Journal of Medicinal Chemistry

Volume 45, Number 4 February 14, 2002

Letters

Inhibitors of Human Histone Deacetylase: Synthesis and Enzyme and Cellular Activity of Straight Chain Hydroxamates

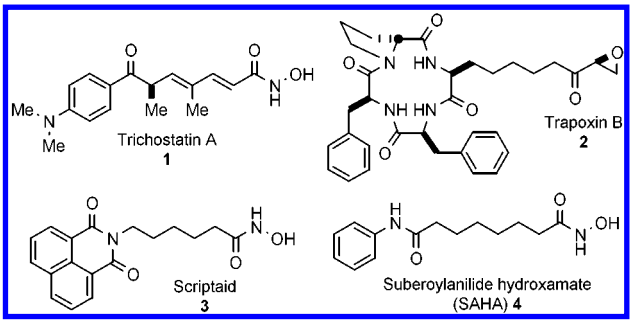
Stacy W. Remiszewski,*,§ Lidia C. Sambucetti,† Peter Atadja,§ Kenneth W. Bair,§ Wendy D. Cornell,‡ Michael A. Green,§ Kobporn Lulu Howell,§ Manfred Jung,⊥ Paul Kwon,† Nancy Trogani,§ and Heather Walker§

Oncology Research and Core Technology, Novartis Institute for Biomedical Research, 556 Morris Avenue, Summit, New Jersey 07901-1398, and Department of Pharmaceutical Chemistry, Westfälische Wilhelms-Universität Münster, Hittorfstrasse 58-62, 48149, Münster, Germany

Received September 5, 2001

Abstract: Inhibitors of histone deacetylase (HDAC) have been shown to induce terminal differentiation of human tumor cell lines and to have antitumor effects in vivo. We have prepared analogues of suberoylanilide hydroxamic acid (SAHA) and trichostatin A and have evaluated them in a human HDAC enzyme inhibition assay, a $p21^{waf1}$ (p21) promoter assay, and in monolayer growth inhibition assays. One compound, 4-(dimethylamino)-*N*-[7-(hydroxyamino)-7-oxoheptyl]-benzamide, was found to affect the growth of a panel of eight human tumor cell lines differentially.

Introduction. Reversible acetylation of nuclear histones is a major regulator of gene expression which may act by altering accessibility of transcription factors to DNA through conformational changes in the nucleosome[1] or by altering interactions with protein bromodomains.[2] A balance between histone acetyl transferase (HAT) activity and histone deacetylase (HDAC) activity exists in normal cells resulting in cell specific patterns of gene expression. Perturbation of this balance has been linked to cancer.[3] Natural product HDAC inhibitors (HDAIs) such as trichostatin A (**1**) and trapoxin B (**2**) have antiproliferative effects on tumor cells,[4,5] and reviews of synthetic and natural HDAIs as potential anticancer agents have been published.[6] The reports of the discovery of scriptaid[7] (**3**) as an HDAI and of the in vivo antitumor efficacy of the HDAI suberoylanilide hydroxamic acid[8] (SAHA, **4**) prompted us to report on the synthesis and structure−activity relationship (SAR) of analogous HDAIs in our $p21^{waf1}$ (p21) promoter assay[9] and in human tumor cell antiproliferation assays.



Chemistry. Scheme 1 outlines the preparation of SAHA analogues. Monomethyl suberate (**5**) was treated with *i*-butyl chloroformate followed by arylamines **6a**−**d**. The intermediate methyl esters were treated with $HONH_2$ in KOH/MeOH to afford hydroxamates **7a**−**d**.

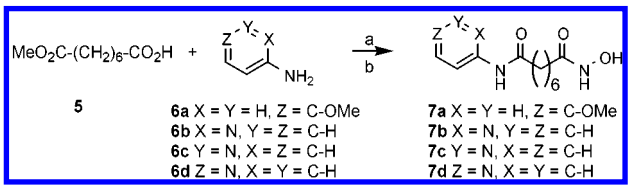
Synthesis of *N*-methyl amide **10** was accomplished by methylation of amide **8**,[10] one-pot ester hydrolysis, and coupling of the resulting acid with *O*-benzylhydroxylamine followed by *O*-debenzylation (Scheme 2). *O*-methyl hydroxylamine **11** was obtained via a similar hydrolysis/coupling scheme. Hydrazide **12** was obtained using the standard methods.

