Perspective

Histone Deacetylase Inhibitors

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Introduction

Histone deacetylase enzymes, which have been divided into three distinct structural classes, operate by zinc-dependent (class I/II) or NAD-dependent (class III) mechanisms. Class I/II histone deacetylase (HDAC) enzymes are an emerging therapeutic target for the treatment of cancer and other diseases.[1-5] These enzymes, as part of multiprotein complexes, catalyze the removal of acetyl groups from lysine residues on proteins, including histones. HDAC inhibitors have been shown to bind directly to the HDAC active site and thereby block substrate access, causing a resultant accumulation of acetylated histones.[1,3,6] These agents possess diverse biological activities and can affect differentiation, growth arrest, and/or apoptosis in transformed cell cultures. In vivo xenograft studies have further demonstrated many of these agents to be effective in the inhibition of tumor growth.

A wide range of structures have been shown to inhibit the activity of class I/II HDAC enzymes, and with few exceptions, these can be divided into structural classes including small-molecule hydroxamates, carboxylates, benzamides, electrophilic ketones, and cyclic peptides. Despite the variety of structural characteristics, all of these HDAC inhibitors can be broadly characterized by a common pharmacophore that includes key elements of inhibitor–enzyme interactions. In addition to the availability of crystal structures, homology models have further aided in the identification and rational design of new HDAC inhibitors for use as chemical tools and potential therapeutics. The high level of interest in developing efficacious HDAC inhibitors and the avail-

ability of design tools have led to an expansive group of agents that target class I/II HDACs. This review encompasses the medicinal chemistry and structure–activity relationships (SAR) underlying advances in HDAC class I/II inhibitor discovery, design, and optimization.[5,7,8]

1. HDAC Structural Studies

The structural details of the class I/II HDAC inhibitor–enzyme interactions were elucidated by Finnin et al. in 1999.[9] The crystal structure of histone-deacetylase-like protein (HDLP, Figure 1), a homologue of mammalian class I/II HDAC with ~35% sequence identity, was solved separately with the HDAC inhibitors SAHA (**1**) and (*R*)-trichostatin A (TSA, **2**) (Figure 2) bound to the active site. The structural findings revealed by these studies led to a proposed mechanism of the hydrolysis of acetyllysine by the class I/II HDAC enzymes based on the observation that the HDLP active site has features of both metallo and serine protease active sites and the hypothesis that these hydroxamate inhibitors act as substrate analogues. These studies demonstrated that both SAHA (**1**) and TSA (**2**) make contact with the same residues in the rim, channel, and active site regions of the protein. In addition, hydrogen bonding between the inhibitor hydroxamic acid functionality and the imidazole groups in the histidine (H131, H132)–aspartate (D166, D173) salt bridges, along with hydrogen bonding of the active site tyrosine (Y297) to the hydroxamate carbonyl, provides further rationale for the potent inhibitory activity of these agents (Figure 3).

The SAR of the HDAC inhibitors reported to date further validates key features found in the X-ray crystal structure. Particularly, the direct interaction of the

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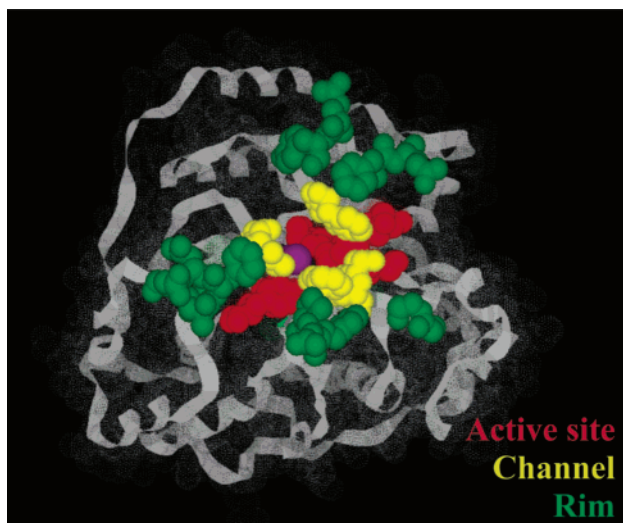
Figure 1. Crystal structure of HDLP. Binding regions are in CPK and are color-coded (zinc in purple) to correspond to the model pharmacophore domains for HDAC inhibitors (Figure 4) and the corresponding HDAC sequence alignments (Figure 27).
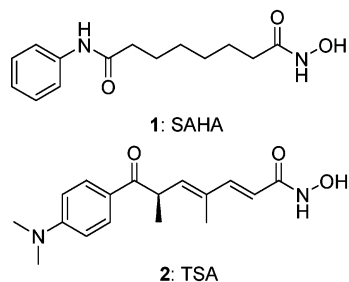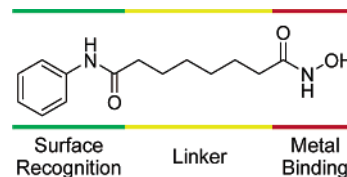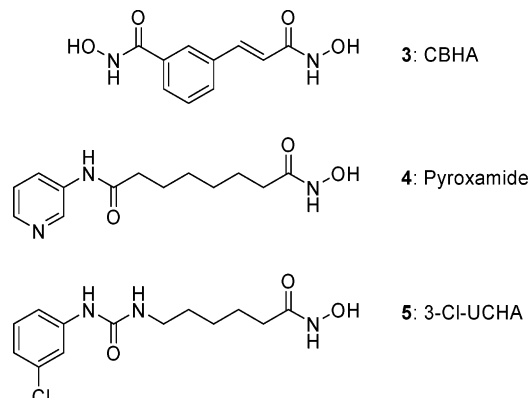

Figure 2.

inhibitor with the active site zinc appears to be a prerequisite to inhibitory activity. Given the X-ray crystallographic findings and the SAR of the various inhibitor classes, the structural characteristics of HDAC inhibitors reported to date can be summarized as depicted in Figure 4. This pharmacophore consists of a metal binding domain, which interacts with the active site, a linker domain, which occupies the channel, and a surface recognition domain, which interacts with residues on the rim of the active site. Homology models of human HDAC enzymes have enabled the identifica-
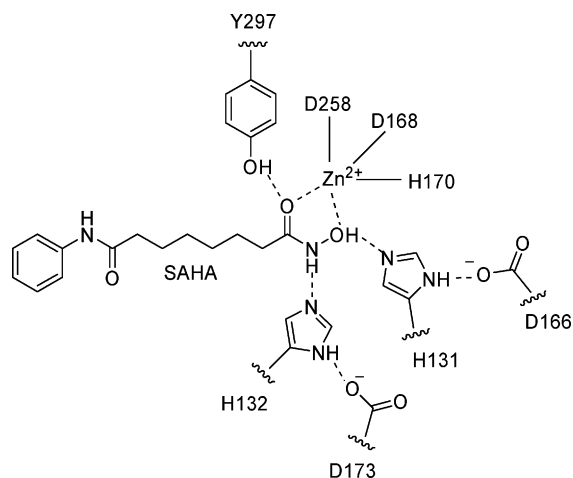
Figure 3. Inhibitor−HDLP enzyme hydrogen bond and chelation interactions in the active site.

Figure 4. Pharmacophoric summary of HDAC inhibitor structural characteristics. Pharmacophore domains are color-coded to correspond to binding regions in the HDLP crystal structure (Figure 1) and the corresponding HDAC sequence alignments (Figure 27).

Figure 5.

tion of new inhibitors, the development of predictive models, and the advancement of rational design efforts.[10−14]

2. Small-Molecule Hydroxamic Acids

Hydroxamic acid based differentiating and antiproliferative agents were among the first compounds to be identified as histone deacetylase inhibitors, and these agents helped to define the model pharmacophore for HDAC inhibitors (Figure 4). The linker domain can consist of linear or cyclic structures, either saturated or unsaturated, and the surface recognition domain is generally a hydrophobic group, most often aromatic. Hydroxamic acids carrying cyclic peptide surface recognition domain structures are described in section 6.

TSA (**2**), a natural product isolated from a *Streptomyces hygroscopicus* strain, was identified as a fungistatic antibiotic in 1976, and its activity was linked to HDAC inhibition (K_i = 3.4 nM) by Yoshida and coworkers in 1990 (Figure 2).[15,16] Trichostatic acid, the corresponding carboxylate, was shown to be ineffective as an HDAC inhibitor, indicating that the hydroxamate is required for activity.[17] Furthermore, the enantiomer (*S*)-TSA, which was obtained by total synthesis, was demonstrated to be inactive.[17,18]

The HDAC inhibitor SAHA (**1**) was reported by Breslow and co-workers.[19−21] Also reported were other hydroxamates (Figure 5), including CBHA (**3**), pyroxamide (**4**), and 3-Cl-UCHA (**5**).[19,20] These agents have been shown to be effective HDAC inhibitors and antiproliferative agents, both in vitro and in vivo. SAHA (**1**) has recently entered phase II clinical trials.[3,22,23]

SAHA (**1**), TSA (**2**), and CBHA (**3**), which represent the paradigmatic hydroxamate HDAC inhibitors, have been instrumental in guiding the design of hydroxamate-derived HDAC inhibitors. Constituents of the hydroxamate class are herein divided into two categories based on the structures of their linkers: inhibitors with

2: TSA
HDAC IC_{50} = 12 nM

6
HDAC IC_{50} = 152 nM

7
HDAC IC_{50} = 359 nM

8
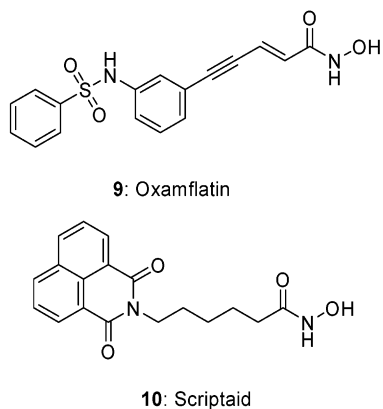HDAC IC_{50} = 12187 nM
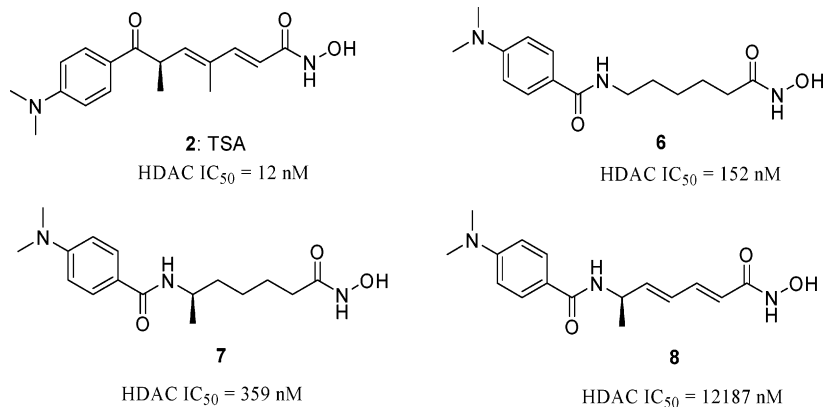
Figure 6.

linear linkers (TSA and SAHA-like) and inhibitors with linkers consisting of carbo- or heterocycles (CBHA-like).

2.1. Inhibitors Containing Linear Linker Domain Structures. In an effort to understand the importance of the methyl-substituted olefinic linker in TSA hybrids, the synthesis and evaluation of the compounds **7** and **8** has been performed.[14] These agents, along with related analogue **6** (Figure 6), demonstrate a highly sensitive and limited SAR profile. For example, the sequential addition of a methyl group (**7**) and two double bonds (**8**) caused a 2.3- and 33-fold reduction in activity, respectively, relative to the linear alkyl (**6**).

Oxamflatin (**9**, Figure 7) was prepared by researchers at Shionogi Laboratories in 1996.[24,25] Oxamflatin was found to be a potent HDAC inhibitor of partially purified mouse HDAC (IC_{50} = 15.7 nM), although it was found to be less potent than TSA (**2**) (IC_{50} = 1.44 nM) in the same assay.

Scriptaid (**10**, Figure 7), which was identified via high-throughput screening for transcriptional activators, was also found to inhibit HDAC and cause the induction of histone hyperacetylation.[26] A series of tricyclic molecules related to scriptaid (**10**) and corresponding to the general formula **11** were recently reported (Figure 8). The most potent analogue, **12**, has IC_{90} = 10 nM.[27]

Jung and co-workers synthesized a group of phenyl-alanine-containing SAHA analogues, including **13** and other derivatives (Table 1).[28,29] These agents were tested against both maize histone deacetylase (HD-2) and partially purified rat liver HDAC. This series provides insight into the nature of inhibitor–enzyme interactions. The trends in activity against HD-2 are different from those observed in mammalian HDAC. For ex-
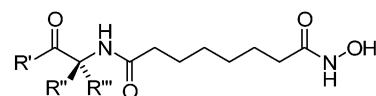
ample, compound **14k** is the most active of the series against HD-2 but does not outperform the others against mammalian HDAC. In addition, the homologous series **14b–d** shows opposite activity trends for the two enzymes. Other findings include the observation that SAHA (**1**) appears to be a poor inhibitor of HD-2, but it is still among the most active differentiating agents in the panel, with the notable exception of TSA (**2**). Compounds **14h–k**, with bulky hydrophobic side chains, appear to be among the best inhibitors and differentiating agents. Finally, inversion of the phenylalanine chiral center (**14d,f** vs **13** and **14g**) did not affect inhibitor activity against the enzyme or in cells.
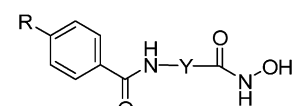
The same group also reported reverse amide SAHA derivatives (Table 2).[30,31] This study confirmed the earlier observation that compounds with five- and six-methylene spacers (**6** and **15c**) are the most active.[21] It is interesting to note the difference in activity trends between HD-2, which does not discriminate between the five- and six-methylene linkers, and HDAC, which favors the six-methylene linker. Substituted aromatics (**6**, **15g–i**) were found to be more active against HD-2 than unsubstituted analogue **15f**, although activity was independent of the electron-withdrawing and electron-donating properties of the substituents. The carboxylic acids corresponding to compounds **15a–i** were also prepared and found to be inactive at 40 μM. This series of compounds was expanded further to include scriptaid analogues **16** and **17** and SAHA analogue **18**.
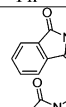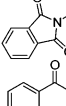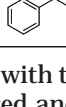
Other examples of TSA–SAHA hybrids have also been reported (Table 3).[32] These agents were evaluated as inhibitors of recombinant human HDAC1, antiproliferative agents against a panel of human cell lines, and inducers of histone hyperacetylation and p21 expression. This series also demonstrated that a six-methylene spacer is optimal (**19a–d**). Heterocyclic

9: Oxamflatin

10: Scriptaid

Figure 7.

