Development of Potential Antitumor Agents. Synthesis and Biological Evaluation of a New Set of Sulfonamide Derivatives as Histone Deacetylase Inhibitors

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Received September 4, 2002

A series of sulfonamide hydroxamic acids and anilides have been synthesized and studied as histone deacetylase (HDAC) inhibitors that can induce hyperacetylation of histones in human cancer cells. The inhibition of HDAC activity represents a novel approach for intervening in cell cycle regulation. The lead candidates were screened in a panel of human tumor and normal cell lines. They selectively inhibit proliferation, cause cell cycle blocks, and induce apoptosis in human cancer cells but not in normal cells. The structure−activity relationships, the antiproliferative activity, and the in vivo efficacy are described.

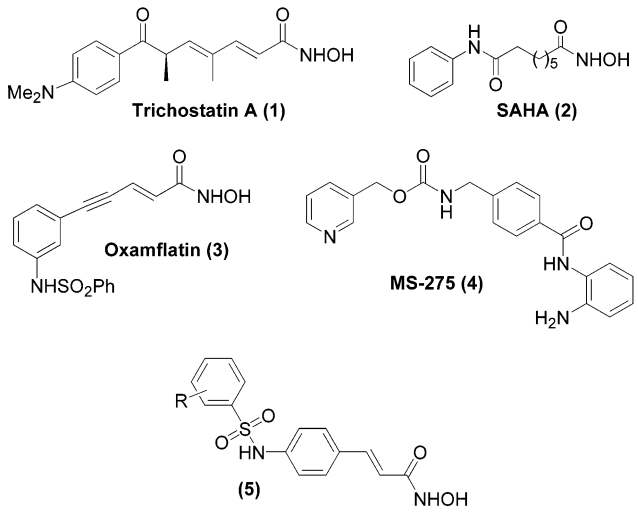
Introduction

The reversible modification of histones by acetylation, carried out by histone acetyltransferase (HATs) and histone deacetylase (HDACs) enzymes, is involved in chromatin remodeling and plays a crucial role in gene expression.[1]

Hyperacetylated histones have been associated with transcriptionally active genes. The neutralization of positively charged lysine residues in the *N*-terminal tails of core histones weakens the histone−DNA interaction, which facilitates the accessibility of a variety of factors to DNA. Conversely, HDACs mediate transcriptional repression by condensing the structure of chromatin and restricting the access of transcription factors.[2] The enzymes are involved in many cellular processes, such as cell cycle progression and differentiation, and the deregulation is associated with several types of human cancers. The modulation of HDAC activity might lead to new therapeutic opportunities against cancer.[3]

To date only a few inhibitors of histone deacetylase are known. Trichostatin A (TSA)[4] (**1**), a natural product, and other synthetic compounds such as suberoylanilide hydroxamic acid (SAHA)[5] (**2**) and analogues,[6] oxamflatin[7] (**3**), or MS-275[8] (**4**) have been reported as antitumor agent which inhibits proliferation of tumor cells in human or animal by inducing terminal differentiation of tumor cells. Inhibition of HDAC activities in cancer cells also leads to cell cycle arrest and induction of apoptosis. The cocrystal structures of the bacterial HDAC enzyme with TSA and SAHA revealed an active site consisting of a tubular pocket and a zinc binding site at the bottom of the pocket.[9] It has been shown that the hydroxamic acid moiety of these compounds is responsible for HDAC inhibition by coordinating to the zinc ion in the catalytic site. Thus, a need to identify additional HDAC inhibitors having more druglike structural features is required to achieve potent in vivo HDAC inhibitory activity.

Chart 1



Recently, the synthesis of a series of sulfonamides analogues of **5**, developed as a new class of potent HDAC inhibitors, has been published.[10] In this study, we showed that substitution on the sulfonamide or on the double bond α to the hydroxamic acid function was insufficient to confere measurable activity, whereas substitution on the aryl group provided an increase in HDAC inhibition. Herein, we focused our investigation on the design, characterization, and structure−activity relationships of these lead candidates, which were optimized through variation of the aryl substituents, sulfonamide functionality, and the zinc-chelating moieties. Poor pharmacokinetic properties associated with the hydroxamic acid group in matrix metalloproteinases inhibitors[11] prompted us to explore anilide-based sul-

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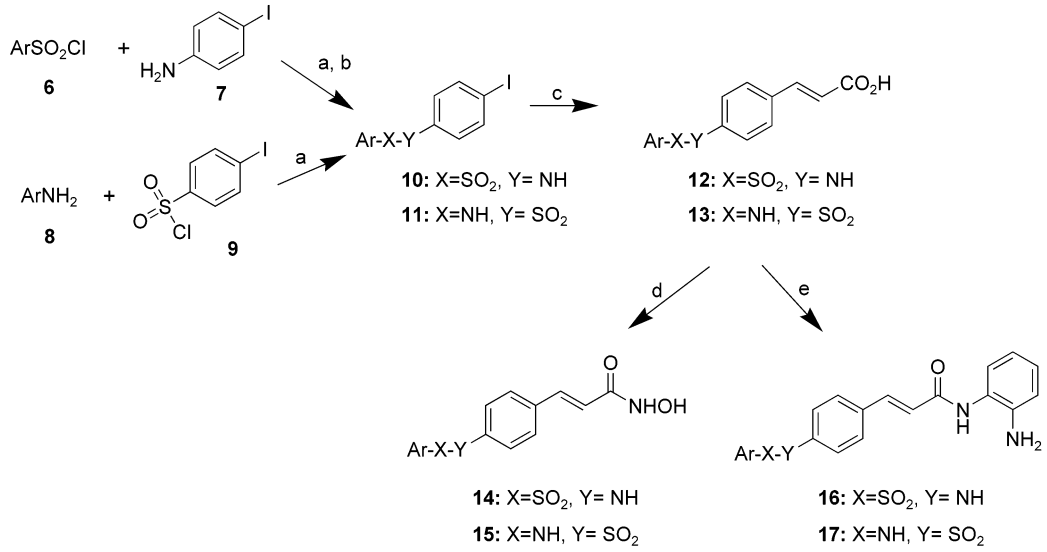
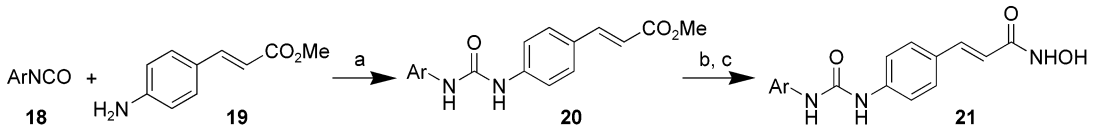
10.1021/jm020377a CCC: $25.00 © 2003 American Chemical Society
Published on Web 01/30/2003

Scheme 1[a]



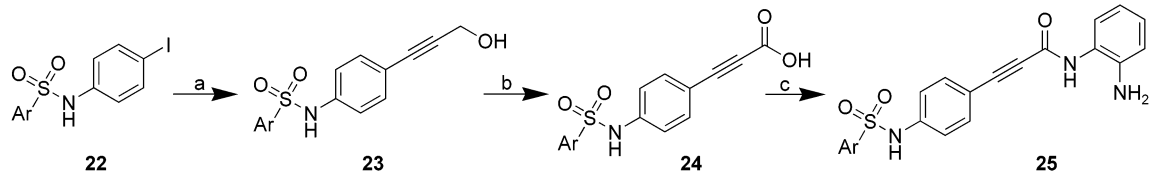
[a] Conditions: (a) Et_3N, DCM; (b) NaOMe, MeOH; (c) $CH_2{=}CHCO_2H$, $Pd_2(dba)_3$, POT, Et_3N, DMF, 100 °C; (d) i. NH_2OTHP, EDC, HOBt, DMF, 50 °C, ii. CSA, MeOH; (e) *o*-phenylenediamine, BOP, Et_3N, DMF, rt, 12 h.

Scheme 2[a]



[a] Conditions: (a) DCM; (b) NaOH, H_2O/THF; (c) EDC, HOBt, $NH_2OH{\cdot}HCl$, Et_3N.

Scheme 3[a]



[a] Conditions: (a) $(PPh_3)_4Pd$, CuI, propargyl alcohol, pyrrolidine, rt, 20 h, quantitative yield; (b) i. Dess-Martin/MeCN, rt, 16 h; ii. $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, quantitative yield; (c) *o*-phenylenediamine, BOP, Et_3N, DMF, rt, 12 h.

fonamides in order to develop more pharmaceutically desirable HDAC inhibitors.