Amides **16a** and **16b** were obtained using standard methodology (Scheme 3). Benzamide **15a** was transformed to hydroxamate **16a** via the *O*-benzylhydroxamate while **15b** was converted to hydroxamate **16b** using NH_2OH in MeOH/NaOMe. Hydrogenation of **15b** followed by hydroxamate formation afforded **17**.

* To whom correspondence should be addressed. Tel: 908-277-3983. Fax: 908-277-4374. E-mail: stacy.remiszewski@pharma.novartis.com.
§ Oncology Research, Novartis.
† Present address: Xenogen Corporation, 869 Atlantic Avenue, Alameda, CA 94501.
‡ Core Technology, Novartis.
⊥ Westfälische Wilhelms-Universität.

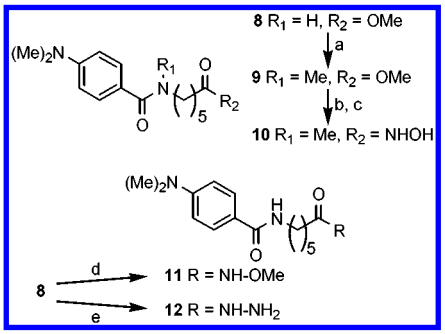
10.1021/jm015568c CCC: $22.00
Published on Web 01/17/2002

Scheme 1[a]



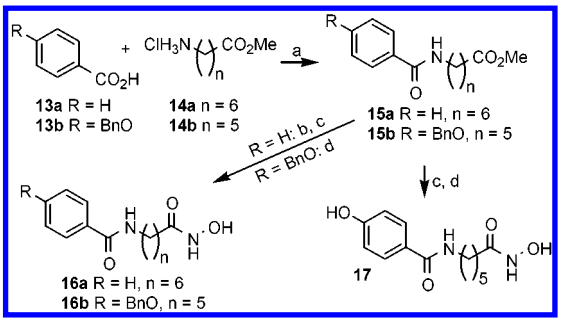
[a] Reagents: (a) **6a** → **7a**: EDCI, HOBT, *N*-methylmorpholine (NMM); all others: *i*-BuOCOCl, NMM, THF; (b) $HONH_3Cl$, KOH, MeOH.

Scheme 2[a]



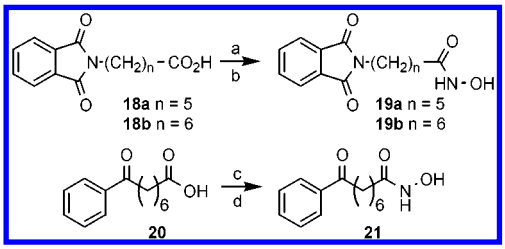
[a] Reagents: (a) NaH, MeI, THF, 0 °C to reflux; (b) LiOH, H_2O, THF then $BnONH_3Cl$, NMM, HOBT, EDCI (one pot); (c) 10% Pd/C, H_2; (d) LiOH, H_2O, THF then $MeONH_3Cl$, NMM, HOBT, EDCI (one pot); (e) H_4N_2, EtOH, reflux.

Scheme 3[a]



[a] Reagents: (a) EDCI, HOBT, NMM; (b) LiOH, H_2O, THF then $BnONH_3Cl$, NMM, HOBT, EDCI (one pot); (c) 10% Pd/C, H_2; (d) $HONH_3Cl$, NaOMe/MeOH.

Scheme 4[a]



[a] Reagents: (a) *i*-BuOCOCl, NMM; (b) pyridine, $HONH_3Cl$; (c) EDCI, HOBT, *O*-tritylhydroxyl-amine resin; (d) 30% HCO_2H/THF.