11

12
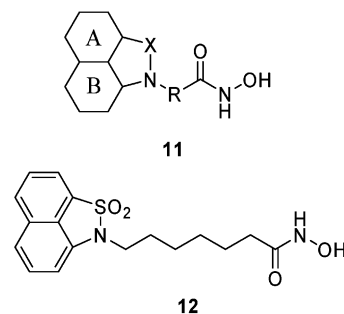
Figure 8.

Table 1. Structure and Activity of Phenylalanine-Derived HDAC Inhibitors

	R′	R″	R‴	IC_{50} (nM), maize HD-2	IC_{50} (nM), rat liver HDAC	IC_{50} (μM), Friend cells proliferation
2 (TSA)				3 ± 0.1	12 ± 1	0.04
1 (SAHA)				1000 ± 80	165 ± 25	0.99
13	H_3CO-	Bn−	H−	500 ± 5	800 ± 130	15
14a	H_2N-	Bn−	H−	1100 ± 35	840 ± 95	64
14b	PhNH−	Bn−	H−	80 ± 5	790 ± 70	15
14c	BnNH−	Bn−	H−	140 ± 5	460 ± 30	69
14d	$Ph(CH_2)_2NH$	Bn−	H−	330 ± 15	260 ± 20	72
14e	$Ph(CH_2)_3NH$	Bn−	H−	380 ± 10	1010 ± 90	58
14f	$Ph(CH_2)_2NH-$	H−	Bn−	340 ± 10	390 ± 25	76
14g	H_3CO-	H−	Bn−	760 ± 15	880 ± 85	28
14h	H_3CO-	1-naphthyl-methyl-	H−	360 ± 20	130 ± 15	22
14i	H_3CO-	2-naphthyl-methyl-	H−	40 ± 2	510 ± 40	5.2
14j	H_3CO-	3-indolyl-methyl-	H−	120 ± 10	320 ± 20	13
14k	$Ph(CH_2)_2NH-$	1-naphthyl-methyl-	H	35 ± 5	310 ± 10	15

Table 2. HDAC Inhibitory Activity of Linear Hydroxamic Acids

	R	Y	HD-2 IC_{50} (nM)	HDAC IC_{50} (nM)
2			3 ± 0.09	26 ± 10
6	Me_2N	$(CH_2)_5$	100 ± 20	244 ± 38
15a	Me_2N	$(CH_2)_3$	>40000	-
15b	Me_2N	$(CH_2)_4$	2000 ± 95	>10000
15c	Me_2N	$(CH_2)_6$	100 ± 4	46 ± 9
15d	Me_2N	$(CH_2)_7$	300 ± 8	145 ± 61
15e	MeO	$(CH_2)_5$	140 ± 10	-
15f	H	$(CH_2)_5$	900 ± 25	-
15g	Cl	$(CH_2)_5$	150 ± 5	369 ± 27
15h	O_2N	$(CH_2)_5$	180 ± 15	1566 ± 250
15i	Ph	$(CH_2)_5$	100 ± 4	265 ± 21
16			-	515 ± 97
17			-	357 ± 17
18			-	270 ± 25

derivatives with the optimized linker (**19e** and **19f**) were also prepared and found to be active. Para-substituted aryl derivatives (**19h−j**) were more active than ortho-substituted derivative **19g**, and it was also shown that increased hydrophobicity in the recognition domain could substantially enhance activity (**19k** and **19l**). The ketone functionality was also replaced with oxime (**19m,n**), alcohol (**19s**), alkene (**19o,q**), and alkane (**19r**) linkages. All of these compounds were shown to be effective inhibitors of HDAC, with those carrying sp³ centers (**19r,s**) displaying attenuated activity relative to the sp²-bearing analogues (**19k,m,o,q**). The corresponding carboxylic acids were also tested and found to be inactive with the notable exception of **19t**.

20: A-161906
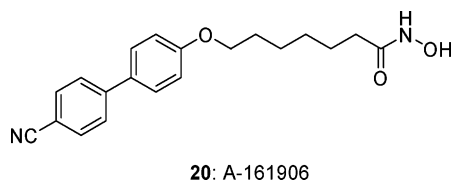
Figure 9.

Biaryl ether hydroxamate **20** was identified by Glaser and co-workers as a mimic of transforming growth factor β (TGF-β) with proliferation inhibitory properties (Figure 9). This agent was further shown to inhibit a mixture containing human HDAC1, HDAC2, and HDAC3 (IC_{50} = 9 nM) and to cause histone hyperacetylation and p21 induction.[33]

In a more recent paper, Curtin and co-workers reported the synthesis and HDAC1 inhibitory activity of **21** and reaction byproduct succinimide **22**, both of which contain a macrocyclic surface recognition domain (Figure 10).[34] A related series of succinimide hydroxamic acids were prepared and assayed against human HDAC1 (Table 4). The number, identity, and disposition of macrocycle substituents appear to be critical for activity. The removal of the succinimide substituents (**23b**) or the phenylalanine side chain (**23f**) depresses activity. Replacement of the succinimide with a lactam or phthalimide also led to reduced activity. The length of the linker domain alkyl group was also found to be critical, with the five-methylene analogue showing maximal activity.

21

HDAC IC_{50} = 2100 nM

22

HDAC IC_{50} = 38 nM
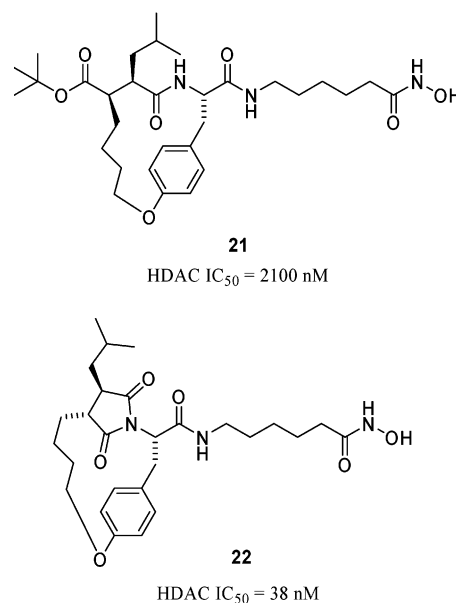
Figure 10.

Table 3. HDAC Inhibitory Activity of Linear Hydroxamic Acids

	R	HDAC1 IC$_{50}$, nM		R	HDAC1 IC$_{50}$, nM
19a		1500 ± 500	**19k**		5 ± 1
19b		500 ± 100	**19l**		5 ± 1
19c		65 ± 5	**19m**		8.5 ± 1.5
19d		135 ± 65	**19n**		4 ± 0.1
19e		153 ± 93	**19o**		8 ± 1
19f		50 ± 20	**19p**		2 ± 1
19g		95 ± 5	**19q**		6 ± 1
19h		15 ± 5	**19r**		25 ± 15
19i		45 ± 15	**19s**		35 ± 15
19j		45 ± 15			
2	TSA	5 ± 0.1	**19t**		200 ± 100

Table 4. Structure and Activity of Succinimide Hydroxamic Acids

	R	R′	R″	HDAC IC$_{50}$ (nM)	HT1080 prolif IC$_{50}$ (nM)	MDA435 prolif IC$_{50}$ (nM)
1 (SAHA)				120	2400	1900
22				38	250	150
23a	Bz	*i*-Bu	H	99	2300	670
23b	Bz	H	H	660	14000	10000
23c	Bz	*i*-Bu	*n*-Pr	51	400	570
23d	cyclohexyl-CH$_2$	*i*-Bu	H	640		
23e	4-MeO-Bz	*i*-Bu	*n*-Pr	38	530	180
23f	H	*i*-Bu	H	5000		

The synthesis of a series of heterocyclic-amide hydroxamic acids (Table 5) demonstrated the highly potent HDAC inhibitory activity of indole–amide structure **24d**.[35] Further study of the indole–amide series revealed that 2-substituted indole–carboxamides are the most active of the agents studied (**24g–k**). Finally, the SAR of monosubstituded indole–amide hydroxamic acids (Table 6) led to the discovery of low nanomolar HDAC inhibitors with potent cellular activity.

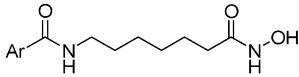Schreiber and co-workers reported the synthesis of a library of SAHA-derived HDAC inhibitors (Figure 11) including hydroxamates (**26**), benzamides (section 4), and carboxylates.[36] This set of compounds was aimed at the exploration of the chemical space in the rim region of the HDAC enzymes (section 8).

Finally, active compounds derived from α-amino-suberic acid, including **27**, were also recently disclosed (Figure 12).[8,37]
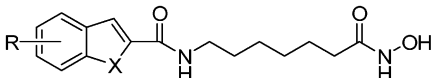
2.2. Inhibitors Containing Cyclic Linker Domain Substructures. One of the earliest papers reporting compounds with aromatic linkers presented the activity of compounds **28** and **29** (Figure 13) together with compounds with linear linkers (Table 2).[28] These studies demonstrate the importance of the proper orientation of the linker, underscoring the importance of conformation and arrangement of substructures for HDAC inhibition.

The discovery of CBHA (**3**) as an HDAC inhibitor demonstrated toleration of aromatic rings in the linker domain (Figure 5).[19,20] This observation, and the olefin–methyl arrangement found in TSA (**2**), suggests an isosteric relationship between these agents (Figure 14).

Oxamflatin–CBHA derived compounds have also been disclosed (Table 7).[38] Compounds containing cinnamic (**30e**) and hydrocinnamic (**30c**) linkers were determined to be the best in a series consisting of various lengths (**30a–e**). This result was interpreted in terms of effect of the chain length but could also stem

Table 5. HDAC Inhibitory Activity of Arylamide Hydroxamic Acids

	Ar	HDAC IC$_{50}$ (nM)		Ar	HDAC IC$_{50}$ (nM)
24a		1680	**24b**		1006
24c		524	**24d**		14.6
24e		732	**24f**		29.6
24g		37.5	**24h**		80.3
24i		31.4	**24j**		56.5
24k		41.0			

Table 6. SAR of Indole- and Benzofuran-Derived HDAC Inhibitors

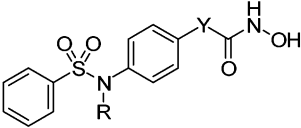|	R	X	HDAC IC$_{50}$ (nM)	HT1080 prolif IC$_{50}$ (nM)	MDA435 prolif IC$_{50}$ (nM)
24d	H	NH	14.6	140	150
25a	4-OCH$_3$	NH	3.1	120	130
25b	5-OCH$_3$	NH	6.5	720	1200
25c	5-F	NH	5.4	480	250
25d	5-Br	NH	3.5	480	470
25e	4-OCH$_3$, 6-OCH$_3$	NH	3.1	260	600
24f	H	O	29.6	760	490
25f	5-OCH$_3$	O	15	380	300
25g	5-Cl	O	15	180	190

from the geometries the compounds can assume, as seen for **28** and **29**. The presence of the double bond has little effect on activity, while substitution α to the hydroxamate dramatically decreased activity (**30f**–**h**). A 3-fold decrease in HDAC inhibitory activity was observed upon methylation of the sulfonamide nitrogen (**30i**). Structural analogues of **30e** have also been disclosed.[38]
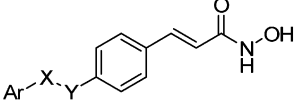

Figure 11.

27
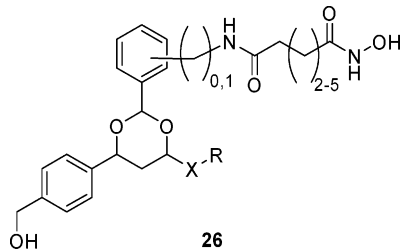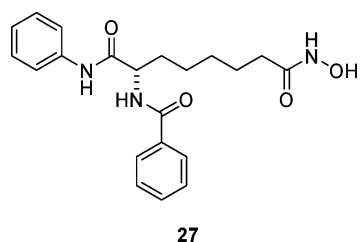
Figure 12.

The oxamflatin–CBHA design has been extended to include CBHA-derived sulfonamides, sulfonylanilides, and ureas (Table 8).[39] Compounds with low nanomolar activity were obtained by substitution on the phenyl ring of **30e**. In particular, the biphenyl compound **31d** was found to be a 10 nM inhibitor. Inversion of the sulfonamide moiety in **31e**–**g** did not affect activity relative to **31a**, **31c**, and **31d**. However, replacement of the sulfonamide with a urea structure resulted in a 20-fold loss in inhibitory activity (**31h** vs **31f**).