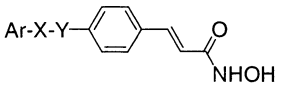
Chemistry

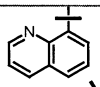
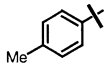
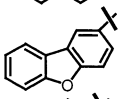
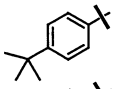
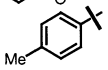
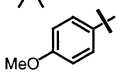
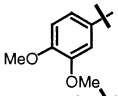
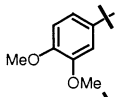
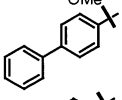
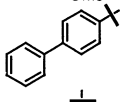
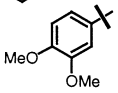
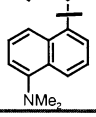
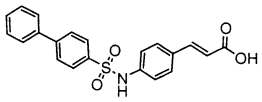
The general synthetic strategies shown in Schemes 1 and 2 were employed for the preparation of the new hydroxamic acids and anilides analogues. The arylsulfonamide **10** was prepared by treating iodoaniline **7** with an excess of aryl sulfonyl chloride **6** in the presence of triethylamine, followed by basic methanolysis. Heck coupling reaction on **10** with acrylic acid in the presence of $Pd_2(dba)_3$ and tri(*o*-tolyl) phosphine gave the requisite carboxylic acid **12** quantitatively. Under typical peptide coupling conditions (EDC/HOBt), **12** was treated with THP-protected hydroxylamine to form, after removal of the protecting group under acidic condition, the hydroxamic acid **14**. In a similar manner, the sulfanilide **16** was obtained by reacting the carboxylic acid with *o*-phenylenediamine in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) and triethylamine.

To evaluate the contribution of the sulfonamide functionality, the reverse sulfonamides **15** and **17** as well as the urea analogue **21** were synthesized. Compounds **15** and **17**, were prepared using routine procedures, starting with the condensation of arylamine **8** with sulfonyl chloride **9**. The urea derivative **21** was readily obtained by reaction of arylisocyanate **18** with the commercially available intermediate **19**, followed by ester saponification and conversion into the corresponding hydroxamic acid **21**.

To access the alkyne analogue outlined in Scheme 3, the sulfonamide **22** was subjected to a Pd−Cu catalyzed coupling reaction with propargyl alcohol to give **23** quantitatively. The primary alcohol was oxidized to the corresponding acid **24** in two steps: Dess−Martin periodinane oxidation to afford the intermediate aldehyde, followed by treatment with sodium chlorite in buffered aqueous media in the presence of a chlorine scavenger. The derivatization of **24** into the anilide **25** was performed using standard conditions.

Table 1. HDAC-1 Inhibition and Histone Acetylation Data for Compounds **14**, **15**, and **21**



Cpds	Ar	X-Y	HDAC-1 IC_{50}, μM[a]	H4-Ac EC, μM[b]	Cpds	Ar	X-Y	HDAC-1 IC_{50}, μM[a]	H4-Ac EC, μM[b]
14a		SO_2NH	0.2	5	**14i**		SO_2NH	0.5	25
14b		SO_2NH	0.3	10	**14j**		SO_2NH	0.04	1
14c		SO_2NH	0.1	5	**15a**		$NHSO_2$	0.2	1
14d		SO_2NH	0.06	5	**15b**		$NHSO_2$	0.05	1
14e		SO_2NH	0.09	1	**15c**		$NHSO_2$	0.04	1
14f		SO_2NH	0.01	1	**21**		NHCONH	1	>25
14g		SO_2NH	0.05	5		TSA		0.005	4
14h		SO_2NH	0.2	5				>25	

[a] Values are means of three separate experiments. [b] Human bladder carcinoma T24 cells treated with escalating doses of compounds **14**, **15**, and **21**. The relative effective concentration (EC, in μM) of a compound to induce the same level of the histone H4 acetylation of human T24 cells by MS-275 at 1 μM is indicated.

Results and Discussion

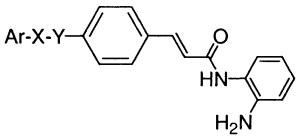
First, we report the preparation of a series of HDAC inhibitors incorporating arylsulfonamide hydroxamate as well as arylurea hydroxamate. The compounds synthesized and tested in this study for inhibitory activity on partially purified recombinant human HDAC-1 and induction of histone acetylation in human bladder T24 cancer cells are presented in Table 1.[12]

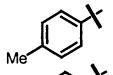
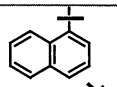
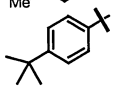
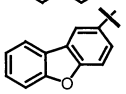
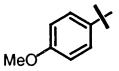
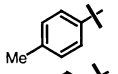
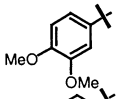
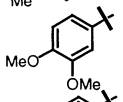
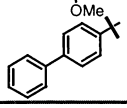
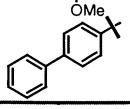
The SAR studies which focused on the effects of substitution on the aromatic ring of **5** showed that the *p*-methyl and *p*-*tert*-butyl analogues (**14b**, **14c**) as well as substitution with an electron-donating group (e.g., *p*-methoxy **14d**) were equipotent in HDAC-1 inhibitory activity with the parent compound **14a**. Replacement of the phenyl ring with a polycyclic aromatic system was well tolerated, with the biphenyl group (**14f**) slightly increasing HDAC activity. The sulfonamide functionality was also studied and it appears that similar activity is observed with the "reverse" sulfonamide derivatives **15a**, **15b**, and **15c**. Since replacement of the sulfonamide function with the urea (**21**) decreased enzymatic activity and is detrimental for histone acetylation, this series was not investigated further. All hydroxamates were more active as HDAC inhibitors as compared to the corresponding carboxylic acids, probably due to the enhanced Zn(II) coordinating properties of the hydroxamate moiety (bidentate vs monodentate binding). The use of hydroxamates as the zinc ligand provides the most effective HDAC inhibitors. However, hydroxamates are often found to be biologically labile involving a limited in vivo efficacy that has prompted additional efforts toward the discovery of new chelating groups suitable for use in HDAC templates.[11] The benzamide derivative (MS-275), which diverges structurally from known HDAC inhibitors, exhibits a decrease in efficacy in enzymatic assay compared to TSA (IC_{50} = 5 nM vs 2 μM). However, MS-275 is still active as an histone acetylation inhibitor and shows good antitumor activity against several human tumors in vivo.[8] The amine group of the benzanilide moiety might act as a hydrogen bond donor or could be involved in other electrostatic interactions, which are necessary for inhibitory activity.

The hydroxamic acid moiety of **5** has been replaced by a benzanilide function in compounds **16** and **17**, which were prepared by coupling of the carboxylic acid derivatives (**12** and **13**) with 1,2-phenylenediamine in the usual conditions. Compounds **16** and **17** were submitted for enzymatic inhibition and induction of histone acetylation and the IC_{50} values are provided in Table 2.

Substitution on the aromatic ring in **16** with electron-donating groups (**16c**, **16d**) or replacement of the aromatic ring with a polycyclic ring (**16e**−**g**) was well tolerated, although these benzamide derivatives exhibited a weaker inhibitory activity in both HDAC-1 and

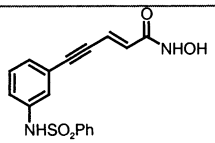
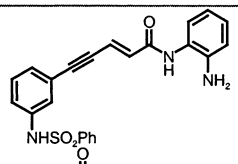
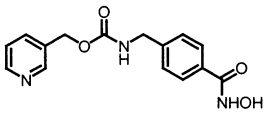
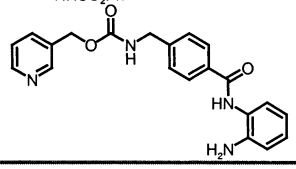
Table 2. HDAC-1 Inhibition and Histone Acetylation Data for Compounds **16** and **17**



Cpds	Ar	X-Y	HDAC-1 IC_{50}, μM[a]	H4-Ac EC, μM[b]	Cpds	Ar	X-Y	HDAC-1 IC_{50}, μM[a]	H4-Ac EC, μM[b]
16a		SO_2NH	3	5	**16f**		SO_2NH	3	10
16b		SO_2NH	1	5	**16g**		SO_2NH	0.4	10
16c		SO_2NH	1	1	**17a**		$NHSO_2$	2	2
16d		SO_2NH	4	10	**17b**		$NHSO_2$	3	3
16e		SO_2NH	1	15	**17c**		$NHSO_2$	1	5

[a] Values are the means of three separate experiments. [b] Human bladder carcinoma T24 cells treated with escalating doses of compounds **16** and **17**. The relative effective concentration (EC, in μM) of a compound to induce the same level of the histone H4 acetylation of human T24 cells by MS-275 at 1 μM is indicated.

Table 3. HDAC-1 Inhibition Data for Known Inhibitors and Analogues

Cpds	Structure	HDAC-1 IC_{50}, μM[a]	Cpds	Structure	HDAC-1 IC_{50}, μM[a]
3[b] Oxamflatin		0.1	**26**[c]		5
27[c]		3	**4**[b] (MS-275)		2

[a] Values are means of three separate experiments. [b] The synthetic method[7,8] followed a literature procedure. [c] The synthetic method was modified by derivatizing the corresponding acids into the hydroxamic acid or the anilide, respectively, using the usual conditions.

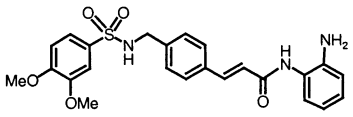
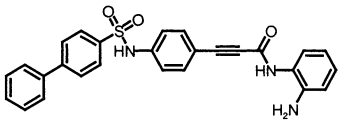
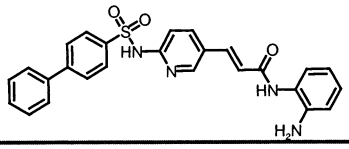
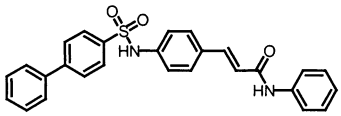
histone acetylation than the hydroxamate analogues. Similar to the hydroxamic acid derivatives, the "reverse" sulfonamide showed a comparable activity (**17a**−**c**).