Starting from the known phthalimides **18a** and **18b**,[11] hydroxamates **19a** and **19b** were prepared via the mixed anhydride (Scheme 4). Hydroxamate **21** was synthesized using *O*-tritylhydroxylamine resin.[12]

Results and Discussion. We have profiled these compounds using partially purified HDAC enzyme obtained from H1299 cell lysate,[13] in antiproliferative assays[14] and in a p21 promoter induction assay.[9] Induction of p21 has been demonstrated using various HDAIs in several cell types.[15] The p21 promoter assay results are reported as AC_{50}, the compound concentration that results in 50% of the maximal promoter activation of a reference compound, psammaplin A.[16]

Table 1. HDA Enzyme and p21 Promoter and Growth Inhibition Data for SAHA Analogues, Trichostatin A and Trapoxin B[a]

cpd	enzyme IC_{50} (μM)	AC_{50} (μM)	H1299 IC_{50} (μM)	HCT116 IC_{50} (μM)
4[b]	0.194 (0.068)	3.1 (1.3)	7.2 (0.7)	1.9 (0.6)
7a	0.279 (0.019)	2.4 (0.8)	>10	>10
7b	0.248 (0.032)	2.9 (0.4)	>10	2.0 (1.2)
7c	0.200 (0.056)	7.9 (3.1)	>10	6.5 (0.5)
7d	0.306 (0.040)	8.9 (2.2)	>10	5.4 (0.7)
1	0.026 (0.010)	0.4 (0.3)	0.10 (0.01)	0.013 (0.004)
2	<0.005	0.010 (0.010)	0.004 (0.001)	0.002 (0.002)

[a] Values are the means of a minimum of six experiments. Numbers in parentheses are standard deviations. [b] Prepared as described in ref 17.

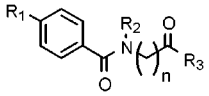
The activity for **4** and its analogues **7a**−**d** in the enzyme assay is comparable (Table 1). The p21 promoter activity of **4** and its 4-methoxy analogue, **7a**, are similar, but there is a difference in the monolayer growth inhibition data for the analogues. The methoxy compound **7a** is inactive in both the H1299 and HCT116 cells, whereas **4** is a 7.27 μM inhibitor of H1299 cell growth and a 1.91 μM inhibitor of HCT116 cell growth. The 4-pyridyl isomer **7b** is essentially equipotent to **4** in the promoter assay, 3-fold less potent in HCT116 growth inhibition, and >10 μM in H1299 growth inhibition. The 3- and 4-pyridyl isomers **7c** and **7d** are approximately 2.5-fold less potent than **4** in promoter induction, >10 μM in H1299 growth inhibition, and approximately 8.5-fold less potent in HCT116 growth inhibition. The reason for the difference in cellular activity among these compounds is unclear, but may be due to differences in cellular permeability or intracellular metabolism of the compounds.

The enzyme potency of the amide analogues of **1** is directly related to chain length, cf., Table 2, entries 1−4; the most potent compound is **23** (entry 3), where $n = 6$, at 46 nM. A variety of substitution is tolerated at the 4-position of the aryl when $n = 5$, cf., entries 6−10, except for NO_2, which resulted in a 6.4-fold loss of enzyme potency when compared to the parent 4-dimethylamino compound **22** (entry 2). Amide N-methylation (entry 5) resulted in a 1.8-fold loss of enzyme potency. Changing the hydroxamate to *O*-methylhydroxamate (entry 12) or hydrazide (entry 13) resulted in inactive compounds. In the homologous 6-carbon chain series, replacement of 4-dimethylamino (entry 3) by H (entry 11) resulted in 12-fold loss of enzyme potency.

There is a distinct dependence of p21 promoter activation on chain length within the 4-dimethylamino compound series, with $n < 5$ resulting in inactive compounds. However, **23**, where $n = 6$ (entry 3), and **24**, where $n = 7$ (entry 4), are essentially equipotent in promoter activation, despite a 3-fold difference in enzyme activity. These compounds also have similar potency in H1299 growth inhibition, ca. 1.9 μM, and are sub-micromolar in HCT116 growth inhibition.