Other related agents containing urea-, amide-, and sulfonamide-hydroxamic acids were also recently reported (Table 9).[40] In this study, unsaturated amides (**33a**–**c**) were found to be more active than the corresponding saturated analogues (**32a**–**c**), although this trend was not observed for sulfonamides (**32d** vs **33d**) or ureas (**32d** vs **33d**). The most active compound in this series is 2-naphthoylamide derivative **33a**. Sulfonamide and urea derivatives were shown to be significantly less active than their amide counterparts, and substitution

28

HD-2 IC$_{50}$ = 180 nM

29

HD-2 IC$_{50}$ >40000 nM
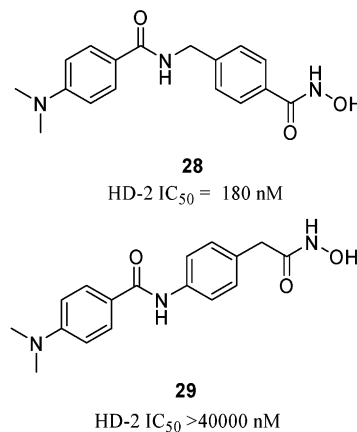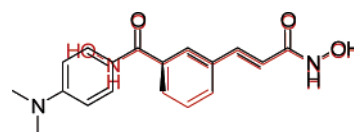
Figure 13.

Figure 14. Isosteric relationship between TSA (**2**, black) and CBHA (**3**, red).

Table 7. Structure and Activity of Sulfonamide Hydroxamates

Compound	Y	R	HDAC1 IC$_{50}$ (μM)	MTT EC$_{50}$ (μM)	H4-Ac EC$_{50}$ (μM)
2 (± TSA)			0.005	1	1
30a	-	H	0.9	2	5
30b	CH$_2$	H	1	22	>25
30c	(CH$_2$)$_2$	H	0.1	2	5
30d	(CH$_2$)$_3$	H	1	8	>25
30e	(vinylene)	H	0.2	3	5
30f	(CH$_3$)	H	2	15	>25
30g	(CH$_3$)	H	17	>50	>25
30h	(i-Pr)	H	>25	30	>25
30i	(vinylene)	CH$_3$	0.6	4	>25

Table 8. SAR of 4-Arylsulfonamidocinnamic Hydroxamic Acids

	Ar	X-Y	HDAC1 IC$_{50}$ (μM)	H4-Ac EC$_{50}$ (μM)
2 (±)	-	-	0.005	4
30e	(phenyl)	SO$_2$NH	0.2	5
31a	(H$_3$C-phenyl)	SO$_2$NH	0.3	10
31b	(H$_3$CO-phenyl)	SO$_2$NH	0.06	5
31c	(H$_3$CO, OCH$_3$-phenyl)	SO$_2$NH	0.09	1
31d	(biphenyl)	SO$_2$NH	0.01	1
31e	(H$_3$C-phenyl)	NHSO$_2$	0.2	1
31f	(H$_3$CO, OCH$_3$-phenyl)	NHSO$_2$	0.05	1
31g	(biphenyl)	NHSO$_2$	0.04	1
31h	(H$_3$CO, OCH$_3$-phenyl)	NHCONH	1	>25

on the phenyl ortho position in compounds **32b** and **33b** reduced HDAC inhibitory activity by more than an order of magnitude relative to that of **32a** and **33a**.
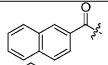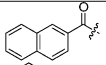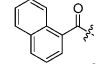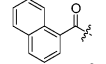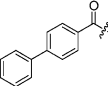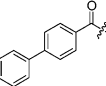
The groups of Massa and Mai reported the design of a series of keto-pyrrole containing CBHA-like hydrox-

amates.[41] These compounds were later found to be micromolar inhibitors of maize HD-2.[42] Inhibitory activity showed little dependence on the nature of the substituents on the phenyl ring (Table 10, compounds **34a**−**g**), and agen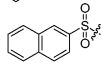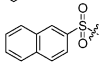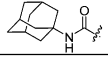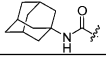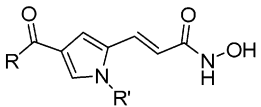ts containing a double bond in the linker domain were found to be more active than the corresponding saturated derivative **34m**. The binding mode of these agents to the HDAC catalytic site was modeled using the TSA−HDLP crystal structure as a starting point.[11,12] The reduction in HDAC inhibitory activity for compounds such as **34a**, relative to SAHA (**1**) and TSA (**2**), was attributed to the nonideal geometry of binding of its hydroxamic acid to the zinc ion. This, in turn, was postulated to be due to the steric hindrance of the *N*-methyl group on the pyrrole. In subsequent work, compound **34h**, in which the benzoyl group on the pyrrole had been replaced by a phenylacetyl, showed a 38-fold increase in enzyme inhibitory activity.[12] The gain in activity was ascribed to the additional flexibility in the surface recognition domain of the inhibitor, which allows overall better contact and an improved binding geometry for the hydroxamate in the active site.
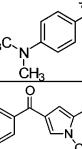
Finally, screening of a compound library yielded CBHA derivative **35** (Figure 15), which was found to inhibit HDAC, induce p21, and inhibit proliferation. A series of agents based on **35** led to **36** (NVP-LAQ824).[43] This compound, which showed activity both in vitro and in vivo, is currently in phase I clinical trials.[44]

Hydroxamic acids constitute the largest class of HDAC inhibitors, with new examples being added at a rapid pace both in the literature and in patent disclosures. These agents are among the most potent HDAC inhibitors, with many examples of low nanomolar activity, and the search for even more potent compounds in

Table 9. Structure and Activity of HDAC Inhibitors: Cyclic Linker Structures

	R	HCT116 IC$_{50}$(nM)	HDAC IC$_{50}$ (nM)		R	HCT116 IC$_{50}$(nM)	HDAC IC$_{50}$ (nM)
32a		6600	2000	**33a**		700	44
32b		90100	-	**33b**		9700	830
32c		4700	310	**33c**		3200	240
32d		9400	1100	**33d**		22500	-
32e		20900	-	**33e**		112500	-
2 (TSA)		-	3.3				

Table 10. SAR of Pyrrole-Containing HDAC Inhibitors

	R	R'	HDAC IC$_{50}$, μM		R	R'	HDAC IC$_{50}$, μM
34a		CH$_3$	3.8 ± 0.15	**34h**		CH$_3$	0.1 ± 0.004
34b		CH$_3$	3.8 ± 1.1	**34i**		CH$_3$	1 ± 0.03
34c		CH$_3$	2.4 ± 0.07	**34j**		H	5 ± 0.15
34d		CH$_3$	3.9 ± 0.19	**34k**		*i*-Pr	53 ± 2.1
34e		CH$_3$	1.9 ± 0.06	**34l**		Ph	110 ± 5.5
34f		CH$_3$	2.9 ± 0.06	**2**	-	-	7.2 ± 0.2 nM
34g		CH$_3$	2.4 ± 0.07	**1**	-	-	50 ± 1.5 nM
34m			36.8 ± 1.1				

this class will presumably continue for the foreseeable future. In addition, in vivo efficacy studies have demonstrated that the hydroxamate class has great therapeutic potential. There are currently two HDAC inhibitors from the hydroxamate class in clinical trials, the most advanced of which is SAHA (phase II).

	35	**36**: NVP-LAQ824
HDAC IC$_{50}$	64 nM	32 nM
HCT116 GI$_{50}$	50 nM	10 nM
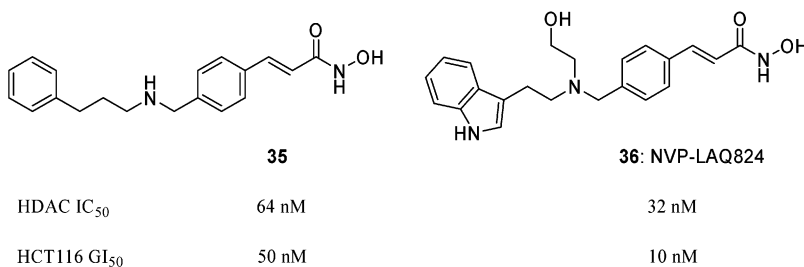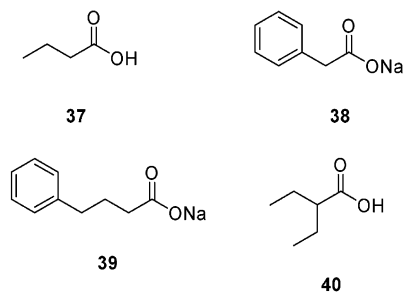
Figure 15.

Figure 16.

3. Carboxylates[45]

The study of the SAR of the carboxylate class, which is defined as possessing a carboxylate in the metal binding domain, has been limited as a result of poor HDAC inhibitory activity. With the notable exception of compound **19t** (Table 3) and a CHAP carboxylic acid analogue (see section 6) corresponding to trapoxin B (IC$_{50}$ = 100 nM), these agents possess millimolar HDAC inhibitory activities. The activity of the CHAP carboxylic acid analogue illustrates the importance of the surface recognition domain to HDAC inhibitory activity. However, most carboxylates reported to date have been limited to simple alkyl chains. Nonetheless, these agents are being studied in the clinic for the treatment of cancer, albeit at doses required to achieve high concentrations of drug. For example, butyric acid (BA, **37**, Figure 16), a natural product generated in man by metabolism of fatty acids and bacterial fermentation of fiber in the colon, has long been known to be an antiproliferative and differentiating agent.[45,46] The first report of anticancer activity of BA on solid tumors in 1933 was followed by the observation that it possesses differentiating activity at high micromolar concentrations on several cancer cell types.[47] It was later determined that BA is a millimolar HDAC inhibitor, and this observation is thought to explain its mechanism of action. Other short-chain fatty acid analogues of BA, such as sodium phenylacetate (**38**), sodium phenylbutyrate (**39**), and the anticonvulsant valproic acid (**40**), have also been identified as antiproliferative agents and HDAC inhibitors.[45,48−50]

Despite poor enzymatic inhibitory activity, a number of carboxylates, including butyric (**37**) and phenylbutyric acid (**39**), are in clinical trials for cancer treatment alone and in combination with other agents.[3,51] A combination of phenylbutyrate and all-trans retinoic acid (ATRA), for example, induced histone hyperacetylation and complete remission in a case of highly resistant promyelocytic leukemia before relapse occurred after 7 months.[2,52]

The high concentration of BA and analogues needed for clinical use, coupled with poor bioavailabilty and rapid metabolic degradation, has led to the exploration of prodrugs such as **41** and **42** (Figure 17).[53] Compound **41**, also known as tributyrin, can deliver 3 equiv of BA upon hydrolysis. Compound **43** is a prodrug of both BA and ATRA. These agents show better absorption and have a more favorable metabolic profile than the corresponding parent compounds.[53]

Overall, the carboxylate class possesses limited HDAC inhibitory activity. Advances in generating carboxylates with improved HDAC inhibitory potency have provided

Table 11. MS-275 Analogues and HDAC Inhibition

compd	R$_1$	R$_2$	R$_3$	R$_4$	HDAC IC$_{50}$ (μM)
44a: MS-275	NH$_2$	H	H	H	4.8
44b	H	H	H	H	>100
44c	H	NH$_2$	H	H	>100
44d	H	H	NH$_2$	H	>100
44e	NHAc	H	H	H	>100
44f	OH	H	H	H	2.2
44g	NH$_2$	CH$_3$	H	H	>100
44h	NH$_2$	H	CH$_3$	H	>100
44i	NH$_2$	H	OCH$_3$	H	44
44j	NH$_2$	H	Cl	H	40
44k	NH$_2$	H	H	CH$_3$	2.8
44l	NH$_2$	H	H	OCH$_3$	4.6
44m	NH$_2$	H	H	Cl	7.7
44n	NH$_2$	H	H	F	6.0
37: sodium butyrate					140
2: TSA					0.0046

important insights into the field of HDAC inhibitor design. However, a great deal of work remains to be done before potent carboxylates can be routinely generated as HDAC inhibitors. Nonetheless, the clinical utility of modestly active constituents of the carboxylate class is under investigation and promising results have been obtained, thereby accentuating the validity of HDAC inhibition as a potential treatment for cancer.

4. Benzamides

The benzamide class, which is generally less potent than the corresponding hydroxamate and cyclic tetrapeptide classes, includes MS-275[54] (**44a**, Table 11) and CI-994[55,56] (**45**, Figure 18). In 1999, Suzuki and coworkers identified MS-275 (**44a**) from a series of synthetic benzamide-based HDAC inhibitors.[54] The SAR of the benzanilide functionality revealed that a 2′-amino
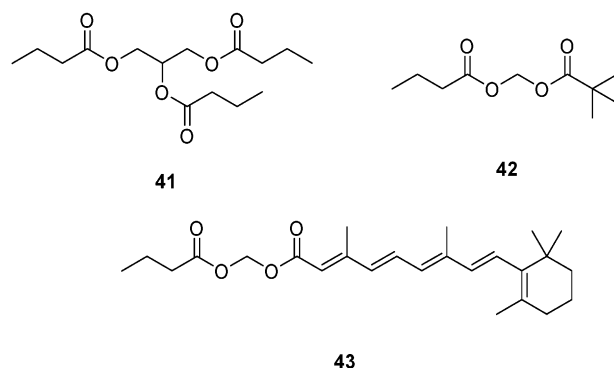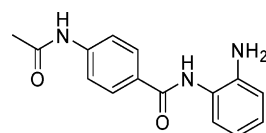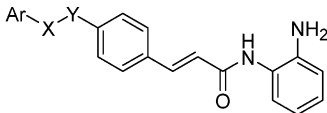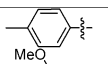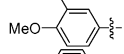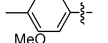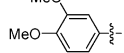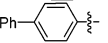

Figure 17.

45: CI-994

Figure 18.

Table 12. SAR of Sulfonamide Anilides[a]

Cpds	Ar	X-Y	HDAC1 IC$_{50}$ (µM)	H4-Ac EC (µM)	HCT116 IC$_{50}$ (µM)	T24 IC$_{50}$ (µM)
46a	(MeO-phenyl)	SO$_2$NH	3	5	4	8
46b	(MeO,MeO-phenyl)	SO$_2$NH	4	10	1	6
46c	(Ph-phenyl)	SO$_2$NH	1	15	15	>36
46d	(MeO-phenyl)	NHSO$_2$	2	2	3.5	6.5
46e	(MeO,MeO-phenyl)	NHSO$_2$	3	3	4	7.5
46f	(Ph-phenyl)	NHSO$_2$	1	5	N.T.	N.T.