To compare the effect of the benzamide group with the hydroxamate, we have compared two known histone deacetylase inhibitors (Table 3). For this purpose, the anilide derivative of oxamflatin **26** and the hydroxamate derivative of MS-275 **27** have been synthesized. Surprisingly the oxamflatin derivative, **26**, showed a 50-fold decrease in potency compare to oxamflatin whereas the hydroxamate analogue, **27**, was equipotent to the parent compound MS-275.

From these results it would appear that the bulkiness of a phenyl group in the hydroxamate **27** near the zinc binding site is less tolerated. The compounds are incorporated into the active-site pocket of the HDAC, but require a specific length to fit in the narrow, tubelike pocket. The benzanilide, which might act as a zinc-chelating moiety, or the amino substituent which is able to form a hydrogen bond or other electrostatic interaction with key amino acids, seems to be well positioned at the specific binding site. Although there is no clear evidence that benzanilide is acting at the zinc active site and an allosteric binding site cannot be ruled out. Additional studies are required to understand the exact mechanism of action of anilide-based HDAC inhibitors.

The evaluation of the distance between the sulfonamide and the anilide on enzymatic inhibition has been accomplished by the synthesis of the compounds reported in Table 4. The replacement of the phenyl ring with pyridine (**16i**) or the alkyne (**25**) were 3- to 4-fold weaker than the parent compound **16e**. Introduction of a methylene group next to the sulfonamide (**16h**) showed a 2-fold lower activity than **16d**. This result suggests that the length between the two functions of the moiety and the steric parameters would affect the enzyme inhibitory activity. Lack of an amino group at the ortho position of the benzamide (**28**) resulted in an

Table 4. HDAC-1 Inhibition Data for Compounds **16** Analogues

Cpds	Structures	HDAC-1 IC_{50}, μM[a]	Cpds	Structures	HDAC-1 IC_{50}, μM[a]
16h		9	**25**		3
16i		4	**28**		>25

[a] Values are means of three separate experiments.

Table 5. Cytotoxic Assay Results of Lead Compounds against Human Tumor and Normal Cells (IC_{50} in μM)[a]

	cell line	MS-275	TSA	**14c**	**14e**	**15b**	**16a**	**16b**	**16c**	**16d**	**16e**	**17a**	**17b**	**28**
tumor type														
bladder	T24	2.5	4	2.5	2	1	8	17	8	6	>36	6.5	7.5	>37
colon	HCT116	0.6	0.9	0.9	0.2	0.8	4	6	2.5	1	15	3.5	4	>36
	SW48	5	5	2.5	0.8	0.7	10	12	4	7	30	6.5	4.5	>37
lung	A549	8	4.5	3	2	1.5	8	7	10	9	18	9.5	12	>36
	H446	1	1.5	0.85	0.4	0.35	1	3	2	2	8	2	2.5	>37
prostate	Du145	1	3.5	2	0.75	1	2	3	5	3	15	7	6.5	>36
breast	MDAmb231	3	3.5	0.8	0.7	0.4	2		6	5	18	9	5.5	>37
	MCF-7	5	10	2.5	1.5	0.7	6	7	8	6	29	11.5	11	>37
normal cell														
fibroblast	MRHF	>37	36	11	5	3	>37	>37		>37	>25	34	>37	>36
epithelial	HMEC	>37	32	9.5	20	16	>37	37	38	>37	>36	>41	>37	>37

[a] Values are means of two experiments. [b] The IC_{50} values represent the drug concentrations producing a 50% decrease in tumor cells colony formation after 72 h of incubation.

inactive compound, suggesting that aniline is essential for inhibitory activity in this class of compounds.

We have profiled the more active compounds in antiproliferative assays and in a $p21^{WAF1/Cip1}$ promoter induction assay. The inhibitory potencies (IC_{50} values) of compounds **14**−**17** and **28**, against a variety of human tumor and normal cells, are compared with those of MS-275 in Table 5. The antiproliferative activities were determined by MTT assays.

In the hydroxamic series, compounds **14e** and **15b** exhibited potent antiproliferative activity against human cancer cells with IC_{50} values from 0.2 to 2 μM. Thus, no difference was observed between the sulfonamide **14e** and the "reverse" sulfonamide derivative **15b**. Overall, these compounds show selective growth inhibition of human cancer cells over all types of human normal cells. In the anilide series, compounds **16a** and **16d** exhibited weaker but still significant antiproliferative activity with IC_{50}'s ranging from 1 to 10 μM. A similar growth inhibition is observed with the "reverse" sulfonamide derivatives **17a** and **17b**. It is interesting that the biphenyl derivative **16e** reduced the antiproliferative activity of human cancer cells by 2- to 15-fold, while **28** could not inhibit the growth of any of the human cancer cell lines. All the sulfonamide derivatives (**16** and **17**) displayed selective growth inhibitory activities against all eight human cancer cell lines compared to normal human cell lines. Thus, these compounds have specific antiproliferative activity caused by HDAC inhibition and not by their nonspecific toxicity.

Known HDAC inhibitors were reported to transcriptionally induce $p21^{WAF1/Cip1}$, a well-known tumor suppressor.[13] Treatment of human HCT116 cancer cells with compound **16a** caused a dose-dependent increase of $p21^{WAF1/Cip1}$, while expression of cyclin A and B1 was downregulated (Figure 1). A time course study showed that the protein $p21^{WAF1/Cip1}$ was induced 8 h after addition of compound **16a** (5 μM), reached its maximum at around 48 h after the treatment and remained elevated for at least 72 h posttreatment.

The induction of histone acetylation in human bladder T-24 cancer cells is presented in Figure 2 for three of the more potent compounds HDAC-1 inhibitors from Table 2.

Compounds **16a** and **16d** induced core histone H3 and H4 acetylation at doses as low as 1 μM in a dose-dependent manner. Compound **16e** was less potent on induction of histone acetylation in whole cells. Compound **28**, which showed no activity in HDAC-1 inhibition (Table 4), did not induce histone acetylation even at 25 μM. The ability of these compounds to induce histone acetylation correlated well with their ability to inhibit HDAC-1 in vitro.

The effects of compounds **16a** and **16d** on the cell cycle were measured by flow cytometric analysis against human HCT116 cancer cells and HMEC normal cells after 16 h exposure (Figure 3). The population of G2/M-phase cells increased in a dose-dependent manner. Induction of G2/M arrest by these compounds appeared to be specific to cancer cells, since no induction of cell cycle arrest was observed in normal cells. Other HDAC inhibitors including TSA, SAHA, and FR901228 induce gene expression of $p21^{WAF1/Cip1}$ and cause G1 and G2/M cell cycle arrest. In contrast to the G1 arrest, the G2/M

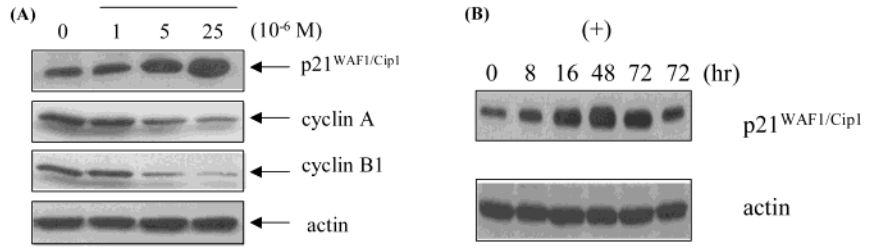


Figure 1. (A) Protein expression of $p21^{WAF1/Cip1}$, cyclin A, and cyclin B1 genes in HCT116 cells treated with compound **16a**. Human cancer HCT116 cells were treated with compound **16a** at indicated concentrations or DMSO alone for 16 h before whole cell lysates were harvested and analyzed by Western Blot. (B) Time course of $p21^{WAF1/Cip1}$ protein induction by compound **16a** at 5 μM in human cancer HCT116 cells. In both panels, the expression level of actin was analyzed to reveal protein loading in each lane.