The 6-carbon chain phthalimide **19b** is slightly more potent in enzyme inhibition than its shorter homologue **19a**, but these compounds are essentially equipotent in promoter activation (Table 3). This is in contrast to **22**

Table 2. HDA Enzyme and p21 Promoter and Growth Inhibition Data for Amides[a]



entry	cpd	R_1	R_2	R_3	*n*	enzyme IC_{50} (μM)	AC_{50} (μM)	H1299 IC_{50} (μM)	HCT116 IC_{50} (μM)
1	**21**[b]	$N(Me)_2$	H	NHOH	4	>10	>10	NT	NT
2	**22**[c]	$N(Me)_2$	H	NHOH	5	0.244 (0.038)	>10	NT	NT
3	**23**[b]	$N(Me)_2$	H	NHOH	6	0.046 (0.009)	2.1 (0.7)	1.62 (0.03)	0.21 (0.08)
4	**24**[b]	$N(Me)_2$	H	NHOH	7	0.145 (0.061)	1.6 (0.6)	2.18 (0.031)	0.50 (0.15)
5	**10**	$N(Me)_2$	Me	NHOH	5	0.443 (0.193)	>10	NT	NT
6	**25**[b]	Ph	H	NHOH	5	0.265 (0.021)	>10	NT	NT
7	**15b**	BnO	H	NHOH	5	0.220 (0.075)	>10	NT	NT
8	**16**	OH	H	NHOH	5	0.149 (0.064)	>10	NT	NT
9	**26**[b]	Cl	H	NHOH	5	0.369 (0.027)	>10	NT	NT
10	**27**[b]	NO_2	H	NHOH	5	1.566 (0.250)	>10	NT	NT
11	**15a**	H	H	NHOH	6	0.568 (0.240)	5.7 (3.4)	>10	1.04 (0.22)
12	**11**	H	H	NHOMe	5	>10	>10	NT	NT
13	**12**	H	H	$NHNH_2$	5	>10	>10	NT	NT

[a] Values are the means of a minimum of six experiments. Numbers in parentheses are standard deviations. NT = not tested. [b] Prepared as described in ref 18. [c] Prepared as described in ref 10.

Table 3. HDA Enzyme and p21 Promoter and Growth Inhibition Data for Miscellaneous Compounds[a]

cpd	enzyme IC_{50} (μM)	AC_{50} (μM)	H1299 IC_{50} (μM)	HCT116 IC_{50} (μM)
19a	0.515 (0.097)	7.5 (2.3)	NT	NT
19b	0.357 (0.017)	6.9 (1.4)	NT	NT
20	0.270 (0.025)	4.1 (1.4)	>10	1.72 (0.89)

[a] Values are the means of a minimum of six experiments. Numbers in parentheses are standard deviations. NT = not tested.

Table 4. p53 Status, Tissue Type, and Growth Inhibition for Cells Treated with **23**[a]

cell line	tissue	p53 status	IC_{50} (μM)
HCT116	colon	wt[b]	0.2 (0.08)
SW 620	colon	mutant	0.7 (0.05)
A549	lung	wt	0.8 (0.02)
MDA-MB-435S	breast	mutant	1.3 (0.04)
LS 174T	colon	mutant	1.5 (0.17)
A431	epidermoid	mutant	2.3 (0.12)
T24	bladder	mutant	3.8 (0.18)
H1299	lung	deleted	3.9 (0.51)

[a] Values are the means of a minimum of three experiments. Numbers in parentheses are standard deviations. [b] wt = wild type.

and **23** (Table 2, entries 2 and 3), where the 5-carbon chain homologue was inactive in promoter activation. Hydroxamate **21**, having an all-carbon spacer, is relatively potent in enzyme inhibition, 0.270 μM, and in promoter activation, 4.1 μM. It is 1.72 μM in HCT116 growth inhibition but >10 μM in H1299 cells.