[a] N.T. = not tested.

(**44a**) or 2′-hydroxyl (**44f**) moiety is critical for inhibitory activity against partially purified histone deacetylase, as illustrated in Table 11. Removing or repositioning the amino group on the aryl substitutent (**44b**–**d**) or capping with acetate (**44e**) generates inactive agents. Substitution at the 3′, 4′, and 5′ positions attenuated enzymatic activity (**44g**–**j**), apparently because of increased steric interactions, given that electronic contributions of substituents at these positions appeared to have little effect. Additional evaluation of MS-275 (**44a**) demonstrated that it could inhibit partially purified human HDAC preparations and cause hyperacetylation of nuclear histones in various cell lines.[57,58] When administered orally to nude mice implanted with tumor lines, seven out of eight lines were strongly inhibited.[57] Importantly, an inactive MS-275 structural analogue was devoid of both in vitro and in vivo activity, further suggesting that the beneficial biological effects are a result of HDAC inhibition. MS-275 is currently in phase I clinical trials.

CI-994[55] (**45**, Figure 18) is an acetylated derivative of dinaline initially developed as a potential anticonvulsive agent.[56] The mechanism for the antitumor activity of CI-994 is currently unclear but may involve the modulation histone deacetylation, although no information on HDAC inhibitory activity has been published to date. Phase II trials for non-small-cell lung cancer revealed activity, and trials comparing gemcitabine alone to gemcitabine in combination with CI-994 (**45**) have been initiated.[59,60]

A series of CBHA-derived benzamides and the corresponding hydroxamic acid analogues were recently reported.[39,61] The benzamides were found to possess reduced activity in HDAC1 enzymatic inhibition assays and in the induction of histone acetylation in human bladder T24 cancer cells relative to the corresponding hydroxamic acid analogues (Tables 12 and 8), which were ≥10-fold more potent in all cases.[39] Moreover, these agents exhibited reduced antiproliferation activity in HCT116 and T24 cancer cells, with IC$_{50}$ values ranging from 1 to 10 µM, relative to hydroxamic acid analogues, which were active in the 0.2–2 µM range. No significant differences in inhibitory activity were observed on inversion of the sulfonamide functionality (**46a**–**c** vs **46d**–**f**). It is interesting that for the benz-

amides, the IC$_{50}$ values for HDAC inhibition closely resemble the antiproliferation values, whereas the hydroxamic acids are typically 10-fold more active in the enzymatic assay.

The benzamide moiety of MS-275 (**44a**) was also explored in a library of SAHA derivatives (Figure 19). These inhibitors, which were based on a 1,3-dioxane diversity structure, were synthesized on polystyrene beads and assayed for biological activity in a "one bead–one stock solution format".[36] These elegant studies led to the identification of molecules that appear to selectively inhibit HDAC1 over HDAC6 (section 8).[62]

The exact mechanism by which the benzamides exert their antiproliferative effects has not been fully elucidated. However, it has been clearly demonstrated that certain members of the benzamide class possess antitumor activity that correlates with HDAC inhibitory activity, although the nature of this activity appears to differ significantly from that of the small-molecule hydroxamates, as measured by changes in gene expression.[63] Additional unanswered questions include whether benzamide-induced HDAC inhibition occurs at the active site, as demonstrated for hydroxamates, and if so, the exact nature of the benzamide moiety interaction with the active site zinc of the enzyme remains to be characterized. Additional studies will be required in order to understand the mechanistic differences between the benzamide and hydroxamic acid classes with regard to HDAC inhibition.
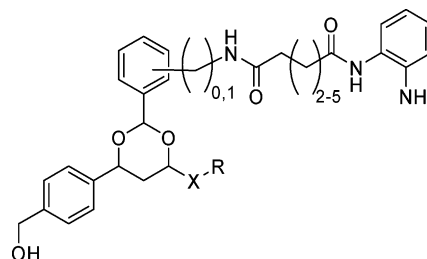
Figure 19.

5. Electrophilic Ketones

Numerous electrophilic ketones are known inhibitors of proteases, including metal-dependent hydrolytic enzymes such as carboxypeptidase A and metallo-β-

Figure 20.

47

Figure 21.

lactamase.[64,65] Studies have demonstrated that the hydrated form of the ketone acts as a transition-state analogue and coordinates the zinc ion in th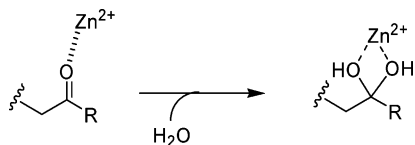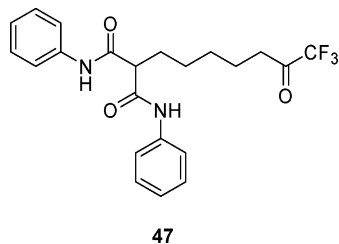e active site of carboxypeptidase A (Figure 20).[64] It seems likely that a similar mechanism is operative for HDAC inhibition by the eletrophilic ketone class.

The first constituent discovered in this newly defined class of HDAC inhibitors was trifluoromethyl ketone **47** (Figure 21).[8,37] The synthesis of various linear molecules based on this structure comprising an aromatic in the surface recognition domain, connected via ether or amide bonds to an aliphatic chain in the linker domain and carrying a trifluoromethyl ketone in the metal binding domain, was recently disclosed (Table 13).[66] These agents were tested against a mixture of HDAC1 and HDAC2 and showed inhibition at low micromolar/high nanomolar concentrations. These agents also possess antiproliferative activities in vitro and induce histone hyperacetylation and p21 gene expression. Both the five- and six-methylene linkers (**48a**,**b**) showed optimal activity in the ether series. Unsaturation (**48d**,**f**), a small ring (**48e**), or heteroatoms such as S and O were also tolerated in the linker. Submicromolar activities against HDAC were achieved with aromatic amide trifluoromethyl ketones (Table 14). Compounds carrying

meta or para substituents were found to be significantly more potent than the corresponding ortho analogues (**49a**−**f**), and an aromatic moiety can be accommodated in the linker. The trifluoromethyl ketone is essential for the inhibitory activity of these compounds. A simple ketone, a trifluoromethylcarbinol and a pentafluoroethyl ketone showed no inhibition up to 50 μM, although some simple ketones in the cyclic peptide class possess potent HDAC inhibitory properties (section 6). The trifluoromethyl ketones studied exhibit short half-lives both in vivo and in vitro by rapid reduction to the corresponding alcohol, which occurs in vitro in the presence of whole blood or cells.

The same group recently presented α-ketoamides as HDAC inhibitors.[67] The compounds of general structure **50** (Figure 22) were chosen as the best candidates among other electrophilic ketones, including various α-ketoacids and ketoesters. Compound **51** is an HDAC inhibitor (IC$_{50}$ = 9 nM) that shows antiproliferative activity in vitro, as well as efficacy in a tumor model in vivo. This series of compounds, as might be expected, was also rapidly metabolized to the corresponding alcohol both in vivo and in vitro in whole blood. The antiproliferative activity and in vivo efficacy of electrophilic ketones, despite their metabolic instability, were rationalized by the hypothesis that a brief exposure to HDAC inhibitors can induce a biological response.[67]
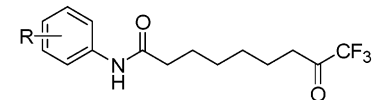
The electrophilic ketone class represents a new design that has not yet been fully exploited. Current unmet challenges include the need to develop additional structural variants in order to assess optimal design parameters and tolerances, as well as the application of the wealth of information available about the linker and surface recognition domains from other structural classes to the electrophilic ketone class. Nonetheless, the electrophilic ketone class contains potent inhibitors of HDAC that, despite apparent poor stability, possess antitumor effects in animal models.
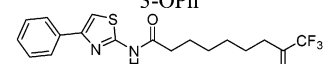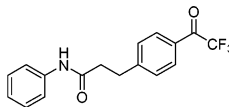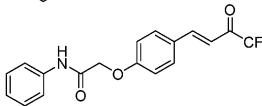
6. Cyclic Peptide Inhibitors

Cyclic peptide containing HDAC inhibitors, which constitute the most structurally complex class of HDAC

Table 13. Activity of Ether Trifluoromethyl Ketones

	X	HDAC IC$_{50}$(μM)	MDA 435 IC$_{50}$(μM)	HT 1080 IC$_{50}$(μM)
48a	-CH$_2$-	2.6	-	13
48b	-CH$_2$CH$_2$-	2.9	30	20
48c	-CH$_2$CH$_2$CH$_2$-	72% @ 50	-	21
48d		5.0	-	-
48e		2.9	-	-
48f		8.6	>50	>50
48g		>50	-	-

Table 14. Activity of Amide Trifluoromethyl Ketones

	R	HDAC IC$_{50}$(μM)	MDA 435 IC$_{50}$(μM)	HT 1080 IC$_{50}$(μM)
49a	H	6.7	15	2.2
49b	4-Ph	3.0	12	7.0
49c	3-Ph	0.38	23	16
49d	2-Ph	16	-	-
49e	4-OPh	0.30	15	12
49f	3-OPh	3.2	-	-
49g		0.31	3.4	4.2
49h		>50	-	-
49i		2.2	-	-

inhibitors, are herein divided into two subclasses: inhibitors bearing (*S*)-2-amino-9,10-epoxy-8-oxodecanoic acid (L-Aoe), which contains an epoxy ketone, and inhibitors without the L-Aoe moiety. All of the agents from each subclass conform to the model pharmacophore for HDAC inhibitors (Figure 4) and possess a macrocycle containing hydrophobic amino acids in the surface recognition domain, an alkyl chain in the linker domain, and a functional group in the metal binding domain. Constituents of the cyclic peptide class typically possess nanomolar levels of HDAC inhibitory activity; however, their general utility in the treatment of cancer remains largely unproven, with the possible exception of FK-228 (**52**, Figure 23, aka depsipeptide).[68,69]

It is believed that cyclic peptide HDAC inhibitors bind to the HDAC enzyme in a manner consistent with the X-ray crystallographic findings with hydroxamates (Figures 1 and 3). By this model, the aliphatic linker passes

50

51
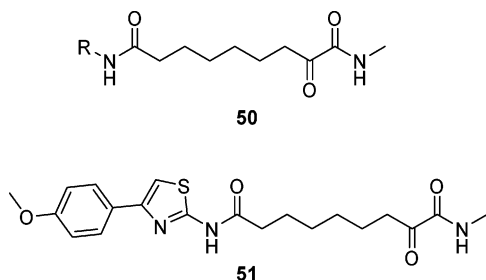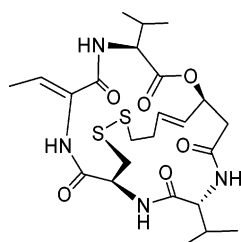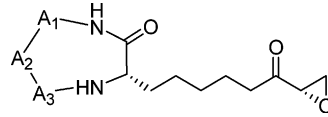
Figure 22.

52: FK228

Figure 23.

down the enzyme's tubelike channel, positioning the binding moiety in proximity to the active site zinc, while the macrocyclic portion binds to the rim of the active site. However, the mechanism of action differs between the Aoe-containing inhibitors, which are typically classified as irreversible HDAC inhibitors, and those without the Aoe moiety, which are reversible inhibitors. However, the irreversible nature of Aoe-containing inhibitors is still in question and mechanistic possibilities include both direct covalent modification via nucleophilic attack on the reactive epoxy ketone in the active site and noncovalent binding with slow dissociation of the inhibitor from the active site. [5,70]

The use of X-ray crystallographic and NMR techniques has led to a better understanding of the spatial orientations of the cyclic tetrapeptide macrocycle found in several natural products as discussed in a recent review by Meinke.[5] The macrocycle in these agents is arranged with a D-amino acid and a cyclic amino acid (Pro or Pip) flanking the amino acid bearing the linker moiety, which generates a constrained 12-membered cyclic structure with extensive internal hydrogen bonding. It is postulated that the D-configuration of the amino acid is necessary for tight association with the rim of the active site, thereby allowing the linker to align and insert into the enzyme's tubelike channel.[71]

The first examples of the cyclic peptide class arose from screening natural products for antiparasitic or antiproliferation activity, with the Aoe-containing cyclic peptides being the first subclass isolated. These compounds include HC-toxins (**53**),[72–75] Cyl-1/2 (**54**, **55**),[76–78] WF-3161 (**56**),[79,80] trapoxin A/B (**57**, **58**),[81] and chlamydocin (**59**)[82] (Table 15). The isosteric nature of the Aoe side chain with acetylated lysine suggests it mimics the natural substrate.