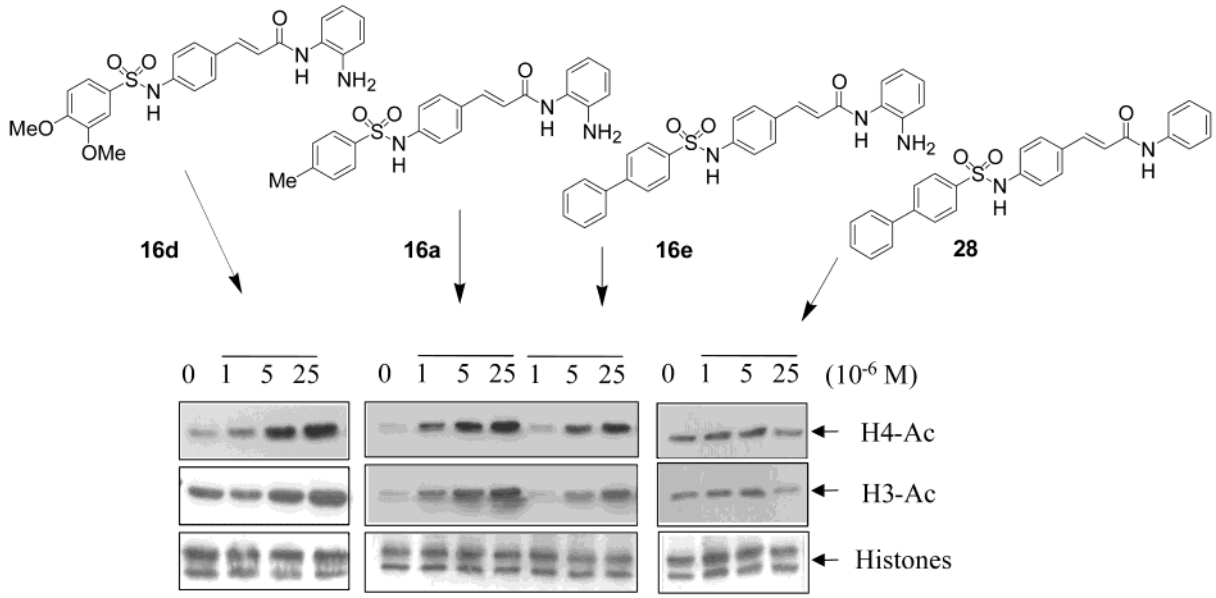


Figure 2. Human T24 cells were treated with compounds **16a**, **16d**, **16e**, and **28** at 0, 1, 5, 25 μM for 16 h. Cells were harvested, and histones were acid-extracted. Histones were analyzed by SDS−PAGE and immunoblotting with antibodies specific for either acetylated H4 or acetylated H3 histones. Histones were also stained by Coomassie Blue to reveal the amount of histones loaded on the blots.

arrest is p21-independent but is associated with significant cytotoxicity.[5,14]

Thus, these observations suggest that compounds **16a** and **16d** transcriptionally induced $p21^{WAF1/Cip1}$ through acetylation of histones and blocked tumor cell cycle progression.

Some of the HDAC inhibitors emerging from the SAR studies were evaluated for in vivo antitumor activity (Table 6). Nude mice bearing established human lung cancer tumors (A549) or human colon cancer tumors (HCT116) were used. The compounds were administered daily intraperitoneally at doses of 40 mg/kg or 50 mg/kg for 21 days. Compound **14e** showed marked antitumor effect against A549 tumors (57% of tumor growth inhibition), and compounds **16a** and **16d** suppressed tumor growth by 55% and 45%, respectively, compared with mice receiving vehicle alone. The results suggest that these sulfonamide anilides inhibit cancer cell growth and may be useful for the treatment of cancer.

In summary, a new series of sulfonamide anilide-based histone deacetylase inhibitors were discovered. Our studies demonstrate that sulfonamide anilides induce hyperacetylation of histones resulting in increased $p21^{WAF1/Cip1}$ expression and G2/M arrest of cancer cell cycle progression. This provides selective inhibition of cancer cell proliferation.

Experimental Section

Chemistry. Nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were measured on Varian 300 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS peak. MS spectra were obtained on HP1100 instrument. Elemental analyses were within ±0.4% of calculated values. Flash column chromatography were performed with silica gel (230−400 mesh). Analytical HPLC were performed on HP1100 instrument equipped with a Zorbax 50 × 4.6 mm C-8 column. The mobile phases used were A: H_2O with 0.01M EDTA + formic acid to pH = 5, B: MeOH with 0.05% formic acid, using a solvent gradient of A/B: 80/20 to 5/95. The analyses were performed at rt with a constant flow rate of 1 mL/min using a gradient elution of 0−10 min.

Synthesis and Characterization. Compounds **14**, **15**, **16**, and **17** were essentially prepared according to the same procedure. The sequence is illustrated for **14a**, followed by analytical data for **14b**−**j**, **15a**−**c**, **16a**−**i**, and **17a**−**c**.

4-(Benzenesulfonylamino)phenyl Iodide (10). To a solution of 4-iodoaniline **7** (10 g, 45.66 mmol) in CH_2Cl_2 (150 mL) were added at room-temperature Et_3N (15.88 mL, 114.15 mmol) followed by benzenesulfonyl chloride (12.19 mL, 95.89 mmol). After the mixture was stirred 4 h, a saturated sodium bicarbonate solution was added, and the phases were separated. The aqueous layer was extracted with methylene

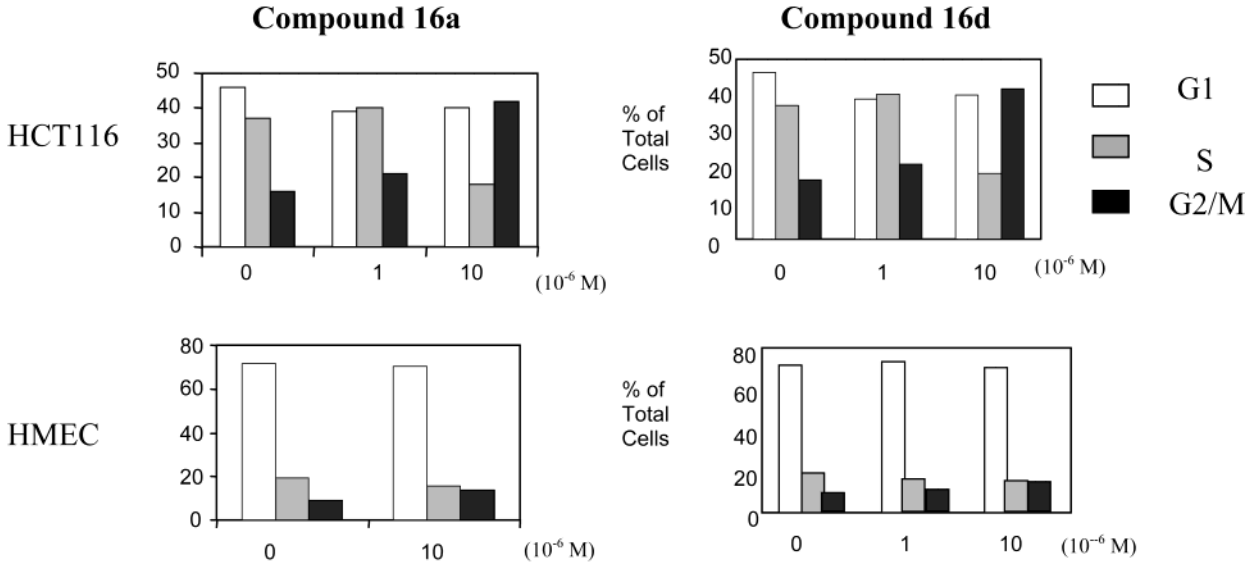


Figure 3. Cell cycle analysis of human HCT116 cancer cells and HMEC normal cells treated with compounds **16a** and **16d** for 16 h. Compound **16a** treated cells are shown in upper left (HCT116) and bottom left (HMEC) panels. Compound **16d** treated cells are shown in upper right (HCT116) and bottom right (HMEC) panels. The final concentration of DMSO in cell culture media is 1%. Cells were harvested by trypsinization. Propidium iodide stained DNA contents in fixed cells were analyzed by flow cytometry.

Table 6. In Vivo Antitumor Activity of Compounds **14**, **15**, **16**, and **17**

compd	**14e**	**15b**	**16a**	**16d**	**17a**
tumor type	A549	A549	HCT116	HCT116	HCT116
dose ip (mg/kg/day)	50	50	40	40	40
tumor growth inhibn (%)	57	37	55	45	37

chloride, and the combined organic fractions were dried over $MgSO_4$ then evaporated. The crude mixture was redissolved in methanol (100 mL) and treated with sodium methoxide (12 g, 228 mmol). The mixture was heated 1 h at 60 °C, cooled to room temperature, and quenched with 1 N HCl. The mixture was concentrated under reduced pressure, extracted with methylene chloride, dried over $MgSO_4$, and concentrated. The crude material was purified by chromatography on silica gel using methylene chloride to give the title compound (15.96 g, 97%) as yellow solid. ^{1}H NMR ($CDCl_3$) δ 9.15 (bs, 1H), 7.82 (d, J = 8 Hz, 2H), 7.68−7.51 (m, 5H), 7.05 (d, J = 8 Hz, 2H). Anal. ($C_{12}H_{10}NO_2S$) C, H, N, S.