The differential sensitivity of H1299 cells and HCT116 cells to the growth inhibitory effects of these compounds led us to test the most potent enzyme inhibitor, **23**, on a panel of eight human tumor cell lines. Table 4 lists the IC_{50}s and p53 status of the cell lines used. The effects range from growth inhibition for H1299 and MDA-MB-435S cells to cell kill for HCT116 and A549 cells (Figure 1). Annexin V and propidium iodide staining of cells with % growth < 0 indicate the cells undergo apoptosis (data not shown). These effects appear to be p53 independent, since two cell lines having wild-type p53, HCT116 and A549, undergo cell kill, as do two lines having p53 mutations, SW 620 and MDA-MB-435S. While this supports the hypothesis that p21 induction plays a role in mediating the antiproliferative effects of HDAC inhibition,[15] since p21 functions downstream of

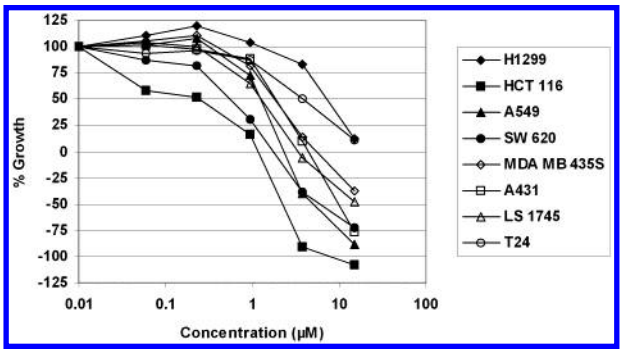


Figure 1. Percent growth as compared to vehicle treated control (% Growth) for cells treated with **23** for 72 h. Error bars were omitted for clarity; see Table 4 for an indication of reproducibility. Values below 0% cell growth indicate percentage of cell death.

p53, these data do not rule out the activation of other genes which lead to growth arrest or cell death, nor the possibility that differential HDAC isoform expression among the cell lines may contribute to the sensitivity differences.

We have developed a homology model of human HDAC-1 based on the crystal structure of a bacterial HDAC homologue (PDB code 1C3R) and have docked **22**, **23**, and **24** into this model using the program QXP (Figure 2).[19] The hydroxamic acid and alkyl chain of each molecule are essentially superimposed in the binding pocket. Differences are observed in the interactions of the substrates at the surface of the binding pocket. Compounds **23** and **24** readily achieve conformations where the amide proton of each compound forms a H-bond with the terminal carboxy group of Asp-92 (HDLP numbering) of the protein. A higher energy conformation of **22** (cf., **23** and **24**) is required for the amide proton of compound **22** to form a H-bond to the carboxy of Asp-92. Additionally, the phenyl groups of **23** and **24** have a lipophilic interaction with the alkyl chain of Glu-91, while the phenyl group of **22** is more solvent exposed. These differences may account for the differences in enzyme potency among the compounds.

While the role of HDAC-1 has not been elucidated at this time, the modeling illustrates one possible role isoform specific inhibition may play in cell growth

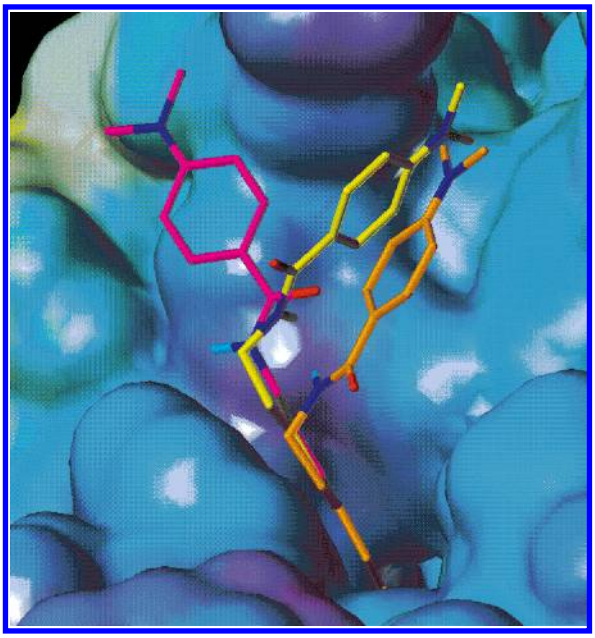


Figure 2. Model of **22** (orange), **23** (magenta), and **24** (yellow) in the human HDAC-1 homology model. The Asp-92 terminal carboxy group lies under the amide nitrogens of **23** and **24**.

inhibition. Although **22**, **23**, and **24** have similar potencies in our enzyme assay, the effect may be from each compound inhibiting a different HDAC isoform (or isoforms) in the enzyme mixture isolated from the H1299 cell lysate. In the cell, compounds that specifically inhibit HDAC isoforms affecting cell growth regulatory pathways may be more effective at inhibiting cell growth or inducing apoptosis.