Some Aoe-containing inhibitors appear to operate exclusively by an irreversible mechanism and require the epoxy ketone functionality for their effects. For example, the 9,10-diol analogue of HC-toxin (**53**) is devoid of activity.[83] Reduction of the C8-ketone of chlamydocin (**59**) to the alcohol or replacement of the

Table 15. Aoe-Containing Cyclic Tetrapeptide HDAC Inhibitors

compd		A_1	A_2	A_3
53	HC-toxin I	D-Pro	L-Ala	D-Ala
54	Cyc-1	D-Tyr(OMe)	L-Ile	L-Pro
55	Cyc-2	D-Tyr(OMe)	L-Ile	L-Pip
56	WF-3161	D-Phe	L-Leu	L-Pip
57	trapoxin A	L-Phe	L-Phe	D-Pip
58	trapoxin B	L-Phe	L-Phe	D-Pro
59	chlamydocin A	Aib	L-Phe	D-Pro

epoxide with $CH_2=CH_2$, CH_2CH_3, or CH_2CH_2OH leads to reduced HDAC inhibitory activity.[82]
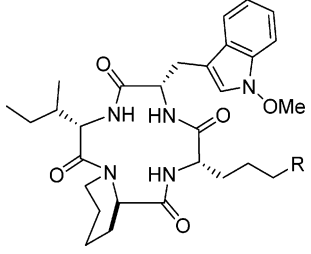
Building on the hypothesis that trapoxin B (**58**, Table 15) exhibits irreversible inhibition via reaction of the epoxy ketone with the target enzyme, Schreiber and co-workers synthesized trapoxin analogues (Figure 24) to identify and characterize its receptor.[84,85] Initially, tritiated and iodinated versions of trapoxin (**60** and **61**, respectively) were synthesized and presented to crude nuclear extracts of bovine thymus. Irreversible inhibition of HDAC activity apparently occurred with both

compounds, but attempted autoradiography visualization of covalently modified proteins failed. It was speculated that the epoxide undergoes nucleophilic attack, generating an intermediate prone to β-elimination or ring closure under denaturing conditions. Subsequently, an affinity matrix derived trapoxin analogue, **62**, with in vitro HDAC inhibitory levels comparable to those of **60** and **61**, was used to isolate a novel protein named HD1 (HDAC1) using affinity chromatography. Moreover, **62** was shown to retain HDAC2 and HDAC3 proteins as well.[86]

Within the subclass of cyclic tetrapeptides classified as reversible inhibitors are active variants of the Aoe-containing natural products lacking the epoxy ketone functionality (Figure 25). These agents demonstrate that the potentially reactive epoxy ketone moiety is not required for HDAC inhibition. Apicidin (**63**), a fungal metabolite, contains an (*S*)-2-amino-8-oxodecanyl side chain lacking an epoxide.[87] It has broad spectrum activity (ranging from 4 to 125 ng/mL) against the apicomplexan family of parasites, presumably via inhibition of protozoan histone deacetylases ($IC_{50} = 1-2$ nM), and demonstrates efficacy against *Plasmodium berghei* malaria in mice.[88] Other fungal metabolites devoid of the reactive epoxy ketone have been charac-

58: Trapoxin B; R = H
60: [³H]Trapoxin B; R = ³H

61: R_1 = ... ; R_2 = Ph

62: R_1 = Ph; R_2 = ...

Figure 24.

63: Apicidin

64: FR-225497

65: 9,10-Desepoxy-9-hydroxy-chlamydocin; $R_1 = R_2$ = H
66: $R_1 = R_2$ = Me

67: TAN-1746s
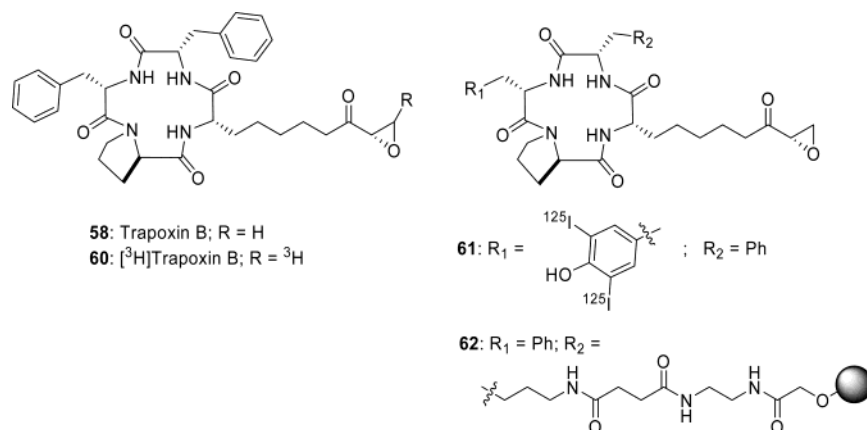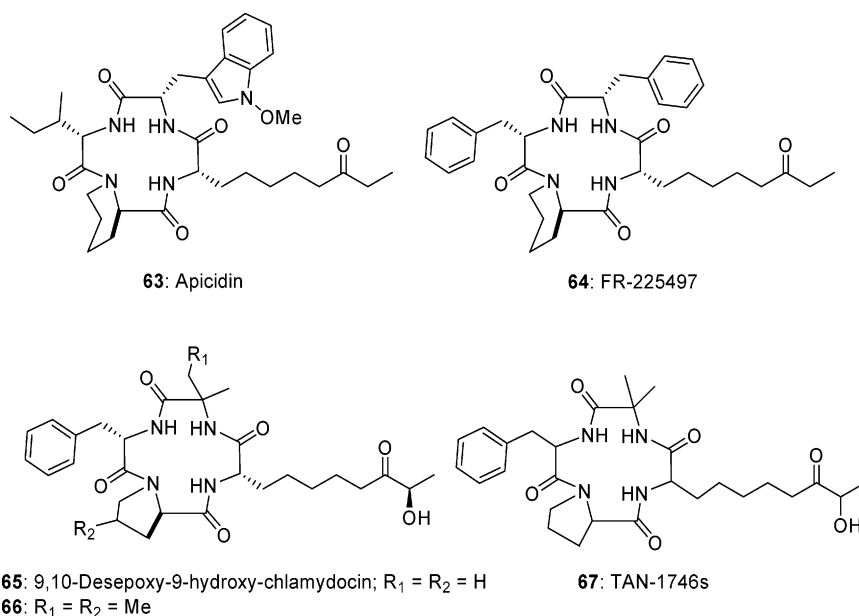
Figure 25.

Table 16. Side Chain Analogues of Apicidin[a]

compd	R	HeLa IC$_{50}$ (nM)	*E. tenella* IC$_{50}$ (nM)	*P. falciparum* IC$_{50}$ (ng/mL)
		HDAC		
68a	$-CH_2CH_2C(O)Et$	1	1	58
68b	$-CH_2CH_2C(O)OMe$	0.4	0.8	210
68c	$-CH_2CH_2C(O)OH$	15	30	>1000
68d	$-CH_2CH_2C(O)CH(OH)Me$	0.3	4	22
68e	$-CH_2CH_2C(O)NHOH$	0.2	4	0.24
68f	$-CH_2CH_2C(O)-$oxirane	<0.1	1	<15
68g	$-CH_2C(O)OMe$	74	N.T.	>1000
68h	$-CH_2CH_2CH_2C(O)OMe$	7	N.T.	500
68i	$-CH_2CH_2C(O)CH(F)Me$	1	N.T.	28
68j	$-CH_2CH(F)C(O)CH_2Me$	110	N.T.	700

[a] N.T. = not tested.

terized, including numerous apicidin analogues,[89,90] desepoxy-trapoxin analogue **64**,[91] and structurally related chlamydocin analogues **65**,[92] **66**,[93] and **67**.[94] Compounds **64** and **66** were characterized in vitro using antiproliferation assays with human Jurkat and HT-29 tumor cells lines. Compound **64** exhibited IC$_{50}$ values of 152 and 158 ng/mL, respectively, and **66** exhibited IC$_{50}$ values of 11 and 14 ng/mL, respectively, in these assays.

A systematic study into side chain derivatives of apicidin revealed several picomolar HDAC inhibitors.[95] These derivatives were tested for HDAC inhibition using partially purified extracts of both human HeLa cells and *Eimeria tenella* protozoa. Selected HDAC and in vitro malaria functional assay data are shown in Table 16. The C-8-ketone of apicidin was apparently necessary for HDAC activity. Reduction to epimeric

alcohols, formation of olefin or epoxide, or conversion to CH_2 depressed activity (data not shown). The relevance of the precise register of side chain functionality was evident (**68b** vs **68g** and **68h**), and external C9 substitution was more tolerated than C7 internal substitution (**68i** vs **68j**). The α-hydroxy ketone (**68d**), hydroxamic acid (**68e**), and epimeric epoxide (**68f**) showed improved HDAC activity over apicidin. The hydroxamic acid analogue (**68e**) was found to be >240-fold more active than apidicin in the in vitro malaria assay. Methyl ester **68b** was also found to be active, illustrating the importance of the macrocyclic tetrapeptide.
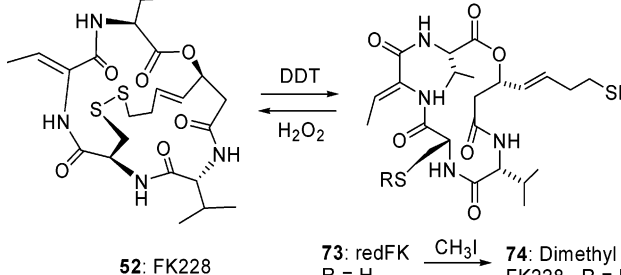
Potent HDAC inhibitors maintaining a tetrapeptide macrocycle and linear linker, but incorporating the known zinc chelating ability of hydroxamic acids, have been explored extensively by Yoshida and co-workers.[71,96] These synthetic hybrids of the hydroxamate and cyclic tetrapeptide classes, termed CHAPs, resulted in reversible inhibitors of HDAC1 that are active at low nanomolar concentrations. Studies of chain length between the macrocycle and the hydroxamic acid revealed that the optimal linker for HDAC1 inhibitory activity was five-methylene units, relative to four- and seven-methylene units. It is interesting to note that a CHAP carboxylic acid analogue corresponding to trapoxin B had significant activity (IC$_{50}$ = 100 nM).

During the optimization of the CHAP molecules, Komatsu et al. explored combinations of chiral amino acids in hybrids of TPX A/B and Cyl-1/2.[71,96] These agents were evaluated for the inhibition of partially purified mouse HDAC, growth inhibition of B16/BL6 cells, and the ability to cause a 2-fold increase in the expression of MHC class-I in B16/BL6 melanoma cells (Table 17). The inhibitory activity of the LDLD, LLLD, and LDLL combinations were similar and were 2 orders of magnitude greater than LLDL. However, MHC class I expression and growth inhibition data revealed a trend among the various combinations. For TPX type, the order of activity was LDLD > LLLD > LDLL ≫ LLDL, and for Cyl-1/2 type, the order was LDLD > LDLL. The

Table 17. CHAP Analogues Based on Sequence of Cyclo-(L-Asu(NHOH)-A-B-C)[a]

compd	analogue	configuration	A-B-C	HDAC IC$_{50}$ (nM)	MHC C$_{X2}$ (nM)	GI IC$_{50}$ (nM)
	TPX B type					
69a	CHAP1	LLLD	L-Phe-L-Phe-D-Pro	6.03 ± 1.51	98.2 ± 23.3	257 ± 35
69b	CHAP27	LDLD	D-Phe-L-Phe-D-Pro	3.44 ± 0.63	3.01 ± 1.26	18.0 ± 2.5
69c	CHAP38	LDLL	D-Phe-L-Phe-L-Pro	5.32 ± 1.64	558 ± 97	3757 ± 1165
69d	CHAP39	LLDL	L-Phe-D-Phe-L-Pro	226 ± 63	65 800 ± 6050	>100 000
69e	CHAP131	LDLD	D-Phe-L-Pro-D-Phe	91.6 ± 57.7	6740 ± 1780	N.T.
69f	CHAP132	LDLD	D-Pro-L-Phe-D-Phe	25.5 ± 18.2	323 ± 55	N.T.
69g	CHAP80	LLLD	L-Ala-L-Phe-D-Pro	1.95 ± 0.90	325 ± 10	N.T.
69h	CHAP88	LDLD	D-Ala-L-Phe-D-Pro	3.23 ± 0.87	75.5 ± 9.6	N.T.
69i	CHAP45	LDLD	D-Phe-L-Ala-D-Pro	1.69 ± 0.54	23.1 ± 3.5	N.T.
	TPX A type					
70a	CHAP57	LDLD	D-Phe-L-Phe-D-Pip	2.91 ± 0.81	2.75 ± 0.54	33.7 ± 17.6
70b	CHAP56	LLLD	L-Phe-L-Phe-D-Pip	4.78 ± 0.49	29.9 ± 5.1	89.0 ± 13.0
70c	CHAP58	LDLL	D-Phe-L-Phe-L-Pip	4.18 ± 1.37	250 ± 48	1048 ± 265
	Cyl-1 type					
71a	CHAP30	LDLL	D-Tyr(Me)-L-Ile-L-Pro	3.31 ± 0.32	16.9 ± 8.2	112 ± 3
71b	CHAP31	LDLD	D-Tyr(Me)-L-Ile-D-Pro	3.32 ± 1.47	1.41 ± 0.51	5.43 ± 0.39
71c	CHAP44	LDLD	D-Tyr(Me)-L-Ala-D-Pro	3.38 ± 0.34	59.6 ± 10.9	N.T.
71d	CHAP77	LDLD	D-Tyr-L-Ile-D-Pro	3.38 ± 0.34	59.6 ± 10.9	N.T.
	Cyl-2 type					
72a	CHAP50	LDLD	D-Tyr(Me)-L-Ile-D-Pip	3.96 ± 1.17	1.41 ± 0.44	5.44 ± 0.37
72b	CHAP49	LDLL	D-Tyr(Me)-L-Ile-L-Pip	4.81 ± 1.71	5.30 ± 2.16	65.9 ± 14.1

[a] N.T. = not tested.

Table 18. HDAC Activity of FK228 and Analogues

52: FK228   **73**: redFK, R = H   **74**: Dimethyl FK228, R = Me

compd	IC$_{50}$ ± SD (nM)				
	HDAC1	HDAC2	HDAC4	HDAC6	HDAC1 (C151S)
52: FK228	36 ± 16	47 ± 18	510 ± 340	14000 ± 3100	280 ± 75
73: redFK	1.6 ± 0.9	3.9 ± 2.7	25 ± 7.3	790 ± 110	57 ± 12
74: dimethyl FK228	6300 ± 1900	3300 ± 1000	110 000 ± 53 000	>100 000	>100 000

increased activity of the various configurations was attributed to higher membrane permeability of the isomers with greater hydrophobicity, as indicated from their longer HPLC retention times. Consistent with other cyclic tetrapeptides, the amino acid bearing the linker and metal binding domains is flanked by a Pip or Pro for optimal HDAC inhibitory activity. Proline in the fourth position (**69b**) is best for cell-free HDAC inhibition and cellular MHC class I expression compared with the third (**69e**) and second positions (**69f**). Positions 2 and 3 are best occupied with hydrophobic L- and D-amino acids for improved MHC-inducing ability. However, a significant influence on HDAC inhibitory activity is not observed in this regard except for **69a** vs **69g** and **69b** vs **69i**, where activity is reduced only 2- to 3-fold.