3-[4-(Benzenesulfonylamino)phenyl]acrylic Acid (12). To a solution of sulfonamide **10** (500 mg, 1.39 mmol) in DMF (10 mL) were added tris(dibenzylidene acetone)dipalladium (0) (38 mg, 0.091 mmol), tri-*o*-tolylphosphine (25 mg, 0.083 mmol), Et_3N (483 μL, 3.48 mmol), and finally acrylic acid (114 μL, 1.67 mmol). The resulting solution was degassed and purged with nitrogen then heated overnight at 100 °C. The mixture was filtered through a Celite pad, and then the filtrate was evaporated. The residue was purified by flash chromatography using CH_2Cl_2/MeOH (95/5) as eluent yielding the title compound (415 mg, 99%) as yellow solid. ^{1}H NMR (acetone-d_6) δ 7.88−7.85 (m, 2H), 7.62−7.55 (m, 6H), 7.29 (d, J = 9 Hz, 2H), 6.41 (d, J = 16 Hz, 1H). ^{13}C NMR (acetone-d_6): 167.69, 144.31, 140.66, 140.44, 133.70, 131.26, 129.94, 129.87, 127.75, 120.73, 118.27.

***N*-Hydroxy-3-[4-(benzenesulfonylamino)phenyl]acrylamide (14a).** To a solution of **12** (200 mg, 0.66 mmol) in DMF (10 mL) were added 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (151 mg 0.79 mmol) and hydroxybenzotriazole hydrate (134 mg, 0.99 mmol). The mixture was stirred 20 min at room temperature, and then NH_2OTHP (116 mg, 0.99 mmol) was added. The resulting mixture was heated at 50 °C for 24 h, DMF was removed under reduced pressure, and the residue was dissolved in methylene chloride and washed with a saturated solution of $NaHCO_3$. The organic extract was dried over $MgSO_4$ then condensed. The crude compound was purified by flash chromatography using hexane/acetone (7/3) as solvent mixture. The residue was dissolved in methanol (10 mL), and then 10-camphorsulfonic acid (77 mg, 0.33 mmol) was added. The mixture was stirred 2 h at room temperature, and then the solvents were evaporated under reduced pressure at room temperature to avoid thermal decomposition. The crude product was purified by flash chromatography using CH_2Cl_2/MeOH (9/1) as eluent giving compound **14a** (116 mg, 55% yield) as an orange solid. ^{1}H NMR (DMSO-d_6) δ 10.69 (s, 1H), 10.54 (s, 1H), 8.98 (bs, 1H), 7.78 (d, J = 6.9 Hz, 2H), 7.65−7.53 (m, 3H), 7.42 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 16.2 Hz, 1H), 7.12 (d, J = 8.4 Hz, 2H), 6.30 (d, J = 15.6 Hz, 1H). LRMS (ESI) 319 (MH^+).

***N*-Hydroxy-3-[4-(toluene-4-sulfonylamino)phenyl]acrylamide (14b).** ^{1}H NMR (DMSO-d_6) δ 10.7 (bs, 1H), 10.45 (bs, 1H), 8.96 (bs, 1H), 7.64 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.32−7.29 (m, 3H), 7.09 (d, J = 8.4 Hz, 2H), 6.29 (d, J = 16.2 Hz, 1H), 2.30 (s, 3H). LRMS (ESI) 333 (MH^+).

***N*-Hydroxy-3-[4-(4-*tert*-butylbenzenesulfonylamino)phenyl]acrylamide (14c).** ^{1}H NMR (DMSO-d_6) δ 10.54 (s, 1H), 7.73 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 15.6 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.30 (d, J = 15.9 Hz, 1H), 1.25 (s, 9H). LRMS (ESI) 375 (MH^+).

***N*-Hydroxy-3-[4-(4-methoxybenzenesulfonylamino)phenyl]acrylamide (14d).** ^{1}H NMR (DMSO-d_6) δ 10.66 (bs, 1H), 10.37 (bs, 1H), 8.56 (bs, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 16.2 Hz, 1H), 7.10−7.03 (m, 4H), 6.27 (d, J = 15.9 Hz, 1H), 3.77 (s, 3H). LRMS (ESI) 349 (MH^+).

***N*-Hydroxy-3-[4-(3,4-dimethoxybenzenesulfonylamino)phenyl]acrylamide (14e).** ^{1}H NMR (DMSO-d_6) δ 10.70 (bs, 1H), 10.33 (bs, 1H), 8.99 (bs, 1H), 7.44−7.26 (m, 5H), 7.12 (d, J = 8.7 Hz, 2H), 7.06 (d, J = 8.4 Hz, 1H), 6.30 (d, J = 16.2 Hz, 1H), 3.78 (s, 3H) 3.75 (s, 3H). LRMS (ESI) 379 (MH^+).

***N*-Hydroxy-3-[4-(biphenyl-4-sulfonylamino)phenyl]-acrylamide (14f).** ^{1}H NMR (DMSO-d_6) δ 10.60 (s, 1H), 9.00 (s, 1H), 7.82−7.10 (m, 14H), 6.20 (d, J = 15.9 Hz, 1H). LRMS (ESI) 395 (MH^+).

***N*-Hydroxy-3-[4-(1-naphthylsulfonylamino)phenyl]-acrylamide (14 g).** ^{1}H NMR (DMSO-d_6) δ 10.94 (bs, 1H), 10.65 (bs, 1H), 8.95 (bs, 1H), 8.73−8.71 (m, 1H), 8.24−8.21 (m, 2H), 8.08−8.05 (m, 1H), 7.74−7.63 (m, 3H), 7.33−7.23 (m, 3H), 7.06−7.04 (m, 2H), 6.24 (d, J = 15.3 Hz, 1H). LRMS (ESI) 369 (MH^+).

***N*-Hydroxy-3-[4-(5-Dimethylaminonaphthalene-1-sulfonylamino)phenyl]acrylamide (14h).** ^{1}H NMR (DMSO-d_6) δ 10.91 (s, 1H), 10.62 (bs, 1H), 8.45 (d, J = 8.1 Hz, 1H), 8.36 (d, J = 8.7 Hz, 1H), 8.25 (d, J = 6.9 Hz, 1H), 7.65−7.59 (m,

2H), 7.37−7.34 (m, 2H), 7.29−7.23 (m, 2H), 7.06 (d, J = 8.7 Hz, 2H), 6.25 (d, J = 15.9 Hz, 1H), 2.80 (s, 6H). LRMS (ESI) 412 (MH^+).

***N*-Hydroxy-3-[4-(quinoline-8-sulfonylamino)phenyl]-acrylamide (14i).** ^{1}H NMR (DMSO-d_6) δ 10.63 (s, 1H), 10.36 (bs, 1H), 9.13−9.12 (m, 1H), 8.93 (bs, 1H), 8.51 (d, J = 8.1 Hz, 1H), 8.40 (d, J = 7.2 Hz, 1H), 8.28 (d, J = 8.4 Hz, 1H), 7.75−7.70 (m, 2H), 7.30−7.20 (m, 3H), 7.09 (d, J = 8.4 Hz, 2H), 6.21 (d, J = 15.9 Hz, 1H). LRMS (ESI) 370 (MH^+).

***N*-Hydroxy-3-[4-(dibenzofuran-3-sulfonylamino)phenyl]-acrylamide (14j).** ^{1}H NMR (DMSO-d_6) δ 10.63 (bs, 1H), 10.56 (s, 1H), 8.67 (s, 1H), 8.29 (d, J = 6.9 Hz, 1H), 7.89−7.85 (m, 2H), 7.75 (d, J = 8.4 Hz, 1H), 7.59 (t, J = 7.2 Hz, 1H), 7.47−7.38 (m, 3H), 7.27 (d, J = 15.6 Hz, 1H), 7.15 (d, J = 8.7 Hz, 2H), 6.25 (d, J = 15.9 Hz, 1H). LRMS (ESI) 409 (MH^+).

***N*-Hydroxy-3-(4-*p*-tolylsulfamoylphenyl)acrylamide (15a).** ^{1}H NMR (CD_3OD) δ 7.74−7.54 (m, 5H), 7.07−6.96 (m, 4H), 6.55 (d, J = 15.7 Hz, 1H), 2.25 (s, 3H). ^{13}C NMR (CD_3OD) δ 165.4, 141.6, 140.4, 139.5, 136.1, 135.9, 130.6, 129.0, 128.8, 123.1, 121.7, 20.8. HRMS (EI) calcd for $C_{16}H_{16}N_2O_4S$ 314.0725, found 314.0734.

***N*-Hydroxy-3-[4-(3,4-dimethoxyphenylsulfamoyl)phenyl]acrylamide (15b).** ^{1}H NMR (DMSO-d_6) δ 10.82 (bs, 1H), 9.95 (bs, 1H), 9.12 (bs, 1H), 7.70 (bs, 4H), 7.46 (d, J = 15.9 Hz, 1H), 6.79 (d, J = 8.7 Hz, 1H), 6.68 (s, 1H), 6.56−6.51 (m, 2H), 3.65 (s, 3H), 3.62 (s, 3H). LRMS (ESI) 379 (MH^+).

***N*-Hydroxy-3-[4-(biphenyl-4-sulfamoyl)phenyl]acrylamide (15c).** ^{1}H NMR (CD_3OD) δ 10.50 (s, 1H), 9.10 (s, 1H), 7.82 (d, J = 6.6 Hz, 2H), 7.67 (bs, 2H), 7.65−7.48 (m, 4H), 7.39 (bs, 2H), 7.30 (d, J = 7.1 Hz, 2H), 7.20 (bs, 2H), 6.56 (d, J = 15.7 Hz, 1H). ^{13}C NMR (CD_3OD-d_4) δ 165.4, 141.6, 141.4, 140.5, 139.5, 139.0, 137.9, 129.8, 129.2, 128.7, 128.6, 128.2, 127.6, 122.7, 121.7. LRMS (ESI) 395 (MH^+). HRMS (EI) calcd for $C_{21}H_{17}N_2O_4S$ 350.1089, found 350.1095 ($M - CO_2$).

