.8	4	1.6	260	2500	45	4200	200
Cell line							IC$_{50}$[a] (nM)	
HeLa							12	
Colon-HCT116							64	
Lung-A549							87	
U937							<8	
A2780							110	
G401							100	
MCF-7							130	
Human renal epithelial cells							930	

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

Investigations into the pentyl chain and around the two amide groups (β-amino acids and alkylated derivatives) revealed these to be preferred (data not shown), accordingly a robust synthesis of the key amino acid was required. This was achieved using Schöllkopf chemistry (Scheme 1).[20] Alkylation of the protected alkyl iodide **32**, readily synthesized without chromatography, gave homo-chiral amino acid **35** in 58% yield after hydrolysis and Boc-protection. SAR into the capping groups was then achieved by standard coupling methodology.

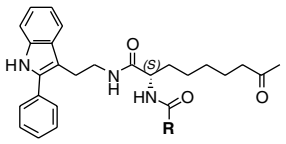
Extensive SAR work to find a replacement for the 2-methyl-5-methoxyindole group of **1** identified small heterocyclic derivatives, such as 5-carboxythiazole **10** or the thiadiazole **11**, as suitable alternatives (Table 3). These groups maintained HDAC activity but lost a few fold in the anti-proliferation assay. Small cyclic amino groups, such as *N*-methyl piperidines **12** + **13** and pyrrolidine **14**, were also well tolerated, resulting in modest improvements in HDAC inhibition. These substantially reduced MWt and furthermore improved microsomal stability. Pendant amines such as the 3-piperid-1-ylpropanamide **15** also behave similarly, improving both enzyme and cellular activities.

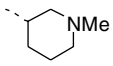
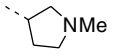
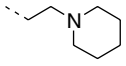
Finding alternatives to the large 2-(2-phenyl-1*H*-indol-3-yl)ethanamine moiety was more problematic and most replacements, with the exception of (hetero)anilides, resulted in a dramatic loss of activity (data not shown).



Scheme 1. Synthesis of key amino acid building block.

Table 3. SAR of replacements of the 2-methyl-5-methoxyindole group



Compound	R	HDAC 1 IC$_{50}$[a] (nM)	PRO(HeLa) IC$_{50}$[a] (nM)
9	Me	930	7600
10		590	3000
11		480	3400
12		540	2500
13		200	1200
14		220	2000
15		190	<390

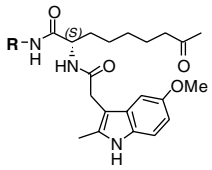
[a] Values are means of three or more experiments.

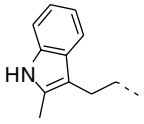
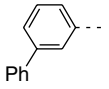
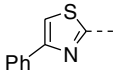
Fortunately, anilide **18** gave rise to a 3-fold improvement in enzymatic activity compared to **1** whilst reducing the MWt by 140 amu (Table 4). Further SAR around this group revealed a more dramatic improvement in enzymatic activity could be achieved by substitution in the *meta*-position. Chlorine (**20**), methoxy (**22**), and phenyl (**23**) groups were well tolerated at these positions resulting in inhibitors with double-digit nanomolar potencies and sub-micromolar anti-proliferation activities. Likewise, heteroaromatic amines were also tolerated, especially those bearing groups in the distal position, such as phenyl thiazole **24**.

After this initial SAR the most interesting substituents were combined and incorporated for a more extensive SAR around this series in a 2-D array. From this work small, low molecular weight, potent HDACis were identified with sub-micromolar activities against HeLa cell proliferation (Table 5). The best of these derivatives show activities comparable to those of **SAHA** and **MS-275**. Interestingly, the results from this work revealed that the SAR into the two separate amide groups was not additive and binding to one part of the surface clearly influenced binding of the second amide group. Nevertheless, **29** displayed IC$_{50}$ = 55 nM against HDAC 1 with HeLa anti-proliferation activity of 430 nM and improved microsomal stability (human Cl$_{int}$ = 54 μL/min/mg compared to >300 μL/min/mg for **1**) so warranted further profiling on the other HDAC isoforms and a panel of cancer cell lines (Table 6).

Extensive profiling revealed that **29** was indeed a isoform selective HDAC inhibitor displaying potent inhibi-

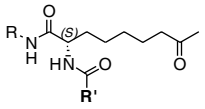
Table 4. SAR of replacements of the 2-phenylindole group

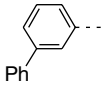
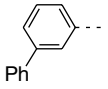
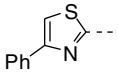


Compound	R	HDAC 1 IC_{50}[a] (nM)	PRO(HeLa) IC_{50}[a] (nM)
16		560	3800
17	Et	>5000	>25,000
18	Ph	180	1500
19	*o*-Cl–Ph–	3300	27,700
20	*m*-Cl–Ph–	44	520
21	*p*-Cl–Ph–	230	2000
22	*m*-MeO–Ph–	23	110
23		18	230
24		25	<390

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

Table 5. Simultaneous SAR into both amide groups



Compound	R	R′	HDAC1 IC_{50}[a] (nM)	PRO(HeLa) IC_{50}[a] (nM)
25	*m*-Cl–Ph–		100	1600
26	*m*-MeO–Ph–		33	880
27			79	500
28			55	720
29			55	430

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

tion of HDACs 1, 2, and 3 + 6, whilst only weak or no activity on HDACs 4, 5, 7 + 8. Furthermore, **29** maintained activity on a wide range of cancer cell lines and showed sub-micromolar activity against: colon HCT116, myeloid U937, kidney G401, and breast MCF7 lines. Moreover, this and other of these novel HDACis show good selectivity over normal cells as demonstrated in human renal epithelial cells where

Table 6. Activity of **29** on the HDAC isoforms and various cell lines

HDAC	1	2	3	4	5	6	7	8
IC_{50}[a] (nM)	55	170	14	NA at 5 μM	1900	13	NA at 5 μM	3000

Cell line	IC_{50}[a] (nM)
Cervical–HeLa	430
Colon-HCT116	670
Lung-A549	2000
Myeloid-U937	450
Ovarian-A2780	1700
Kidney-G401	250
Breast-MCF7	230
Human renal epithelial	>20,000

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

concentrations of 20 μM were well tolerated without significant growth inhibition.

In summary, a novel class of potent, low molecular weight, non-hydroxamic acid histone deacetylase inhibitors has been developed. These HDACis are equipotent with current clinical candidates.

Acknowledgments

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16. C-terminally Flag tagged HDACs 1, 2, and 3 + 6 were expressed in HEK293 cells and affinity purified using anti-FLAG antibody affinity resin and eluted with a 3× FLAG peptide. The HDACs 4, 5, and 7 + 8 were expressed in *Escherichia coli* (an organism devoid of endogenous HDACs to overcome the problem of co-purification of endogenous HDACs). Enzymes were purified to apparent homogeneity (Coomassie-stained SDS–PAGE) by nickel affinity then anion-exchange (MonoQ) chromatographies, and finally gel filtration. The IC_{50} were determined using the HDAC Fluorescent Activity Assay, BioMol Research Laboratories (Plymouth Meeting, PA).
17. Anti-proliferation assays were conducted for 72 h using the Promega (Madison, WI) CellTiter-Blue™ Cell Viability Assay. Cells + 10% FCS were grown in 96-well plates, 5000–15,000 cells/well, in the presence of HDACi for 72 h. CellTiter-Blue™ was added, cells were incubated for an hour and read (ex. 550 nm, em. 590 nm).
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