Other studies investigating the biological effects of synthetically modified macrocyclics have been performed.[97,98] In their efforts to identify parasite-selective HDAC inhibitors, Merck researchers generated indole-modified apidicin analogues possessing picomolar enzyme activity, as well as tryptophan-replacement analogues displaying 20- to 100-fold parasite selectivity.[98]

FK228 (**52**, Table 18), which was discovered from a fermentation broth of *Chromobacterium violaceum*,[99,100] differs structurally from other constituents of the cyclic peptide class. FK228 contains a unique bicyclic structure with four amino acids and a β-hydroxyamide moiety, which collectively form a 16-membered lactone with a disulfide bridge. FK228 is currently the only member of the cyclic peptide class under clinical investigation.[68,69]

Furumai and co-workers have investigated the long-standing question of FK228's mode of action.[101] It has been postulated that the disulfide bond is reduced in the cellular environment, releasing the free thiol analogue as the active species. To address this issue, FK228 (**52**) was converted to its reduced form, redFK (**73**), in the presence of DDT and evaluated for its inhibitory activity against various HDACs prepared from 293T cells (Table 18). The reduction of the intramolecular disulfide bond of FK228 greatly enhanced its inhibitory activity (**73** vs **52**). This reduction was shown to occur in the cellular environment via participation of glutathione. HDAC activity is significantly lowered after capping of the two sulfhydryl groups via methylation
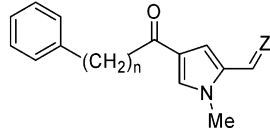
(**74**), lending further support to the active species of FK228 being **73**. RedFK (**73**) is more active against HDAC1 and HDAC2 compared to HDAC4 and HDAC6. HDAC1 is reversibly inhibited by **73**, and a cysteine to serine HDAC1 mutant is still sensitive to **73**, suggesting the **73** is not covalently bound to the conserved Cys-151 in the active site. Computer modeling suggests that the thiol of the four-carbon side chain passes down the HDAC tubelike channel toward the active site residues and interacts with the zinc via a water molecule. Therefore, FK228 (**52**) acts as a potential prodrug by entering the cell and then being reduced in the cellular environment to **73**, generating the sulfhydryl groups responsible for inhibiting HDAC activity.
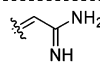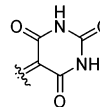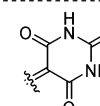
Cyclic peptides comprise a diverse class of HDAC inhibitors possessing modes of enzyme inactivation ranging from reversible to irreversible inhibition. This class of HDAC inhibitors has aided in the understanding of the factors governing HDAC inhibitor activity and selectivity. Further investigations probing binding interactions on the outer rim of the HDAC enzymes will hopefully lead to a greater understanding of essential inhibitor−enzyme contacts and ultimately to the identification of selective inhibitors with more druglike qualities.
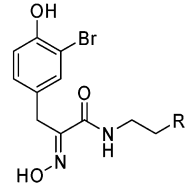
7. Miscellaneous
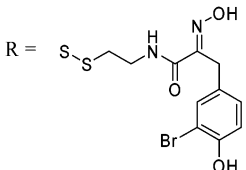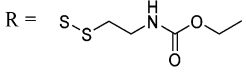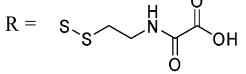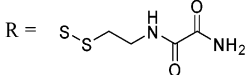
There is a growing number of HDAC inhibitors that do not fit the standardized structural classifications adopted in this review and by others.[5,7,8] Without exception, these agents possess some or all of the predicted structural subunits required for HDAC inhibi-

75: Depudecin

76: Radicicol
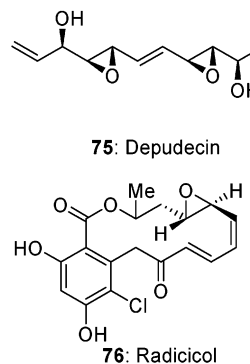
Figure 26.

Table 19. Alternative Metal Binding Groups

	Z =	n =	IC$_{50}$ (μM)
77a	(nitrile)	1	0% at 30 (μM)
77b		0	70
77c	(amidine NH$_2$, NH)	0	23
77d	(barbiturate)	0	0% at 30 (μM)
77e		1	104
77f	(thiobarbiturate)	0	0% at 30 (μM)
77g		1	85

Table 20. Psammaplins

ID	Psammaplin		IC$_{50}$(nM)
78a	A	R = (oxime)	~4
78b	D	R = (carbamate, methyl)	~44
78c	H	R = (carbamate, ethyl)	~79
78d	F	R = (oxalic acid, OH)	~2
78e	E	R = (oxalic acid, NH$_2$)	~330

tory activity and therefore adhere to the model pharmacophore for HDAC inhibitors (Figure 4).

Depudecin (**75**, Figure 26) was shown to inhibit HDAC in 1998.[102] This study further demonstrated that another epoxide (**76**) with similar biological properties did not possess HDAC inhibitory activity.

A recent study reported the identification of alternative metal binding domains linked to CBHA-like scaffolds (Table 19).[12] These agents have metal chelators that include nitrile (**77a** and **77b**), amidine (**77c**), barbiturate (**77d** and **77e**), and thiobarbiturate (**77f** and

77g) functional groups. The best of these agents is active at low micromolar concentrations.

Another recent report disclosed naturally occurring agents with unique metal binding domains and structures. These agents possess nanomolar inhibitory potencies against isolated HDAC enzyme preparations (Table 20).[103] Active groups include an oxime derivative (**78a**), carbamates (**78b**,**c**), and oxalic acid derivatives (**78d**,**e**). These agents further demonstrate the wealth of structural diversity tolerated in the HDAC active site.

8. Selectivity

Because the class I/II family of mammalian HDAC enzymes currently includes 11 constituents, exclusive of splice variants, and are thought to have differential functional and spatial disposition within biological systems, a great deal of focus has recently been put on selectively targeting individual HDAC enzymes. These efforts have led to the identification of several classes of agents that exhibit differential inhibition of specific HDAC enzymes. Recent work by Yoshida demonstrated that even relatively simple structures, as well as more complex cyclic tetrapeptide-CHAP compounds, differentially inhibit HDAC1 over HDAC6 in partially purified enzyme preparations.[104] For example, butyrate (**37**) and MS-275 (**44a**) possess ~50-fold and 40000-fold

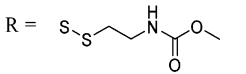**Figure 27.** HDAC sequence alignments of the HDLP crystal structure regions: (a) active site; (b) channel; (c) rim. HDAC sequence alignments are color-coded to correspond to regions in the HDLP crystal structure (Figure 1) and the HDAC inhibitor model pharmacophore domains (Figure 4).

Table 21. Selective Inhibition

compd	structure	analogue	HDAC6 IC$_{50}$/HDAC1 IC$_{50}$
2 (TSA)			1.4
44a (MS-275)			40000
37 (butyrate)			59
57	cyclo(Aoe-Phe-Phe-D-Pip)		640
58	cyclo(Aoe-Phe-Phe-D-Pro)		3300
59	cyclo(Aoe-Aib-Phe-D-Pro)		7300
55	cyclo(Aoe-D-Tyr(Me)-Ile-Pip)		57000
70b	cyclo(Asu(NHOH)-Phe-Phe-D-Pip)	TPX A	25
69a	cyclo(Asu(NHOH)-Phe-Phe-D-Pro)	TPX B	10
72b	cyclo(Asu(NHOH)-D-Tyr(Me)-Ile-Pip)	Cyl-2	30
79	cyclo(Asu(NHOH)-Aib-Phe-D-Pro)	chlamydocin	86
52	FK-228		389
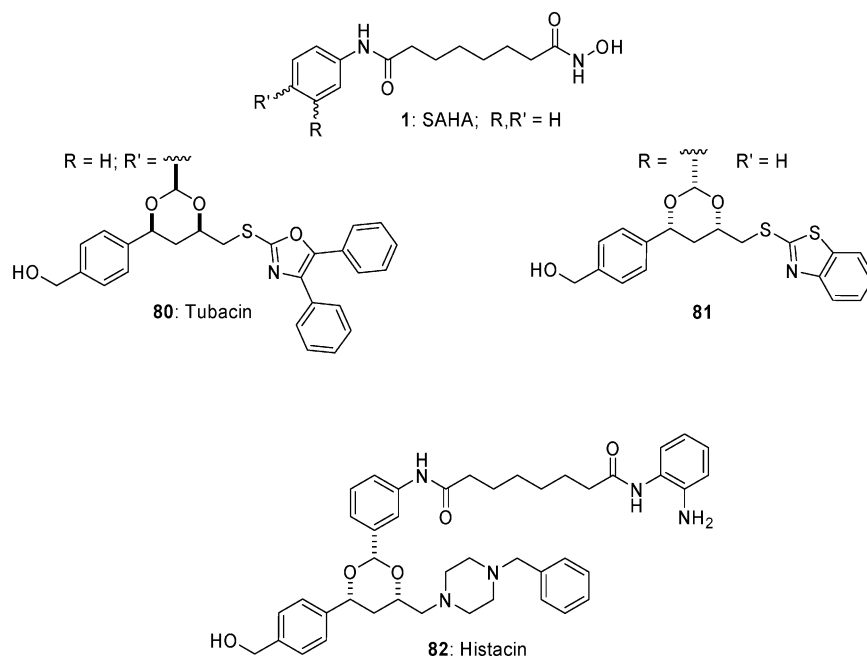
Figure 28.

differential activities, respectively, while TSA (**2**) exhibited little or no preference (Table 21). These observations can be considered in light of sequence alignments of the mammalian HDAC enzymes (Figure 27a,b), which demonstrate that residues in the channel and active site domains are not completely conserved. In addition, given sequence differences and available structural information, the interior space available within each HDAC active site is expected to vary.

In 2001, Yoshida et al. reported the differential inhibitory activities of a series of CHAP compounds and a number of natural products for class I/II HDACs (Table 21).[96] This work demonstrated for the first time that the surface recognition domain of the pharmacophore (Figure 4) affects not only inhibitory potency but selectivity as well. Unlike the simpler, less potent agents butyrate (**37**) and MS-275 (**44a**), all of the agents reported are nanomolar inhibitors of HDAC1. Although only modest levels of difference were seen for HDAC1 (class I) relative to HDAC4 (class II), a 57000-fold differential was observed for HDAC1 (class I) over HDAC6 (class II) for the natural product Cyl-2 (**55**). Compounds **70b**, **69a**, **72b**, and **79** (Table 21), which are hydroxamic acid variants of the natural products TPX A, TPX B, Cyl-2, and chlamydocin, respectively, also possess lower IC$_{50}$ values for HDAC1 over HDAC6, although less strikingly. Similarly, FK228 (**52**) has been

shown to differentially inhibit HDAC1 over HDAC6 by ∼400-fold. FK228 also shows a more modest 14-fold preference for the inhibition of HDAC1 over HDAC4.

The structural diversity on the periphery of the catalytic site (Figure 27c) provides a rationale for the selectivity results of the CHAP compounds and has led Schreiber et al. to synthesize a series of SAHA analogues.[36] One of these agents appears to favor the inhibition of HDAC6 over HDAC1 (Figure 28, **80**), as evidenced by cellular assays.[105] However, related analogue **81** does not discriminate between HDAC1 and HDAC6 in enzyme assays. These workers have also extended earlier findings with MS-275 (**44a**)[104] by creating a SAHA−benzamide hybrid that appears to inhibit HDAC1 over HDAC6 in cellular assays (Figure 28, **82**).[62]

The ability to selectively inhibit single HDAC enzymes is currently a major focus of HDAC inhibitor design. This approach may be an avenue by which improved agents are identified and specific diseases can be targeted. However, only modest progress toward selective inhibitor design has been made to date and much work remains to be done. Nonetheless, the current structures that exhibit differential HDAC inhibitory activities available in the literature provide not only lead structures on which to build but also important tools for the elucidation of HDAC function.

Conclusion

Early HDAC inhibitor lead structures have provided effective platforms for further optimization, created design paradigms that have afforded HDAC inhibitors with subnanomolar enzyme inhibitory activities, and led to the creation of an expansive group of agents that target class I/II HDACs. Most structures that inhibit the activity class I/II HDAC enzymes can be divided into distinct structural classes including small-molecule hydroxamates, carboxylates, benzamides, electrophilic ketones, and cyclic peptides. Despite the variety of structural characteristics, all HDAC inhibitors can be broadly characterized by a common pharmacophore.

HDAC inhibitors have been shown to possess diverse biological activities and can induce differentiation, growth arrest, and/or apoptosis in transformed cell cultures. In vivo xenograft studies have demonstrated many of these agents to be effective in the inhibition of tumor growth. On the basis of these results, HDAC inhibitors from four of the major inhibitor structural classes that target class I/II HDACs have been promoted to clinical trials. The most advanced agents include constituents from the small-molecule hydroxamate (SAHA, phase II) and cyclic peptide (FK-228, phase II) structural classes. Promising results from these studies will continue to drive the exploration of HDAC function and inhibitor design.

Biographies

Thomas A. Miller is the Director of Chemistry and Research Operations at Aton Pharma, Inc. After receiving an undergraduate degree in chemistry from Indiana University Southeast, he moved to Charlottesville, VA, to study with Professor and Chairman Timothy L. Macdonald. While at the University of Virginia, he obtained his Ph.D. in bioorganic chemistry, authored numerous publications, patents, and patent applications and was recognized as a University of Virginia Inventor of the Year. In 1998, he moved to Columbia University to work with Professor Ronald Breslow, where he began contributing to the Patent Estate of Aton Pharma, Inc. In 2001, he joined Aton Pharma as Director of Chemistry and began directing the growth and productivity of the company's discovery and development chemistry programs.