***N*-(2-Aminophenyl)-3-[4-(toluene-4-sulfonylamino)phenyl]acrylamide (16a).** To a solution of acid **12a** (1.34 g, 4.22 mmol), BOP (2.05 g, 4.64 mmol), and 1,2-phenylenediamine (502 mg, 4.64 mmol) in DMF (20 mL) was added Et_3N (2.34 mL, 16.88 mmol). The mixture was stirred at room temperature under nitrogen overnight. DMF was removed under reduced pressure. The crude product was purified by flash chromatography using hexane/EtOAc (1/1) as eluent giving compound **16a** (607 mg, 35%). ^{1}H NMR (CD_3OD) δ 7.68 (d, J = 8.2 Hz, 2H), 7.55 (d, J = 15.9 Hz, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 7.19−7.12 (m, 3H), 7.03 (t, J = 7.1 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.75−6.69 (m, 2H), 2.37 (s, 3H). HRMS (EI) calcd for $C_{22}H_{21}N_3O_3S$ 407.1304, found 407.1293.

***N*-(2-Aminophenyl)-3-[4-(4-*tert*-butylbenzenesulfonylamino)phenyl]acrylamide (16b).** ^{1}H NMR (acetone-d_6) δ 9.25 (bs, 1H), 8.77(bs, 1H), 7.79 (d, J = 8.5 Hz, 2H), 7.61−7.57 (m, 3H), 7.53 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.5 Hz, 1H), 7.30 (d, J = 8.5 Hz, 2H), 6.96 (t, J = 8.2 Hz, 1H), 6.85 (d, J = 9.3 Hz, 1H), 6.82 (dd, J = 8.0, 1.4 Hz, 1H), 6.65 (dd, J = 7.7, 1.4 Hz, 1H), 4.63 (bs, 2H), 1.30 (s, 9H).HRMS (EI) calcd for $C_{25}H_{27}N_3O_3S$ 449.1773, found 449.1767.

***N*-(2-Aminophenyl)-3-[4-(4-methoxyphenylsulfonylamino)phenyl]acrylamide (16c).** ^{1}H NMR (DMSO-d_6) δ 7.77 (d, J = 8.8 Hz, 2H), 7.51 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 16.0 Hz, 1H), 7.34 (d, J = 8.8 Hz, 1H), 7.18 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.8 Hz, 2H), 6.94 (t, J = 7.4 Hz, 1H), 6.77 (m, 2H), 6.6 (t, J = 7.4 Hz, 1H), 4.95 (bs, 1H), 3.83 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 162.5, 141.5, 139.2, 138.8, 130.9, 130.2, 128.9, 128.6, 125.7, 124.7, 119.4, 116.2, 115.9, 114.5, 55.6. LRMS (ESI) 424.5 (MH^+). HRMS (EI) calcd for $C_{22}H_{21}N_3O_4S$ 423.1253, found 423.1235.

***N*-(2-Aminophenyl)-3-[4-(3,4-dimethoxybenzenesulfonylamino)phenyl]acrylamide (16d).** ^{1}H NMR (CD_3OD) δ 7.50 (d, J = 16.0 Hz, 1H), 7.41 (d, J = 7.9 Hz, 2H), 7.35 (d, J = 8.2 Hz, 2H), 7.20 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 7.9 Hz, 2H), 6.97−6.95 (m, 1H), 6.82 (d, J = 6.8 Hz, 1H), 6.69−6.65 (m, 2H), 3.81 (bs, 3H), 3.80 (bs, 3H).^{13}C NMR (CD_3OD) δ 167.0, 154.4, 150.5, 143.1, 141.9, 141.0, 132.5, 132.3, 129.9, 128.2, 126.7, 125.2, 122.4, 121.8, 120.8, 119.6, 118.7, 111.9, 110.9, 56.6. HRMS (EI) calcd 453.1358, found 453.1351.

***N*-(2-Aminophenyl)-3-[4-(biphenyl-4-sulfonylamino)-phenyl]acrylamide (16e).** ^{1}H NMR (DMSO-d_6) δ 7.91−7.81 (m, 4H), 7.63−7.58 (m, 5H), 7.48−7.43 (m, 2H), 7.39−7.33 (m, 2H), 7.24 (d, J = 8.5 Hz, 2H), 6.97 (dd, J = 9.9, 7.1 Hz, 2H), 6.79 (d, J = 7.7 Hz, 1H), 6.61 (dd, J = 7.7, 7.1 Hz, 1H), 5.01 (bs, 2H). ^{13}C NMR (DMSO-d_6) δ 162.9,141.9, 141.6, 139.8, 139.2, 137.6, 136.9, 135.8, 128.9, 128.3, 127.4, 127.3, 127.2, 126.3, 126.0, 125.5, 124.8, 123.2, 120.4, 116.2, 115.9. HRMS (EI) calcd for $C_{27}H_{23}N_3O_3S$ 469.1460, found 469.1452.

***N*-(2-Aminophenyl)-3-[4-(1-naphthylsulfonylamino)-phenyl]acrylamide (16f).** ^{1}H NMR (acetone-d_6) δ 8.81 (d, J = 8.4 Hz, 1H), 8.34 (d, J = 7.2 Hz, 2H), 8.20 (d, J = 8.1 Hz, 1H), 8.05 (d, J = 7.5 Hz, 1H), 7.75−7.59 (m, 4H), 7.53−7.41 (m, 3H), 7.23−7.07 (m, 3H), 6.89−6.86 (m, 1H), 6.75 (d, J = 15.3 Hz, 1H). LRMS (ESI) 444 (MH^+).

***N*-(2-Aminophenyl)-3-[4-(dibenzofuran-3-sulfonylamino)phenyl]acrylamide (16g).** ^{1}H NMR (DMSO-d_6) δ 9.66 (s, 1H), 8.72 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 7.94−8.00 (m, 2H), 7.82 (d, J = 8.0 Hz, 1H), 7.61 (m, 1H), 7.50−7.40 (m, 5H), 7.32 (d, J = 8.0 Hz, 1H), 7.22 (d, J = 8.0 Hz, 2H), 7.00 (m, 1H), 6.94 (m, 1H), 6.72 (d, J = 15.0 Hz, 1H). LRMS (ESI) 484 (MH^+).

***N*-(2-Aminophenyl)- 3-{4-[(3,4-dimethoxy-benzenesulfonylamino)methyl]phenyl}acrylamide (16h).** ^{1}H NMR (DMSO-d_6) δ 8.06 (d, J = 6.3 Hz, 1H), 7.55 (dd, J = 8.5, 6.8 Hz, 3H), 7.43−7.32 (m, 4H), 7.12 (d, J = 8.5 Hz, 1H), 6.97−6.59 (m, 4H), 4.99 (bs, 2H), 4.01 (d, J = 6.0 Hz, 2H), 3.86 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 163.5, 151.8, 148.6, 141.6, 139.4, 133.7, 132.1, 128.1, 127.5, 124.7, 122.7, 120.2, 116.3, 115.9, 111.1, 109.4, 55.8, 55.6, 45.9. HRMS (EI) calcd for $C_{24}H_{25}N_3O_5S$ 467.1515, found 467.1508.

***N*-(2-Aminophenyl)-3-[6-(biphenyl-4-sulfonylamino)-pyridin-3-yl]-acrylamide (16i).** ^{1}H NMR (CD_3OD) δ 8.23 (d, J = 1.9 Hz, 1H), 8.03 (bd, J = 8.5 Hz, 2H), 7.96 (dd, J = 9.1, 1.9 Hz, 1H), 7.76 (bd, J = 8.5 Hz, 2H), 7.63 (dd, J = 8.2, 1.4 Hz, 2H) 7.53 (d, J = 15.5 Hz, 1H), 7.48−7.36 (m, 3H), 7.29 (d, J = 9.1 Hz, 1H), 7.18 (dd, J = 8.0, 1.4 Hz, 1H), 7.03 (dt, J = 7.8, 1.4 Hz, 1H), 6.86 (d, J = 7.9, 1.4 Hz, 1H), 6.76 (d, J = 15.6 Hz, 1H), 6.75−6.69 (m, 1H), 4.85 (bs, 4H). ^{13}C (CD_3OD) δ 166.4, 154.7, 146.9, 146.2, 143.1, 141.1, 140.6, 138.6, 137.9, 130.1, 129.5, 128.8, 128.5, 128.3, 126.7, 125.6, 125.0, 122.1, 120.8, 119.5, 118.6, 114.9. LRMS (ESI) 471 (MH^+).