Summary. For substituted benzamide analogues of trichostatin A (**1**), we have shown that enzyme and cellular potency is related to chain length, with $n = 6$ optimal. Substitution at the 4-position of the benzamide affects enzyme potency, with a 10-fold difference between the most potent and least potent homologous hydroxamates. There is a differential response among eight human tumor cell lines treated with the most potent enzyme inhibitor, **23**, in a p53 independent manner, with effects ranging from growth inhibition to cell death via apoptosis.

The analogues of SAHA (**4**) tested were all fairly potent enzyme inhibitors, and all induced the p21 promoter. However, there was a clear difference in monolayer growth inhibition among them, with **4** being the best compound.

The differences in cellular potency of compounds with similar enzyme activity may be due to one or more of a number of causes. There may be differences in cellular permeability or intracellular metabolism of the compounds. Cell-line-specific induction of pro-apoptotic genes may occur due to differential HDAC isoform expression among the cell lines. We are currently investigating the role of HDAC isoforms in regulating cell growth to determine if isoform selectivity of our inhibitors may contribute to these effects.

Supporting Information Available: Experimental procedures including characterization data for new compounds, biological methods, and a brief description of the molecular modeling are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wu, J.; Grunstein, M. 25 years after the nucleosome model: chromatin modifications. *Trends Biochem. Sci.* **2000**, *25*, 619−623.

(2) Winston, F.; Allis, C. D. The bromodomain: a chromatin-targeting module? *Nat. Struct. Biol.* **1999**, *6*, 601−604.

(3) (a) Kouzarides, T. Histone acetylases and deacetylases in cell proliferation. *Curr. Opin. Genet. Dev.* **1999**, *9*, 40−48. (b) Archer, S. Y.; Hodin, R. A. Histone acetylation and cancer. *Curr. Opin. Genet. Dev.* **1999**, *9*, 171−174. (c) Cress, W. D.; Seto, E. Histone deacetylases, transcriptional control, and cancer. *J. Cell. Physiol.* **2000**, *184*, 1−16. (d) Mahlknecht, U.; Ottmann, O. G.; Hoelzer, D. When the band begins to play: histone acetylation caught in the crossfire of gene control. *Mol. Carcinog.* **2000**, *27*, 268−271. (e) Kuo, M.-H.; Allis, C. A. Roles of histone acetyltransferases and deacetylases in gene regulation. *Bioessays* **1998**, *20*, 615−626.

(4) 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.

(5) 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.

(6) (a) Saunders: N. A.; Popa, C.; Serewko, M. M.; Jones, S. J.; Dicker, A. J.; Dahler, A. L. Histone deacetylase inhibitors: novel anticancer agents. *Expert Opin. Invest. Drugs* **1999**, *8*, 1611−1621. (b) Weidle, U. H.; Grossmann, A. Inhibition of histone deacetylases: a new strategy to target epigenetic modifications for anticancer treatment. *Anticancer Res.* **2000**, *20*, 1471−1485.

(7) 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.

(8) Butler, L. M.; Agus, D. B.; Scher, H. I.; Higgins, B.; Rose, A.; Cordon-Cardo, C.; Thaler, H. T.; Rifkind, R. A.; Marks, P. A.; Richon, V. M. Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo. *Cancer Res.* **2000**, *60*, 5165−5170.

(9) Sambucetti, L. C.; Fischer, D. D.; Zabludoff, S.; Kwon, P. O.; Chamberlin, H.; Trogani, N.; Xu, H.; Cohen, D. Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects. *J. Biol. Chem.* **1999**, *274*, 34940−34947.

(10) Schmidt, K.; Gust, R.; Jung, M. Inhibitors of histone deacetylase suppress the growth of MCF-7 breast cancer cells. *Arch. Pharm. (Weinheim)* **1999**, *332*, 353−357.