David J. Witter originates from Kitchener, Ontario, Canada, and in 1989 received his B.S. in chemistry from the University of Waterloo. He entered graduate studies in bioorganic chemistry with Professor John C. Vederas at the University of Alberta, Canada, and in 1994, he obtained his Ph.D. degree. He moved to Salt Lake City, UT, for a 2-year postdoctoral fellowship at the University of Utah with C. Dale Poulter, and in 1996, he joined Cadus Pharmaceutical Corp. in New York, investigating signal transduction pathways, including those involved in asthma and inflammation. He moved to OSI Pharmaceuticals in 1999 and studied small-molecule therapeutics for diabetes. Since 2002, he has worked as a Principal Scientist at Aton Pharma Inc., exploring the arena of chromatin remodeling.

Sandro Belvedere received his undergraduate education in chemistry at the University of Rome "La Sapienza" (cum laude). In 1996, he began pursuing graduate studies as a Sherman Clarke Fellow in the laboratory of Professor E. Kool. In 1997, after having received his M.S. from Rochester, he joined Professor R. Breslow at Columbia University and continued his studies in bioorganic and biomimetic chemistry and began contributing to the patent estate of Aton Pharma, Inc. He has coauthored several publications, patents, and patent applications, was awarded the first Arun Guthikonda Memorial Fellowship, and received his Ph. D. "with distinction" in 2001. He had worked in the chemistry group at Aton Pharma since 2001 as a Scientist, exploring the arena of chromatin remodeling.

References

(1) Marks, P. A.; Rifkind, R. A.; Richon, V. M.; Breslow, R.; Miller, T.; Kelly, W. K. Histone deacetylases and cancer: causes and therapies. *Nat. Rev. Cancer* **2001**, *1*, 194−202.

(2) Kelly, W. K.; O'Connor, O. A.; Marks, P. A. Histone deacetylase inhibitors: from target to clinical trials. *Expert Opin. Invest. Drugs* **2002**, *11*, 1695−1713.

(3) Marks, P. A.; Richon, V. M.; Breslow, R.; Rifkind, R. A. Histone deacetylase inhibitors as new cancer drugs. *Curr. Opin. Oncol.* **2001**, *13*, 477−483.

(4) Marks, P. A.; Rifkind, R. A.; Richon, V. M.; Breslow, R. Inhibitors of histone deacetylase are potentially effective anticancer agents. *Clin. Cancer Res.* **2001**, *7*, 759−760.

(5) Meinke, P. T.; Liberator, P. Histone deacetylase: a target for antiproliferative and antiprotozoal agents. *Curr. Med. Chem.* **2001**, *8*, 211−235.

(6) Grozinger, C. M.; Schreiber, S. L. Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. *Chem. Biol.* **2002**, *9*, 3−16.

(7) Jung, M. Inhibitors of histone deacetylase as new anticancer agents. *Curr. Med. Chem.* **2001**, *8*, 1505−1511.

(8) Curtin, M. L. Current patent status of histone deacetylase inhibitors. *Expert Opin. Ther. Pat.* **2002**, *12*, 1375−1384.

(9) Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; et al. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* **1999**, *401*, 188−193.

(10) Park, H.-J. Y. J.; Kim, Y. K.; Han, J.-W.; Lee, H.-W. Discovery of novel histone deacetylase inhibitors by molecular docking and database screening. MEDI-120 In *Abstracts of Papers*, 224th National Meeting of the American Chemical Society, Boston, MA; American Chemical Society: Washington, DC, 2002 (CODEN: 69CZPZ Conference, meeting abstract written in English).

(11) Mai, A.; Massa, S.; Ragno, R.; Esposito, M.; Sbardella, G.; et al. Binding mode analysis of 3-(4-benzoyl-1-methyl-1*H*-2-pyrrolyl)-*N*-hydroxy-2-propenamide: a new synthetic histone deacetylase inhibitor inducing histone hyperacetylation, growth inhibition, and terminal cell differentiation. *J. Med. Chem.* **2002**, *45*, 1778−1784.

(12) Mai, A.; Massa, S.; Ragno, R.; Cerbara, I.; Jesacher, F.; et al. 3-(4-Aroyl-1-methyl-1*H*-2-pyrrolyl)-*N*-hydroxy-2-alkylamides as a new class of synthetic histone deacetylase inhibitors. 1. Design, synthesis, biological evaluation, and binding mode studies performed through three different docking procedures. *J. Med. Chem.* **2003**, *46*, 512−524.

(13) Remiszewski, S. W.; Sambucetti, L. C.; Atadja, P.; Bair, K. W.; Cornell, W. D.; et al. Inhibitors of human histone deacetylase: synthesis and enzyme and cellular activity of straight chain hydroxamates. *J. Med. Chem.* **2002**, *45*, 753−757.

(14) Van Ommeslaeghe, K. E. G.; Brecx, V.; Papeleu, P.; Iterbeke, K.; Geerlings, P.; Tourwe, D.; Rogiers, V. Amide analogues of TSA: synthesis, binding mode analysis and HDAC inhibition. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1861−1864.

(15) Tsuji, N.; Kobayashi, M.; Nagashima, K.; Wakisaka, Y.; Koizumi, K. A new antifungal antibiotic, trichostatin. *J. Antibiot.* **1976**, *29*, 1−6.

(16) Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J. Biol. Chem.* **1990**, *265*, 17174−17179.

(17) Yoshida, M.; Hoshikawa, Y.; Koseki, K.; Mori, K.; Beppu, T. Structural specificity for biological activity of trichostatin A, a specific inhibitor of mammalian cell cycle with potent differentiation-inducing activity in Friend leukemia cells. *J. Antibiot.* **1990**, *43*, 1101−1106.

(18) Mori, K.; Koseki, K. Synthesis of trichostatin A, a potent differentiation inducer of Friend leukemic cells, and its antipode. *Tetrahedron* **1998**, *44*, 6013−6020.

(19) Richon, V. M.; Webb, Y.; Merger, R.; Sheppard, T.; Jursic, B.; et al. Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5705−5708.

(20) Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; et al. A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3003−3007.

(21) Breslow, R.; Belvedere, S.; Gershell, L. Development of cyto-differentiating agents for cancer chemotherapy. *Helv. Chim. Acta* **2000**, *83*, 1685−1692.

(22) Richon, V. M.; Zhou, X.; Rifkind, R. A.; Marks, P. A. Histone deacetylase inhibitors: development of suberoylanilide hydroxamic acid (SAHA) for the treatment of cancers. *Blood Cells, Mol. Dis.* **2001**, *27*, 260−264.

(23) Marks, P. A.; Richon, V. M.; Rifkind, R. A. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. *J. Natl. Cancer Inst.* **2000**, *92*, 1210−1216.

(24) Kim, Y. B.; Lee, K. H.; Sugita, K.; Yoshida, M.; Horinouchi, S. Oxamflatin is a novel antitumor compound that inhibits mammalian histone deacetylase. *Oncogene* **1999**, *18*, 2461−2470.

(25) Ohtani, M.; Matsuura, T.; Shirahase, K.; Sugita, K. (2*E*)-5-[3-[(Phenylsulfonyl)amino]phenyl]-pent-2-en-4-ynohydroxamic acid and its derivatives as novel and potent inhibitors of ras transformation. *J. Med. Chem.* **1996**, *39*, 2871−2873.

(26) Su, G. H.; Sohn, T. A.; Ryu, B.; Kern, S. E. A Novel Histone Deacetylase Inhibitor Identified by High-Throughput Transcriptional Screening of a Compound Library. *Cancer Res.* **2000**, *60*, 3137−3142.

(27) Tricyclic lactam and sultam derivatives as histone deacetylase inhibitors: Wo02062773. *Expert Opin. Ther. Pat.* **2003**, *13*, 387−391.

(28) Jung, M.; Hoffmann, K.; Brosch, G.; Loid, P. Analogues of trichostatin A and trapoxin B as histone deacetylase inhibitors. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1655−1658.

(29) Wittich, S.; Scherf, H.; Xie, C.; Brosch, G.; Loidl, P.; et al. Structure−activity relationships on phenylalanine-containing inhibitors of histone deacetylase: in vitro enzyme inhibition, induction of differentiation, and inhibition of proliferation in Friend leukemic cells. *J. Med. Chem.* **2002**, *45*, 3296−3309.

(30) Jung, M.; Brosch, G.; Kölle, D.; Scherf, H.; Gerhäuser, C.; et al. Amide Analogues of Trichostatin A as Inhibitors of Histone Deacetylase and Inducers of Terminal Cell Differentiation. *J. Med. Chem.* **1999**, *42*, 4669−4679.

(31) Breslow, R.; Marks, P. A.; Rifkind, R. Potent inducers of terminal differentiation and methods of use thereof. U.S. Patent 5,700,-811, 1997.

(32) Woo, S. H.; Frechette, S.; Abou Khalil, E.; Bouchain, G.; Vaisburg, A.; et al. Structurally simple trichostatin A-like straight chain hydroxamates as potent histone deacetylase inhibitors. *J. Med. Chem.* **2002**, *45*, 2877−2885.

(33) Glaser, K. B.; Li, J.; Aakre, M. E.; Morgan, D. W.; Sheppard, G.; et al. Transforming growth factor beta mimetics: discovery of 7-[4-(4-cyanophenyl)phenoxy]-heptanohydroxamic acid, a biaryl hydroxamate inhibitor of histone deacetylase. *Mol. Cancer Ther.* **2002**, *1*, 759−768.

(34) Curtin, M. L.; Garland, R. B.; Heyman, H. R.; Frey, R. R.; Michaelides, M. R.; et al. Succinimide hydroxamic acids as potent inhibitors of histone deacetylase (HDAC). *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2919−2923.

(35) Dai, Y.; Guo, Y.; Guo, J.; Pease, L. J.; Li, J.; et al. Indole amide hydroxamic acids as potent inhibitors of histone deacetylases. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1897−1901.

(36) Sternson, S. M.; Wong, J. C.; Grozinger, C. M.; Schreiber, S. L. Synthesis of 7200 small molecules based on a substructural analysis of the histone deacetylase inhibitors trichostatin and trapoxin. *Org. Lett.* **2001**, *3*, 4239−4242.

(37) Breslow, R.; Belvedere, S.; Gershell, L.; Miller, T. A.; Marks, P. A.; et al. Class of cytodifferentiating agents and histone deacetylase inhibitors, and methods of use thereof. U.S. Patent 6,511,990-B1, 2003.

(38) Lavoie, R.; Bouchain, G.; Frechette, S.; Woo, S. H.; Khalil, E. A.; et al. Design and synthesis of a novel class of histone deacetylase inhibitors. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2847−2850.

(39) Bouchain, G.; Leit, S.; Frechette, S.; Khalil, E. A.; Lavoie, R.; et al. Development of potential antitumor agents. Synthesis and biological evaluation of a new set of sulfonamide derivatives as histone deacetylase inhibitors. *J. Med. Chem.* **2003**, *46*, 820−830.

(40) Uesato, S.; Kitagawa, M.; Nagaoka, Y.; Maeda, T.; Kuwajima, H.; et al. Novel histone deacetylase inhibitors: *N*-hydroxycarboxamides possessing a terminal bicyclic aryl group. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1347−1349.

(41) Massa, S.; Artico, M.; Corelli, F.; Mai, A.; Di Santo, R.; et al. Synthesis and antimicrobial and cytotoxic activities of pyrrole-containing analogs of trichostatin A. *J. Med. Chem.* **1990**, *33*, 2845−2849.

(42) Massa, S.; Mai, A.; Sbardella, G.; Esposito, M.; Ragno, R.; et al. 3-(4-Aroyl-1*H*-pyrrol-2-yl)-*N*-hydroxy-2-propenamides, a new class of synthetic histone deacetylase inhibitors. *J. Med. Chem.* **2001**, *44*, 2069−2072.

(43) Perez, L. B.; Remiszewski, S. W.; Sambucetti, L. C.; Atadja, P.; Blair, K. W.; et al. Discovery and SAR of NVP-LAQ824: novel histone deacetylase inhibitor with in vitro and in vivo antitumor activity. Presented at the Meeting of the American Association for Cancer Research, San Francisco, CA, 2002; Abstract 3671.

(44) Remiszewski, S. W.; Bobadilla, M.; Bontempo, J.; Brouillard, J. F.; Cesarz, D.; et al. Preclinical efficacy, toxicology and pharmacokinetics of NPV-LAQ824, a novel histone deacetylase inhibitor. Presented at the 14th EORTC-NCI-AACR, Frankfurt, Germany, 2002; Abstract 326.

(45) Chen, J. S.; Faller, D. V. Short-chain fatty acid inhibitors of histone deacetylases: promising anticancer. *Curr. Cancer Drug Targets* **2003**, *3*, 219−236.

(46) Cummings, J. I. Short chain fatty acids in the human colon. *Gut* **1981**, *22*, 763−779.

(47) Watson, J.; Glasg, M. B. Butyric acid in the treatment of cancer. *Lancet* **1933**, 746−748.

(48) Gottlicher, M.; Minucci, S.; Zhu, P.; Kramer, O. H.; Schimpf, A.; et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J.* **2001**, *20*, 6969−6978.

(49) Jeong, M. R.; Hashimoto, R.; Senatorov, V. V.; Fujimaki, K.; Ren, M.; et al. Valproic acid, a mood stabilizer and anticonvulsant, protects rat cerebral cortical neurons from spontaneous cell death: a role of histone deacetylase inhibition. *FEBS Lett.* **2003**, *542*, 74−78.