***N*-(2-Aminophenyl)- 3-(4-*p*-tolylsulfamoyl-phenyl)-acrylamide (17a).** ^{1}H NMR (DMSO-d_6) δ 7.77 (bs, 4H), 7.57 (d, J = 15.7 Hz, 1H), 7.35 (d, J = 6.9 Hz, 1H), 7.03−6.94 (m, 6H), 6.76 (d, J = 7.1 Hz, 1H), 6.59 (d, J = 6.9 Hz, 1H), 4.98 (bs, 2H), 2.19 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 162.9, 141.6, 139.8, 139.0, 137.6, 134.8, 133.6, 129.6, 128.1, 127.3, 125.9, 125.4, 124.7, 123.2, 120.7, 116.2, 115.9, 20.3. HRMS (EI) calcd for $C_{22}H_{21}N_3O_3S$ 407.1304, found 407.1301.

***N*-(2-Aminophenyl)-3-[4-(3,4-dimethoxyphenylsulfamoyl)phenyl]acrylamide (17b).** ^{1}H NMR (CD_3OD) δ 7.72 (bs, 5H), 7.20 (d, J = 8.0 Hz, 1H), 7.05 (dd, J = 7.1, 8.0 Hz, 1H), 6.93 (d, J = 15.9 Hz, 1H), 6.87 (d, J = 7.9 Hz, 1H), 6.80 (d, J = 8.7 Hz, 2H), 6.73 (d, J = 8.1 Hz, 1H), 6.60 (d, J = 8.5 Hz, 1H), 3.77 (bs, 3H), 3.74 (bs, 3H). ^{13}C NMR (CD_3OD) δ 166.2, 150.7, 148.5, 143.2, 141.7, 140.6, 140.5, 131.9, 129.2, 128.9, 128.4, 126.7, 124.9, 119.5, 118.6, 116.4, 113.2, 108.9, 56.6, 56.4. HRMS (EI) calcd for $C_{23}H_{23}N_3O_5S$ 453.1358, found 453.1351.

***N*-(2-Aminophenyl)- 3-[4-(biphenyl-4-sulfamoyl)phenyl]acrylamide (17c).** ^{1}H NMR (DMSO-d_6) δ 7.91−7.81 (m, 4H), 7.63−7.58 (m, 5H), 7.48−7.43 (m, 2H), 7.39−7.33 (m, 2H), 7.24 (d, J = 8.5 Hz, 2H), 6.97 (dd, J = 9.9, 7.1 Hz, 2H), 6.79 (d, J = 15.9 Hz, 1H), 6.61 (dd, J = 7.7, 7.1 Hz, 1H), 5.01(bs, 2H). ^{13}C NMR (DMSO) δ 162.9, 141.9, 141.6, 139.8, 139.2, 137.6, 136.9, 135.8, 128.9, 128.3, 127.4, 127.3, 127.2, 126.3, 126.0, 125.5, 124.8, 123.2, 120.4, 116.2, 115.9. HRMS (EI) calcd for $C_{27}H_{23}N_3O_3S$ 469.1460, found 469.1452.

***N*-Hydroxy-3-[4-(3-biphenyl-4-ylureido)phenyl]acrylamide (21). (i) 3-{4-[3-(3,4-Dimethoxyphenyl)ureido]phenyl}acrylic Acid Methyl Ester (20).** To a solution of 4-isocyanato-1,2-dimethoxybenzene 18 (250 mg, 1.4 mmol) in

anhydrous dichloromethane (20 mL) was added 3-(4-aminophenyl)acrylic acid methyl ester **19** (248 mg, 1.4 mmol) at room temperature and under nitrogen. The mixture was stirred at room temperature for 16 h, quenched with a solution of ammonium chloride, and extracted with dichloromethane. The organic layer was washed with water and brine and then dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (9/1) to give the desired product **20** in 75% yield (377 mg). ^{1}H NMR: (CD_3OD) δ 7.60−7.40 (m, 4H), 7.20 (s, 1H), 7.00−6.82 (m, 3H), 6.43 (d, J = 16.0 Hz, 1H), 3.99 (s, 3H), 3.80 (s, 3H), 3.75 (s, 3H). LRMS (ESI) 358 (MH^+).

(ii) *N*-Hydroxy-3-[4-(3-biphenyl-4-ylureido)phenyl]acrylamide (21). To a solution of compound **20** (786 mg, 2 mmol) in methanol (10 mL) was added at room temperature a 5% sodium hydroxide solution (10 mL). The mixture was stirred at room temperature for 4 h. The solvent was evaporated, and the residue was diluted with water. The mixture was washed with dichloromethane, acidified with 10% HCl, extracted with ethyl acetate, and then dried over $MgSO_4$. The solvent was evaporated to give the product in 73% yield (554 mg). LRMS (ESI) 341(M − H).

To a solution of carboxylic acid (316 mg, 1 mmol) in DMF (10 mL) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (248 mg 1.3 mmol) and hydroxybenzotriazole hydrate (162 mg, 1.2 mmol). The mixture was stirred at room temperature for 2 h. Hydroxylamine hydrochloride (348 mg, 5 mmol) was added followed by triethylamine (696 μL, 5 mmol). After the mixture was stirred at room-temperature overnight, the solvent was evaporated. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with saturated solution of $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (9/1) to give **21** in 35% yield. ^{1}H NMR (CD_3OD) δ 7.62−7.40 (m, 4H), 7.20 (s, 1H), 7.03−6.80 (m, 3H), 6.45 (d, J = 16 Hz, 1H), 3.80 (s, 3H), 3.75 (s, 3H). LRMS (ESI) 358 (MH^+).

***N*-(2-Aminophenyl)- 3-[4-(biphenyl-4-sulfonylamino)-phenyl]propynamide (25). (i) 4-(4-Biphenylsulfonylamino)phenyl Iodide (22).** A solution of biphenylsulfonyl chloride (3.35 g, 13.26 mmol) and 4-iodoaniline (**7**) (1.79 g, 8.15 mmol) in a mixture of acetonitrile/dichloromethane/pyridine (4/2/1, 35 mL) stirred at 0 °C under nitrogen was treated with triethylamine (4 mL) and slowly warmed to 10 °C during 16 h. Aqueous saturated sodium bicarbonate solution was then added and the mixture stirred at room temperature for 3 h, extracted with ethyl acetate, dried ($MgSO_4$), filtered, and concentrated. The crude residue was suspended in dry methanol (100 mL), warmed to 60 °C, treated with 4.4 M NaOMe solution in methanol (41 mmol), and stirred at the same temperature for 4 h. After being cooled to 0 °C, 2 N HCl was carefully added until pH = 2 and extracted with ethyl acetate, and the combined organic layers were washed (sat. $NaHCO_3$), dried, and concentrated. The crude was purified through chromatographic column on silica gel (elution 50% to 100% ether in hexanes) to afford 2.96 g (6.8 mmol, 83% yield) of sulfonamide **22**. ^{1}H NMR ($CDCl_3$) δ 7.85 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 7.58−7.56 (m, 2H), 7.55 (d, J = 8.7 Hz, 2H), 7.46−7.43 (m, 3H), 6.90 (d, J = 8.7 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 146.1, 138.9, 138.4, 137.2, 136.3, 129.9, 129.0, 128.6, 127.8, 127.7, 127.3, 123.2. LRMS (ESI): 436 (MH^+), 458 (M + Na).

(ii) Biphenyl-4-sulfonic Acid [4-(3-Hydroxyprop-1-ynyl)phenyl]amide (23). A mixture of iodosulfonamide **22** (2.63 g, 6.05 mmol), CuI (228 mg, 1.19 mmol), and $Pd(PPh_3)_4$ (376 mg, 0.325 mmol) was dissolved in degassed pyrrolidine (22 mL) at room temperature, under nitrogen and at dark. After the mixture was stirred 8 min, propargyl alcohol (1.2 mL, 20.5 mmol) was added and the mixture stirred for 20 h, then poured on saturated NH_4Cl, and extracted with ethyl acetate. The organic layer was dried (Na_2SO_4), filtered, concentrated, and purified by column chromatography on silica gel (elution 5% MeOH in DCM) to give **23** as a yelow solid (2.2 g, 100%). ^{1}H NMR (acetone-d_6) δ 7.89 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 8.7 Hz, 2H), 7.58 (d, J = 7.5 Hz, 2H), 7.50−7.20 (m, 5H), 7.39 (d, J = 7.5 Hz, 2H), 6.22 (bs, 2H), 4.37 (s, 2H). ^{13}C NMR (acetone-d_6) δ 146.4, 139.5, 139.2, 138.7, 133.3, 129.7, 129.2, 128.4, 128.1, 127.8, 120.8, 119.6, 89.5, 84.1, 50.9. LRMS (ESI) 364 (MH^+), 386 (M + Na).