(11) Itoh, K.; Kori, M.; Inada, Y.; Nishikawa, K.; Kawamatsu, Y.; Sugihara, H. Synthesis and angiotensin converting enzyme-inhibitory activity of 1,5-benzothiazepine and 1,5-benzoxazepine derivatives. II. *Chem. Pharm. Bull.* **1986**, *34*, 2078−2089.

(12) Bauer, U.; Ho, W.-B.; Koskinen A. M. P. A novel linkage for the solid-phase synthesis of hydroxamic acids. *Tetrahedron Lett.* **1977**, *38*, 7233−7236.

(13) Kolle, D.; Brosch, G.; Lechner, T.; Lusser A.; Loidl, P. Biochemical methods for analysis of histone deacetylases. *Methods: A Companion to Methods in Enzymology* **1998**, *15*, 323−331.

(14) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589−601.

(15) Representative articles: (a) Kim, J. S.; Lee, S.; Lee, T.; Lee, Y. W.; Trepel, J. B. Transcriptional activation of p21(WAF1/CIP1) by apicidin, a novel histone deacetylase inhibitor. *Biochem. Biophys. Res, Commun.* **2001**, *281*, 866−871. (b) Burgess, A. J.; Pavey, S.; Warrener, R.; Hunter, L. J.; Piva, T. J.; Musgrove, E. A.; Saunders: N.; Parsons, P. G.; Gabrielli, B. G. Up-regulation of p21WAF1/CIP1 by histone deacetylase inhibitors reduces their cytotoxicity. *Mol. Pharmacol.* **2001**, *60*, 828−837. (c) Richon, V. M.; Sandhoff, T. W.; Rifkind, R. A.; Marks, P. A. Histone deacetylase inhibitor selectively induces $p21^{waf1}$ expression and gene-associated histone acetylation. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10014−10019. (d) Sandor, V.; Senderowicz, A.; Mertins, S.; Sackett, D.; Sausville, E.; Blagosklonny, M. V.; Bates, S. E. p21-dependent G_1 arrest with downregulation of cyclin D1 and upregulation of cyclin E by the histone deacetylase inhibitor FR901228. *Br. J. Cancer* **2000**, *83*, 817−825. (e) Kim, Y. B.; Ki, S. W.; Yoshida, M.; Horinouchi, S. Mechanism of cell cycle arrest caused by histone deacetylase inhibitors in human carcinoma cells. *J. Antibiot.* **2000**, *53*, 1191−1200. (f) Xiao, H.; Hasegawa, T.; Isobe, K. Both Sp1 and Sp3 are responsible for $p21^{waf1}$ promoter activity induced by histone deacetylase inhibitor in NIH3T3 cells. *J. Cell. Biochem.* **1999**, *73*, 291−302. (f) Archer, S. Y.; Meng, S.; Shei, A.; Hodin, R. A. p21WAF1 is required for butyrate-mediated growth inhibition of human colon cancer cells. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6791−6796.

(16) Perez, L. B.; Bair, K. W.; Dean, K.; Green, M.; Lamberson, C.; Remiszewski, S. W.; Sambucetti, L. Unpublished results. See Supporting Information for representative data.
(17) Stowell, J. C.; Huot, R. I.; Van Voast, L. The synthesis of *N*-hydroxy-*N*′-phenyloctanediamide and its inhibitory effect on proliferation of AXC rat prostate cells. *J. Med. Chem.* **1995**, *38*, 1411−1413.
(18) Jung, M.; Brosch, G.; Koelle, D.; Scherf, H.; Gerhaeuser, C.; Loidl, P. Amide analogues of trichostatin A as inhibitors of histone deacetylase and inducers of terminal cell differentiation. *J. Med. Chem.* **1999**, *42*, 4669−4679.
(19) QXP is developed and distributed by Colin McMartin of Thistlesoft in Colebrook, CT 06021; version 98.2S (1998) was used. McMartin C.; Bohacek R. S. QXP: powerful, rapid computer algorithms for structure-based drug design. *J. Comput. Aided Mol. Des.* **1997**, *11*, 333−344.

JM015568C