(50) Phiel, C. J.; Zhang, F.; Huang, E. Y.; Guenther, M. G.; Lazar, M. A.; et al. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J. Biol. Chem.* **2001**, *276*, 36734−36741.

(51) Johnstone, R. W. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat. Rev. Drug Discovery* **2002**, *1*, 287−299.

(52) Warrell, R. P., Jr.; He, L. Z.; Richon, V.; Calleja, E.; Pandolfi, P. P. Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. *J. Natl. Cancer Inst.* **1998**, *90*, 1621−1625.

(53) Rephaeli, A.; Zhuk, R.; Nudelman, A. Prodrugs of butyric acid from bench to bedside: synthetic design, mechanism of action, and clinical applications. *Drug Dev. Res.* **2000**, *50*, 379−391.

(54) Suzuki, T.; Ando, T.; Tsuchiya, K.; Fukazawa, N.; Saito, A.; et al. Synthesis and histone deacetylase inhibitory activity of new benzamide derivatives. *J. Med. Chem.* **1999**, *42*, 3001−3003.

(55) El-Beltagi, H. M.; Martens, A. C. M.; Lelieveld, P.; Haroun, E. A.; Hagenbeek, A. Acetyldinaline: a new oral cytostatic drug with impressive differential activity against leukemic cells and normal stem cells−preclinical studies in a relevant rat model for human acute myelocytic leukemia. *Cancer Res.* **1993**, *53*, 3008−3014.

(56) Seelig, M. H.; Berger, M. R. Efficacy of dinaline and its methyl and acetyl derivatives against colorectal cancer in vivo and in vitro. *Eur. J. Cancer* **1996**, *32A*, 1968−1976.

(57) Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; et al. A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4592−4597.

(58) Jaboin, J.; Wild, J.; Hamidi, H.; Khanna, C.; Kim, C. J.; et al. MS-27-275, an inhibitor of histone deacetylase, has marked in vitro and in vivo antitumor activity against pediatric solid tumors. *Cancer Res.* **2002**, *62*, 6108−6115.

(59) Prakash, S.; Foster, B. J.; Meyer, M.; Wozniak, A.; Heilbrun, L. K.; et al. Chronic oral administration of CI-994: a phase I study. *Invest. New Drugs* **2001**, *19*, 1−11.

(60) Ardizzoni, A.; Loprevite, M. Histone deacylation inhibitors. *Tumori* **2002**, *88* (Suppl.), S52−S54.

(61) Fournel, M.; Trachy-Bourget, M.-C.; Yan, P. T.; Kalita, A.; Bonfils, C.; et al. Sulfonamide anilides, a novel class of histone deacetylase inhibitors, are antiproliferative against human tumors. *Cancer Res.* **2002**, *62*, 4325−4330.

(62) Wong, J. C.; Hong, R.; Schreiber, S. L. Structural biasing elements for in-cell histone deacetylase paralog selectivity. *J. Am. Chem. Soc.* **2003**, *125*, 5586−5587.

(63) Glaser, K. B.; Staver, M. J.; Waring, J. F.; Stender, J.; Ulrich, R. G.; et al. Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: Defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. *Mol. Cancer Ther.* **2003**, *2*, 151−163.

(64) Christianson, D. W.; Lipscomb, W. N. The complex between carboxypeptidase A and a possible transition-state analogue: mechanistic inferences from high-resolution X-ray structures of enzyme−inhibitor complexes. *J. Am. Chem. Soc.* **1986**, *108*, 4998−5003.

(65) Walter, M. W.; Felici, A.; Galleni, M.; Soto, R. P.; Adlington, R. M.; et al. Trifluoromethyl alcohol and ketone inhibitors of metallo-beta-lactamases. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2455−2458.

(66) Frey, R. R.; Wada, C. K.; Garland, R. B.; Curtin, M. L.; Michaelides, M. R.; et al. Trifluoromethyl ketones as inhibitors of histone deacetylase. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3443−3447.

(67) Frey, R. R.; Curtin, M. L.; Garland, R. B.; Wada, C. K.; Vasudevan, A.; et al. Electrophilic ketone-based histone deacetylase (HDAC) inhibitors as cancer chemotherapeutic agents. Presented at the 224th National Meeting of the American Chemical Society, Boston, MA, 2002.

(68) Marshall, J. L.; Rizvi, N.; Kauh, J.; Dahut, W.; Figuera, M.; et al. A phase I trial of depsipeptide (FR901228) in patients with advanced cancer. *J. Exp. Ther. Oncol.* **2002**, *2*, 325−332.

(69) Sandor, V.; Bakke, S.; Robey, R. W.; Kang, M. H.; Blagosklonny, M. V.; et al. Phase I trial of the histone deacetylase inhibitor, depsipeptide (FR901228, NSC 630176), in patients with refractory neoplasms. *Clin. Cancer Res.* **2002**, *8*, 718−728.

(70) Kijima, M.; Yoshida, M.; Sugita, K.; Horinouchi, S.; Beppu, T. Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. *J. Biol. Chem.* **1993**, *268*, 22429−22435.

(71) Komatsu, Y.; Tomizaki, K.-Y.; Tsukamoto, M.; Kato, T.; Nishino, N.; et al. Cyclic hydroxamic-acid-containing peptide 31, a potent synthetic histone deacetylase inhibitor with antitumor activity. *Cancer Res.* **2001**, *61*, 4459−4466.

(72) Gross, M. L.; McCrery, D.; Crow, F.; Tomer, K. B. The structure of the toxin from *Helminthosporium carbonum*. *Tetrahedron Lett.* **1982**, *23*, 5381−5384.

(73) Liesch, J. M.; Sweeley, C. C.; Staffeld, G. D.; Anderson, M. S.; Weber, D. J.; Scheffer, R. P. Structure of HC-toxin, a cyclic tetrapeptide from *Helminthosporium carbonum*. *Tetrahedron Lett.* **1982**, *38*, 45−48.

(74) Kim, S.-D.; Knoche, H. W. Structure of an amino acid analog of the host-specific toxin from *Helminthosporium carbonum*. *Tetrahedron Lett.* **1985**, *26*, 969−972.

(75) Tanis, S. P.; Horenstein, B. A.; Scheffer, R. P.; Rasmussen, J. B. A new host specific toxin from *Helminthosporium carbonum*. *Heterocycles* **1986**, *24*, 3423−3431.

(76) Hirota, A.; Suzuki, A.; Suzuki, H.; Tamura, S. Isolation and biological activity of Cyl-2, a metabolite of *Cylindrocladium scoparium*. *Agric. Biol. Chem.* **1973**, *37*, 643−647.

(77) Hirota, A.; Suzuki, A.; Suzuki, H.; Aizawa, K.; Tamura, S. Structure of Cyl-2, a novel cyclotetrapeptide from *Cylindrocladium scoparium*. *Agric. Biol. Chem.* **1973**, *37*, 955−956.

(78) Hirota, A.; Suzuki, A.; Suzuki, H.; Tamura, S. Characterization of four amino acids constituting Cyl-2, a metabolite from *Cylindrocladium scoparium*. *Agric. Biol. Chem.* **1973**, *37*, 1185−1189.

(79) Kawai, M.; Pottorf, R. S.; Rich, D. H. Structure and solution conformation of the cytostatic cyclic tetrapeptide WF-3161, cyclo-[L-leucyl-L-pipecolyl-L-(2-amino-8-oxo-9,10-epoxydecanoyl)-D-phenylalanyl]. *J. Med. Chem.* **1986**, *29*, 2409−2411.

(80) Umehara, K.; Nakahara, K.; Kiyoto, S.; Iwami, M.; Okamoto, M.; Tanaka, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. Studies on WF-3161, a new antitumor antibiotic. *J. Antibiot.* **1983**, *36*, 478−483.

(81) Itazaki, H.; Nagashima, K.; Sugita, K.; Yoshida, H.; Kawamura, Y.; Yasuda, Y.; Matsumoto, K.; Ishii, K.; Uotani, N.; Nakai, H.; Terui, A.; Yoshimatsu, S.; Ikenishi, Y.; Nakawaga, Y. Isolation and structural elucidation of new cyclotetrapeptides, trapoxin A and B, having detransformation activities as antitumor agents. *J. Antibiot.* **1990**, *63*, 1524−1532.

(82) Closse, A.; Huguenin, R. Isolation and structural clarification of chlamydocin. *Helv. Chim. Acta* **1974**, *57*, 533−545.

(83) Ciuffetti, L. M.; Pope, M. R.; Dunkle, L. D.; Daly, J. M.; Knoche, H. W. Isolation and structure of an inactive product derived from the host-specific toxin produced by *Helminthosporium carbonum*. *Biochemistry* **1983**, *22*, 3507−3510.

(84) Taunton, J.; Hassig, C. A.; Schreiber, S. L. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* **1996**, *272*, 408−411.

(85) Taunton, J.; Collins, J. L.; Schreiber, S. L. Synthesis of natural and modified trapoxins, useful reagents for exploring histone deacetylase function. *J. Am. Chem. Soc.* **1996**, *118*, 10412−10422.

(86) Hassig, C. A.; Tong, J. K.; Fleischer, T. C.; Owa, T.; Grable, P. G.; et al. A role for histone deacetylase activity in HDAC1-mediated transcriptional repression. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3519−3524.

(87) Singh, S. B.; Zink, D. L.; Polishook, J. D.; Dombrowski, A. W.; Darkin-Rattray, S. J.; Schmatz, D. M.; Goetz, M. A. Apicidins: novel cyclic tetrapeptides as coccidiostats and antimalarial agents from *Fusarium pallidoroseum*. *Tetrahedron Lett.* **1996**, *37*, 8077−8080.

(88) Darkin-Rattray, S. J.; Gurnett, A. M.; Myers, R. W.; Dulski, P. M.; Crumley, T. M.; et al. Apicidin: a novel antiprotozoal agent that inhibits parasite histone deacetylase. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13143−13147.

(89) Singh, S. B.; Zink, D. L.; Liesch, J. M.; Dombrowski, A. W.; Darkin-Rattray, S. J.; et al. Structure, histone deacetylase, and antiprotozoal activities of apicidins B and C, congeners of apicidin with proline and valine substitutions. *Org. Lett.* **2001**, *3*, 2815−2818.

(90) Singh, S. B.; Zink, D. L.; Liesch, J. M.; Mosley, R. T.; Dombrowski, A. W.; et al. Structure and chemistry of apicidins, a class of novel cyclic tetrapeptides without a terminal α-keto epoxide as inhibitors of histone deacetylase with potent antiprotozoal activities. *J. Org. Chem.* **2002**, *67*, 815−825.

(91) Abe, F.; Hino, M.; Mori, H.; Yoshimura, S.; Takase, S. Inhibitor of histone deacetylase. WO200008048-A2, 2000.

(92) Gupta, S.; Peiser, G.; Nakajima, T.; Hwang, Y.-S. Characterization of a phytotoxic cyclotetrapeptide, a novel chlamydocin analogue, from *Verticillium coccosporum*. *Tetrahedron Lett.* **1994**, *35*, 6009−6012.

(93) Motohiro, H.; Hiroaki, M.; Kazutoshi, S.; Shigehiro, T.; Yasuhisa, T.; et al. Cyclic Tetrapeptide and Use Thereof. WO200021979, 2000.

(94) Yoshimura, K.; Tsubotani, S.; Okazaki, K. Tan1746 compounds, their production and use thereof. JP 7196686, 1995.

(95) Colletti, S. L.; Myers, R. W.; Darkin-Rattray, S. J.; Gurnett, A. M.; Dulski, P. M.; et al. Broad spectrum antiprotozoal agents that inhibit histone deacetylase: structure−activity relationships of apicidin. Part 1. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 107−111.

(96) Furumai, R.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Yoshida, M.; et al. Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 87−92.

(97) Nishino, H.; Tomizaki, K.-Y.; Kato, T.; Nishino, N.; Yoshida, M.; et al. Synthesis of cyclic tetrapeptides containing non-natural imino acids. *Peptide Sci.* **1999**, 189−192.

(98) Colletti, S. L.; Myers, R. W.; Darkin-Rattray, S. J.; Gurnett, A. M.; Dulski, P. M.; et al. Broad spectrum antiprotozoal agents that inhibit histone deacetylase: structure−activity relationships of apicidin. Part 2. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 113−117.

(99) Ueda, H.; Nakajima, H.; Hori, Y.; Fujita, T.; Nishimura, M.; Goto, T.; Okuhara, M. FR901228, A novel antitumor bicyclic depsipeptide produced by *Chromobacterium violaceum* no. 968. *J. Antibiot.* **1994**, *47*, 301−310.

(100) Shigematsu, N.; Ueda, H.; Takase, S.; Tanaka, H. FR9011228, a novel antitumor bicyclic depsipeptide produced by *Chromobacterium violaceum* no. 968. *J. Antibiot.* **1994**, *47*, 311−314.

(101) Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K. H.; Nishiyama, M.; et al. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. *Cancer Res.* **2002**, *62*, 4916−4921.

(102) Kwon, H. J.; Owa, T.; Hassig, C. A.; Shimada, J.; Schreiber, S. L. Depudecin induces morphological reversion of transformed fibroblasts via the inhibition of histone deacetylase. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3356−3361.

(103) Pina, I. C.; Gautschi, J. T.; Wang, G. Y.; Sanders, M. L.; Schmitz, F. J.; et al. Psammaplins from the sponge *Pseudoceratina purpurea*: inhibition of both histone deacetylase and DNA methyltransferase. *J. Org. Chem.* **2003**, *68*, 3866−3873.

(104) Matsuyama, A.; Shimazu, T.; Sumida, Y.; Saito, A.; Yoshimatsu, Y.; et al. In vivo destabilization of dynamic microtubules by HDAC6-mediated deacetylation. *EMBO J.* **2002**, *21*, 6820−6831.

(105) Haggarty, S. J.; Koeller, K. M.; Wong, J. C.; Grozinger, C. M.; Schreiber, S. L. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4389−4394.