(iii) Biphenyl-4-sulfonic Acid [4-(3-oxoprop-1-ynyl)-phenyl]amide. To a solution of alcohol **23** (1.37 g, 3.77 mmol) in dry acetonitrile was added Dess−Martin reagent (1.87 g, 4.41 mmol) under nitrogen and the mixture stirred at room temperature in the dark for 4 h. The reaction mixture was diluted with ethyl acetate and the oxidant quenched with a solution of $Na_2S_2O_3$ (4.8 g) in water (25 mL). The organic layer was washed (sat. $NaHCO_3$), dried ($MgSO_4$), filtered, and concentrated. Upon purification by flash chromatography on silica gel (elution 50% EtOAc in hexane), a yellow crystalline solid (930 mg, 68% yield) corresponding to the aldehyde was obtained. ^{1}H NMR (acetone-d_6) δ 9.64 (bs, 1H), 9.34 (s, 1H), 7.98 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.53−7.50 (m, 2H), 7.49 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.7 Hz, 2H), 7.39−7.27 (m, 3H). ^{13}C NMR (acetone-d_6) δ 177.4, 146.1, 141.5, 139.2, 138.7, 135.2, 129.6, 129.1, 128.3, 128.1, 127.6, 119.8, 114.8, 94.5, 89.2. HRMS (EI) calcd for $C_{21}H_{15}NO_3S$: 361.0773, found: 361.0771.

(iv) [4-(Biphenyl-4-sulfonylamino)phenyl]propynoic Acid (24). A solution of aldehyde precursor (1.03 g, 2.85 mmol) in *tert*-butyl alcohol/dichloromethane (3/1, 60 mL) was treated with 2-methyl-2-butene (15 mL) followed by a freshly prepared solution of NaH_2PO_4 (2.53 g, 18.3 mmol) and $NaClO_2$ (2.53 g, 28 mmol) in water (24 mL), which was added dropwise during 12 min. The mixture was allowed to react for 17 h at room temperature, diluted with ethyl acetate, and extracted with saturated $NaHCO_3$ and the organic layer discarded. The aqueous layer then was acidified to pH = 2 with solid $KHSO_4$ and extracted with EtOAc, and the organic phase was dried with $MgSO_4$ and concentrated, to afford the acid **24** (1.075 g, 100% yield). ^{1}H NMR (acetone-d_6) δ 9.51 (bs, 1H), 7.92 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.69 (d, J = 8.7 Hz, 2H), 7.51 (d, J = 8.7 Hz, 2H), 7.46−7.31 (m, 5H). ^{13}C NMR (acetone-d_6) δ 154.4, 146.4, 141.2, 139.7, 139.2, 134.9, 129.9, 129.8, 129.3, 128.5, 128.4, 128.0, 120.3, 115.5, 85.7. HRMS (EI) calcd for $C_{21}H_{16}NO_4S$: 378.0800, found: 378.0809.

(v) *N*-(2-Aminophenyl)- 3-[4-(biphenyl-4-sulfonylamino)phenyl]propynamide (25). A mixture of acid **24** (442 mg, 1.17 mmol) and BOP (728 mg, 1.65 mmol) was dissolved in DMF (12 mL) and treated with triethylamine (0.7 mL, 5 mmol), followed by 1,2-phenylenediamine (295 mg, 2.73 mmol). After 17 h at room temperature, the mixture was diluted with ethyl acetate and washed with aqueous 5% $KHSO_4$/brine then saturated $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude mixture was purified by flash chromatography on silica gel (elution 50% to 100% EtOAc in hexane), to give 330 mg of **25** (0.69 mmol; 59% yield). ^{1}H NMR (acetone-d_6) δ 9.43 (bs, 1H), 8.02 (d, J = 8.5 Hz, 2H), 7.93 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.47−7.34 (m, 7H), 7.21−7.17 (m, 2H). ^{13}C NMR (acetone-d_6) δ 167.2, 158.6, 146.3, 141.3, 140.9, 139.8, 139.5, 134.2, 131.0, 129.9, 129.8, 129.3, 128.7, 128.6, 128.4, 128.0, 126.8, 125.1, 122.7, 103.6, 99.1. HRMS (EI) calcd for $C_{27}H_{21}N_3O_3S$: 467.1304, found: 467.1307.

5-(3-Benzenesulfonylaminophenyl)-pent-2-en-4-ynoic Acid (2-Aminophenyl)amide (26). ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.51 (t, J = 7.5 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 7.29−6.95 (m, 7H), 6.86 (d, J = 15.6 Hz, 1H), 6.73 (d, J = 8.6 Hz, 2H), 6.50 (d, J = 15.6 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 163.61, 140.45, 138.97, 137.26, 132.79, 132.28, 129.17, 128.87, 128.04, 127.24, 126.80, 125.29, 125.22, 123.81, 123.13, 121.90, 121.54, 119.38, 117.95, 96.19, 86.99. LRMS (ESI) 418 (MH^+).

(4-Hydroxycarbamoylbenzyl)carbamic Acid Pyridin-3-ylmethyl Ester (27). ^{1}H NMR (DMSO-d_6) δ 8.57 (bs, 1H), 8.52−8.50 (m, 1H), 7.94−7.86 (m, 2H), 7.76 (bd, J = 8.1 Hz, 1H), 7.69−7.65 (m, 1H), 7.42−7.37 (m, 1H), 7.30−7.25 (m, 2H), 5.70 (s, 2H), 4.23 (d, J = 8.7 Hz, 2H). LRMS (ESI) 302 (MH^+).

***N*-Phenyl-3-[4-(biphenyl-4-sulfonylamino)phenyl]acrylamide (28).** ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 8.5 Hz, 2H), 7.65−7.55 (m, 5H), 7.50−7.30 (m, 6H), 7.10 (d, J = 8.8 Hz, 3H), 6.90 (d, J = 8.8 Hz, 3H), 6.45 (bd, J = 15.0 Hz, 1H). LRMS (ESI) 455 (MH^+).

Biology. Production of Recombinant HDAC1 Enzyme. Human HDAC1 cDNA was generated by RT-PCR reactions using primers complementary to the 5′ and 3′ coding sequence of human HDAC1 gene (Genbank accession number U50079). Error-free HDAC1 cDNA were inserted either into pcDNA3.1 vector (Invitrogen) or into the pBlueBAC (Invitrogen) vector, both with Flag epitope tagged at the C-terminus of the protein. Insect High Five cells (Invitrogen) were used to produce recombinant HDAC1. HDAC1 recombinant enzymes were partially purified by a Q-Sepharose column (Pharmacia) followed by purification using a column with anti-Flag M2 affinity gels (Sigma), according to manufacturer's instructions.

HDAC Enzyme Assay. [^{3}H]-Labeled acetylated histones were prepared in Jurkat-T cells as described[12] and used as HDAC enzyme substrates. Assay was performed as in the literature.[12] Briefly, small molecule inhibitors diluted in DMSO at various concentrations were preincubated with recombinant HDAC1 enzyme for 30 min at 4 °C in buffer containing 40 mM Tris-Cl, pH 7.6, 20 mM EDTA, and 50% glycerol. At 37 °C, [^{3}H]-labeled acetylated histones were added into the reaction mixture. Incubation time for HDAC1 enzyme reaction was 10 min. Reaction was stopped, and released [^{3}H] acetic acid was extracted and quantified by scintillation counting. The 50% inhibitory concentrations (IC_{50}) for inhibitors were determined by analyzing dose−response inhibition curves.

Western Blot Analysis. Whole cell extracts or acid extracted histones prepared from inhibitor-treated cells were analyzed by SDS−PAGE. Proteins were transferred on the PVDF membrane and probed with various primary antibodies. Primary antibodies were ordered from Santa Cruz Biotechnology except for acetylated H4 or acetylated H3 antibodies (Upstate Biotechnology), or antibodies against $p21^{WAF1/Cip1}$ (Transduction Laborotories). Horseradish peroxidase-conjugated secondary antibodies (Sigma) were used and the enhanced chemiluminescence (ECL, Amersham) was followed for detection.

MTT Assay. The MTT assay is based on the reduction of yellow colored 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide by mitochondrial dehydrogenase of metabolically active cells to a purple formazan that can be measured spectrophotometrically. Compounds at various concentrations were added to cells plated in 96-well plates. Cells were incubated for 72 h at 37 °C in 5% CO_2 incubator. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide, Sigma) was added at a final concentration of 0.5 mg/mL and incubated with the cells for 4 h before an equal volume of solubilization buffer (50% *N*,*N*-dimethylformamide, 20% SDS, pH 4.7) was added onto cultured cells. After overnight incubation, solubilized dye was quantified by colorimetric reading at 570 nM using a reference at 630 nM. OD values were converted to cell numbers according to a standard growth curve of the relevant cell line. The concentration which reduces cell numbers to 50% of those of DMSO-treated cells is determined as MTT IC_{50}.

In Vivo Antitumor Activity. CD1 immune-deficient (nude) mice were housed in microisolator cages and received food and water ad libitum. A549 human nonsmall cell lung cancer and HCT116 human colon tumors were maintained by serial passage in CD1 nude mice. In each experiment test mice weighing 18−22 g were implanted with tumor fragments (30 mg) from a single donor tumor bearing animal. When tumors reached an average volume of 100 mm^3, animals were randomized and separated into treatment groups of six animals per group and dosing initiated. Compounds dissolved in DMSO were administered daily (7 days/week) by intraperitoneal injection for 21 days. Body weights and tumor volumes were monitored three times weekly. Calculations of tumor growth inhibition (%T/C) were performed as described previously.[15]

Acknowledgment. The authors would like to thank Isabelle Dupont and Eric Chan for cloning and expression of the HDAC enzyme.

Supporting Information Available: HPLC purity data on final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM020377A